UNIVERSITÉ DE LILLE

École Doctorale Biologie-Santé

THÈSE

Pour l'obtention du grade de

DOCTEUR DE L'UNIVERSITÉ DE LILLE

Spécialité : Neuroscience

# Genetic and diet-induced disruption of tanycyte transcytosis: molecular mechanisms, metabolic consequences and pharmacological therapy

*Metformin strikes again*

Eleonora Deligia

Thèse présentée et soutenue à Lille, le 18 juin 2024

Composition du jury:

| | | |
|---|---|---|
| Dr. Catherine Postic | Directeur de Recherche, CNRS UMR8104, Paris | Presidente |
| Dr. Serge Luquet | Directeur de Recherche, CNRS UMR8251, Paris | Rapporteur |
| Dr. Daniela Cota | Directeur de Recherche, INSERM U862, Bordeaux | Examinatrice |
| Dr. Amandine Gautier-Stein | Chercheur, INSERM U1213, Lyon | Examinatrice |
| Dr. Marc Baroncini | Neurochirurgien, CHU, Lille | Directeur de Thèse |
| Dr. Monica Imbernon | Senior Lecturer in Metabolism, Queen Mary University London, London UK | Co-encadrant de Thèse |
| Dr. Vincent Prevot | Directeur de Recherche, Lille Neuroscience & Cognition, INSERM U1172, Lille | Co-Directeur de Thèse |

# Table of content

**Table of content……...…1**
**Abbreviations……..……7**
**Abstract……9**
**Resume……...10**

***Chapter 1: Brain orchestrates energy homeostasis ……...12***

**Preface……..……13**
**I. Energy homeostasis……...13**
1. Energy intake…….....…….…14
2. Energy expenditure…….....…….…….14
3. Nutrient partitioning…….....…….15

**II. Central regulation of energy homeostasis……..……16**
1 Homeostatic control of metabolism…….....……..……17
1.1. Hypothalamus…….....……...……17
1.1.1. Arcuate nucleus of the hypothalamus (ARH ) …….....……18
1.1.1.i Melanocortin system……..……19
1.1.1.i.i. AgRP neurons……..……...……19
1.1.1.i.ii. POMC neurons …….……20
1.1.1.i.iii. Other ARH neurons……...…21
1.1.2. Paraventricular nucleus of the hypothalamus (PVN) …....……21
1.1.3. Ventromedial nucleus of the hypothalamus (VMN) …….....…….…22
1.1.4. Dorsomedial nucleus of the hypothalamus (DMN) …….....……..…22
1.1.5. Lateral hypothalamus (LH) …….....…….……22
1.2. Brainstem.....…….....……..……23
2. Hedonic control of metabolism…….....……..……24

***Chapter 2: The dialogue between brain and body…….……26***

**I. CNS to periphery communication……27**
1. The autonomic nervous system…….....…...……27
1.1. Sympathetic nervous system…….....……..……27
1.2. Parasympathetic Nervous System …….....……..……28
1.3. Enteric Nervous System (ENS) …….....……..……28
2. ANS implication in energy homoeostasis…….....……..……29
2.1. Communication with the liver…….....……29
2.2. Communication with the pancreas…….....……..……30
2.3. Communication with the muscle…….....……30
2.4. Communication with the white adipose tissue …….....……..…31
2.5. Communication with brown adipose tissue…….....……..……31

**II. Periphery to CNS communication……..33**
1. Nutrients……...……33
1.1. Glucose ……..……33
1.2. Fatty acids ……..……34
1.3. Amino acids ……...……...……34
2. Hormones playing a role in metabolism……35
2.1. Gastro-intestinal-derived hormones……...…35
2.1.1. Ghrelin……..……....…35
2.1.2. Cholecystokinin.....……36
2.1.3. Peptide YY…..……...……...……36
2.1.4.Gastric inhibitory polypeptide……....…….……36
2.1.5. Glucagon like peptide-1…….……...……...……37
2.1.6. Oxyntomodulin……...……37
2.2. Pancreas-derived hormones……...……...…….……38
2.2.1. Insulin……..…….....……38
2.2.2. Glucagon……..…….....……38
2.3. Adipose-derived hormones……..…….....……39
2.3.1. Adiponectin……..…….....……39
2.3.2. Resistin…….....……39
2.3.3. Leptin…….....……40

**III. Leptin……..……42**
1. Structure and production of leptin…….....42
2. Leptin Receptor…….....……43
2.1. JAK/STAT3……...……44

2.2. SHP2/MAPK…… 44
2.3. PI3K……45
2.4. AMPK……45
3. Role of Leptin in Central Energy Metabolism Regulation……46
3.1. Leptin action on ARC neuronal population……46
3.2. Leptin action on PVH neuronal population……47
3.3. Leptin action on VMH and the DMH neuronal population……47
3.4. Leptin action on LH neuronal population……47
3.5. Leptin action on extrahypothalamic areas neuronal population……48
4. Leptin in memory and cognition……48

***Chapter 3: Blood-borne molecules access to the brain……49***

**I. How do the metabolic signals access the brain? ……50**
1. Blood-Brain Barrier……50
1.1. Blood-brain barrier passage: transcytosis……52
2. Blood-cerebrospinalfluid barrier……53
2.1. Choroid Plexus……54
2.2. Circumventricular organs……54
2.2.1. Median eminence, knocking on brain's door……56

**II. Tanycytes……58**
1. Classification……58
1.1. Tanycytes of the dorsomedial part of the ARC, VMH and DMH……58
1.2. Tanycytes in the ventromedial part of the ARC……59
1.3. Median eminence tanycytes……59
2. Tanycytes and CSF clearance……60
3. Tanycytes orchestrate central metabolic control……60
3.1. Neural progenitor cells involved in energy metabolism: tanycytes……61
3.2. Control of neuroendocrine TRH secretion……61
3.3. Tanycyte plasticity involved in seasonal metabolic changes……62
3.4. Tanycytes as metabolic sensors and processors……62
3.5. Tanycytes architects of the median eminence……64
3.6. Tanycytes as hormone transporters: focus on leptin……65

*Chapter 4: Metabolic disorders and the brain…….....……69*

**I. Obesity……70**

1. Genetic factors…….....…….....……71
2. Obesity and brain inflammation…….....…….....……71
3. Obesity and leptin…….....…….....……72
    3.1. Leptin resistance…….....…….....……73
4. Tanyctes and obesity…….....…….....……75

**II. Diabetes……....……76**

1. Inflammation role…….....…….....……77
2. Diabetes and the brain…….....…….....……77
3. Tanycytes and diabetes…….....…….....……78

**III. Anti-obesity treatments……....……80**

1. The long road to an effective treatment: historical perspective……..…….....……80
    1.1. Greco-Roman Medicine – Hindu tradition – Islamic tradition…….....…..……80
    1.2. A renaissance of anti-obesity treatments…….....……81
    1.3. Contemporary medicine……...…….....……81
        1.3.1. Thyroid hormones……..…….....……82
        1.3.2. Dinitrophenol.…….....…….....……82
        1.3.3. Amphetamine: 1932-1968…….....……82
        1.3.4. Anorectic Sympathomimetic Drugs: 1950-1997…….....……83
        1.3.5. Lorcaserin…….....……84
        1.3.6. Cannabinoid Receptor Antagonists…….....……84
        1.3.7. Dopamine D1/D5 Receptor Modulators for Weight Loss.…....……..84
        1.3.8. Drugs that modify nutrients absorption ……....……85
2. Anti-diabetic drugs against obesity…….....……85
    2.1. Metformin…….....……85
    2.2. Alpha-Glucosidase Inhibitors…….....……88
    2.3. SGLT-2 Inhibitors…….....……89
    2.4. GLP-1 Receptor Agonists…….....……89
        2.4.1. Liraglutide…….....……90
        2.4.2. Semaglutide…….....……90
        2.4.3. Tirzepatide…….....……92

3. Tanycytes: novel therapeutic targets for metabolic diseases' treatment…….....……93
3.1. Rescue of tanycytic leptin transport reverses the DIO mice phenotype.....…….....……93
3.2. Endozepines…….....……94
3.3. Tanycytes transport Liraglutide…….....……94
3.4. Tanycytes play a role in the brain access of GLP1R agonist dependent on glycaemia……95

***Chapter 5: Objectives …….....……96***

***Chapter 6: Material and methods …….....……98***

Animals……...…….99
Stereotaxic delivery of TAT-Cre and AAVs…….....……...…….99
Pharmacological treatments……...…….....…..100
Isolation of hypothalamic tanycytes using fluorescence-activated cell sorting…….……100
Quantitative RT-PCR analyses...……...……100
Indirect calorimetry study……..... 100
Hormone measurements ……101
Lipids measurements……..101
Immunoblot analyses of liver and white adipose tissue…….....101
Glucose tolerance tests……102
Insulin tolerance test……...……102
Homa-IR…….……102
Mouse pancreatic islet isolation and culture ……..102
RNA extraction, measurement and profiling of pancreatic islets……..... 103
Immunofluorescence of pancreatic sections…….....103
Immunohistochemistry and analysis……..... 104
Primary culture…….....……..... 104
Western blot …….....……..... .....105
Leptin sensitivity assay…….....……..... 106
In vivo microdialysis…….....……..... 106
Statistics…….....……..... .....106

***Chapter 7: Results ……...……108***

Objective 1: To decipher the physiological consequences in energy homeostasis after the specific impairment of tanycytic exocytosis in chow and DIO mice.……109

1. Botulinum neurotoxin type B expression in adult hypothalamic tanycytes promotes body weight gain while increasing carbohydrate consumption…….111

2. BoNT/B expression in tanycytes blocks blood-borne leptin transport into the hypothalamus…….115

3. BonT/B expression in hypothalamic tanycytes alters peripheral lipid homeostasis……..118

4. Expression of BoNT/B in tanycytes promotes glucose intolerance and insulin resistance associated with pancreatic beta cell compensation……121

Objective 2: To determine whether metformin requires tanycyte function to mediate its anti-obesity and anti-diabetic effect.....125

1.Metformin induces body weight loss by decreasing food intake……..…127

2.Metformin chronic treatments restore leptin transport into the MBH of DIO mice..130

3. Metformin anti-obesity action is dependent on LepR expression in tanycytes……...……..……132

4. Metformin activates ERK and metformin-boosted tanycytic leptin transport is AMPK-independent……...……135

5.Acute but not chronic metformin treatment ameliorates glucose tolerance and insulin sensitivity in 8-week HFD fed mice……138

**Chapter 8: Discussion and conclusion ……...……141**

**I - Inhibition of transcytosis in tanycytes induces feeding, adiposity and glucose intolerance …….....……142**

**II - Metformin anti-obesity effect is dependent on tanycyte-mediated leptin transport…….....……144**

**Conclusion…….....……149**

**Bibliography……150**

**Acknowledgements……208**

## Abbreviations

AD: Alzheimer's Disease
AP: Area postrema
OVLT: Organum vasculosum of the lamina terminalis
ATP: Adenosine tri-phosphate
BBB: Blood-brain barrier
BCSFB: Blood-Cerebrospinal fluid barrier
BoNTB: Botulinum neurotoxin serotype B expressing mouse line
BoNT/B: Botulinum neurotoxin serotype B
BSA: Bovine serum albumin
CP: Choroid plexus
CSF: Cerebrospinal fluid
CVO: Circumventricular organs
DAPI: 4'-6-diamidino-2-phenylindol
DARPP-32: Dopamine and cAMP-regulated phosphoprotein
DIO: Diet-induced obesity
Dio2: Deiodinase 2
Dio3: Deiodinase 3
DMN: Dorsomedial hypothalamic nuclei
DNA: Deoxyribonucleic acid
EEA1: Early Endosome Antigen 1
EGFP: Enhanced green fluorescent protein
EMA: European Medicines Agency
ERK: Extracellular signal-regulated kinase
FDA: Food and Drug Administration
FGF21: Fibroblast growth factor 21
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
GDF15: Growth differentiation facto 15
GFAP: Glial fibrillary acid protein
GFP: Green fluorescent protein
GK: Glucokinase
GLAST: Glutamate Aspartate transporter
GPCR: G protein-coupled receptor
GPR50: G protein-coupled receptor 50
HPA: Hypothalamic-pituitary-adrenal axis
HPT: Hypothalamic-pituitary-thyroid axis
HRP: Horseradish peroxidase
KATP: ATP-dependent potassium channel

ME: Median eminence
MBH: Mediobasal hypothalamus
MRI: Magnetic resonance imaging
mRNA: messenger ribonucleic acid
NMDA: N-methyl-D-aspartate
NPY: Neuropeptide Y
NSC: Neural stem cell
OXT: Oxytocin
PBS: Phosphate buffer saline
PFA: paraformaldehyde
PG: Pineal gland
PGE2: Prostaglandin E2
PLVAP: Plasmalemmal vesicle associated protein
POMC: Pro-opiomelanocortin
RNA: Ribonucleic acid
SCO: subcommissural organ
SFO: Subfornical organ
SNARE: Soluble N-ethymaleimide sensitive factor attachment protein receptor
T3: Tri-iodothyronin
T4: Thyroxin
TBS: Tris buffer saline
TRH: Thyrotropin-releasing hormone
TRHR1: Thyrotropin-releasing hormone receptor 1
TSH: Thyroid stimulating hormone
UPS: Ubiquitin-proteasome system
VAMP1/2/3: Vesicle-associated membrane protein
VEGF-A: Vascular endothelial growth factor A
Wt: wild type
ZO1: Zona occludens 1

## Abstract

Obesity is a growing epidemic in the world, characterized by an imbalance between food intake and energy expenditure. Disruption of the body-brain communication, essential for maintaining glucose and energy homeostasis, arises from obesogenic and genetic factors, leading to metabolic disorders. The adipokine leptin controls energy homeostasis by reducing food intake and increasing energy expenditure through its action in the hypothalamus, however in the context of diet-induced obesity (DIO) circulating leptin quickly fails to reach its target neurons in the mediobasal hypothalamus (MBH). Interestingly it has been shown that tanycytes, which are ependymoglial cells lining the floor of the third ventricle, shuttle leptin into the MBH in an extracellular-regulated kinase (ERK) and a leptin receptor (LepR) dependent manner. Indeed, in DIO mice, the reactivation of ERK rescues leptin central transport and in mice in which leptin receptor is selectively knocked out in tanycytes ($Lepr^{TanKO}$), leptin entry in the brain is impaired.

In addition, we now establish that the inhibition of vesicle-associated membrane proteins (VAMP1-3)-mediated molecules release with targeted expression of botulinum neurotoxin type B (BoNT/B) in tanycytes induces a prediabetic state. In mice on a standard diet, selective expression of BoNT/B in tanycytes blocks leptin transport into the brain, resulting in increased food intake, abdominal fat deposition, and heightened leptin levels. Concurrently, it favors fatty acid storage and leads to glucose intolerance and insulin resistance.

Furthermore, we assessed whether metformin treatment could impact on leptin-transport in DIO mice. Indeed, metformin is a weight loss promoting anti-diabetic drug known both to increase leptin sensitivity and to induce ERK phosphorylation. Firstly, we show that metformin boosts the release of leptin previously taken up by tanycytes in a model of tanycyte primary culture. In vivo, we observe that a chronic metformin treatment significantly induces body weight loss and reduces food intake in DIO mice. Hypothalamic microdialysis approaches showed that this metformin-induced reduction in body weight was associated with the rescue of leptin transport into the brain. Intriguingly, knocking out selectively LepR, but not the AMP-regulated kinase AMPK, a known metformin target, in tanycytes blunted both the ability of metformin to rescue leptin shuttles and to induce weight loss in DIO mice, suggesting that the anti-obesity effects of metformin are mainly mediated by its action on tanycytes.

These findings underscore the central role of tanycytes in brain-periphery communication, highlighting their potential implications in type 2 diabetes and their possible action modulation as a therapeutic strategy against overweight and obesity.

## Résumé

L'obésité est une épidémie mondiale croissante qui se caractérise par un déséquilibre entre l'apport alimentaire et la dépense énergétique. Des facteurs obésogènes et génétiques perturbent la communication entre le corps et le cerveau, essentielle au maintien de l'homéostasie du glucose et de l'énergie, ce qui entraîne des troubles métaboliques. L'adipokine leptine contrôle l'homéostasie énergétique en réduisant la prise alimentaire et en augmentant la dépense énergétique par son action dans l'hypothalamus. Cependant, chez les souris souffrant d'obésité induite par l'alimentation (DIO), la leptine circulante n'atteint pas ses neurones cibles dans l'hypothalamus médiobasal (MBH). Il a été démontré que les tanycytes, cellules épendymogliales qui tapissent le plancher du troisième ventricule, font la navette entre la leptine et le MBH par un mécanisme qui implique la formation d'hétérodimères par les récepteurs de l'EGF et de la leptine (LepR), et la voie de la kinase régulée extracellulaire (ERK). En effet, chez les souris DIO, la réactivation de l'ERK sauve le transport central de la leptine et chez les souris dont le récepteur de la leptine ou de l'EGFR est sélectivement désactivé dans les tanycytes, l'entrée de la leptine dans le cerveau est altérée.

En outre, nous avons maintenant établi que l'inhibition de la libération de molécules médiée par les protéines membranaires associées aux vésicules (VAMP1-3) avec l'expression ciblée de la neurotoxine botulique de type B (BoNT/B) dans les tanycytes induit un état prédiabétique. Chez les souris maigres, l'expression sélective de BoNT/B dans les tanycytes a bloqué le transport de la leptine dans le cerveau, ce qui a entraîné une augmentation de la prise alimentaire, un dépôt de graisse abdominale et une élévation des taux sériques de leptine. Parallèlement, elle favorise le stockage des acides gras et entraîne une intolérance au glucose et une résistance à l'insuline.

En outre, nous avons évalué si le traitement à la metformine pouvait avoir un impact sur le transport de la leptine chez les souris DIO. En effet, la metformine est un médicament amaigrissant et antidiabétique connu pour augmenter à la fois la sensibilité à la leptine et la phosphorylation ERK. Tout d'abord, nous avons montré que la metformine stimule la libération de la leptine précédemment absorbée par les tanycytes dans les tanycytes en culture primaire. Chez les souris DIO, nous avons observé qu'un traitement de 2 semaines à la metformine réduisait significativement le poids corporel et la prise alimentaire. En effectuant une microdialyse dans le MBH, nous avons montré pour la première fois que la metformine rétablissait le transport de la leptine dans le MBH, altéré chez les souris DIO. De manière intrigante, en désactivant sélectivement dans les tanycytes la LepR, mais pas la kinase régulée par l'AMP AMPK, une cible connue de la metformine, la capacité de la metformine à sauver le transport de leptine et à réduire le poids corporel chez les souris DIO a été émoussée. Ces résultats suggèrent que les effets anti-obésité de la metformine sont principalement médiés par son action sur les tanycytes, en améliorant le transport central de la leptine

Ces résultats soulignent le rôle central des tanycytes dans la communication cerveau-périphérie, mettant en évidence leurs implications potentielles dans le diabète de type 2 et l'obésité, et la possibilité de les cibler pour développer de nouvelles stratégies thérapeutiques pour le traitement des troubles métaboliques.

# *Chapter 1: The brain orchestrates energy homeostasis*

## Preface

The fine regulation of energy homeostasis relays on the neuroendocrine dialogue in between the central nervous system and the peripheral organs.
If we interpret this dialogue as a theatre play, it would be a colossal piece of art with hundreds of comedians. There are protagonists and extras, but each role is essential for the success of the piece and if the lines are not well exchanged, the risk of a fiasco is high.
In this manuscript we will explore the actors of energy homeostasis, what happens when things start to get wrong and how to rectify this.

## I. Energy homeostasis

Every living organism has to obey the laws of thermodynamics. According to what is known as the law of conservation of energy, the amount of energy stored in an isolated system does not change unless the energy input or output of the system also changes (Lopez et al., 2016).
The weight of an individual is normally stable for a given amount of time, meaning that the amount of food eaten is balanced by the amount of energy burnt. There is a basic drive in each living organism that monitors the optimal food intake, a biological system that seeks to maintain consistency of body weight, an evolutionary advantage related to our hunter-gatherers past (Pontzer et al., 2012). The set point of weight is part of a larger concept that comprises all the functions of the body. Already in the 19th century, the physiologist Claude Bernard theorized the presence of critical parameters to be kept in a constant physiological range. From the ancient Greek: "standing still" the term homeostasis was first used in the third decade of the 20th century by Walter Cannon (Cannon, 1945). In biology, the term acquires the meaning of a dynamic equilibrium that the living being must preserve to stay alive. The organism encounters both external and internal variations that can induce changes in the healthy parameters range of instances such as pH, temperature, hydration, ion concentration and energy. This leads to a loss of homeostasis, to which the body tries to compensate to restore the right parameters range.
The first to mention a lipostatic control of body weight, was Gilbert Kennedy, who, in the 1950s, understood that energy balance is regulated by continuous hormonal feedback that communicates the energy state of the organism to the central nervous system (Kennedy, 1953).
The energy balance of an organism is the ability to maintain its stable energy store through the regulated exchange of food intake and energy expenditure with the outside world through metabolic processes. When the balance is altered, we can encounter pathological states. An exacerbated state of positive energy balance normally develops into obesity (a subject on which

we will focus in detail later on), while a negative energy balance net can reflect an anorexic phenotype.

## 1.Energy intake

Animals get access to energy by food, catabolizing reduced carbon compounds that serve as substrate for the synthesis of adenosine-triphosphate (ATP), the body's energy currency (Dunn & Grider 2023). Energy is either burnt or stored in the body mainly as disposable glycogen or adipose tissue (Markan et al., 2010). While later in the text we will focus on the regulation of food intake, let's here introduce the presence of nutrients, the actual source of energy that can be differentially stored in the body.

**Carbohydrates** are the major source of energy in the diet, however, due to the chemical characteristics of glycogen (heavy and osmotically active) its storage in the liver and muscle is very limited. Daily carbohydrate intake corresponds to around 50-100 % of daily reserves (compared to 1% for fat or protein), which means that in a short period of hours or days glucose stores fluctuate intensively. When excess carbohydrate intake is very high, activation of de novo lipogenesis can occur. De novo lipogenesis is a highly regulated metabolic pathway restricted to liver and the adipose tissue (Björntorp, & Sjöström, 1978) which converts excess of carbohydrates into fatty acids that are then esterified to store triacylglycerols (TGs). These TGs could later provide energy via β-oxidation.

**Protein** intake is about 15 % of the diet and its storage represents one-third of the total energy stored in the human body. Daily protein intake is equivalent to just over 1 % of the total protein reserves contained in the body (Galgani & Ravussin, 2008). Protein stores increase in size only in response to growth stimuli such as growth hormone, androgens, physical exercise or weight gain.

Unlike all other nutrients, **fat** stores can be virtually unlimited (Schutz et al., 1989, Flatt et al., 1985). As for proteins, daily fat intake represents less than 1% of total fat stores, yet fat storage contains 6 times more energy than protein storage (Bray, 19991). The function of the fat depot is to store energy and to report its energy variation via hormonal signals on which we will focus in following sections.

## 2.Energy expenditure

When we talk about energy expenditure, we need to consider the physical activity, the basal metabolism and the thermogenesis.

Physical activity accounts for 8-15% of total energy expenditure in humans (Ravussin et al., 1986). It encompasses both the involuntary and voluntary physical activity of each organism, the latter being highly variable. The main fuel used for muscle contraction is glucose, however,

during fasting or if the stored glycogen is exhausted, the muscle begins to consume fatty acids mobilized from the white adipose tissue (Kelley et al., 1999).

Basal metabolism is the energy used for the basic sustenance of the organism in a resting state: cellular metabolism and physiological maintenance of organ functions. During a day the basal metabolic rate varies, being higher during the active phase than at the resting phase. Basal metabolism is increased after a meal due to a hormonally controlled process called dynamic action of food (diet-induced thermogenesis) (Frayn, 2013). In fact, the influence of thyroid hormones in modulating basal metabolism has been known for more than a century; dysregulation of the thyroid axis leads to significant alterations in energy balance (Silva, 2003, Lopez et al., 2013).

Although thermogenesis could be considered as part of basal metabolism, the importance of this process in temperature maintenance in homeothermic organisms deserves a separate section because of the fine regulation of the process. Brown adipose tissue is responsible for carrying out the process of dissipation of energy in the form of heat. The uncoupling protein 1 (UCP1), present on the inner membrane mitochondria of different tissues, acts to dissipate the electrochemical gradient associated with the mitochondrial respiratory chain, producing energy in the form of heat, transforming chemical energy in thermic one (Ricquier et al., 1991). Thermogenesis is fundamental for body thermoregulation in newborns, hibernal mammals and probably it plays a role in the case of hypercaloric diets (Cannon & Nedergaard, 2004).

## 3.Nutrient partitioning

Efficient utilization, storage, and conversion of nutrients in peripheral tissues, collectively known as nutrient partitioning, are essential for adapting to nutritional and metabolic demands and are vital for sustaining a healthy energy equilibrium (Manceau et al., 2020). Changes in this process during periods of nutrient surplus can negatively impact glucose and lipid regulation, leading to the onset of obesity and type 2 diabetes. Nutrient partitioning represents a sophisticated, interconnected process regulated by hormonal and neural cues. Neural regulation pivots on the brain's ability to detect circulating metabolic signals and orchestrate adaptive neuroendocrine and autonomic reactions (Denis et al., 2014, Manceau et al., 2020).

## II. Central regulation of energy homeostasis

Energy homeostasis requires fine regulation between energy intake, storage and expenditure. Intake, nutritional partitioning and energy storage are controlled by neuroendocrine circuits located in different areas of the central nervous system (CNS) such as the hypothalamus or the brainstem. This system is based on a constant flow of information from peripheral organs with metabolic activity (gastrointestinal tract, adipose tissue, pancreas, liver, etc.) to the CNS which responds in turn, transmitting an adapted response to the peripheral organs involved.
During food intake, food is perceived by the taste, the smell and the sight, hinting to a connection to somatosensory neurons (Rolls, 2007). Secondly, once food is ingested, neurons receive gut-derived signals and finally hormonal signals that communicate the energy state of the organism. The identification of the central mechanism of energy homeostasis starts exactly with the identification of neurocircuitry targeted by peripheral hormones (Tschöp et al., 2000; Brüning et al., 2000).

Food intake is managed by the dynamic interaction of homeostatic and hedonic systems (Figure 1; McCutcheon & Williams, 2021; Lutter & Nestler, 2009; Campos, Port & Acosta, 2022).
The food intake behaviour by homeostatic circuits is driven by blood-borne molecules and gastrointestinal vagal-afferents, that will inform the hypothalamus and brainstem about energy status and nutrient availability. In addition, homeostatic regulation is controlled by appetitive cues from the gut, adipose tissue and pancreas, conveyed to the brain by the vagus nerve. It controls energy balance by increasing the stimulus to eat when energy stores are depleted.

Conversely, hedonic regulation of food intake is influenced by motivation and reward and it increases the desire to assume highly palatable food in moments of energy abundance (Lutter & Nestler, 2009). It acts through the same dopaminergic neural circuits that are studied for the issue of drug abuse and it can highly cross-talk and overlap with the homeostatic system (Rossi & Stuber, 2018).

In the research projects that complies my doctorate thesis, I mostly covered the physiology of the homeostatic control of energy balance. Thus, the hedonic mechanisms that can also control energy balance will be briefly described, although the interplay between the two systems is not ignored.

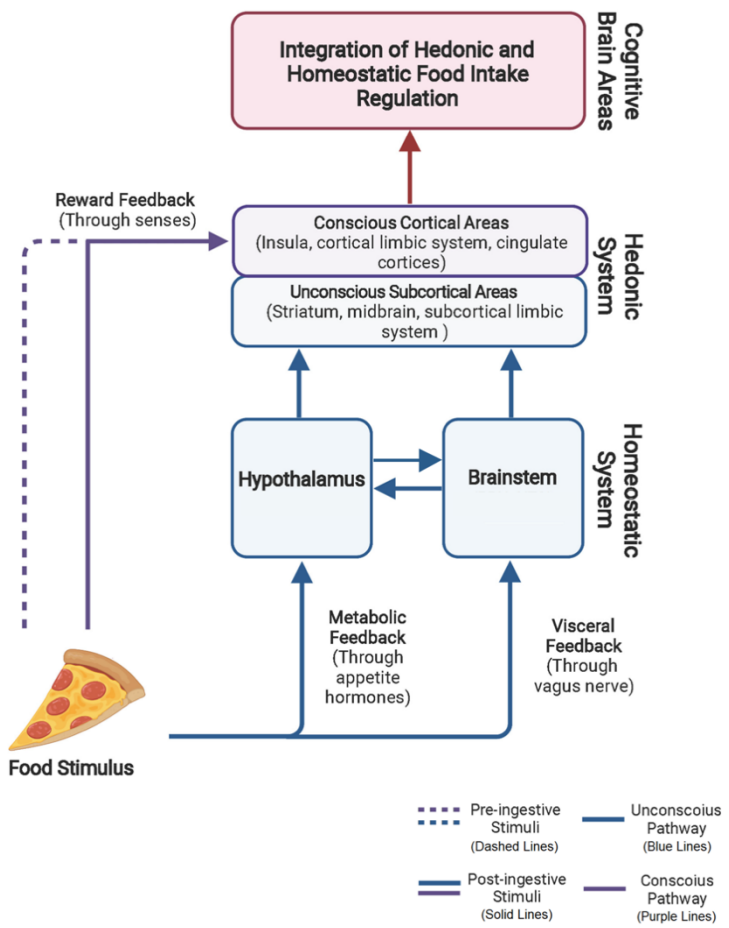



***Figure 1. Homeostatic and hedonic networks of food intake regulation.***
*Food stimuli, perceived during the pre-ingestive and ingestive phases, are conveyed to the CNS through a conscious pathway. In addition, the arrival of the food in the gut lumen, stimulates the release of gut hormones according to the macronutrient composition of the food. This peripheral cue informs the CNS of the energy intake. Furthermore, the distention of the gut is sensed by the vagus nerve and projected to the brainstem.*
*The unconscious metabolic and visceral signals and the conscious reward signal are integrated in brain areas associated and projected to higher cognitive brain centres to regulate eating behaviour (Modified from Campos, Port, & Acosta, 2022).*

## 1. Homeostatic control of metabolism

### 1.1 Hypothalamus

The master neuroendocrine regulator of homeostasis is the hypothalamus.
Literally "beneath the thalamus", it is a region of the diencephalon structurally constituted by distinct nuclei. In the hypothalamic–adenohypophyseal axis, the parvocellular neurons secret releasing/inhibiting hormones that target the anterior pituitary gland through the hypophyseal portal system. The adenohypophysis, in turn, secretes tropic hormones that stimulate the endocrine glands' hormone secretion to regulate normal organs´ functioning. Magnocellular hypothalamic neuroendocrine cells instead, release their neurohormones directly in the general circulation through the posterior lobe of the pituitary gland (Clasadonte et al., 2018, Figure 2). The hypothalamus entertains a feedback relationship with the target organs in the body. Hormones and nutrients are sensed by the hypothalamus. After processing the information, the brain will generate a response driven by efferent nerves to peripheral metabolic organs, modulating their glucose and lipid metabolism to control energy homeostasis.

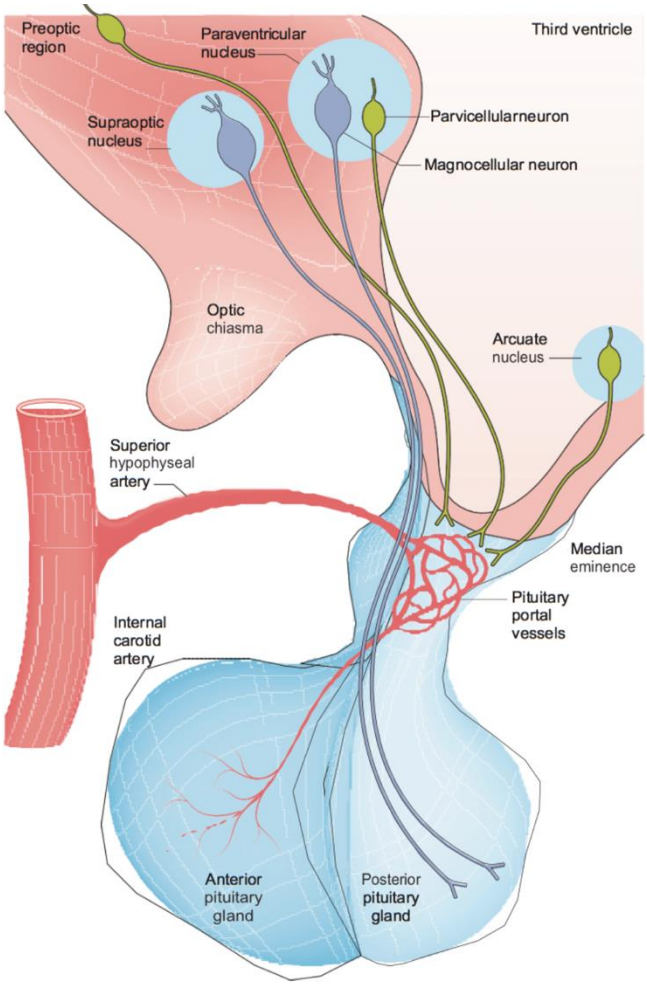



***Figure 2. Hypothalamus and hypophysis.***
*In blue the magnocellular neurons secreting oxytocin and vasopressin with cells body in the paraventricular nucleus and sopraoptic nucleus and axonal terminals in the neurohypophysis. In green the parvicellular neurons secreting thyrotropinreleasing hormone (paraventricular nucleus), corticotropin-releasing hormone (paraventricular nucleus), dopamine (arcuate nucleus), growth hormone-releasing hormone (arcuate nucleus), gonadotropin-releasing hormone (preoptic area) and somatostatin (periventricular nucleus) into the pituitary portal vessel, to reach and stimulate the adenohypophysis (from Clasadonte et al., 2018).*

The discovery of the involvement of the hypothalamus in the regulation of energy homeostasis goes back to the lesion studies conducted in the 40's and 50's of the 20th century. Lesions in the ventromedial (VMN) nucleus of the hypothalamus induced an avid hyperphagia in rats (Brobeck, Tepperman & Long, 1943) defining, back in the days, the VMN as the "satiety centre" of the brain.
The knowledge on the subject massively evolved since that moment. Hundreds of studies tried to describe the circuitries that control metabolism, expanding the involvement in energy balance regulation to several hypothalamic nuclei and neuronal circuits.

### 1.1.1. Arcuate nucleus of the hypothalamus

The arcuate nucleus (ARC) is situated next to the 3rd ventricle in its more ventral portion, next to the median eminence and below the ventromedial nucleus. Its privileged location in close contact with blood-borne molecules (as more deeply discussed later) makes it the home of first-order neurons, meaning the first neurons to respond to circulating signals, and the most studied neurocircuitry controlling metabolism.

### 1.1.1.i Melanocortin system

The complex and still not completely undisclosed neurocircuitry named the melanocortin system is the peripheral neuronal interface of systemic metabolism regulation. The cell bodies of this neurocircuit are located in the ARH and their projections get in contact with other hypothalamic and extrahypothalamic neuronal populations to modulate energy homeostasis (Figure 3).

This neurocircuit is characterized by signalling pathways driven by the five G protein-coupled melanocortin receptors MCR1 to MCR5 (Mountjoy et al., 1992; Cone, 2005). In the CNS, MC4R is broadly expressed, contrarily to MC3R, restricted to POMC and AgRP neurons (Jegou et al., 2000) and its modulation by agonist and antagonist neuropeptides (notably α-MSH and AgRP, respectively) (Figure 4).

Following the Taoist notion of a ying-yang system, two antagonist neuronal populations control the modulation of the MCR4 receptor: the agouti-related peptide (AgRP) and Pro-opiomelanocortin (POMC) expressing neurons. Notably, α-MSH (POMC product) and AgRP act, respectively, as agonist and competitive antagonist of the aforementioned receptor.

#### 1.1.1.i.i. AgRP neurons

Due to their activation by deficient energy state and orexigenic hormones such ghrelin, AgRP neurons promote food intake, whereas anorexigenic hormones such leptin and insulin, inhibit their activity. In addition to AgRP, which is only expressed in the ARH (Broberger at al., 1998), these neurons are also known to co-express and secrete the neuropeptide Y (NPY), a molecule expressed throughout the brain, but most densely present in the ARH (Gehlert et al., 1987). This population is chief among the 'anabolic' effector neurons (Morton & Schwartz, 2001).

The ablation of AgRP neurons in adult mice causes a lean and hypophagic phenotype (Gropp et al., 2005; Bewick et al., 2005) starvation and death (Luquet et al., 2005) and its optogenetic and pharmacogenetic inhibitions negatively modulate food intake (Krashes et al., 2011). Contrarily, the activation of those neurons through the same techniques promotes feeding behaviour (Krashes et al., 2011).

The orexigenic effects of NPY are mediated by postsynaptic receptors Y1 and Y5 within the PVN (Nguyen et al. 2012, Sohn et al. 2013) and directly onto POMC neurons, that express the NPY-Y1 receptor (Broberger et al., 1999).

When active, AgRP neurons release γ-Aminobutyric acid (GABA), the most abundant fast-acting neurotransmitter in the ARH (Horvath et al., 2017; van den Pol, 2003), which directly tonically hyperpolarize the neighbouring POMC neurons, inhibiting their activity (Atasoy et al., 2012) (Figure 4).

AgRP- and NPY-containing fibers synapse mainly onto the same second-order targets of POMC neurons (Broberger et al., 1998). AgRP is a competitive antagonist of MCR4 and it

exerts its orexigenic action partly by impeding POMC product, α-melanostimulating hormone (α-MSH), to bind to its receptor, preventing so the α-MSH anorexic effect (Ollmann et al., 1997; Haskell-Luevano & Monck, 2001; Nijenhuis et al., 2001; Tolle & Low, 2008) (Figure 4).

The presence of several transmitters released by this neuronal population points out to their respective functions. A study from Krashes and colleagues showed the main difference of the orexigenic molecules to be related to food intake induction timing. By the chemogenetic activation of AgRP neurons, the rapid stimulation of feeding by GABA or NPY was pointed out, while AgRP acts on a prolonged period (Krashes et al., 2013).

Food intake is indeed finely managed by the simultaneous bidirectional communication in between AgRP and POMC neurocircuits (De Solis et al., 2024).

Agrp neurons project and receive inputs to and from hypothalamic and extra-hypothalamic areas, largely overlapping with the POMC neurons ones (Wang et al., 2015).

#### 1.1.1.i.ii. POMC

Pro-opiomelanocortin (POMC) neurons are activated when the individual energy balance state is positive, they reduce food intake, and increase energy expenditure.

They co-expresses α-melanocytes stimulating hormone (α-MSH), yielded by the processing of the precursor peptide pro-opiomelanocortin (Benjannet et al., 1991), and cocaine and amphetamine-regulated transcript (CART), which intracerebroventricular (i.c.v.) delivery inhibits food intake (Kristensen et al., 1998) (Figure 4). POMC neurons also co-secrete β-endorphin which mediate the cannabinoid-induced feeding (Koch et al., 2015).

Central α-Msh delivery in the lateral ventricle or in the PVN reduce food intake inducing weight loss (Poggioli et al., 1986 and Wirth et al., 2001). The α-Msh gradually exerts its anorexigenic action by binding the MC4Rs primarily in the PVN (Koch et al., 2015; Wang et al., 2015) POMC neurons also express MC4R, so that α-Msh can act in an autocrine way (Smith et al., 2007). Indeed, ARH POMC neuron-specific deletion of MC4R lowers POMC neurons activation, enhancing food intake and impairing energy expenditure, resulting in weight gain and impaired glucose homeostasis (Guo et al., 2024).

POMC neurons contact and are contacted by an even broader circuitry than Agrp neurons, including both hypothalamic and extra hypothalamic nuclei (Wang et al., 2015). The larger connectivity that regards these neurons, may be explained by the high number of transmitters produced by this population (Millington 2007).

The impressive heterogeneity of this neuronal population has been on the spotlight of numerous studies lately. The differential expression of neurotransmitters (Dicken, Tooker & Hentges, 2012; Hentges et al., 2009; Wittman, Hrabovszky & Lechan, 2013) and hormonal and neurotransmitter receptors (Williams et al., 2010; Sohn et al., 2011; Lam et al., 2017) is the signature of the different, spatially distinguished (Wang et al., 2015; Elias et al., 1998; Baker & Herkenham, 1995) subpopulations of the POMC population.

The functional identity of POMC subgroups that also release GABA, glutamate, or both, has been proven to be regulated by the activity of the mechanistic target of rapamycin complex 1 (mTORC1). Indeed, blocking mTORC1, therby simulating a negative energy state, activates GABAergic-POMC neurons and inhibits glutamatergic-POMC ones and α-Msh release, globally resulting in hyperphagia (Saucisse et al., 2021).
Very intriguingly, a population of "Ghost" POMC neurons was proposed and it defies the concept of developmentally fixed neuronal identities. Indeed, by lineage tracing studies of POMC neurons, the population defined as ghost and found in the brain of adult mice, displays a very low expression of POMC and cannot be detected by classical neuroatomical tools. Notably, the population size is not fixed, indeed it increases when diet induces obesity and decreases with weight loss (Leon et al., 2024).
Further investigations are auspicated to deepen the understanding of POMC divergent and convergent contribution to the control of energy homeostasis (Quarta et al., 2021).

#### 1.1.1.i.iii. Other ARH neurons

Metabolic control is not only portrayed by POMC and Agrp neurons in the ARC, or at least their action can be assisted or impinged by other local neuronal populations. There is indeed evidence that in addition to POMC neurons, a population of ARH neurons has been found to induce satiety through the release of glutamate onto PVN neurons expressing MC4R. This modulation is positively regulated by the ARH POMC neuron-derived α-MSH transmission (Fenselau et al., 2017). On the orexigenic front, optogenetic stimulation of mouse ARH tyrosine hydroxylase (TH) neurons increase food intake, inhibit POMC neurons and activate Agrp neurons. ARH TH neurons also directly inhibit PVN neurons by dopamine and GABA co-release (Zhang et al., 2016).
In addition, the RIP-Cre population exert GABAergic inhibition onto PVN neurons mediating increased energy expenditure, but with no association on food intake (Kong et al., 2012).

### 1.1.2. Paraventricular nucleus of the hypothalamus (PVN)

PVN is more rostral and dorsal with respect to the ARC, and it receives innervation from AgRP and POMC-ARH neurons and extra hypothalamic regions (Stanley et al., 1985). This nucleus acts as an integrator end effector of energy homeostasis.
In the PVN, neurons that express the thyrotrophic releasing hormone (TRH) are stimulated by ARH POMC neurons by α-Msh release and inhibited by Agrp (Fekete et al., 2000). Stimulation of TRH neurons in the PVN boosts the secretion of thyroid hormones (T3 and T4). T3, in turn, induces an increase in basal metabolic rate and thermogenesis in brown adipose tissue. In addition, corticotropin-releasing hormone (CRH)-expressing neurons are present in the PVN.

The binding of α-MSH to MC4R on these neurons activates the hypothalamic–corticotropic axis (HPA) that induces a negative energetic state (Lu et al., 2003) (Figure 3).
Oxytocin (OXT)-expressing neurons negatively control eating behaviour. OXT neurons in the PVH project widely throughout the brain in areas related to energy homeostasis regulation (Jurek & Neumann, 2017; Sofroniew, 1980; Buijs et al., 1978; Liao et al., 2020). α-MSH, activates PVH magnocellular oxytocin neurons (Caquineau et al., 2006) and increases secretion of oxytocin (Maejima et al., 2018). Accordingly, OXT receptor KO mice show an increased food intake (Yamashita et al., 2013), and virally-mediated knockdown of PVH oxytocin augments food intake as well (Zhang et al., 2011).
Recently, a population of Npy1R-expressing neurons in the PVN was found to be activated by the simultaneous activation and inhibition of Agrp and POMC neurons respectively, to promote food consumption (De Solis et al., 2024). MC4Rs expressing neurons in the PVN are mainly involved in the control of food intake.

### 1.1.3. Ventromedial nucleus of the hypothalamus (VMN)

Situated just dorsally to the ARC, the VMN was the first recognized site for the control of body weight and energy homeostasis (Hetherington, 1941). It is considered to be, like the ARH a centre of first-order neurons, i.e., neurons that integrate messages of blood-borne molecules (Bouret, 2017). Steroidogenic factor 1 (Sf1) a transcription factor selectively expressed in VMN neurons, is implied in energy balance control (Majdic et al., 2002; McClellan et al., 2006), programming the regulation of diet-induced thermogenesis and exerting an anorectic action (Kim et al., 2011) (Figure 3). In addition, brain-derived neurotrophic factor (BDNF) is highly expressed in the VMN according to nutritional state and by MC4R signalling (Xu et al., 2003). Its deficiency leads to hyperphagia and obesity (Lyons et al., 1999).

### 1.1.4. Dorsomedial nucleus of the hypothalamus (DMN)

This nucleus, above the VMN, is involved in multiple physiological processes, such as appetite, thermoregulation, stress and circadian rhythms. It receives projections from the ARH and sends projections to the PVN and LH nuclei (Bi et al, 2003). DMN neurons express NPY, and its expression is higher under chronic conditions of hyperphagia (e.g., pregnancy or diet-induced obesity) (Guan et al., 1998).

### 1.1.5. Lateral hypothalamus (LH)

The LH is historically considered an orexigenic centre. Its two neuronal subpopulations secrete neuropeptides that are also involved in the mesolimbic reward system by modulating the

hedonic part of food intake. The main populations of the lateral hypothalamus are orexin neurons and melano-concentrating hormone (MCH) neurons (Figure 3). These glutamatergic neurons project to several hypothalamic nuclei and other brain areas, promoting arousal and food intake (Horvath et al., 1999, Horvath et al., 1999).

### 1.2. The Brainstem

The brainstem is the part of the central nervous system that connects telencephalon and diencephalon to the spinal chord. It provides an anatomical pathway that connects the brain to the rest of the body. It also hosts many specific neural centres that regulate the homeostasis of vital physiological processes, including the integration of energy-related information from the forebrain and midbrain, from circulating hormones and metabolites and from the GI tract.
Short-term satiating signals from the gastrointestinal tract are conveyed by vagus nerve afferents to the brainstem, specifically in the dorsal complex of the vagus nerve in the medulla oblongata, where they are processed and integrated (Schneeberger, Gomis, Claret, 2014). This complex is constituted by the nucleus of the solitary tract (NTS), the area postrema (AP) and the dorsal nucleus of the vagus nerve (DMV).
The NTS acts as a key element for the communication between the encephalon and the brainstem and in the regulation of appetite and satiety states by integrating both ascending signals from the gastrointestinal tract and modulatory signals from the brain (Rui et al., 2013). Luminal distension, nutritive values and locally produced peptides´ information reach the NTS through vagal glutamatergic signalling (Travagli et al., 2006).
The NTS, specifically the preproglucagon (PPG)-neurons, is the major source of central GLP-1 production. Interestingly the ablation of this population only influence feeding in case of fasting and stress, while its chemogenetic activation induces a strong anorectic effect (Holt et al., 2019). These neurons in addition are not contacted by vagal afferent from GLP-1r-expressing vagal neurons, indicating that the satiety effect that they cause is independent on peripheral Glp1 (Brierley et al., 2021).

The NTS itself expresses MC4R (Kishi et al., 2003) and it has been reported that the NTS hosts a POMC population of around 300 neurons (Palkovits et al., 1987). Interestingly, axons from the NTS-POMC neurons have been found to contact the ARC, but not specifically ARC-POMC or AgRP neurons (Wang et al., 2015). The two sister populations (ARH and NTS POMC neurons) respond to different peripheral signals and central signals, with the NTS POMC cells mainly integrating brainstem circuits (Wang et al., 2015). Furthermore, their control of metabolism works with different timings. Chronic or acute activation of ARC- and NTS-POMC neurons, respectively, is needed for the negative regulation of food intake, highlighting the different roles of the two populations in the regulation of long and short-term feeding (Zhan et al., 2013).

NTS receives also projections from orexin- and MCH-expressing neurons from the LH (Ciriello et al., 2003), whose firing increases food intake (Parise et al., 2011). PVN contacts the NTS and disruption of NTS-directed PVN projections causes hyperphagia (Kirchgessner and Sclafani, 1988).

*Disclaimer: not only neurons control energy homeostasis. There is growing evidence that glial cells, play crucial roles in the preservation of physiology. You can find more information in two elegant and exhaustive reviews by García-Cáceres and Nampoothiri (García-Cáceres et al., 2019; Nampoothiri et al., 2022).*

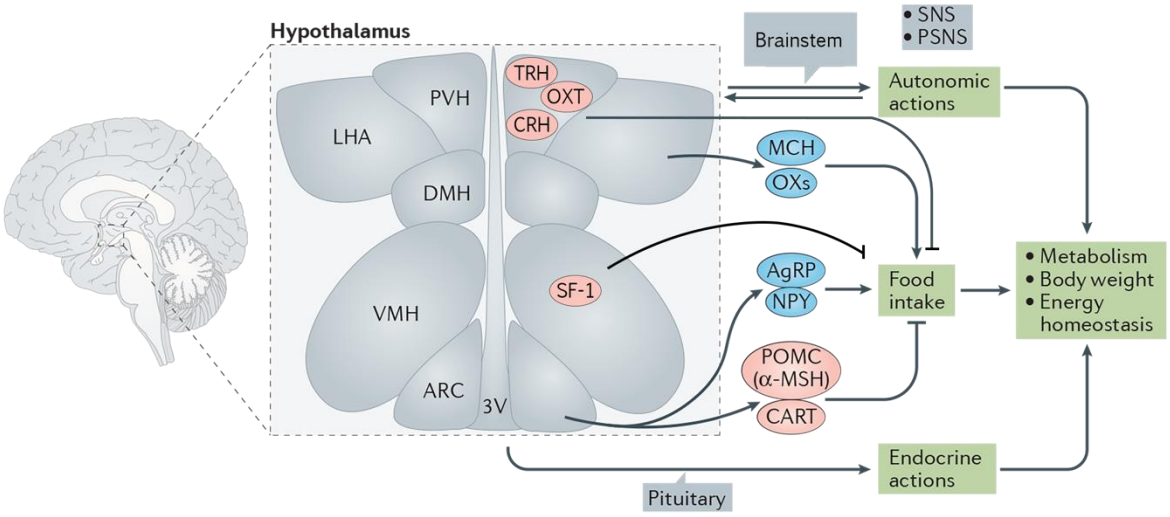



***Figure 3. The anatomically demarcated nuclei of hypothalamus establish interconnected neuronal circuits.*** *Here the main neuronal populations are represented. We find the 2 fist-order neurons of the melanocortin system: the orexigenic agouti-related protein (AgRP) and neuropeptide Y (NPY) neurons and the neurons that secrete the anorexigenic products of proopiomelanocortin (POMC) α-melanocyte stimulating hormone (α-MSH), and cocaine- and amphetamine-regulated transcript (CART). These project widely to other hypothalamic nuclei including the paraventricular nucleus (PVH) which harbours the thyrotropin-releasing hormone-expressing (TRH) neurons, oxytocin (OXT) neurons and corticotropin-releasing hormone (CRH) neurons, all implicated in the downregulation of food intake. On the other hand, the lateral hypothalamic area (LHA) is mainly the place of two neuronal population that increase food intake: the melanin concentrating hormone (MCH)-expressing neurons and the orexin (OX)-expressing neurons. Adjacent to the ARC lies the ventromedial nucleus of the hypothalamus (VMH) with steroidogenic factor-1 (SF-1)-expressing neurons shown to negatively influence eating behaviour. Hypothalamic neurons also extend axonal projections to brainstem areas such as the nucleus tractus solitarius (NTS), dorsal motor nucleus of the vagus (DMV) which control the autonomic nervous system regulating the sympathetic (SNS) and parasympathetic nervous system (PSNS) which govern glucose and lipid homeostasis in peripheral tissues and energy expenditure (Modified from López et al., 2016).*

## 2. Hedonic control of metabolism

Feeding is not only driven by physiological needs but also influenced by hedonic factors—pleasure and reward, ignoring the energy status of the individual (Avena, 2015). The brain's reward systems regulate the hedonic control of feeding based on the desire to eat driven by the enjoyment and sensory pleasure derived from food that can can contrast and even override the

homeostatic feeding controlled by the hormones that mediate the message for body's energy status. This system plays a crucial role in shaping eating habits, integrating cognition and the motor control of feeding (de Araujo, Schatzker, & Small, 2020).

Dopamine (DA) is a monoaminergic neurotransmitter that strongly regulates food intake through the mesolimbic pathway. This circuitry starts in the VTA in the midbrain and projects to both the ventral (nucleus accumbens) and dorsal (caudate nucleus and putamen) striatum (Swanson, 2000; Berridge & Kringelbach, 2015).
DA projections contact cortical regions and limbic regions such as the insula, the hippocampus, and the amygdala. The mesolimbic dopamine pathway projects also to the LH generating the incentive motivation to feeding (Castro et al., 2014). GABAergic projections from LH neurons to the ventral tegmental area drive vigorous feeding (Marino et al., 2020).
Highly palatable food, those with high sugar and fat content, can generate addictive-like behaviours, the release of dopamine reinforces the pleasurable experience and creates a drive to repeat the behaviour (Lenoir et al., 2007; Avena, Rada & Hoebel, 2008; Avena, Rada & Hoebel, 2009), according also to external factors such as, socio-cultural context, visual appeal, and incentives. Peripheral metabolic cues directly sign to the mesolimbic pathway or indirectly to the hypothalamus by projecting then to the VTA, modulate the rewarding feature of food (Figlewicz et al., 2003; Leshan et al., 2010; Fadel& Deutch, 2002; Davis et al., 2011, Alhadeff et al., 2012; Jerlhag et al., 2007; Cason et al., 2010; Dickson, 2012).

The extended amygdala includes nuclei implicated in anxiety, stress, and fear learning (Cassell et al., 1999). Two components of the amygdala, the bed nucleus of the stria terminalis (BNST), and the central amygdala (CeA) are involved in reward and feeding. The BNST is a critical mediator of anxiety and fear learning (Walker et al., 2003), and it is contacted by Agrp projections, increasing food intake. (Betley et al., 2013). In addition, it was found that the activation of BNST neurons projecting to the LH drastically reduces food intake in mice fed with of chow diet, but not HFD (Zhang et al., 2023).

The pontine parabrachial nucelus (PBN) is studied for its role in taste processing, receiving gustatory inputs via the NTS. Lesions of the PBN blunt the ability to learn conditioned taste aversion (Reilly et al., 1993). Electrophysiological recordings show that PBN neurons are reciprocally connected with CeA and LHand respond to several tastants (Li et al., 2005).

Memory also strongly influences food intake. Lesions in the hippocampus, memory centre, promotes hyperphagia and lipid storage (Davidson et al., 2009). Additionally, cognitive controlling centres like the medial prefrontal cortex, affect feeding behaviour. Its neurons connect to somatostatin neurons of the lateral septum and finally to the LH, controlling food-seeking behaviour without affecting food intake (Carus-Cadavieco et al., 2017).

# *Chapter 2: The dialogue between brain and body*

# I. CNS to periphery communication

The autonomic nervous system (ANS) is the part of the peripheral nervous system that controls involuntary physiologic processes comprising respiration, heart rate, blood pressure, sexual arousal, and, the one that we will focus on, metabolism.
The ANS innervates tissues and organs that regulate of energy metabolism, such as liver, pancreas, muscles, and white and brown adipose tissue. The efferent communication is crucial in the understanding of metabolic diseases. Indeed, although peripheral cues can reach the central nervous system through the blood-stream, when the two-way communication between brain and periphery is altered, pathologies such as diabetes and obesity arise with dysfunctions in peripheral organs.

## 1. The autonomic nervous system

The ANS is composed of the sympathetic (SNS), parasympathetic (PNS) and enteric nervous system (ENS), three anatomically separated units. Efferent fibres provide motor output to the periphery, while afferent fibres transport the sensory input to the CNS. The sympathetic and parasympathetic nervous systems are characterized by a two-neuron serial cytoarchitecture, with the preganglionic neuron in the CNS and the post-ganglionic neuron that contacts the peripheral organ off target. Differently, the ENS can function independently from the rest of the nervous system and its structure is like a web that innervates the gastrointestinal tract.
While PNS neurons, both pre- and post-synaptic use acetylcholine as a neurotransmitter, SNS neurons differ in the use of the second effector transmitter which generally is norepinephrine. ENS neurons use several neurotransmitters (e.g., acetylcholine, nitric oxide and serotonin) (Waxenbaum, Reddy & Varacallo, 2023).

### 1.1. Sympathetic nervous system

The cell bodies of the SNS are located in the lateral horns of the spinal cord. The presynaptic fibres exit the spinal cord through anterior roots and enter the spinal nerves. From here, the fibres reach a superior or inferior paravertebral ganglion where pre- and postganglionic neurons synapse. Presynaptic fibres are normally shorter than their postsynaptic ones. Commonly, there are three cervical, twelve thoracic, four lumbar, and five sacral ganglia. Splanchnic nerves convey afferent and efferent fibres between the CNS and the viscera.
Postganglionic sympathetic neurons release norepinephrine (NE) that acts on adrenergic receptors in the target tissue. The subtype of the receptor, alpha-1, alpha-2, beta-1, beta-2, or beta-3, and the tissues in which they are expressed, modify the affinity and the effects of NE for the receptor (Nance & Sanders, 2007).

The sympathetic nervous system contacts the adrenal medulla, forming what is known as a sympathoadrenal system, essential for an organism's physiological response to external stimuli. Adrenal medulla release of norepinephrine and epinephrine directly into the blood enables the "fight-or-flight" response. The sympatho-adrenal axis maintains glucose levels, sodium levels, blood pressure, and various other metabolic pathways (Christensen, 1991).
The SNS is continually active and complements the PNS action.

### 1.2. Parasympathetic Nervous System

Parasympathetic fibres exit the CNS via cranial nerves. There are four pairs of parasympathetic ganglia, all located in the head. Every other presynaptic parasympathetic fibre synapses in a ganglion near the target tissue; according to the fact that presynaptic fibres are longer than the postsynaptic. Postganglionic parasympathetic neurons release ACh that acts on muscarinic and nicotinic receptors (Karemaker, 2017), providing parasympathetic input to most of the thoracic and abdominal viscera.
In the medulla oblongata, the vagal nerve nucleus is constituted of four nuclei:

- Dorsal nucleus: output to the viscera
- Nucleus ambiguus: innervates the heart
- Nucleus solitarius: receives afferents of taste sensation and that from viscera, discussed about in the previous chapter about Extrahypothalamic centres that control metabolism,
- Spinal trigeminal nucleus: receives information of touch, pain, and temperature of the ear, the larynx, and the dura mater.

The vagus nerve, innervating all major organs, is responsible for the "rest and digest" processes, promoting cardiac relaxation (Karemaker, 2017) and digestion. Parasympathetic fibres that contact the ENS lead to increased peristaltic and secretory activity (Siessere et al., 2008; Wood, 1994). Parasympathetic ganglia also express receptors for key cytokines in the inflammatory response (Thayer & Sternber, 2010) which, in turn, activates the hypothalamic-pituitary-adrenal axis and SNS, leading to the release of glucocorticoids and NE, respectively (Karemaker, 2017).

### 1.3. Enteric Nervous System

The ENS is composed of two ganglionated plexuses: the myenteric and the submucosal. The ENS works through local reflex activity (mediated by nicotinic synapses), but also receives input and gives output to the SNS and PNS (Sternini, 1997; Million & Larauche, 2016).
The submucosal plexus regulates the flux of water and electrolytes across the intestinal wall, and the myenteric plexus coordinates peristalsis (Hansen, 2003).

The ENS shows similarities to the CNS in the morphologies of neurons, the excitatory and inhibitory communication and the variety of neurotransmitters used to accomplish it (Hansen, 2003).

## 2. ANS implication in energy homoeostasis

### 2.1. Communication with the liver

The liver has a pivotal role in the regulation of the metabolism of amino acids, lipids and glucose homeostasis. The organ is highly innervated by ANS fibres to receive and provide information from and to, especially, the hypothalamus and the brain stem (LaFleur et al., 2000). Sympathetic information is delivered through of α or β adrenergic receptors, secretion of NPY, galanin, noradrenaline or vasoactive intestinal peptide (VIP) (Ulken et al., 1991; Burcelin et al., 2004; Akiyoshi et al., 1998; el-Salhy et al., 1993).

The involvement of the ANS in the regulation of liver-mediated glucose homeostasis, especially as a response to hypoglycaemia, was first shown by injecting insulin in the cerebral ventricles, removing at the same time the pancreas, spleen and intestinal tract. Despite this, glucose production by the liver was still modified by central insulin injection (Szabo and Szabo, 1975). In addition, when performing a hepatic vagotomy and sympathectomy, the effects of insulin central injections were abolished, demonstrating the importance of ANS mediated brain-liver communication in hepatic glucose production (Van den Hoek et al., 2008).

The stimulation of the sympathetic splanchnic nerve induces hepatic gluconeogenesis and decreases glycogen synthesis through β2 (adenylyl cyclase-linked) adrenergic receptors and via the α1 (phospholipase C-linked) adrenergic receptors.

On the other way, by stimulating the parasympathetic vagus nerve, increased hepatic glycogen production was obtained (Shimazu, 1971).

Several hypothalamic areas are involved in the control of hepatic glucose production.

Norepinephrine injection into the PVN caused an immediate hyperglycaemia and the norepinephrine-induced hyperglycaemia was largely reduced by a ganglionic blocker, independent of corticosterone (Ionescu et al., 1989). The PVN is likely to be one major area of inputs governing sympathetic and parasympathetic neurons.

The LH primarily manages the rhythmicity of hepatic glucose production and requires an intact autonomic nervous system (Tsuneki et al., 2015), decreasing hepatic gluconeogenesis and increasing glycogen production (Shimazu et al., 1975), while VMN stimulation leads to the opposite effect (Shimazu, 1979).

Furthermore, the liver also plays a role in lipid metabolism. It synthesizes fatty acids from a surplus of glucose, it can use them for energy through fatty acid oxidation or mobilize them as triglycerides by a lipoprotein mediated modulation. In addition, the liver is the centre of the synthesis of ketone bodies during fasting.

Sympathetic stimulation of the liver increases triglyceride secretion to the blood, it blocks ketone bodies production and it inhibits the expression of CPT-1, involved in fatty acid β-oxidation (Yamauchi et al., 1998; Carreno and Seelaender, 2004). Moreover, NPY, modulators of MCR3/4 activity and MCH are involved in the central regulation of lipid metabolism by the liver mediated by the ANS (Lam et al., 2007; Leckstrom et al., 2011).

### 2.2. Communication with the pancreas

The pancreas produces and secretes glucagon and insulin via Langherans islets α and β cells respectively. Glucagon induces hyperglycaemia and insulin hypoglycaemia, the right balance of these two hormones assures euglycemia. Conversely, dysfunctional secretion can lead to diabetes. The ANS is involved in the secretion of pancreatic hormones, affecting both α and β cells. The parasympathetic pathway stimulates insulin release to lower glycaemia, by secreting acetylcholine acting on muscarinic receptors on β-cells (Ionescu et al., 1983). When glycaemia drops, the parasympathetic pathway moderately activates α-cells to secrete glucagon and normalize blood glucose levels, most importantly in glucose counter-regulation (Gerich et al., 1979). The sympathetic nervous system acts on both islet types too. By activating β2 adrenergic receptors on α cells it induces glucagon secretion, while stimulation of α2 receptors on β cells inhibits insulin secretion (Rodriguez-Diaz et al., 2012; Thorens, 2014).
AgRP glucose-sensing neurons in the ARH are directly involved in sympathetic regulation of the pancreas (Joly-Amado et al., 2012).
The activity of these fibres is also regulated by neurons in the PVN (Jansen et al., 1997), which in turn is connected to other hypothalamic regions. Lesion of the VMN, able to sense variations in glucose concentration through the expression of the GLUT2 receptor (Dunn et al., 1998), leads to hyperinsulinemia, which can be ameliorated by transection of the vagus nerve (Berthourd et al., 1979). In addition, VMN neurons sensing of insulin variation is essential for the proper functioning of α- and β-cells, and also decreases glucagon secretion via GABA released by VMN neurons (Paranjape et al., 2010; Paranjape et al., 2011).

### 2.3. Communication with the muscle

The muscle can take up glucose via the GLUT4 receptor for its use, but can also store glycogen. Postprandial-induced insulin secretion stimulates glucose uptake by the muscle, which is highly sensitive to insulin actions. However, it is possible for the muscle to capture glucose without insulin intervention, thanks to the VMN-mediated activation of the sympathetic nervous system (Nonogaki et al., 2000), which releasing NE activates peripheral β-adrenergic receptors (Shimazu et al., 1991; Sudo et al., 1991; Minokoshi et al., 1994). Furthermore, a glucose-sensing population of LH neurons that releases orexin A, upon the VMN, triggers the activation of the sympathetic nervous system. Activation of β-adrenergic receptors promotes

insulin-independent glucose uptake by the muscle, as well as glycogen production (Shiuchi et al., 2009).
Muscles are also implicated in the regulation of energy expenditure. Shivering thermogenesis (heat production through muscle contraction) is also under the control of the autonomic nervous system. The preoptic region of the hypothalamus integrates information from thermoreceptors via neurons, modulating the sympathetic tone sent to muscle fibrers (Boulant & Hardy, 1974).

### 2.4. Communication with the white adipose tissue

The white adipose tissue is the lipid main storage site from which the body draws energy in case of need (Ahima & Flier, 2000). It is a proper endocrine organ, because of the secretion of adipokines, hormones with a plethora of functions. Adipose tissue´ functions are controlled by the central nervous system. The sympathetic nervous system conveys the communication between the brain and white adipose tissue, regulating lipolysis, adipocyte number and adipokine secretion (Bartness et al., 2005; Bartness et al., 2010; Nogueiras et al., 2010). Adrenergic receptors, including β1, β2 and β3, stimulate lipolysis, while α2 inhibits lipolysis by modulating the activity of adenylate cyclase and protein kinase A (PKA) (van Baak, 2001; Fruhbeck et al., 2014). The hypothalamus can detect and metabolize lipids, intending to adapt the neural response sent to peripheral organs, such as the liver or white adipose tissue, as a consequence of the body's energy status (Le Foll et al., 2009; Sarruf et al., 2009).
Numerous studies have shown that hypothalamic MCR3/4 receptor´s activity increases the turnover and secretion of noradrenaline to adipocytes, stimulating lipolysis and decreasing fat stores (Hwa et al., 2001; Raposinho et al., 2003; Song et al., 2005; Brito et al., 2007). Similarly, if POMC neurons secrete less α-MSH, lipolysis is inhibited and lipid stores increase in the animal, effects that can be reversed by administration of an α and β adrenergic receptor agonist, suggesting that the SNS mediates the central control of lipid metabolism (Kaushik et al., 2012). Orexin LH neurons positively modulate sympathetic tone, (Shen et al., 2008); MCH, produced in the same nucleus, also mediates white adipose tissue activity increasing adiposity and decreasing lipolysis (Imbernon et al., 2013).

### 2.5. Communication with brown adipose tissue

The brown adipose tissue burns energy by producing heat in response to cold or certain diets (Cannon & Nedergaard, 2004; Morrisson et al., 2014). Its role has always been recognized in rodents, hibernating animals and neonates, but recently it has been detected in adult humans uncovering his role in the regulation of energy metabolism (Nedergaard et al., 2007; van Marken Litchtenbelt et al., 2009).

While only a limited number of deposits are contacted by parasympathetic fibres, a high expression of β-adrenergic receptors is present to sense the message of the highly rich sympathetic nervous system innervation (Cannon & Nedergaard, 2004, Richard et al., 2012). UCP1 gene expression is also influenced by β-adrenergic activation (Cao et al., 2001).
Several hypothalamic regions are involved (Bamshad et al., 1999) in the ANS-mediated control of the brown tissue metabolism.
Central injection of α-MSH also increases sympathetic tone in BAT, suggesting the involvement of MC4R-expressing neurons in this regulation. CART and POMC neurons activation of MC4R receptors, is indeed essential for regulating shiver-free thermogenesis (heat production due to tissue activity not muscle activity) (Berglund et al., 2014). AgRP neurons are also involved, indeed their activation reduces the "browning" of white adipose tissue and activates sympathetic innervation of brown adipose tissue (Shi et al., 2013; Ruan et al., 2014).
VMN SF1-expressing neurons, send terminals to the ARH and PVN, but also to the NTS or locus coeruleus, leading to a decrease in UCP1 expression and energy expenditure (Lindberg et al., 2013; Kim et al., 2011). VMN neurons mediate also the effects of T3 which regulates thermogenesis in BAT and browning of white adipose tissue (WAT) (Martínez-Sánchez et al., 2017).
DMN neurons send glutamatergic projections to the raphe pallidus, which mediates their stimulatory effects on brown adipose tissue activity (Cao & Morrison, 2006). NPY neurons of this nucleus reduce the energy expenditure by blocking the "browning" of inguinal white adipose tissue.

## II. Periphery to CNS communication

The communication between the CNS and the peripheral metabolically active organs is achieved by neuronal interactions as seen above, and also through hormonal interactions. Different peripheral structures secrete hormones that will either go directly to the brain via routes that we will discover later in the text, or activate afferent neurons of the ANS that transmit the signal to the CNS. As hormones, nutrients can also act as messengers, since they are sensed by neurons to integrate the body energy status and participate in neuroendocrine control of energy homeostasis.
In the next section I will focus on those peripheral messengers, essential actors of the energy homeostasis dialogue.

### 1. Nutrients

#### 1.1. Glucose

Glucose is the major source of energy in the CNS and its concentration can be sensed by glucose-sensing neurons. Variations in blood glucose levels lead to changes in the activity of VMN and LH neurons (Silver et al., 1998).
Two glucose-sensing neuronal populations present in the hypothalamus appear to be involved in this regulation: AgRP and POMC neurons are considered as glucose-inhibited (GI) and glucose-excited (GE) neurons, respectively (Parton et al., 2007, Ibrahim et al., 2003). A recent study (Rodríguez-Cortés et al., 2022) has found that the increase of glucose levels in the ARH activate mechanism to reduce glycaemia, which result by the coordinated activity of GE and GI neurons. Intriguingly, the ability of POMC neurons to directly sense glucose is still debated (Fioramonti et al., 2007) and a recent study from the laboratory demonstrates that POMC neurons rely on tanycyte-provided lactate for thei activity (Lhomme et al., 2022). Surely also in this phenomenon, the heterogeneity of POMC neurons plays is a key element in the understanding of POMC neurons glucose sensing and their contribution to general glucose homeostasis.
Regarding food intake, strong glucoprivation elicits hunger and feeding in humans (Heller et al., 1991) and rats (Bernardis et al., 1981) respectively. I.P. (Smith et al 1969) and I.C.V (Miselis et al.,1975) administration of 2-deoxyglucose, a modified glucose molecule that cannot undergo glycoyisis, also prompts food intake in mice and rats, initiated from both hypothalamic (Sanders et al., 2004) and brainstem sites (Ritter, Dinh & Zhang, 2000).
So far, there is no real evidence for a hyperglycaemic satiating effect. In humans, acute hyperglycaemia diminishes hunger after fasting but not in a fed state (Russell et al., 2001),

while 2h of hyperinsulinemic-hyperglycaemic clamping was required to decrease hunger (Gielkens et al., 1998).
In addition to reduced food intake, several days of combined elevations of both blood glucose and insulin levels were required in baboons (Woods et al., 1984).
Whether acute physiologic oscillations in glycaemia influence meal initiation and termination is still unknown.

### 1.2. Fatty acids

Circulating fatty acids are also indicative of the body's metabolic status. Hypothalamic nuclei respond to free fatty acids, which modulate their activity via their receptors (Ichimura et al., 2014). Neurons sensitive to free fatty acids are present in the ARC, VMN, DMN and PVN. Indeed, an ICV injection of oleic acid induces a reduction in the expression of NPY and AgRP peptides in the ARH and inhibits food intake and glucose production (Obici et al., 2002). In addition, inhibition of the transport of free fatty acids to the mitochondria leads to their accumulation in the cytosol and a reduction in food intake.
It has been shown that Oleic Acid, and not the saturated palmitate, in the VTA inhibits food intake and increases locomotor activity. The reduction of food-induced reward was mediated by the inhibition of firing of neurons co-expressing dopamine and fatty acids transporters. (Hryhorczuk et al., 2018).
Polyunsaturated fatty acid derivatives known as endocannabinoids play an important role in eating behaviour (Aguilera Vasquez & Nielsen., 2022). In the central nervous system, the cannabinoid receptor 1 is mainly expressed in presynaptic neurons and it modulates the activity of several neurotransmitters (Schlicker & Kathmann, 2001). To the present knowledge, studies indicate a positive indfucense on food intake by 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine (AEA) via cannabinoid receptor-1 (CNR1) binding, on the other hand oleylethanolamide (OEA) may play a different role (Oveisi et al., 2004; Higuchi et al., 2012; Montelone et al., 2017). The effects of the endocannabinoids are mediated by the neurons of the hypothalamus, they hav been shown to modulate the activity of AgRP/NPY (Verty et al., 2009) and POMC (Koch et al., 2015) neurons promoting feeding behaviour, and in the neurons that control hedonic feeding that I briefly introduced before (Cota et al., 2003).
Altogether, these studies suggest that the concentration of circulating free fatty acids plays a role in the control of satiety.

### 1.3. Amino acids

Unlike glucose and fatty acids, the majority of amino acids are not synthesized in our bodies but come directly from the food we eat and play an essential role in protein synthesis.
An ICV injection of L-leucine, an amino acid with a branched chain, directly into the 3rd ventricle, suppresses food intake by acting on the rapamycin (mTOR) signalling pathway (Cota

et al., 2006). It is thought to act as a satiety factor. In addition, it has been shown that a high concentration of L-leucine in the blood is associated with obesity and insulin resistance (Adeva et al., 2012), making branched amino acids a strong predictive marker for risks associated with obesity (Wang et al., 2011, Würtz et al., 2013).

## 2. Hormones playing a role in metabolism

### 2.1. Gastro-intestinal-derived hormones

#### 2.1.1. Ghrelin

Ghrelin is a 28 amino acid peptide that contains an O-n-octanoic group at serine residue 3, which is produced and secreted by the parietal cells of the stomach (Kojima et al, 1999). It was discovered as a ligand of the growth hormone secretagogue receptor (GHSR-1a) (Kojima et al. 1999, Sun et al. 2003), through which it exerts its orexigenic function.

Circulating levels of ghrelin are increased before the ingestion, decreasing to initial values after the meal in proportion to the calories ingested (Kojima & Kangawa, 2005). Ghrelin expression and secretion vary according to energy status; it increases in fasting, weight loss and insulin-induced hypoglycaemia (Ariyasu et al., 2001 Tschop et al., 2001, McCowen et al., 2002). Central or peripheral administration of ghrelin stimulates food intake and adiposity (Tschop et al., 2000); thus, ghrelin is considered an enteric signal modulator of energy homeostasis.

By binding to GHSR-1a, ghrelin activates Agrp neurons, increasing food intake and it is the only orexigenic peptide hormone in rodents and humans (Tschop et al., 2000, Nakazato et al., 2001 and Wren et al., 2001) (Figure 4). In these neurons, ghrelin activates a pathway involving SIRT1/AMPK/p53 and fatty acids (Lopez et al., 2008, Velásquez et al., 2011) that induces the expression of both AgRP and NPY, mediating the effects of ghrelin on hypothalamic lipid metabolism (Bagnasco et al., 2003, Tamura et al., 2002).

It has been described that the orexigenic effect of ghrelin is not only modulated by AgRP/NPY neurons but is also regulated in through the stimulation of GHSR in the caudal brainstem (Faulconbridge et al., 2003) or afferent signals from the vagus nerve activated by ghrelin secretion in the stomach (Date et al., 2002).

Ghrelin favours the acculmunlation of fat stores not only through the positive modulation of feeding behaviour, since it also exters an adipogenic action on white adipose tissue through the SNS (Nogueiras et al., 2007, Theander-Carrillo et al., 2006, Porteiro et al.,2013).

### 2.1.2. Cholecystokinin

Cholecystokinin (CCK) from Greek chole, "bile"; cysto, "sac"; kinin, "move" = move the bile-sac, is a peptide synthesised in the jejunum and duodenum in response to the presence of nutrients. It has a half-life of 1-2 minutes and its main action is to reduce appetite by activating afferents from the vagus nerve (Holzer et al., 1998). It has local functions such as delaying gastric emptying, stimulating pancreatic enzyme secretion and gallbladder contraction (Berthoud et al., 2002).

### 2.1.3. Peptide YY

The peptide YY (PYY) is localised throughout the intestine with a higher concentration in the colon and rectum. Although there is some controversy, the 1-36 form is orexigenic while PYY 3-36 exerts an anorexigenic effect (Ballantyne et al., 2006). Its post-prandial effects include delayed gastric emptying and reduced gastric secretion (Pappas et al., 1985; Wiley et al., 1991).

### 2.1.4. Glucose-dependent insulinotropic polypeptide

Glucose-dependent insulinotropic polypeptide (GIP) is an incretin, a hormone that activates insulin secretion in the pancreas after food intake, and it is synthesized in the K cells of the duodenum and jejunum, activated by the presence of glucose and fat.
Genome-wide association studies have identified the GIP receptor (GIPR) as a gene involved in the augmentation of the body mass (Berndt et al., 2013; Speliotes et al., 2010).
Gipr is expressed in different nuclei of the brain related to energy metabolism. Central administration of GIP decreases feeding in mice (NamKoong et al., 2017). A recent study (Adriaenssens et al., 2023) shows that chemogenetic stimulation of *Gipr*-expressing neurons int the hypothalamus and in the DVC suppresses food intake, and the sole stimulation of *Gipr*-expressing neurons of the DVC reduced ambulatory activity. In addition, they dimostrate that *Gipr*-expressing neurons in the hypothalamus, AP, and NTS contact different regions, show diverse transcriptomics, and they exert appetite in different ways. The exact molecular mechanism that underlies the GIP-GIPR interaction and leads to anti-obesity action is still debated. Different studies show that both agonism and antagonism of the GIPR can reduce body weight (Mroz et al., 2019; McClean et al., 2007), and one controversial hypothesis also states that the chronic agonis of the receptor actually leads to desensitization of the GIPR, resulting in an antagonistic effect (Killion et al., 2020).

Despite the still undiscovered mechanism of action of this hormone, recently a molecule named Trizepartide, that agonizes both the GIPR and the Glucagon-like peptide-1 receptor, leads to

substantial weight loss, promising a revolutionary approach against obesity (Bradley et al., 2022).

### 2.1.5. Glucagon-like peptide 1

Glucagon-like peptide 1 (GLP-1) is an incretin derived from post-transcriptional processing of the pre-pro-glucagon gene in the L cells of the gut and CNS (Holst et al., 2004), in pancreatic alpha cells (Chambers et al., 2017) and in neurons of the NTS (Holt et al., 2019). The actions of GLP-1 span several tissues, stimulating insulin secretion in the pancreas, delaying gastric emptying and increasing blood pressure in the heart (Schmidt, Siegel & Creutzfeldt, 1985; Horowitz & Nauck, 2006, Barragán, Rodriguéz & Blázquez, 1994).
Postprandial GLP-1 secretion can induce meal termination animals and human and it is associated with neuronal activity of brain areas involved in satiety (Kreymann et al., 1987; Pannacciulli et al., 2007). GLP-1 exerts its metabolic action in, among other areas, the hypothalamus. Here neurons receive GLP-1 by NTS PPG-neurons projections (Holt et al., 2019), acting as a satiety hormone and in glucose metabolism independently of peripheral Glp-1 (Brierley et al., 2021).
Central activation of GLP-1 receptor stimulates glucose uptake into the muscles and promotes glycogen formation in the liver (Knauf et al., 2005). The central actions of GLP-1 also involve lipid metabolism in adipose tissue by reducing TG storage independent of food intake (Nogueiras et al., 2009). Brown adipose tissue is also regulated by GLP-1R-expressing neurons which increase the thermogenic programme, also favouring the transformation of white adipose tissue adipocytes to a brown adipocyte phenotype (Lockie et al., 2012, Beiroa et al., 2014).
GLP-1 analogues represent a huge breakthrough in the fight against obesity and diabetes. Several chemical modifications of the GLP-1 molecule have been developed in order to increase the molecule half-life and to preserve its biological action over its receptor. As stated above the molecule Tirzepatide, co-agonist of the GLP1-R and the GIPRis the last promise in against obesity (Bradley et al., 2022).

### 2.1.6. Oxyntomodulin

Oxyntomodulin (OXM) is a 33-amino acids peptide which, like GLP-1, is the result of post-transcriptional processing of the pre-pro-glucagon gene that is postprandially released by the oxyntic cells of the colon. It exerts its action via the GLP-1 and glucagon receptor but with much lower affinity. OXM reduces acid secretion by the stomach and reduces food intake (Shankar et al., 2018) and body weight (Wynne et al., 2005).

## 2.2. Pancreas-derived hormones

### 2.2.1. Insulin

The quintessential hypoglycaemic hormone, it acts via the insulin receptor tyrosine kinase (IR) by activating the PI3K/AKT pathway in peripheral tissues such as skeletal muscle, fat and liver, allowing glucose uptake by these tissues, reducing glycaemia. In addition, Insulin is the hormone with the greatest anabolic potential, promoting the synthesis and storage of carbohydrates, lipids and proteins. In turn, it inhibits the breakdown and release of fatty acids and glucose into the bloodstream by inhibiting the expression of enzymes that promote fuel degradation, thereby promoting energy storage in peripheral tissues. Insulin stimulates the uptake of glucose, amino acids and fatty acids into cells, and increases the expression or activity of enzymes that catalyse glycogen, lipid and protein synthesis while inhibiting the activity or expression of those that catalyse degradation (Saltiel & Kahn, 2001).

The discovery that insulin modulates neuropeptide expression in the hypothalamus and regulates energy metabolism has shown that the brain is an insulin-sensitive organ. At central level, insulin plays a regulatory role in energy balance, reproduction and neuronal survival (Baskin et al., 1999, Schwartz et al., 1992, Bruning et al., 2000). IR expression in the brain is highly dispersed with high concentrations in the olfactory bulb, cerebral cortex, hypothalamus, cerebellum and hippocampus (Havrankova et al., 1978, van Houten et al., 1979). In the ARC, insulin acts on AgRP/NPY and POMC/CART neurons, it decreases the production and expression of NPY, favouring that of POMC, activating the melanocortin pathway leading to food intake reduction (Sipols et al., 1995, Benoit et al., 2002). In addition to the anorexigenic effect, the hypothalamic action of insulin also modulates glucose metabolism by increasing insulin sensitivity in peripheral tissues and reducing hepatic gluconeogenesis (Obici et al., 2001). Finally, insulin infusion in the mediobasal hypothalamus can also regulate the amount of fat independently of its anorexigenic effect, as central insulin decreases lipogenic protein expression in WAT, it inactivates hormone sensitive lipase (HSL) suppressing lipolysis (Scherer et al., 2011).

### 2.2.2. Glucagon

Glucagon is secreted by the alpha cells of pancreatic islets of Langerhans derived from the pre-pro-glucagon gene, in response to low plasma glucose levels (Quesada et al., 2008). In addition to its involvement in glucose metabolism, glucagon has also been reported to be involved in the regulation of lipid metabolism and energy expenditure. Numerous studies have reported that the effect of glucagon on lipid metabolism results in reduced synthesis and secretion of components involved in energy storage (triglycerides, VLDL, cholesterol) and lipid mobilisation (Penhos et al., 1966, Guettet et al., 1991). Centrally, the glucagon receptor is

distributed in various regions such as the thalamus, olfactory bulb and hypothalamus (Hoosein et al., 1984). Peripheral administration of glucagon has been reported to reduce food intake but this effect is more pronounced when administered centrally (Inokuchi et al., 1984) and the anorexigenic action of glucagon involves the PKA/CAMKK/AMPK pathway in the ARH (Quiñones et al., 2015).

### 2.3. Adipose-derived hormones

#### 2.3.1. Adiponectin

It is a protein secreted and produced exclusively by adipocytes and is one of the most abundant plasma proteins in the bloodstream (Ouchi et al., 2003), hypoadiponectinaemia is also associated with obesity, therefore the secretion of the protein is inversely correlated to the amount of adipose tissue (Ryo et al., 2004). Adiponectin production is inhibited by pro-inflammatory factors such as tumour necrosis factor alpha (TNFα) and interleukin 6, as well as stress and hypoxia (Hosogai et al., 2007). At the metabolic level, adiponectin increases fatty acid oxidation in muscle and reduces plasma levels of glucose, free fatty acids and triglycerides via the AMP kinase (AMPK) pathway (Yamauchi et al., 2002). While in healthy mice the levels of adiponectin in the serum and CSF decrease in the post-prandial state, in obese mice, the lowering effect of feeding is lost indicating a role of adiponectin as a starvation signal in healthy mice (Kubota et al., 2007), which counterintuitively has been shown to potentiate the effects of leptin of the activation of POMC neurons (Sun et al., 2016).

#### 2.3.2. Resistin

Resistin is an adipokine implicated in inflammatory processes and energy homeostasis. It has been described that this protein is also produced by other tissues such as brown adipose tissue, stomach, intestine and muscle (Noguerias et al., 2003). Its name comes from its effect on insulin resistance (Steppan et al., 2001); resistin knock-out mice have lower post-prandial glucose levels and lower hepatic gluconeogenesis than control ones (Banerjee et al., 2004). The ability of resistin to modulate insulin resistance is associated with the activation of suppressor of cytokine signalling 3 (SOCS3), an inhibitor of insulin signalling in adipocytes (Steppan et al., 2005). The main effects of resistin on energy metabolism are mediated by the hypothalamus; central administration of resistin reduces intake and regulates peripheral tissue lipid metabolism (Tovar et al., 2005, Vazquez et al., 2008). In addition, resistin impairs adiponectin central signalling by interacting with the toll like receptor 4 present in the neurons (Benomar et al., 2016).

### 2.3.3. Leptin

Coleman showed in 1973 that obese mice that lacked the ob gene or the diabetic mice deficient for the *db* gene, showed similar phenotypes characterized by hyperphagia, hyperglycaemia, hyperinsulinemia and obesity (Coleman, 1973). In a parabiosis experiment, the circulation systems of two mice were connected, so that the endocrine factors of an animal could reach the other's body. When a *wt/wt* mouse and an $ob^-/ob^-$ were connected, the $ob^-/ob^-$ mouse lost weight while the other animal maintained a constant body weight. When a $db^-/db^-$ mouse was in parabiosis with a *wt/wt*, the *wt/wt* drastically lost weight while the $db^-/db^-$ kept a steady body weight. If the parabionts mice were $db^-/db^-$ and $ob^-/ob^-$, the $ob^-/ob^-$ showed a weight loss and the $db^-/db^-$ weightwas unchanged.

These experiments indicated the presence of a circulating factor implied in the regulation of body weight that was missing in the $ob^-/ob^-$ mice, and on the contrary was present in the $db^-/db^-$ mice, but could not be perceived by them (Coleman, 1973).

A whole section of this manuscript will be dedicated to the molecule in question, that was then discovered to be the master anorectic adipokine: leptin.

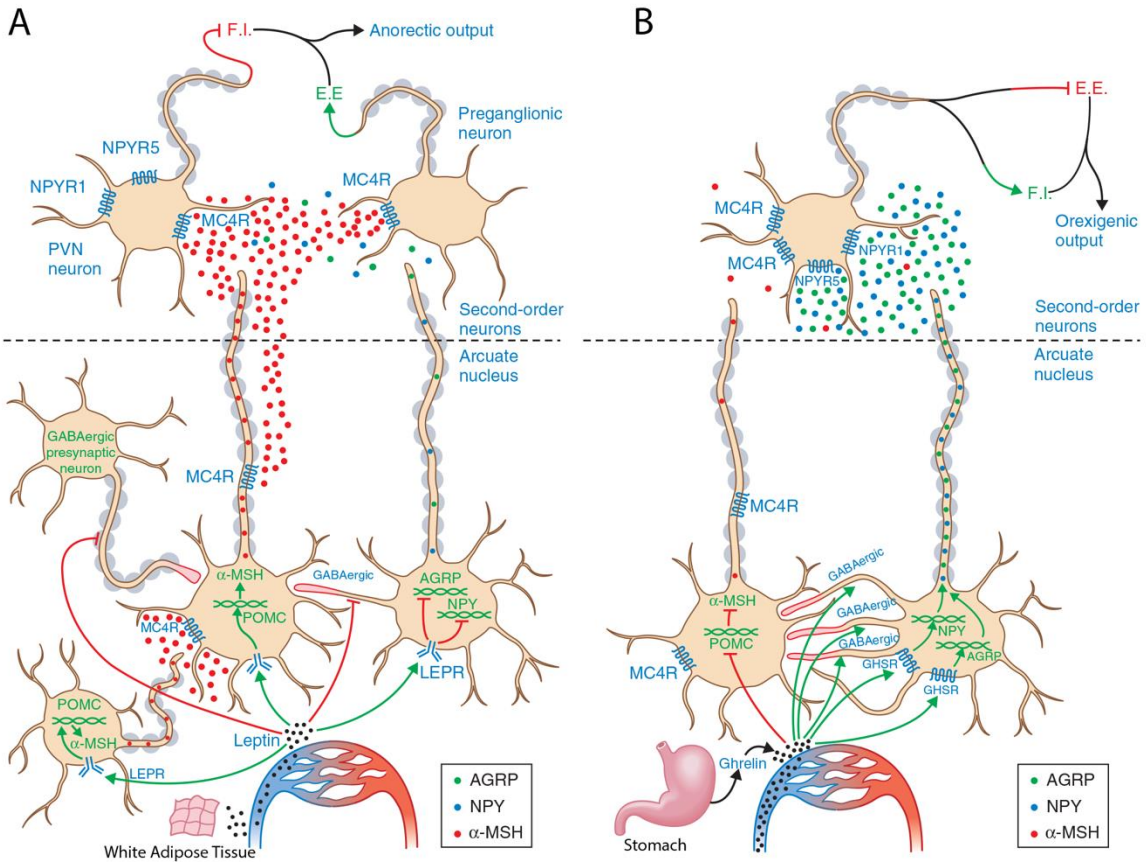



***Figure 4. Schematic representation of the melanocortin system and its regulation by anorexigenic leptin and orexigenic ghrelin.***

*(A) Leptin fuels the activity of POMC neurons in the ARC, hindering the activity of AgRP neurons. The subsequent increase in α-MSH release onto downstream second-order MC4R-expressing neurons in the hypothalamus and in extrahypothalamic regions is the key anorectic signal. α-MSH acts in an autocrine way by acting on POMC neurons which also express MC4R. Leptin also inhibits GABAergic presynaptic neurons that target POMC neurons.*

*(B). Ghrelin augments inhibitory GABAergic contacts onto POMC neurons and increases the expression and release of NPY and AGRP. AGRP acts as an antagonist of MC4R and NPY binds to Y1 and Y5 receptors, exerting the orexigenic effect of ghrelin.*

*Red = inhibition, green = activation (from Schneeberger, Gomis, & Claret, 2014).*

# III. Leptin

## 1. Structure and production of leptin

Leptin, discovered in 1994 (Zhang et al., 2004) and derived from the Greek "leptos" meaning "thin," is a 16 kDa protein produced through the expression of the human LEP or murine ob genes. The protein formed by four antiparallel α-helices akin to long-chain cytokines like ciliary neurotrophic factor (CNTF) or interleukin 6 (IL-6) (Denver et al., 2011; Zhang, 1997).
Studies in human and rodents with an unknown mutation, that a posteriori was identify as leptin deficiency (*e.g.*, ob/ob mice), already highlighted the pivotal role of leptin in metabolism. The absence of leptin results in severe obesity from an early age, accompanied by increased food intake, reduced energy expenditure, infertility, and diabetes among other conditions (Myers and Leibel, 2015). Coleman's 1973 parabiosis experiments showed that a circulating factor could restore normal weight in these obese animals (Coleman, 1973). Furthermore, treating individuals solely with leptin reversed the monogenic disorder in humans (Farooqi et al., 2002).
Leptin is produced in proportion to fat mass body content, thus plasma leptin levels reflects individual long-term energy stores (Frederich et al., 1995, Myers & Leibel, 2015, Friedman & Halaas, 1998). Other organs like stomach mucosa, placenta, muscle, and pituitary gland produce and release leptin to a lesser extent than the WAT (Bado et al., 1998; Mise et al., 2007; Ahima and Flier, 2000; Jin et al., 2000).
Leptin's expression and secretion hinge on many factors. Circadian rhythm master protein BMAL1/CLOCK generates *ob* gene transcription rhythm in WAT via C/EBPα (Kettner et al., 2015), multiple cytokines, acute inflammation, and glucorticoids raise mouse leptin levels (Sarraf et al., 1997, Uddén et al., 2003). Physiologic insulin infusion has been reported to either maintain or increase leptin serum levels (Malmstrom et al., 1996) while activation of 3 Adrenergic Receptors and also melatonin administration suppresses leptin plasma levels (Mantzoros et al., 1996, Rasmussen et al., 1999).
The body's energy status is an important parameter in the regulation of leptin secretion. Fasting reduces circulating leptin levels, while obesity leads to a significant increase in leptinaemia (Ahima et al., 1996). In addition, there's a sexual dimorphism in circulating leptin levels, with higher levels in women due to estrogen stimulation and testosterone inhibition (Moon et al., 2013).

## 2. Leptin Receptor

Coleman's research on *db*$^{-}$/*db*$^{-}$ mice (known for "diabetes") through parabiosis experiments shed light on the structure of leptin receptors, showing that the impact of the same serum factor responsible for obesity in *ob*$^{-}$/*ob*$^{-}$ mice, was mediated by a receptor.

In 1995, in explants from mice choroid plexuses (Tartaglia et al., 1995) the *db* genes were identified as leptin receptors (LepR). LepR is a type I cytokine receptor and exhibits six diverse isoforms resulting from alternative splicing (Lee et al., 1996; Wang et al., 1996), varying their size and structure. Five isoforms have a 34-amino-acid transmembrane portion (LepRa, b, c, d, f). The short forms (LepR a, c, d, f) include an intracellular segment of 32 to 40 amino acids and they are speculated to have a role in leptin transportation (Bjorbaek et al., 1998), the long form LepRb displays an intracellular portion of 303 amino acids can fully transduce leptin message trough several signalling pathways. Additionally, the LepRe isoform, a soluble variant of LepR without a transmembrane domain, is believed to play a role in deactivating and transporting circulating leptin (Li et al., 1998) (FI 5).

A point mutation identified in db/db mice affects the LepRb isoform expression and activity, leading to the expression of a truncated long receptor form lacking its intracellular part unable to activate STAT (Chen et al., 1996; Ghilardi et al., 1996).

Leptin receptors (LepRs) are type I cytokine receptors that lack inherent kinase activity, thus they need other proteins to lead the activation of the signalling pathway after leptin binds its receptor. When leptin binds to these complexes, it induces a structural change, facilitating the recruitment and optimal activation of mediator proteins (Hegyi et al., 2004). First, the kinase Janus Kinase 2 (JAK2) binds to an intracellular Leprb region known as the proline-rich "box 1" motif, and it is activated through autophosphorylation. Once active, it phosphorylates tyrosine residues (Tyr979, Tyr985, Tyr 1077, and Tyr 1138), leading to the recruitment of other proteins and the leptin signal intracellular transmission (Myers, 2004; Banks et al., 2000).

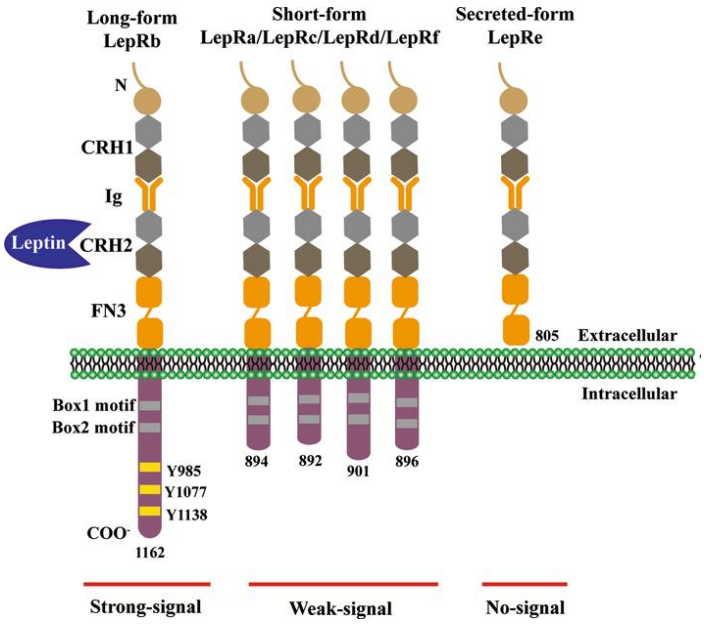



***Figure 5. Schematic representation of 6 different spliced isoforms of the LepR: LepRa–LepRf.***
*The extracellular ligand-binding domain (the second segment of cytokine receptor homology (CRH)) and the fibronectin type III domain (FN3) are common to all the isoforms, excluding the soluble receptor LepRe. All other LepRs have a different cytoplasmic portion and only the LepRb, the long form of the receptor, contains three tyrosine conserved regions (Y985, Y1077, and Y1138) enabling the leptin-induced activation of the JAK-STAT pathway together with Box1 and Box2. Y985 interacts with the SH2-containing protein tyrosine phosphatase 2, Y1077 with STAT5, and Y1138 with STAT3. Ig, immunoglobulin domain; JAK, Janus kinase (from Liu et al., 2021).*

### 2.1. JAK/STAT3 signalling

STAT3 (Signal transducer and activator of transcription 3) protein is a transcription factor. Its phosphorylation is used as a proxy for LepR activation. When in the cytoplasm, it binds to the proline-rich "box3" motif through its SH2 ("Src homology 2") domain when LepRb Tyr1138 is phosphorylated by JAK2. Subsequently, JAK2 phosphorylates STAT3 (Banks et al., 2000) leading to the detachment of pSTAT3 from the receptor, and its association with another STAT family proteins (pSTAT1, pSTAT5, or pSTAT6). Once the homo or heterodimer complex it is formed, it will be translocated to the nucleus to act as transcription factor for genes crucial in the control of energy homeostasis such as Socs3, Ptp1b, POMC, Cart, Npy, and AgRP (Allison et al., 2014) (Figure 6).

### 2.2. SHP2/MAPK signalling

The protein phosphatase SHP2 binds to LepRb through its SH2 domain, when the receptor in phosphorylated on Tyr 985. This triggers the activation of ERK1 and ERK2 (Extracellular signal-regulated kinases 1/2) signalling pathways (Zhang et al., 2004, Rahmouni et al., 2009). Activation of this signalling pathway by leptin is required for recruitment of cytosolic LepR antagonists such as Suppressor of cytokine signalling 3 (Socs3). This signalling cascade is also involved in decreased body mass, reduced food intake, and increased energy expenditure, promoting thermogenesis due the activation of sympathetic nervous system in the BAT (Rahmouni et al., 2009). Additionally, this pathway is recognized for its neurotrophic effect in

the development of the hypothalamic network involved in regulating food intake (Bouret et al., 2004) (Figure 6).

### 2.3. PI3K

Leptin-induced LepRb activation can also influence proteins linked to the insulin signaling pathway. JAK2 indeed, can phosphorylates insulin receptor substrate (IRS) ½ that in turn activates PI3K, leading to the production of phosphatidylinositol 3, 4, 5-triphosphate (PIP3). PIP3-mediated Akt activation blocks Forkhead box O (FoxO)-1 from exerting its orexigenic role in mealanocortin system first order neurons (Wauman & Tavernier, 2011; St-Pierre & Tremblay, 2012) (Figure 6).

### 2.4. AMPK

AMPK (adenosine monophosphate-activated protein kinase) is a serine/threonine kinase that plays a crucial role in governing energy homeostasis by acting as a sensor for intracellular ATP concentrations. It integrates metabolic signals both peripherally and centrally (Minokoshi et al., 2004; Hardie et al., 2012).
Leptin suppresses appetite also through the inhibition of AMPK, inducing p70S6K-mediated phosphorylation at S491 of the α2-subunit (Dagon et al., 2012) (Figure 6). Conversely, in peripheral tissues like skeletal muscles, leptin activates AMPK through two pathways: directly, by elevating the AMP/ATP ratio, and indirectly, via the hypothalamus-central nervous system axis involving α-adrenergic signaling leading to increased fatty acid oxidation (Minokoshi et al., 2002). However, the precise mechanisms governing leptin's differential regulation of AMPK in the hypothalamus versus peripheral tissues need to be elucidated.

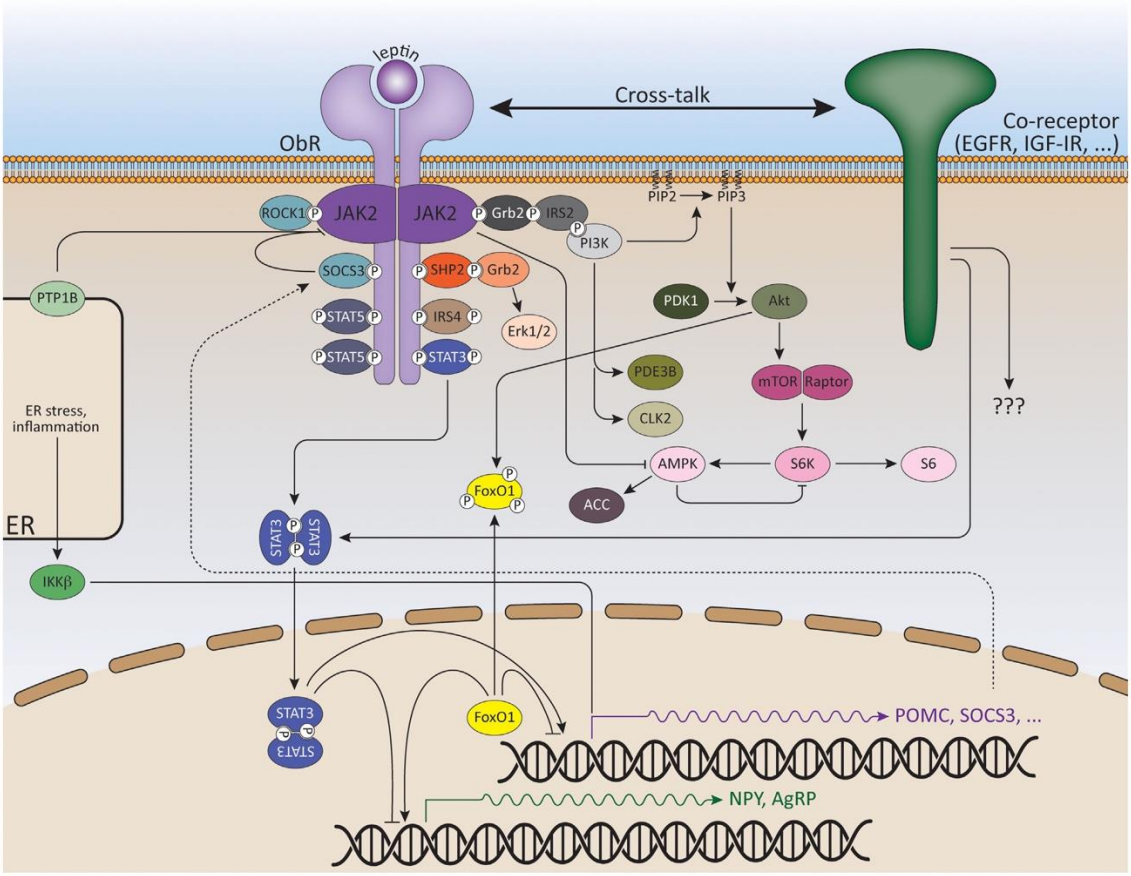



***Figure 6. Leptin receptor long isoform and its sinalling pathway.***
*LepR dimerization and following phosphorylation of associated JAK2 kinases. Recruitment and phosphorylation of secondary signalling molecules MAPK, PI3K, AMPK, and mTOR to exert leptin action (from Wauman, Zabeau & Tavernier, 2017).*

## 3. Role of Leptin in Central Energy Metabolism Regulation

The re-introduction of LepRb in the brains of *db*$^{-}$/*db*$^{-}$ mice completely rescues the metabolic phenotype of these animals (de Luca et al., 2005), underscoring the pivotal role of the brain as the primary site for leptin action. We will now focus in the specific brain areas which integrate leptin message.

### 3.1. Leptin modulation of the ARC

POMC/CART and NPY/AgRP neurons express LepRb (Lam et al., 2017). When leptin binds its receptor in POMC neurons, it triggers their depolarization (Cowley et al., 2001) and stimulates POMC expression through the PI3K/FOXO1 signalling pathway (Kwon et al., 2016). On the other hand, leptin inhibits orexigenic NPY/AgRP neurons, impeding their inhibitory effect over POMC neurons (Krashes et al., 2013; Aponte et al., 2011). Leptin inhibits

presynaptic GABAergic neurons (not Agrp-expressing) that modulate POMC neurons firing, giving an extra anorexigenic contribute (Vong et al., 2011).
Postprandial state or intracerebroventricular (ICV) leptin injection deactivates AMPK in the ARH and PVN regions, subsequently reducing food intake and weight gain. Leptin induces the phosphorylation of serine 491 on the α2 subunit of AMPK, diminishing its activity (Minokoshi et al., 2004).

### 3.2. Leptin modulation of the PVN

Glutamatergic neurons of the PVN express LepR (Shi et al., 2020). In addition, direct leptin activation of neurons in the paraventricular hypothalamus (PVN) was demonstrated in brain slices (Powis et al., 1998). Neurons expressing thyrotropin-releasing hormone (TRH) are stimulated by leptin, and TRH inhibits food intake via the melanocortin system (Guo et al., 2004; Harris et al., 2001). Similar effects occur in oxytocin neurons (Hâkansson et al., 1998). Remarkably, a population of LepRb neurons in the ARC, responding to leptin by producing α-MSH, projects to and stimulates PVN neurons, including TRH neurons (Kim et al., 2000).

### 3.3. Leptin modulation of the VMN and the DMN

Leptin administration activates neurons in the ventromedial hypothalamus (VMN) and dorsomedial hypothalamus (DMN). A majority of LepRb-expressing VMN neurons are SF1 (Steroidogenic Transcription Factor 1). Leptin prompts depolarization of these neurons, which moderately affect basal energy balance but regulate energy expenditure during high-fat diets (Dhillon et al., 2006; Hawke et al., 2009). Microinjection of leptin into the VMN increases glucose uptake into muscle via activation of the sympathetic nervous system, leading to stimulation of the AMPK pathway in muscle (Minokoshi et al., 1999; Minokoshi et al., 2002). Dorsal DMN neurons that express LepRb, regulate diurnal patterns of feeding, and locomotion. In addition, by activating prolactin secretion, promote energy expenditure by increasing heat production through UCP1 in brown adipose tissue (Rezai-Zadeh et al., 2014; Faber et al., 2021).

### 3.4. Leptin modulation of the LH

Leptin injection phosphorylates STAT3 in neurons within the lateral hypothalamic area (LH), aiming to reduce food intake and weight. LH neurons expressing LepRb are mainly GABAergic and distinct from LH predominantly orexigenic neurons (Leinninger et al., 2009). LepR-expressing LH neurons, along with neurotensin, regulate dopaminergic activity in the VTA (Lenninger et al., 2011), driving the hedonic-mediated feeding behaviour.

In addition, Lepr-expressing LH neurons target the opioid peptide proenkephalin (Penk) neurons present in the PAG, affecting binge-eating like behaviours (Shin et al., 2023).

### 3.5. Leptin modulation of extrahypothalamic areas

Leptin does not only modulate the hypothalamus, but other several brain areas.
Leptin directly influences parasympathetic control of the gastrointestinal tract by hyperpolarizing DMV neurons involved in visceral regulation and gastric related neurons (Williams et al., 2007).
NTS, major sensory nucleus in the dorsal medulla is also sensitive to leptin, which heightens the responsiveness to satiety factors. 20% of POMC neurons present in the NTS express LepRb and are sensitive to elevations in endogenous or exogenous leptin even when blocking synaptic transmission in ex vivo slices, suggesting a direct action of the adipokine on this neuronal population (Georgescu et al., 2020).
VTA dopamine neurons expressing Lepr respond negatively to leptin through the intracellular JAK-STAT3 pathway. Leptin administration to the VTA decrease food intake while Lepr ablation in the VTA increases food intake, locomotor activity, and sensitivity to highly palatable food. (Hommel et al., 2006).
Leptin potent role in the mesolimbic pathway regulation was also depicted by studying the leptin-responsive VTA neurons projecting to nucleus accumbens (NAc). In ob/ob mice indeed the locomotor response to amphetamine is dramatically reduced and can be reversed by leptin administration. In addition, electrically stimulated dopamine release from NAc was reduced in ob/ob *ex vivo* slices, highlighting the role of the adipokine in the integration of motivated behaviour (Fulton et al., 2006).

## 4. Leptin and cognition

The identification of LepRb in the cortex and hippocampus implies the relevance of leptin signalling in cognitive processes. Indeed, administering leptin into the hippocampus enhances rodents' learning ability (Farr et al., 2006). How? It enhances synaptic transmission mediated by glutamatergic NMDA receptors, augmenting the synaptic vesicle exocytosis triggered by the rapid intracellular calcium concentration increase (Shanley & Harvey, 2001). This mechanism underlies long-term potentiation and depression (LTP and LTD), vital for memory processes, and impaired in hippocampal neurons (Amnon's Horn 1) in ob/ob or db/db mice (Li et al., 2002). Leptin could possibly be the link that connects nutrition and cognition (Morrison 2009). In addition to the metabolic impairments associated to a loss of leptin entrance into the brain in the progression of obesity, individuals that during their middle life become obese are also more susceptible to the onset of cognitive decline and dementia later in life (Fitzpatrick et al., 2009), hinting to a correlation in between loss of leptin entrance in the brain and cognition.

# *Chapter 3: Blood-borne molecules access to the brain*

# I. How do metabolic signals access the brain?

Although nowadays we have a large and growing knowledge of the influence of peripheral cues on the brain areas that control energy homeostasis, the route that those molecules undertake to reach their target neurons, is still not completely unveiled. We will now deepen this subject and finally introduce the cellular population that has been in the spotlight of my studies: the tanycytes.

## 1. Blood Brain Barrier

The communication between blood and brain is strictly regulated to keep a constant and protected environment essential for the brain functioning. Studies from the end of the 19th century and the beginning of the 20th showed that an *in vein* (i.v.)-injected tracer is unable to reach the brain parenchyma (Ehrlich, 1885). Later on, it was shown that, on the other end, a marker infused in the CSF can stain the parenchyma (Goldmann, 1909), meaning that molecules can diffuse from the ventricles, but not from the vessels. The existence of a morphological barrier, the blood-brain barrier (BBB), constituted by brain capillaries was proposed by one of Ehrlic's students, but it was confirmed only after Reese and Karnovsky electron microscopy investigations (Reese & Karnovsky, 1967; Dyrna et al., 2013).
The BBB allows the transport of selected molecules towards and from the brain tissue, preventing pathogens to enter and damage the precious environment needed for the functioning of the central nervous system.

The BBB is formed by:
-**The endothelial cells** organize their tight junctions to close the vessels in a continuous endothelium (Reese and Karnovsky 1967). The presence of tight junctions allows the absence of intercellular pores, very low pinocytosis, polarization of endothelial cells (Begley & Brightman, 2003; Wolburg et al., 2009) and electrical resistance (Butt et al., 1990). These structures are form by different transmembrane proteins such as claudins (the isoform 5 essential for the BBB integrity (Nitta et al., 2003)), occludins and junctional adhesion molecules (JAMs), that can interact with the cytoplasmic protein e.g., ZO-1. Cell membranes are brought together by adherens junctions which comprise cadherins and β-catenins, α-actinins and vinculins (Figure 7).
The endothelial cells united by the tight junctions form a continuous endothelium layer. In addition, to increase the barrier properties, the abluminal side of the vessels is juxtaposed by a basement membrane, extracellular matrix composed of IV collagen, laminin and glycoproteins (Shepro & Morel, 1993);

-**The perycytes**: in contact with 1/5 of the external endothelial surface, they overlay the basement membrane together with smooth muscle cells. They play a role in vasoconstriction and angiogenesis (Peppiat et al., 2006; Gonul et al., 2002) (Figure 7);
-**The astrocytes**: in contact with almost the entirety of the brain vessels and oftentimes with the perycytes, they regulate the properties of the BBB and possibly integrate neuronal signals (Janzer & Raff, 1987) (Figure 7).

The passage of molecules through the BBB is highly specific (Figure 7). With the exclusion of gases and small lipophilic molecules which cross freely in a non-saturable transport (Clark, 2003) the endothelial sheet made up of a layer of cellular membranes, polar molecules require the expression of active transport effectors by the endothelial cells.
Many drugs and metabolites transport require energy, indeed they need transporters known as ABC (ATP-binding cassette). Other peripheral molecules can be transported into the CNS, and vice versa, by receptor-mediated or adsorptive transcytosis though the endothelium (Abbott et al., 2010).
The mechanism of transcytosis is important in the context of my thesis, so I will briefly describe it in the following section.

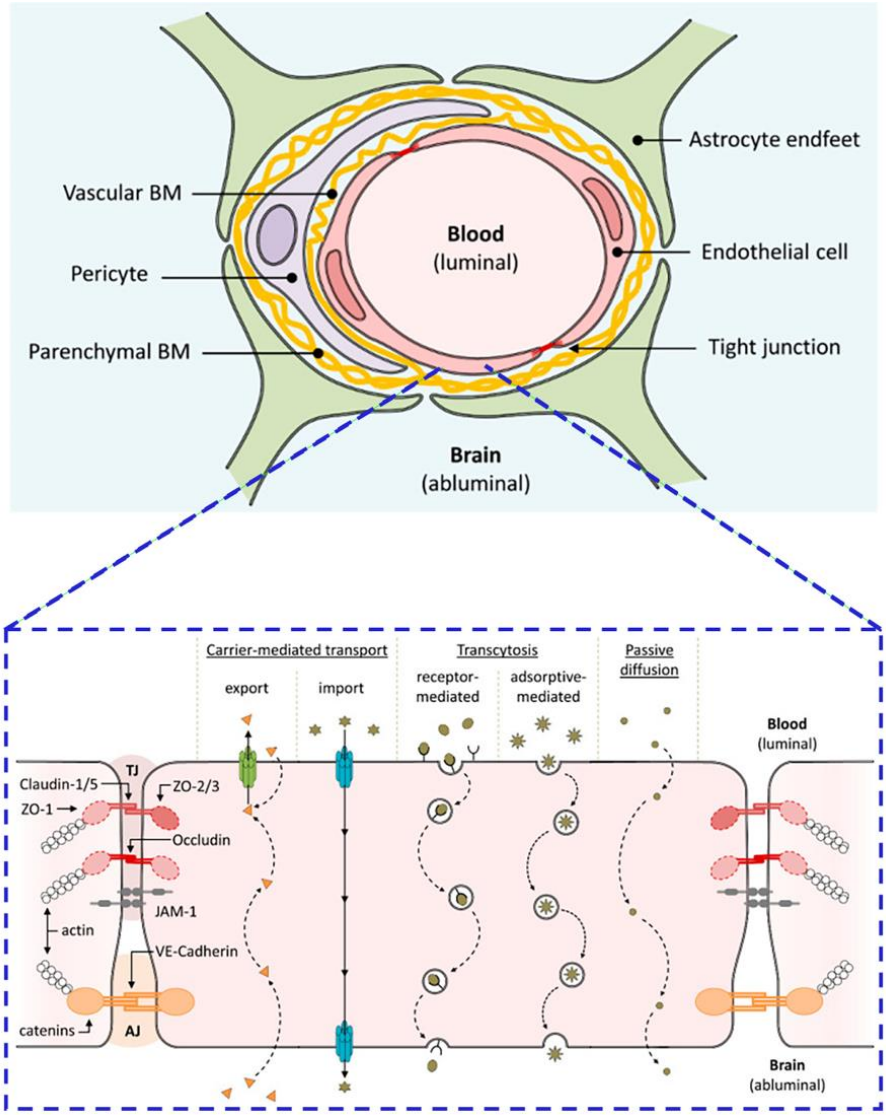



***Figure 7. BBB structure***.
*In the upper image we can appreciate the thin monolayer of endothelial cells joined by tight junctions forms the wall of brain capillaries, acting as a physical, transport and metabolic barrier that impedes the paracellular diffusion of random blood-born molecules. The capillaries are surrounded by a vascular basement membrane (BM), pericytes, a parenchymal BM and astrocytes, all of which contribute to the good functioning of the barrier. In the zoom image, the molecular structure of the tight junctions and the different way of transport are shown. Adherens junctions (AJ), formed by interaction of vascular endothelial (VE)-cadherin from adjacent cells and anchored to the actin cytoskeleton through catenins, provide structural support. The real barrier is made up by tight junctions (TJ), shaped by junctional adhesion molecules like JAM-1 and by the interaction of claudins, occludins, anchored to the actin cytoskeleton through membrane-associated zonula occludens (ZO) proteins.*
*The transport of xenobiotics and drugs is carried out by ATP-dependent efflux transporters present in the luminal membrane. Transfer of nutrients and other compounds can occur through carrier-mediated import, receptor- or adsorptive-mediated transcytosis or passive diffusion for gases and lipophilic molecules. (Modified from Neumaier, Zlatopolskiy & Neumaier, 2021).*

### 1.1. Blood-brain barrier passage: transcytosis

Transcytosis is a process of vesicular transport that enables macromolecules to be transported from one pole of the cell to the other, as just stated, it is an essential transport for the crossing of the BBB through endothelial cells, but is also found in other cell populations such as neurons

and glial cells (Barford et al., 2018; Slezak et al., 2012). To achieve this, 3 important stages can be distinguished: endocytosis, intracellular trafficking and finally exocytosis.
Transcytosis can be of two types, absorption or receptor-dependent. Receptor-dependent transcytosis involves a macromolecule, binding specifically to its receptor at the plasma membrane to trigger the formation of endocytosis vesicles by invagination of the plasma membrane. Two types of vesicles have been identified that may be involved in this stage of transcytosis: clathrin vesicles and caveolin vesicles (Cahudary et al., 2014).
After internalisation, the vesicles will cross the endothelial cytoplasm via multiple endosomal fusions and then, once the "final" intracellular compartment has been reached, the vesicular membrane will fuse with the opposite cell plasma membrane, resulting in the release of the ligand into the extracellular environment. For transcytosis to be effective, the internalised molecule must be released and functional after being transported into the cell.
Exocytosis is the final transport process and involves vesicular fusion with the plasma membrane to release the vesicular contents outside the cell. The exocytosis process involves a family of proteins, the soluble N- ethylmaleimide- sensitive-factor attachment protein receptors (SNAREs) (Söllner et al., 1993). These proteins are specifically involved in the fusion of vesicles with target membranes (Lafont et al.,1999; Low et al., 1998; Steegmaier et al.,2000). There are two types of SNAREs, depending on their location. The proteins present on vesicles are called v-SNAREs and those on the target membrane are called t-SNAREs (Söllner et al., 1993). The interaction of the v-SNARE protein with the t-SNARE leads to the formation of a complex that fuses the vesicles with the target membrane (Schiavo, Stenbeck & Rothman, 1997; Söllner et al., 1993). However, fusion efficiency is modest and the SNAREs complex requires the intervention of several partners and regulators for fusion to be effective (Grosshans, Ortiz & Novick, 2006; Zerial & McBride, 2001).
Exocytosis can be of two types, calcium-regulated or constitutive. In regulated exocytosis, the final stage in the fusion of the two membranes occurs following the entry of calcium into the neurons. Synaptotagmin proteins are membrane proteins located on lysosomes, the plasma membrane and secretory granules. Synaptotagmin acts as a calcium sensor, thanks in particular to the presence of two C2 domains (C2A and C2B) capable of binding calcium. Once bound to synaptotagmin, calcium reverses the action of a protein, complexin, and triggers vesicle fusion (Fernández-Chacón et al., 2001).

## 2. Blood-cerebrospinal fluid barrier

Not all brain areas harbour the BBB. The circumventricular organs (CVO) and the choroid plexus (CP) lack the BBB, indeed in these regions that line the 3$^{rd}$ and 4$^{th}$ ventricles the vasculature is fenestrated and molecules can relatively freely diffuse. The brain, however, is still protected by a different kind of barrier: the blood-cerebrospinal fluid barrier (BCSFB)

(Abbott et al., 2010). Unlike the BBB, these structures are more permissive and allow for facilitated communication between the cerebrospinal fluid (CSF) and blood compartments. We will now see the special function and structural organization of these areas.

### 2.1. Choroid Plexus

The cerebrospinal fluid functions as a buoyant that allows the brain not to collapse and it protects the brain by mechanical injuries. It is then essential for the distribution of substances and neuroendocrine factors to keep homeostasis, and it plays a role in waste clearing, being part of the glymphatic system, the waste clearance system composed of perivascular channels, formed by astroglial cells (Jessen et al., 2015).
The structures that produce the CSF are known as choroid plexus (CP), basically a layer of epithelial cells that overlies a richly vascularized stroma present inside the ventricles. The plasma filtration put up in order to produce CSF is made possible by the active transport of Na+, Cl- and HCO3- ions by the Na+/K+ ATPase pump and by the carbonic anhydrase activity. Fluid secretion by the choroidal epithelium is then possible thanks to the presence of aquaporin-like channels at the apical pole of the cuboidal CP cells (Brown et al., 2004).
As stated, here the endothelium that forms the vessels is fenestrated and expresses MECA32 (Ciofi et al., 2011; Mullier et al., 2010). The vascular and subepithelial basement membrane, primarily consisting of type IV collagen, serves as an initial "filter" for large cationic molecules while remaining highly permeable to numerous hydrophilic molecules (Peress & Thompkins, 1981). In addition, the tight junctions that in the BBB are present at the level of the endothelium, here are translated to the epithelial cells. TJs here are primarily formed by type 2 claudins and adherens junctions (Rosenthal et al., 2017) (Figure 8).
The diffusion of molecules within epithelial cells is predominantly influenced by fat-solubility parameters. With the presence of tight junctions, epithelial cells exhibit polarity, preventing the paracellular diffusion of small soluble molecules and promoting the selective expression of transporters on their basal surface (Spector & Johanson, 2006).
A significant portion of compounds (such as glucose, vitamins, ions, nucleotides, peptides, etc.) are transported bidirectionally via active or passive transport mechanisms, allowing for the retrieval and release of metabolites and other molecules (Brinker et al., 2014).

### 2.2. Circumventricular organs

The circumventricular organs are regions that line the 4 ventricles. They were first acknowledged in the 1950s (Hofer, 1958; Davson et al., 1987), and they are extremely studied in the context of metabolic homeostasis and hormonal exchanges. In these regions the endothelium is fenestrated like in the CP, indeed an i.v.-injected molecule can diffuse in these

areas (Ueno et al., 2000). Like in the CP, the barrier is translocated from the endothelial to another type of cells, more specifically the ependymoglial cells that line the ventricles.
The CVOs comprise the median eminence (ME), the subfornical organ (SFO), the pineal gland (PG), the neurohypophysis, the area postrema (AP), the organum vasculosum of the lamina terminalis (OVLT), the subcomissural organ (SCO) (even though the lack of fenestration make the definition of the SCO as a CVO controversial). Here a rapid neurohormonal exchange is permitted, allowing the CVOs to act like sensors, secretors or transporters.
Depending on their main role, CVOs are categorized into two groups:

- The CVOs classified as "sensor" organs include the SFO, the organum vasculosum of OVLT, the SCO and the AP. In these structures, neurons come into direct contact with blood vessels to detect information conveyed through the bloodstream.
- The CVOs categorized as "secretory" consist of the median eminence (ME) (although recent findings support its strong sensory role as well), the pineal gland (PG) and the neurohypophysis. In these neurosecretory regions, brain cells release their products directly into the bloodstream.

CVOs also play crucial roles in mediating communication between blood and CSF. They participate in various functions related to information detection and transmission. Consequently, their disruption may be implicated in numerous pathological conditions (Sisó et al., 2010).
Several studies suggest that molecules up to 10,000 Da can extravasate in sensory CVOs, while the size increases up to 40 kDa in the median eminence (Schaeffer et al., 2013; Ciofi et al., 2011). High-molecular-weight molecules tend to remain within the central portion of the CVO vessels and do not extravasate.
The CVOs harbour populations of glial cells that prominently express Glial fibrillary acidic protein (GFAP). Tight junctions have been observed in these cells using electron microscopy (Krisch et al., 1978; Petrov et al., 1994). Similar to the choroid plexus and their epithelial cells, it appears that the barrier function of the CVOs is localized to these ependymoglial cells (Figure 8). Referred to as tanycytes, the presence of tight junctions between each tanycyte effectively prevents paracellular diffusion, establishing them as a barrier between the peripheral blood and the CSF of the third ventricle (Mullier et al., 2010; Langlet et al., 2013).

Considering the privileged location next to the hypothalamic ARC, master metabolic regulator, we will now focus on a specific CVO extremely studied for its role in hormonal control and homeostasis: the median eminence.

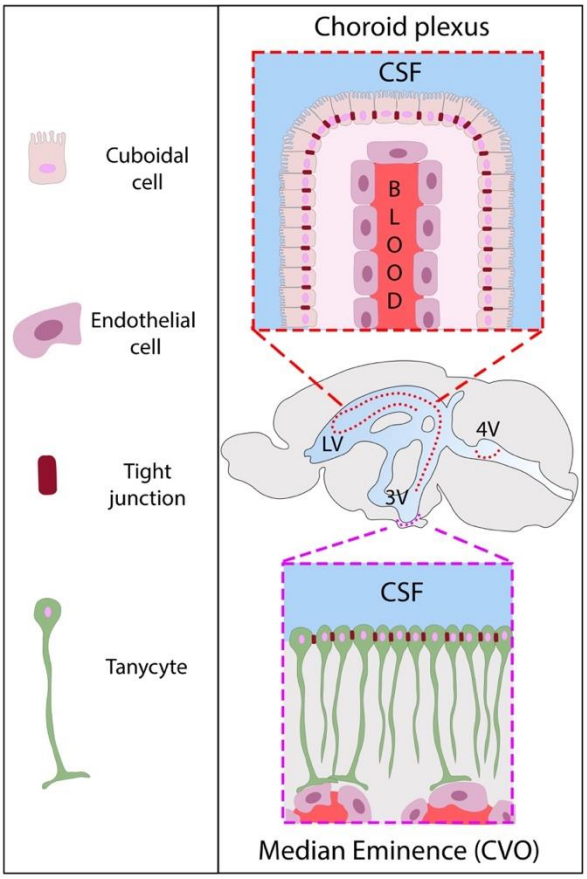



***Figure 8. Blood-cerebrospinal fluid barrier.***
*Schematic representation of the cytoarchitecture of the choroid plexus and the median eminence (a circumventricular organ). Notably, in both region vessels present fenestration in between endothelial cells. Here the tight junctions are translocated in between the cuboidal cells and the tanycytes in the CP and in the ME respectively, cellular population that bulid the interface in between clood and CSF and that avoid the free diffusion of molecules both ways. Designed by me.*

### 2.2.1. Median eminence, knocking on brain's door

The median eminence delineates the ventral boundary of the third ventricle and it is situated at the base of the hypothalamus. It serves as a crucial junction between the hypothalamic neurons responsible for regulating reproduction, lactation, stress, growth, and the pituitary gland.
Median eminence permeable vasculature carries the blood coming from hypothalamic arteries. The capillaries form two plexuses: the primary capillary and the secondary capillary plexus. The primary capillary plexus contact the outer part of the median eminence and it receives neurohormones released by neuronal axons. It also gives rise to long capillary coils that can enter deeper into the median eminence or even the arcuate nucleus. The deep capillary plexus is in contact with the inner part of the median eminence, at a slow rate of flow, suggesting exchanges between blood and CSF (Talanti & Kivalo, 1961). Finally, the primary plexus drains into the secondary capillary plexus to join the pituitary gland (Duvernoy & Risold, 2007).
The vascular endothelial growth factor (VEGF) is involved in vascular plasticity in the median eminence, as it is known to increase MECA- 32 expression in ME vessels. Low blood-glucose levels induce by a 24h-fast results in an increase in the number of MECA32-positive vascular loops in the ME and ARH (Langlet et al., 2013), allowing a fast access of blood-borne molecules into the hypothalamus.

This vascular structure enables the secretion of neurohormones into the pituitary portal circulation and opens the gates to blood-born molecules into its tissue. While peripheral hormones can freely diffuse into the median eminence tissue, they are unable to penetrate further into the hypothalamus or the CSF. Similarly, CSF components cannot freely enter the median eminence (Mullier et al., 2010). This stringent regulation of exchange, pivotal to brain-blood communication, is attributed to the ependymoglial cells of the median eminence, known as tanycytes.

## II. Tanycytes

Observation of the morphology of the cells lining the cerebral ventricles has shown the existence of several types of ependymal cells. Cell size, shape and the presence of cilia are all factors that allowed their characterisation. In the early 20th century, Sterzi looked at the base of the EM folds and found 2 types of ependymal cells: those with long processes corresponding to tanycytes, and those resembling cubic ependymal cells found mainly on the dorsal part of the 3V (Sterzi, 1909).

First observed by Ramon y Cajal, tanycytes were described as resembling embryonic radial glia cells (Ramon y Cajal, 1909). In 1954, Horstmann first used the term "tanycyte" ("tanus" in Greek, meaning elongated) to refer to these bipolar cell-shaped with a round apical pole facing the CSF and a long basal extension towards the brain parenchyma (Horstmann, 1954). Unlike cubic ependymal cells, tanycytes have few or no cilia. The flow of CSF contained in the ventral part of the 3V is therefore relatively calm, suggesting that the site is conducive to multiple exchanges (Kutcuoglu et al., 2007). Electron microscopy observations of the ME have shown that tanycytes contact both fenestrated vessels in the ME and BBB-type vessels in the parenchyma or neurons (Wittkowski, 1967; Güldner and Wolff, 1973). Tanycytes also display tight junction markers, notably at their apical pole. In the median eminence, tanycytes play this gatekeeper role (Mullier et al., 2010; Langlet et al., 2013b).

### 1. Classification

Depending on their position around the third ventricle, their function, their morphology, the cell type in contact with their basal extension, they have long been classified into 4 groups: the α1, α2, β1 and β2 (Akmayev et al., 1973) (Figure 9). Thanks to new techniques, this classification is evolving.

#### 1.1. Tanycytes of the dorsomedial part of the ARC, VMN and DMN

This group includes α1 and α2 tanycytes. These cells are arranged along the ventricle and project towards the nuclei of the DMN, VMN and dorsomedial part of the ARC (Figure 9). Their cell bodies contain abundant euchromatin, a developed sarcoplasmic reticulum and an important Golgi-endoplasmic reticulum-lysosome complex. These elements are associated with multiple vesicles and mitochondria suggesting the entry and exit of numerous molecules, calcium signalling and lipid biosynthesis (Rodriguez et al., 2019; Geller et al., 2019).

The apical membrane features microvilli as well as 2 non-motile cilia (Mirzadeh et al., 2017). The terminal part appears in contact with vessels present in the parenchyma. These tanycytes do not display a linear barrier-like organization. Indeed, the expression of ZO1 and occludin

being rather random at the apex may explain the diffusion of tracers between the CSF and these hypothalamic nuclei (Mullier et al., 2010).

### 1.2. Tanycytes in the ventromedial part of the ARC

This group represents the tanycytes that serve as the interface between the ME and the ARC, classified as the β1 type (Figure 9). They project into the ventral part of the ARH and the infundibulum of the ME. They have been grouped through the common expression of cornifin A (SPRR1A) (Campbell et al., 2017).
They possess transport and secretion machinery and multiple vesicles. The tight junctions of these tanycytes have a special feature. The expression of ZO1 and occludin is always present, but their organization depends on the type of vessel they are in contact with, which plasticity is dependent on the energy status of the subject. When contacting a BBB-type vessel, junctions are diffuse, whereas when contacting a fenestrated vessel, junctions take on a honeycomb structure (Mullier et al., 2010; Langlet et al., 2013b).

### 1.3. Median eminence tanycytes

Median eminence tanycytes correspond to the β2 type forming the floor of the 3$^{rd}$ ventricle (Figure 9). They present a long extension towards the fenestrated vessels and the basal lamina of the median eminence. Unlike the other 2 tanycyte groups, ME tanycytes do not derive from cells expressing *Shh* (Rizzoti et al., 2017).
The machinery required for endocytosis and exocytosis is present, with clathrin and caveolin-1 expressed at the two poles of the cells (Peruzzo et al., 2004). It is therefore highly likely that they have a transport role in addition to their structural role within the ME.
The presence of a network of tight junctions around each tanycyte cell-body is supported by the strong, uniform expression of ZO- 1, occludin, claudin 1 and claudin 5. This "honeycomb" structure creates a true barrier, preventing paracellular diffusion following the extravasation of molecules via fenestrated vessels and at the same time forming the junction between the bloodstream and the CSF (Mullier et al., 2010; Langlet et al., 2013b).

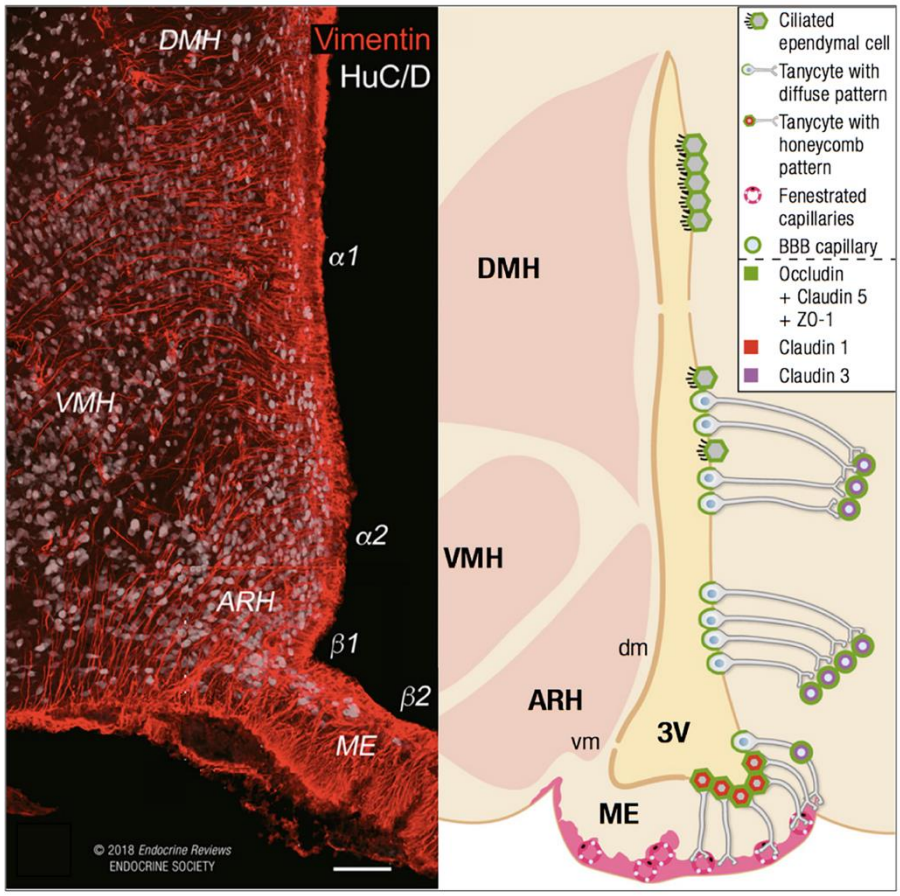



***Figure 9. Tanycytes.***
*On the left: Confocal image of a rat mediobasal hypothalamus. Tanycytes in red, are labelled for vimentin, neurons in white, for the neuronal marker HuC/D. Tanycyte subtype classical classification is indicated with Greek letters. Scale bar: 100 µm.*
*On the right: Illustration of the distribution of tight junction proteins in the MBH and ME of a fed animal. In both images: 3V: third ventricle; ARH: arcuate nucleus of the hypothalamus; DMH: dorsomedial nucleus of the hypothalamus; VMH: ventromedial nucleus of the hypothalamus (from Prevot et al., 2018).*

## 2. Tanycytes and CSF clearance

Hypothalamic tanycytes, were shown in a preprinted work of the laboratory (Sauve et al., 2023) to shuttle Tau from the cerebrospinal fluid (CSF), which accumulation plays a significant role in Alzheimer's disease. Tanycytes then release Tau into nearby capillaries, which then journey into the bloodstream. Blocking the vesicular trafficking of tanycytes leads to the build-up of fluorescent Tau in the CSF of mice. In Alzheimer's disease the structure of tanycytes is degraded, with a high fragmentation of the cytoskeleton. These findings prompt crucial inquiries into the function of tanycytes in natural clearance processes and their involvement in the onset of neurodegenerative conditions.

## 3. Tanycytes orchestrate central metabolic control

The variety of the tanycytic population reflects their different functions. Considering the unique location in between the fenestrated vessels, the CSF (playing the role of a bascule bridge) and the proximity to neurons controlling metabolism, it is not surprising to discover a role of tanycytes in the control of metabolism. Let's see how.

### 3.1. Neural progenitor cells involved in energy metabolism: tanycytes

The hypothalamus has emerged as an area of adult neurogenesis, alongside the subventricular zone of the lateral ventricle (SVZ) and the subgranular zone of the hippocampal dentate gyrus (SGZ). Tanycytes, expressing various stem and progenitor cell markers, possess slow proliferation capabilities, undergo turnover, and produce neurons and astrocyte-like cells. While all tanycytes express the Nestin protein and predominantly generate neurons, different tanycyte subtypes exhibit varying stem cell marker expression and functions (Robins et al., 2013; Haan et al., 2013).

β-tanycytes are considered progenitor cells, expressing doublecortin and responding to fibroblast growth factor 10 (FGF10) by producing neurons (Haan et al., 2010). In contrast, α-tanycytes renew their pool, generating more glial cells and potentially functioning as stem cells (Robins et al., 2013).

Inhibition of hypothalamic neurogenesis disrupts central energy balance control, tanycytic neurogenesis contributes to this process (Recabal et al., 2017), with neurons originating from tanycytes in the arcuate nucleus (ARC) responding to metabolic signals via STAT3 phosphorylation (Haan et al., 2013).

### 3.2. Control of neuroendocrine TRH secretion

The thyrotropic axis neurons of the hypothalamus produce thyrotropic hormone (TRH) and secrete it at the level of the ME for it then to be transported by the blood to activate the thyroid-sensitive cells of the pituitary gland. The production and secretion of thyroid stimulating hormone (TSH) from the pituitary into the bloodstream leads to the thyroid gland synthesis of triiodothyronine (T3, the active form of thyroid hormone) and thyroxine (T4, the inactive form of thyroid hormone), which in turn feedback to the hypothalamic TRH-neurons. Organs and tissues respond to thyroid hormones, generally favouring catabolic metabolism. Deiodinase 1 (Dio1) and deiodinase 2 (Dio2), enable the formation of T3 from T4. Deiodinase 3 (Dio3) transforms T3 into T4.

It would appear that tanycytes play an important role in regulating the thyrotropic axis. The tanycyte feet of the median eminence are in direct contact with the axons of TRH neurons before they secrete it into the circulation (Sanchez et al., 2009). Tanycytes also express the enzyme for TRH degradation (TRH-DE), suggesting that they may affect TRH bioavailability. Interestingly, tanycytes are the brain cells that most express Dio2 (Diano et al., 2003; Guadano-Ferraz et al., 1997) and they also express T3 and T4 transporters: the monocarboxylate transporter 8 MCT8 and the anion-transporting polypeptide OATP1C1 (Roberts et al., 2008). Tanycytes could therefore capture T4 from the circulation or CSF, convert it to T3 and induce feedback control of TRH secretion (Fekete & Lechan, 2014).

A study int the laboratory of Markus Schwaninger showed that activation of the TRH type 1 receptor in tanycytes leads to an increase in intracellular calcium via the Gαq/11 calcium channel. By genetically ablating this calcium channel in tanycytes, plasma TSH levels are higher. Indeed, the calcium increase enhances the growth of tanycyte feet at the level of their contact with TRH neurons, as well as the activity of the TRH-DE enzyme demonstrating the interaction between tanycytes and the thyroid axis (Müller-Fielitz et al., 2017).

### 3.3. Tanycyte plasticity involved in seasonal metabolic changes

In seasonal animals, the regulation of energy metabolism depends on day length or photoperiod. Suprachiasmatic nuclei control circadian rhythms by modulating the secretion of melatonin by the pineal gland. Melatonin GPR50 receptor appears to be involved in the management of thermogenesis, as in its absence the animal enters significant hypothermia after fasting (Bechtold et al., 2012). These same animals are also resistant to fatty diets (Ivanova et al., 2008). GPR50 is highly expressed in tanycytes of the median eminence and its expression there varies with photoperiod, thyrotropic axis activity and leptinaemia (Sidibe et al., 2010; Barrett et al., 2006; Betchtold et al., 2012). This suggests, once again, that tanycytes are involved in the regulation of energy metabolism.

### 3.4. Tanycytes as metabolic sensors and processors

Tanycytes are glucosensors and sensors of changes in CSF composition.
Tanycytes express proteins involved in glucose sensing such as GLUT2, glucokinase and the ATP-dependent potassium channel (Garcia et al., 2003; Thomzig et al., 2005; Millan et al., 2010). GLUT2 expression occurs at the feet of ME tanycytes, and rapid cellular transport of glucose is dependent on GLUT6. Moreover, the absence of these 2 proteins leads to a reduction in CSF glucose concentration (Martinez et al., 2019). ARH tanycytes express GLUT1 and can sense an increase in extracellular glucose, to which respond with an increase in intracellular calcium concentration, associated also with the secretion of ATP in the extracellular medium (Frayling, Britton & Dale, 2011).
Intracellular calcium waves in tanycytes are triggered by ATP binding to purinergic receptors (P2Y1). Moreover, ATP has a paracrine effect, which could represent a possible amplifying mechanism by propagating the calcium waves to the neighbouring tanycytes communicating through hemidesmosomes formed by connexins 43. (Orellana et al., 2012).
The ATP released can supply adenosine, its metabolite, and thus serve as a modulator of neuronal activity, thus modifying neuronal activity and also affecting feeding behaviour (Yang et al., 2015; Xu et al., 2016; Kittner et al., 2006). The calcium signal propagation mechanism again requires ATP release from tanycytes and activation of P2Y1 purinergic receptors (Frayling et al., 2011).

Tanycyte also express sweet-taste receptors (Tas1r2 and Tas1r3) through which they are capable of sensing glucose and artificial sweeteners inducing the same intracellular calcium response (Benford et al., 2017, Masuda et al., 2012). This set of reports support a role for glucose-induced tanycyte ATP release in the regulation of food.

In addition, the destruction of 3V walls modifies the response induced in the hypothalamus after hypoglycemia. This response is restored when these cells are functional again (Sanders et al., 2004).

Recently it was shown that tanycytes are involved also directly in the access of glucose in ARH and in the maintenance of physiological fluctuations of glycaemia during the circadian cycle. Suprachiasmatic nucleus (SNC) releases vasopressin at the beginning of the sleep phase, which causes an increase of GLUT1 in tanycytes. This promotes glucose entrance into the ARC, thereby lowering glycemia (Rodriguéz-Cortés at al., 2022). It was recently proven that tanycytes are able to sequester glucose-6-phosphate (G6P) into the endoplasmatic reticulum (ER) and can release it in case of hypoglycaemia. The G6P knockdown in tanycytes has also been shown to reduce food intake and NPY expression in fasted mice, highlighting the importance of the tanycyte-mediate glucose central transport for the physiological response to fasting (Barahona et al., 2024).

Other glucose metabolites such as lactate can be released by tanycytes to modulate neuronal metabolism and action (Clasadonte et al., 2017). Indeed, tanycytes express monocarboxylate transporters (MCT) 1 and 4 enabling lactate efflux. AgRP neurons on the other side, express MCT2 which allows lactate entry into these neurons. Genetically ablating the expression of MC1 or glucokinase selectively in tanycytes results in increased food intake and weight gain (Elizondo- Vega et al., 2015; Uranga et al., 2017).

The dialogue between tanycytes and energy homeostasis-controlling neurons has been highlighted in a study in which tanycytes, when engineered to express channelrohodopsin (light-activated membrane channels), can activate arcuate neurons to induce hyperphagia when activated by light (Bolborea et al., 2020). In addition, a recent study from the laboratory has revealed that tanycytes provide lactate, a glucose metabolite, to fuel the neuronal activity of glucose-insensitive POMC neurons. Specifically, the study demonstrated that, in response to elevated peripheral glucose levels, tanycytes produce lactate which is transported to POMC neurons via MCT 1 and 4. Consequently, POMC neurons sense the increase in lactate concentration and adjust their firing rate to maintain metabolic balance (Lhomme et al., 2022).

In addition to glucose sensing, tanycytes also appear to be involved in the detection of monoamines (Lazutkaite et al., 2017) and fatty acids (Hofmann et al., 2017). A recent study shows that fibroblast growth factor 21 (FGF21), considered an energy stress signal, whose expression is increased during fasting in peripheral tissues, is produced and secreted by tanycytes. During fasting the presence of free fatty acids in the blood increases, inducing increased FGF21 expression in tanycytes. Upon detection of palmitate, tanycytes would

activate FGF21 production which in turn would pass into the CSF, and could impact energy expenditure (Geller et al., 2019).
A very recent study also proves the mediation of tanycytes in the heat-induced food intake reduction. Thermosensing neurons of the brainstem convey the heat message to tanycytes that in turn secrete VEGF-A in the ARH. Here the growth factor reduces the excitability of AgRP neurons, resulting in the anorectic effect (Benevento et al., 2024).
Overall, tanycytes are able to sense metabolic cues and respond to adapt their function or influence the function of nearby neurons that regulate energy balance.

### 3.5. Tanycytes architects of the median eminence

Depending on nutritional status, several modifications have been observed within the ME. This plasticity relies on the aforementioned nutrient-detection ability of tanycytes.
Fasting for 24h promotes a significant increase in vessel fenestration selectively in the median eminence and ventromedial arcuate nucleus (vmARC) (Langlet et al., 2013) (Figure 9). These changes are accompanied by an increase in the organization of tight junctions in vmARH tanycytes in order to limit the diffusion of blood-born molecules into the parenchyma. These changes in the vessels and tanycytes are thought to be due to increased tanycytic VEGF-A expression that acts on VEGF receptors 1 and 2 in the endothelial cells of the median eminence. Functionally, this structural modification of the median eminence would facilitate access of molecules from the blood to the ARH given the presence of more permeable vessels in the region.
The remodulation of the vasculature architecture of the base of the hypothalamus also favors access to the orexigenic ghrelin to the ARC's orexigenic Agrp neurons. Leptin can also pass more easily. An increase in pSTAT3 labelling in vmARH was observed, but not dmARC. The latter is eliminated if treated with LAN (leptin receptor antagonist), suggesting that the increase in p-STAT3 is dependent on LepR activity (Langlet et al., 2013; Schaeffer et al., 2013).

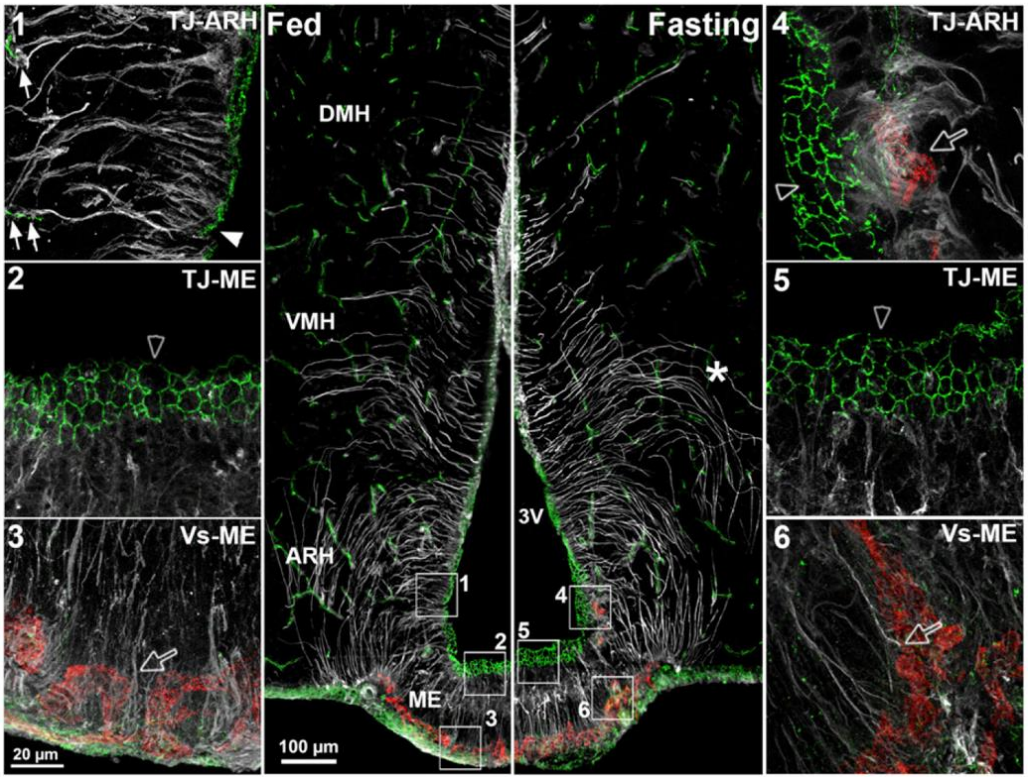



***Figure 10. ME BCSFB organization variation in accordance to energy status.***
*Vimentin in white, ZO-1 in green, and MECA- 32 in red. Fenestration of ME vessel loops that reach the vmARH after 24 of fasting is shown, in addition TJ proteins reorganize in ARH tanycytes. When in contact with ZO-1-positive vessels, tanycytic TJ show a diffuse pattern (inset 1) while when in contact with MECA-32-positive vessels (arrows, insets 3, 4, and 6) TJ are organized in a honeycomb pattern (arrowheads, insets 2, 4, and 5). 3V, third ventricle; DMH, dorsomedial nucleus; VMH, ventromedial nucleus, ARH arcuate nucleus, TJ-vmARH: ventromedial arcuate nucleus tight junctions, TJ-ME: median eminence tight junctions, Vs-ME: median eminence vessels (from Langlet et al., 2013)*

### 3.6 Tanycytes as hormone transporters: focus on leptin

As just discussed, energy status can influence tanycyte-mediated neuronal modulation and access of blood-borne molecules to the ARC, however we still don't have a clear view of how hormones can access area of the brain that control metabolism far from the fenestrated vessels. Tanycytes appear to transport hormones such as ghrelin (Collden et al., 2014).

In addition, the insulin receptors expressed in tanycytes, rather than brain endothelial cells, was shown to be essential for regulating insulin access to the hypothalamic arcuate nucleus. Furthermore, tanycytic insulin receptors are crucial for mediating the orexigenic effects of ghrelin, but not for the anorexic effects of leptin. (Porniece-Kumar et al., 2021).

Our focus will be on the transport of leptin, the queen adipokine of the regulation of long-term energy metabolism. The transport of leptin is still a subject of debate.

The presence of LepRb in the hypothalamus suggests that peripheral leptin is transported from the blood to its targets (Caron et al., 2010).

Because of its size (16KDa), leptin is unable to be transported by diffusion through the BBB. Peripheral injection of radiolabelled leptin has shown that the CP, ARH and ME are the areas

where leptin is most present (Banks et al., 1996). The high expression of LepR in the choroid plexuses suggested that this is where leptin transport was predominant (Burguera et al., 2000; Merino et al., 2006; Banks, 2019). However, decreasing LepR expression in the CP decreases leptin uptake and increases weight gain, but the receptors appeared not to be mediating the homeostatic control of feeding mainly carried out by hypothalamic nuclei. Indeed, animals appeared to be more sensitive to food-associated reward (Di Spieizo et al., 2018).

Which route does leptin undertake in order to reach its target neurons?

The detection of the hormone in CSF, suggests that peripheral leptin transport involves the BCSFB (Hagan et al., 1999). In physiology, plasma leptin concentrations correlate with CSF leptin concentrations. It would therefore appear that leptin transport to the CSF follows a saturable-type transport model, requiring a receptor (Banks, 2004). Neutralization of leptin in the CSF via the use of a neutralizing antibody results in decreased P-STAT3 (proxy for LepR activation) signalling in the dmARC, VMN and DMN indicating that leptin can reach these areas via the CSF (Balland et al., 2014).

It has been demonstrated that the accessibility of leptin to the various hypothalamic nuclei varies in terms of kinetics after peripheral injection, but also in terms of the amplitude of leptin-dependent activation (Faouzi et al., 2007). Indeed, the hypothalamic arcuate nucleus has a faster and stronger response to peripheral injection of leptin, even at very low levels. However, when leptin is injected centrally, successive access to the different nuclei is abolished (Faouzi et al., 2007). This suggests that leptin has different access to different hypothalamic nuclei.

The location and morphology of tanycytes as a bridge in between peripheral blood and CSF and their capacity for transcytosis justifies the interest in these cells in hormone transport to the CSF and hypothalamus (Mullier et al., 2010; Perruzo, 2004). Moreover, it has been shown that leptin injected peripherally induces a progressive basal to dorsal activation of the hypothalamic neurons starting from the vmARC, next to the ME fenestrated vessels, and ascending towards the more dorsal nuclei (Faouzi et al., 2007). The study of the alumni of the laboratory Balland in 2014, showed that 5 minutes after administration, tanycytes in the median eminence are activated (displaying pSTAT3 immunopositivity) by leptin. Bioactive fluorescent leptin injected in the circulation was shown to be present in ME fenestrated vessels and was then captured by tanycytes (Balland et al., 2014) (Figure 11). Interestingly, leptin doesn't appear to extravasate BBB-harbouring vessels present in the brain parenchyma. The uptake of leptin appears to be LepR-dependent, as a mutated leptin capable of binding to receptors without inducing their activities (LAN) is not captured by tanycytes and does not enter the ME (Figure 11). Leptin internalization into tanycytes occurs via clathrin vesicles at the basal pole of tanycytes traversing the cell towards the apical pole (Peruzzo et al., 2004; Balland et al., 2014). Leptin is then released into the CSF via a mechanism dependent on activation of the ERK signalling pathway (Balland et al., 2014).

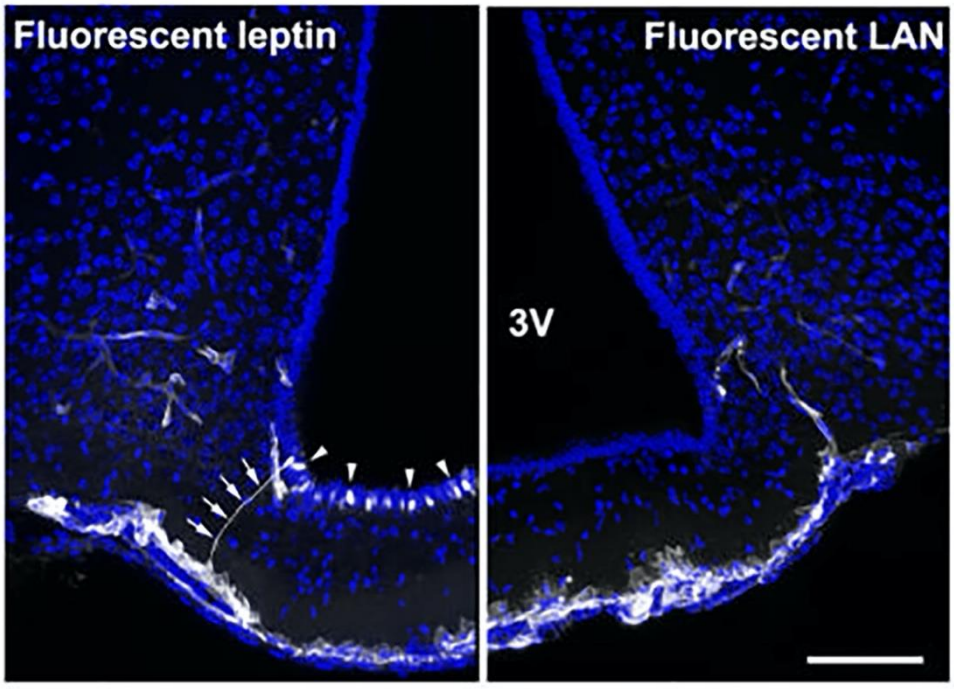



***Figure 11. Tanycytes transport leptin.*** *Immunofluorescent picture representing the presence of a fluorescent leptin (white) in tanycytic processes (arrows) and cell bodies (arrowheads). On the other hand, a fluorescent LAN (leptin receptor antagonist) only labels blood vessels in the ventral of the ME and in the ARH. Scale bar: 100 µm. (From Balland et al., 2014).*

Another more recent study of the laboratory conducted by Duquenne et al. (I also contributed to this work) confirms that ME tanycytes indeed express functional LepR and that leptin triggers tanycytes Ca2+ waves in tanycytes. LepR–EGFR complex activation by leptin and EGF is needed for the transcytotic transport of leptin. Furthermore, selective genetic ablation of LepR in tanycytes shut off leptin entry into the brain (Duquenne et al., 2021) (Figure 12).
Once leptin is transported into the CSF, leptin is able to reach these target neurons in the hypothalamus as well as higher structures via the flow of CSF into the ventricular system (Faouzi et al., 2007; Balland et al., 2014), supposedly via paracellular diffusion through the epithelium of the ventricles.
Interestingly, the tanycyte mediated leptin central transport is strongly debated taking into account that some authors have failed to detect LepR expression in tanycytes (Yoo et al., 2019), which seems to be caused by solid methodological differences between studies.

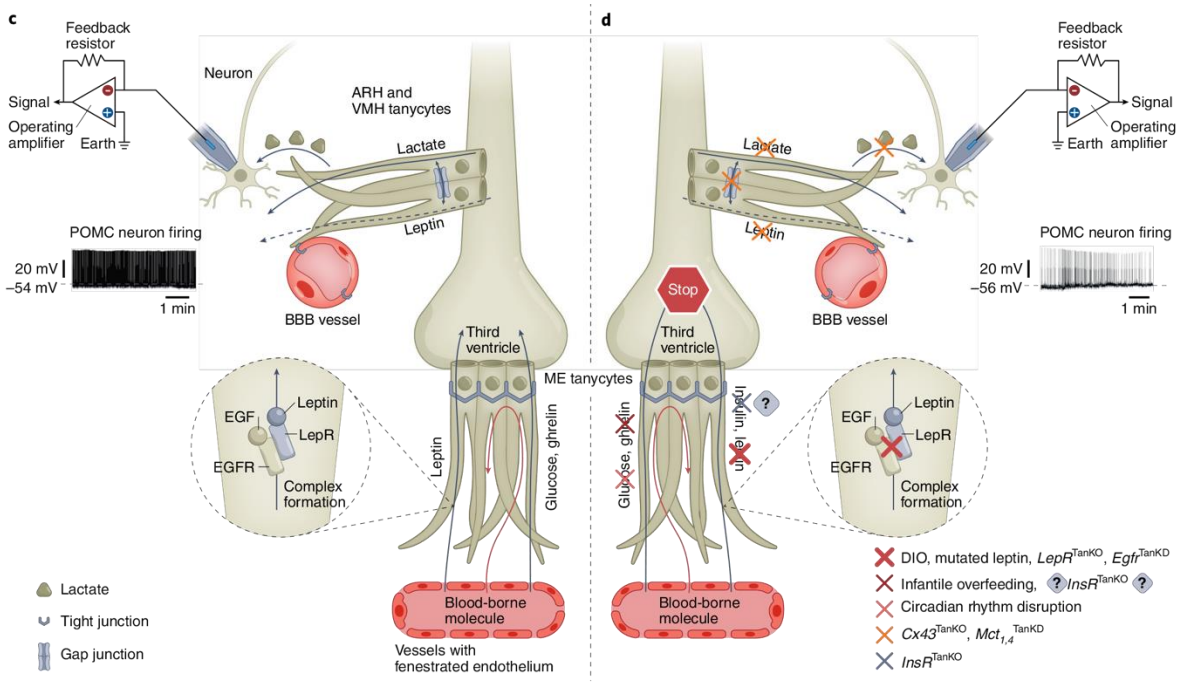



***Figure 12. The shuttling function of tanycyte in physiology, pathology and in genetically mutated models.***
*ME tanycytes shuttle molecules from the fenestrated vessels to the CSF. Notably, leptin is transported thanks to the formation of a LepR-leptin-EGFR-EGF complex formation. Tanycytes within the arcuate nucleus of the hypothalamus (ARH) detect molecules carried by the CSF, and relay this information to neurons influenced by the tanycytic network.*
*Altered physiological states or experimental conditions wherein mechanisms associated with leptin transport, insulin action or transport, ghrelin transport, or action are modified within tanycytes. The metabolic networks facilitated by Cx43 gap junctions among tanycytes are crucial for transporting lactate, generated and released by tanycytes POMC neurons that cannot catabolize glucose. Additionally, tanycytes contribute to the regulation of daily circadian fluctuations (from Nampoothiri et al., 2022).*

Tanycytes play a significant role in the regulation of metabolic and hormonal functions. Understanding their involvement in metabolic and hormonal diseases, is an important area of investigation. It is now the time to focus on the metabolic disorders that mostly affect people, their correlation with the neuroendocrine control of metabolism to then go back to the role of the tanycytes in these conditions and their possible role in therapeutic interventions.

# *Chapter 4: Metabolic disorders and the brain*

# I. Obesity

Beginning in the early Paleolithic era between 25,000 and 35,000 years ago, is when we appreciate the first representations of obesity in human statues, including the Venus of Hohle Fels (Conard, 2009), and 10,000 years later the Venus of Willendorf (Weber et al., 2022). The problem of obesity was already known to physicians in the Greco-Roman era since 500 BCE. "An Egyptian pharaoh was said to have a middle wider than the span of his slave's outstretched arms." Nichomachus of Smyrna "…was so huge that he couldn't even get up from his bed" (Foxcroft, 2011). The enormous size of a Roman Senator was such that he could only walk when two of his slaves carried his belly for him.

Soranus of Ephesus, a 2nd century CE Greek physician described obesity as a disease where the body keeps gaining additional flesh beyond the necessary. Nowadays overweight and obesity are described as abnormal or excessive fat accumulation that can present a threat to health. The BMI (Body mass index), calculated by dividing weight by height squared, is still today the main diagnostic test for obesity, despite the limitations of this formula that do not measure body fat and it also ignores age, sex and race (Prillaman, 2023).

**Keys to a healthy diet**

Eating a variety of whole and fresh foods every day helps obtaining the right amounts of essential nutrients, avoiding a diet that high in sugars, fats and salt, which can lead to unhealthy weight gain.

WHO recommends that people eat a combination of different foods, including staple foods, legumes, vegetables, fruit and foods from animals sources.

WHO recommends that people eat a wide variety of vegetables and fruit. Choosing them over foods high in sugars, fat or salt for snacks. Vegetables and fruit are important sources of vitamins, minerals, dietary fibre, plant protein and antioxidants, elements that lower the risk of obesity, heart disease, stroke, diabetes and certain types of cancer.

Moderate amounts of fats and oils are part of a healthy diet. Fats and oils are concentrated sources of energy, and eating too much fat, especially saturated fat and trans-fat, increases the risk of heart disease and stroke.

Industrially produced trans-fat present in various processed foods is the main source.

Use unsaturated vegetable oils rather than animals fats or oils high in saturated fats.

Choose white meat and fish, which are generally low in fats, rather than red meat.

Opt for low-fat or milk and dairy products. Avoid processed, baked and fried foods.

Rates of overweight and obesity keep on growing in adults and children. From 1975 to 2016, the percentage of overweight or obese young people aged 5–19 years increased globally from 4% to 18%, meaning more than four-fold.

With over 4 million people dying each year because of pathologies arisen or worsened by overweight or obesity, the issue has grown to an epidemic scale.

Except sub-Saharan Africa and Asia, today obesity is more prevalent than underweight everywhere, dramatically on the rise in low- and middle-income countries, particularly in urban settings, revealing the main cause of the problem: malnutrition. Perinatal malnutrition (both low and high weight gain) could lead to obesity, insulin resistance, diabetes, high cholesterol, elevated blood pressure, and increased cardiovascular disease risk (Barker et al., 1990; Oken

et al., 2003; Rolland-Cachera et al., 2013; Eriksson et al., 2002; Kuzawa et al., 2003; Adair et al., 2005; Leon et al., 1998; Huxley et al., 2007).
Obesity and diabetes (discussed in the next section) seem linked to a struggle to adapt to the modern world. The human being is genetically "programmed" to handle periods of scarcity, it presents an energy-saving genotype (Neel et al., 1962). Historically, even a slight increase in food intake aimed to stock energy. However, this evolutionary advantage turned into a risk factor for metabolic diseases, now that food is always accessible. Capital-driven modern food industry produces high-caloric foods that are readily available at low prices. This kind of processed and fast food, perfectly combines with the needs of millions of exploited urban workers whose job hours leave them little time for making groceries and cooking. In addition, society of nowadays put people under continuous stress and numerous studies have highlighted the association between stress and weight gain or adiposity, particularly in individuals who are already overweight or have high glucose or insulin levels (Dallman et al., 2005; Torres & Nowson, 2007; Jastreboff et al., 2013).

Nowadays the growing mechanized way of transportation and sedentary habits, together with unhealthy eating, leads to the imbalance in between ingested and consumed calories that causes overweight and obesity. In the following paragraphs we will focus on the purely biological causes and mechanisms underlying the onset of obesity.

**1. Genetic factors**

Polygenic forms of obesity arise from mutations that, individually, would not impact body weight, but collectively, they create a considerable predisposition.
Oligogenic forms with variable penetrance, involve mutations affecting groups of proteins engaged in intracellular signalling, such as receptors, influencing an individual's sensitivity to their environment and most of these genes encode components of the leptin–melanocortin and BDNF–TrkB signalling pathways (Loos & Yeo, 2022).
Certain genetic mutations independently cause severe obesity without behavioural or environmental involvement. These genes significantly regulate energy metabolism and can harbour single-gene mutations with complete penetrance. Severe obesity arises endocrine system disorder, such as mutations in the leptin gene (LEP) (Montague et al., 1997; Strobel et al., 1998) or the leptin receptor (LR) (Clement et al., 1998).

**2. Obesity and brain inflammation**

Chronic exposure to fatty diet and obesity are associated with increased inflammation in peripheral tissues, but also in the central nervous system (Zhang et al., 2005, De Souza et al., 2005). Several cell types present in the hypothalamus are involved. The application of a fatty

diet activates astrocyte recruitment, increasing inflammation from the first days of exposure, in addition to decreasing the accessibility of leptin to target neurons (Horvath et al., 2010). Moreover, activation of the surrounding microglia also leads to disruptions in the activity of POMC neurons (Gao et al., 2017). Toll like receptor 4 (TLR4) receptors, present mainly in hypothalamic microglia and astrocytes is over-expressed on a high-fat diet, leading to the production of pro-inflammatory cytokines (Milanski et al., 2009). These cytokines, will stimulate their respective receptors (IL-6R and TNF-R2) expressed on POMC and AgRP neurons (De Souza et al., 2005) and stimulate pro-inflammatory signalling pathways including IκB Kinase β/nuclear factor-κB (IκKβ/NFκB) and c-Jun N-terminal kinase signalling (JNK) (Milanski et al., 2009), which will lead to the inhibition of POMC expression and the blockade of its anorectic action (Jang et al., 2010; Shi et al., 2013).

## 3. Obesity and leptin

As previously described, leptin receptor-mediated signalling pathway is crucial in energy homeostasis regulation. Animals with a substitution at tyrosine 1138 (s/s) that prevents STAT3 binding and activation display obesity and increased food intake, akin to db/db animals. Moreover, specific loss of STAT3 expression in the rodent brain (Gao et al., 2004) or LepRb-expressing neurons (Piper et al., 2008) results in obesity and increased food intake while maintaining normal blood sugar levels.

Although leptin administration has been shown to reduce food intake in leptin-deficient (ob/ob) mice (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995), this genetic model of obesity is not suited to the study of obesity in humans, in whom very rare cases of obesity are due to single-gene syndromes (Farooqi & O'Rahilly, 2005). Indeed, the majority of obese individuals have markedly elevated serum leptin levels, directly related to adiposity (Maffei et al., 1995).

The most fitting mouse model, is so the one of diet-induced obesity: the diet-induced obesity (DIO) model (Van Heek et al., 1997). It has been shown that these mice, after several weeks of exposure to a high-fat diet high-fat diet (45-60% of caloric intake from lipids), become obese and leptin, although growing in concentration, start to lose its effects (Van Heek et al., 1997; Widdowson et al., 1997). In an animal model that overexpress leptin, hyperleptinemia aggravates HFD-induced weight gain and metabolic abnormalities (Ogus et al., 2003). In addition, administration of exogenous leptin, even at high doses, does not reduce food intake and body weight in obese animals and humans, unless leptin deficiency is present (Bluher, Shah & Mantzoros, 2009).

Following these observations and inspired by the better-known phenomenon of insulin resistance, researchers developed the concept of leptin hormone resistance, which corresponds to the inability of obese individuals to respond adaptively to increase in endogenous or exogenous leptin.

### 3.1. Leptin resistance

The phenomenon of resistance to the effect of leptin on body weight reduction in obesity has been the subject of numerous studies since the first identification of the leptin receptor (Tartaglia et al., 1995), but the mechanisms are still under debate.

Two mechanisms have been identified:

- The first involves impaired transport of leptin from the periphery to the hypothalamus, the critical site of action for leptin's regulation of energy balance (Van Heek et al., 1997).
- The second mechanism concerns leptin signalling at the central level. The ability of leptin infused centrally in DIO to activate STAT3, decreases in these animals (El-Haschimi et al., 2000).

*Peripheral leptin resistance: impaired transport*

A study in humans and minipigs has demonstrated a marked reduction in the ability of peripheral leptin to enter the brain. Peripheral leptin levels (serum) and central leptin levels (CSF) were measured in obese and control individuals. In obese individuals, the CSF leptin/serum leptin ratio was decreased compared with control individuals (Caro et al., 1996 Schwartz et al., 1996; Chmielewski et al., 2019). This suggests that leptin transport from the blood to the brain is impaired in obese individuals. This impaired transport of leptin from the periphery to the brain in obesity is underlined by the fact that peripheral administration of a supraphysiological dose of exogenous leptin fails to activate central leptin signalling pathways in DIO mice (El-Haschimi et al., 2000).

One study decreased the kinetics of the progressive development of leptin transport alterations during the progression of obesity under HFD in C57BL6 mice (Lin et al., 2000). The authors demonstrated that, during the HFD, a progressive increase in body weight, adiposity and leptinaemia takes place in a non-gradual way, reflecting the intensification of leptin resistance. Leptin sensitivity was assessed also by peripheral and/or central injections of leptin with consequent measurement of the variations in body weight and food intake of the DIO mice.

At 8 weeks of HFD mice develop an insensitivity to 2mg/kg leptin injected i.p., while they retain their sensitivity to i.c.v.-administrated 0.1 μg of leptin.

However, ad 19 weeks of HFD, mice also lose their sensitivity to to 0.1μg of i.c.v.-injected leptin, retaining the response to 2 μg of i.c.v.-injected leptin. DIO centrally leptin-injected mice indeed decrease their food intake and body weight, but this response was significantly less than in control mice.

This study clearly demonstrates that the development of leptin resistance is a gradual, intake-related phenomenon linked to weight gain. A recent study on DIO minipigs support this hypothesis and shows how an impaired central leptin transport precedes the onset of morbid obesity (Chmielewski et al., 2019).

The progressive blockage of leptin central transport can be described as follows: firstly mice show an increase in adipocyte fat mass due to the HFD, but still respond to exogenous

peripheral leptin administration by a reduction in food intake, then DIO mice are no longer sensitive to peripheral injection of leptin, but retain the ability to respond to a central leptin injection (El-Haschimi et al., 2000; Lin et al., 2000; Van Heek et al, 1997), until when, in later stages, mice show central resistance to leptin, even at high doses (El-Haschimi et al., 2000; Lin et al., 2000).

*Central leptin resistance: impaired neuronal LepR signalling*

The existence of a mechanism of central leptin resistance is supported by the fact that the response to leptin injection in DIO animals is diminished or absent even subsequently to central administration (El-Haschimi et al., 2000; Lin et al., 2000; Widdowson et al., 1997).

Leptin resistance is not homogeneously established in hypothalamic different nuclei. In fact, the onset of leptin resistance initially develops in the ARC, which is more sensitive than the other nuclei to the same dose of leptin (Faouzi et al., 2007), then later in the VMN, DMN and PVN (Munzberg et al. al., 2004). Interestingly, this sequence of appearance of leptin resistance follows the same pattern as that of leptin accessibility to these same sites, suggesting that the hyperleptinemia could be at the origin of the development of resistance.

Impaired signalling associated with LepRb in obese animals may be due to reduced expression of this receptor on the cell surface (Wilsey & Scarpace, 2004; Wilsey et al., 2003). The decrease in LepRb-associated signalling in the ARH (Munzberg et al., 2004), and particularly in the POMC and AgRP neurons, is associated with altered release of these neuropeptides.

In parallel, increased expression of the leptin signalling inhibitor leptin signalling, SOCS3, is observed (Enriori et al., 2007; Munzberg et al., 2004). Deficiency of SOCS3 in POMC neurons neurons (Kievit et al., 2006) protects mice against the development of obesity induced by an HFD diet. Similarly, deletion of PTP1B phosphatase, another negative regulator of LepRb signalling, increases leptin sensitivity and attenuates weight gain on a HFD diet (Bence et al., 2006). These data therefore suggest that impaired action of leptin plays a critical role in the pathophysiology of leptin resistance and hence in the maintenance of obesity.

Inflammation is also one of the players of central leptin resistance, especially the endoplasmic reticulum (ER) stress, caused by the accumulation of misfolded proteins, beyond the proteasome's capacity to degrade them (Vembar & Brodsky, 2008). ER stress is greatly increased in obesity, particularly in the brain (Zhang et al., 2008).

An excessive food intake stimulates the mTOR signalling pathway, which in turn generates ER stress (Ozcan et al., 2008). Interestingly, ER stress is sufficient to inhibit leptin signalling in vitro, whereas pharmacological inhibition of ER stress increases leptin signalling (Hosoi et al., 2008; Ozcan et al., 2008). Moreover, inhibition of ER stress in the hypothalamus restores leptin sensitivity and reduces food intake in mice (Ozcan et al., 2008).

## 4. Tanycytes and obesity

Tanycytes are increasingly recognized as potential contributors to the pathophysiology of metabolic diseases. Identified through single-cell RNA sequencing among the ARC-ME cell populations, tanycytes exhibit significant enrichment in genes linked to the waist-hip ratio, a marker of fat distribution and correlated health conditions (Campbell et al.,2017).

Research conducted on DIO mouse model has revealed that the impairment in the leptin central transport, first stage of leptin resistance, is actually an impairment of tanycytic leptin transport. In lean mice, peripherally administered leptin reaches the mediobasal hypothalamus within 45 minutes, as measured by the activation of Stat3 and by the actual detection of the hormone in the lysates of the MBH. However, in DIO mice levels of leptin in the MBH are undetectable at the same time point after peripheral injection, indeed the hormone seems to remain stuck in the ME. Interestingly, these mice show no alteration in Stat3 phosphorylation during the same timeframe, but they show a constitutive activity of the translation factor (Balland et al., 2014). The link in between the diet-induced impaired leptin central transport and the impaired leptin tanycytic transport is dilucidated in a following study of the laboratory (Duquenne et al., 2021) where we observed the metabolic phenotype of mice lacking the leptin receptor selectively in tanycytes. The mice in which the leptin receptor is selectively knocked out in tanycytes weighted more, they showed a threefold increase in visceral, but not subcutaneous fat mass, reflected also by higher leptin serum levels. The main cues pointing to the onset of a hypothalamic resistance to peripheral leptin, caused by the impaired tanycytic transport, were mainly the increased food intake even after overnight feeding and despite high circulating leptin levels. Indeed, after an overnight feeding the number of immunopositive cells for the Stat3 phosphorylation was diminished by 30% when compared to the controls. In agreement to this physiological data, the measurement of the concentration of MBH leptin by microdialisys after a leptin peripheral administration displayed the dramatic loss of increase in central leptin levels when Lepr is knocked out in tanycytes. Considering the similarities of this model with DIO models, it can be hypothesized that tanycyte leptin transcytosis is disrupted in DIO mice.

## II. Diabetes

Diabetes is a chronic disease characterized by one of these two phenomena: either the pancreas does not synthetize enough insulin (insulinopenia), or the body cannot use the insulin produced (insulin resistance). Insulin, a hormone produced by beta cells of the islets of Langerhans in the pancreas, is the master regulator of glycaemia. Hyperglycaemia is a hallmark of uncontrolled diabetes and progressively leads to damage the body, especially the nerves and blood vessels.

In 2014, 8.5% of adults had diabetes. In 2019, diabetes was the direct cause of 1.5 million deaths and hyperglycaemia causes around 20% of cardiovascular deaths (Global Burden of Disease) and an 13% increase in the mortality rate in lower-middle-income countries has been reported. Diabetes can progressively cause damages in blood vessels in the heart, eyes, kidneys and nerves and can lead to a higher risk of having heart attack, stroke and kidney failure.
Deficient insulin production is typical of type I diabetes and it often stems from the autoimmune destruction of pancreatic β-cells. The 9 million people with type 1 diabetes, counted in 2017, need a daily administration of insulin for survival.
Type 2 diabetes affects how the body uses glucose for energy, the body becomes resistant to the effects of insulin, which can lead to high glycaemia if not treated. More than 95% of people with diabetes have type 2 diabetes. This type was seen only in adults but its occurrence in children is dramatically increasing. Nearly 80% of type 2 diabetics are either obese or overweight, though it's essential to note that only 20% to 30% of obese individuals develop diabetes (Bernard et al., 2013). Over time, type 2 diabetes can cause serious damage, especially to peripheral nerves and blood vessels (Global Burden of Disease Collaborative Network, 2020).
Disregarding the genetic causes, type 2 diabetes (T2D) is often preventable, by avoiding the onset of obesity by getting enough exercise and having a healthy diet. Higher fat depots, increase dysfunctional metabolism and inflammation. The increase in systemic TNF-α in obesity activates different kinases which directly phosphorylate serine in the insulin receptor substrate (IRS) protein impairing tyrosine phosphorylation and leading to insulin resistance in adipose tissues, muscles, and liver (Nieto-Vazquez et al., 2008). Consequently, people that suffers of obesity faces three times the risk of develop type 2 diabetes compared to people without obesity. Patients with overweight or obesity, by getting regular blood glucose tests, can benefit for an early detection of glucose metabolism disturbances that precedes the development of T2D. Indeed, impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) are intermediate conditions in the transition between physiology and diabetes. If fasting glycaemia levels fall between 1.10 and 1.26 g/L, the individual is considered to suffer pre-

diabetes condition. When blood glucose levels surpass 1.27 g/L in two consecutive tests, a diagnosis of diabetes is confirmed (Sarwar et al., 2010).
One of the most important methods to both prevent and treat T2D is to maintain a healthy lifestyle. In addition, a pharmacological intervention may be necessary to manage blood sugar levels, medication to reduced possible high blood pressure and statins to reduce the small blood vessels damages are often needed (https://www.who.int/news-room/fact-sheets/detail/diabetes).

## 1. Inflammation role in the development of T2D

Research suggests that low-grade inflammation may play a pivotal role in the onset of insulin resistance and T2D associated with obesity (Cefalu, 2006). Biomarkers of inflammation, such as TNF, IL-6, and CRP, are found in higher concentrations in individuals with insulin resistance and obesity. Consequently, these proinflammatory markers could serve predictors of the development of T2D.
Several proinflammatory cytokines, increased SOCS protein expression (inhibitor of leptin and insulin receptors) (Galic et al., 2014), ER stress (Flamment et al., 2012), the IKK pathway of NF-B activation (Zhange et al.,2010) and JNK signalling pathways (Li & Yu, 2013) are all associated with the development of insulin resistance. These studies indicate that proinflammatory mediators released by adipocytes, link the immune system with obesity-related insulin resistance. Intriguingly the activation of these pathways is regulated by leptin, the putative molecule linking the immune system with obesity-related both insulin and leptin resistance.

## 2. Central regulation of glycaemia and insulin resistance in the brain

Neurons of the hypothalamus express insulin receptors (Marks et al., 1990). Specifically, both POMC (at least a part of its population) and AgRP neurons are modulated by insulin (Liu et al., 2012, Hill et al., 2010, Qiu et al., 2018, Varela & Horvath, 2012). The melanocortin system is implicated in blood glucose regulation. Rodents lacking MC4R showed hyperglycaemia and hyperinsulinemia before developing obesity, indicating the involvement of these neurons in the direct blood glucose regulation (Huszar et al., 1997; Ste Marie et al., 2000). Central regulation of blood glucose involves MC4Rs on neurons in the DMV. Additionally, activation of MC4R in the DMV inhibits parasympathetic tone, affecting insulin release without impacting glycemic tone (Berglund et al., 2014). Agrp neurons also play a role in regulating insulin sensitivity in brown adipose tissue via connections with the lateral hypothalamus and the stria terminalis (Steculorum et al., 2016).
Numerous pathways associated to inflammation and leading to insulin resistance have been identified, particularly within the ARH (Jais and Brüning, 2017), including increased

expression of suppressor of cytokine signalling 3 (SOCS-3) (Bjørbaek et al.,1998). Additionally, excessive endoplasmic reticulum (ER) stress contributes to altered hormonal responses in key metabolic regulatory circuits such as POMC and AgRP neurons (Ozcan et al., 2009). Moreover, ectopic lipid accumulation and resulting lipotoxicity have been associated with both leptin and insulin resistance in the ARC.

## 3. Tanycytes and diabetes

Although clinical data regarding the involvement of tanycytes in diabetes are limited, a growing body of evidence suggests that tanycytes may indeed play a role in the context of glucose homeostasis.

Given their role as glucosensors, tanycytes are considered integral to the development of type 2 diabetes. The association between diabetes and tanycytes dates back to the 1980s when Bestetti and Rossi (Bestetti and Rossi., 1983) demonstrated that injections of streptozotocin, a toxic agent for glucose-sensing β cells that leads to diabetes, also led to significant changes in tanycyte morphology. This includes degenerative alterations in the vmARH and the ME, resulting in hypotrophic tanycytes with reduced cytoplasmic processes and a smooth apical surface (Bestetti and Rossi., 1983). Similar effects were observed following injections of alloxan, another agent harmful to insulin-producing β cells, in the third ventricle. The degradation of the tanycyte layer in this case led to disturbances in glucose counterregulatory responses and hyperphagia (Sanders et al., 2004).

Furthermore, recent research showed in mice that tanycytes mediate insulin transport into the hypothalamus and that DIO-mice tanycytes are nonresponsive to exogenous insulin (Porniece Kumar et al., 2021). In addition, when the insulin receptor expression was specifically ablated in tanycytes, it hampered insulin transport to the mediobasal hypothalamus, triggering systemic insulin resistance despite independently of food intake and energy expenditure (Porniece Kumar et al., 2021). Analogous to leptin, individuals without metabolic disorders show a positive correlation between plasma and cerebrospinal fluid (CSF) insulin levels, which becomes disrupted in obese individuals (Kern et al., 2006, Bakker et al., 2022). This disruption suggests that tanycytes might influence insulin transportation in humans and thereby contribute to obesity-related insulin resistance (Porniece-Kumar et al., 2021).

As mentioned, tanycytes are essential for the transport of leptin in the brain (Balland et al., 2014, Duquenne et al., 2021) and the signalling pathway of leptin and insulin cross-talk in between each other (Thon et al., 2016). Intriguingly, mice in which Lepr is selectively ablated in tanycytes (Duquenne et al., 2021), appear to recapitulate some of the metabolic problems that arise with age in the *db*$^{-}$/*db*$^{-}$ mouse model (Hummel Dickie & Coleman, 1966, Wyse et al., 1970) such as the onset of glucose intolerance and the impaired glucose-stimulated insulin secretion, which in mice with leptin receptor tanycytic ablation occurs 12 weeks after knocking down the expression of the receptor (Duquenne et al., 2021). These changes were proposed to

be due to the loss of CNS-controlled and SNS-mediated pancreatic function, because of the impossibility of leptin to enter the hypothalamus. In addition, this mouse model seems to prevent the adaptive neuroendocrine response to fasting, put in place by the corticotropin-releasing hormone (CRH)-mediated rise in glucocorticoids, needed liver mobilization of glucose to maintain glycaemia (Duquenne et al., 2021).

Altogether, these data highlight the relevance of tanycytic intact functionality to maintain glucose metabolism.

In this section we have talked about obesity and type 2 diabetes and how tanycytes are involved in their onset. In the next chapter we will focus on the pharmacological interventions to counteract those metabolic diseases and we will shed a light on whether tanycytes may become a novel therapeutic target.

## III. Anti-obesity treatments

Well intentioned, but often misguided approaches characterise the challenges the scientific community had face to treat obesity. The complexity of the disease, as well as the impaired patient outcome, have made difficult the development of an effective drug.
Besides the typical toxicities associated with any drug development program, a number of other specific toxicities have been identified. Off-target effects can occur when receptor modulation impacts more than one intra-cellular signalling system and when receptor specificity is low. While activation of one receptor can lower food intake, simultaneous activation of another receptor can produce cardiac valvopathy or an increased risk for suicidality.
In this first section we will approach the anorectic drugs that have had to fit in a small therapeutic window which improved appetite control causing however, adverse behavioural effects.

### 1. The long road to an effective treatment: historical perspective

#### 1.1. Greco-Roman Medicine – Hindu tradition – Islamic tradition

The earliest attempts at producing weight loss have been ascribed to the Greek physician Soranus of Ephesus. The Greek model of disease implicated four humours (blood, phlegm, yellow bile, and black bile) being out of balance. The treatment to rebalance them in obesity would cause less intake and more output (Papavramidou & Christopoulou-Aletra, 2008), indeed Soranus' regimen consisted of laxatives and purgatives along with exercise, heat, and massage. Induction of vomiting was also used during Roman times. The emetic for this purpose was half a cup of the hyssop plant ground with three litres of water to which vinegar and salt are added. (Foxcroft, 2011).
Ayurvedic Medicine originated in India more than 3,000 years ago and it was interested in the treatment of obesity as we can read in The Sushruta Samhita, one of the foundational medical texts dating back to between 600 BCE and 100 CE. The text is credited with the recognition of the sugary taste of diabetic urine and that this disease often affected indolent, overweight people who ate excessively, especially sweet and fatty foods. "For treatment of obesity two suggestions are made…The vigorous massage of the body with pea flour...The gullet hair compress and flesh of a wolf remedy against and obesity." (Dorj & Parfionovich, 1992)
In the Islamic culture Abu Ali Ibn Sina (Avicenna) published the most influential medical textbook of the Middle Ages called the Canon (Talbott, 1970; Sarton, 1962). Avicenna describes how to manage patients with obesity: 1) Produce a rapid descent of the food in order to prevent completion of absorption (Castiglioni, 1941); 2) Prefer bulky but feebly nutritious

food (Sigerist, 1961); 3) Take the bath before food, often (Major, 1954); and 4) Hard exercise (Gruner, 1930).

### 1.2. A renaissance of anti-obesity treatments

The spread of American influence on European society from the end of the XV$^{th}$ century resulted in the introduction of novel ingredients into gastronomic culture, as well as new pharmacotherapies based on plants with medicinal qualities.

Tobacco became a treatment for obesity. Nowadays, we know that nicotine is addictive, and that smoking can both reduce food intake and stimulate energy expenditure (Chao et al., 2019). However, obesity was less common in the XVI$^{th}$ century, and maintaining good health through diet and exercise remained the golden standard.

In the XVII$^{th}$ century, obesity gradually increased in prevalence and strategies for its management started to appeared in textbooks for physicians of that time. In (year), Theophile Bonet (Bonetus) referred to several methods for treating "*obesitas"* or *"corpulency*,". Bonet stated that: "*But this* (obesity treatment) *must be done by degrees and slowly, since it is not safe to disturb so much matter violently, lest it should come all at once. Therefore, the best way of Purging is by Pills, of Rheubarb, Aloes, Agarick, Cinnamon, and yellow Sanders. Also, abstention from Wine (…) drinking vinegar would induce weight loss and a long life.*" (Bonet, 1684)

Later, in the XVIII$^{th}$ century, the first two books exclusively about obesity were published in England. In the first book (Short, 1727), Dr. Thomas Short stated that, "*one should live where the air is not too moist or too soggy*" to prevent obesity. Exercise was necessary and that diet should be "*moderate spare and of the more detergent kind*" (Short, 1727). The second book was written by Dr. Malcolm Flemyng in 1760. Flemyng's theory was that sweat, urine, and faeces were made of "*oil*" and in order to lose weight one had to lose "*oil*" by any of these physiological processes (using laxatives, diuretics and exercise). "*Now we are so happy as to be in possession of a diuretic medicine, (…) and is withal so safe, as that it may be taken in large quantities every day for years together: that medicine is soap*" (Flemyng, 1760).

### 1.3. Contemporary medicine

Within the introduction of the concept of the "cell" by Robert Hooke in 1665 (Baluska, Volkmann & Barlow, 2004), the advances in the chemistry field in the XIX$^{th}$ century such as the atomic theory by Dalton and the development of thermodynamics by Clausius and Maxwell triggered a new era in medicine.

Importantly, I would like to point out the predominantly Western-centric perspective of the present assay related to anti-obesity drugs given the limits of scope of this work, which

undoubtedly limits my analysis. In this section I will introduce the different treatments against obesity that had been used from the XIX$^{th}$ century.

### 1.3.1. Thyroid hormones

The introduction of thyroid hormone is dated to 1893, being the first drug used on a rational basis against obesity. In 1888, the Myxedema Commission published a report on a disease called *myxedema*, a form of hypothyroidism (Report on the Subject of Myxoedema, 1888). Patients with *myxedema* were characterise by a subcutaneous lipid hyperplasia that might provoke a dramatic drop in body temperature and coma. When these patients were treated with thyroid extract from sheep, the symptoms, including the weight gain, were reversed. The belief that many patients with obesity had low metabolic rates consistent with hypothyroidism, became popular. "Calorigenic" doses of the hormone were used so that they could raise metabolic rate (Bram, 1937).

Thyroxine, thyroid hormone T4, was isolated by Kendall in 1915 (Kendall, 1915) and then synthesized in 1926 (Harington, 1926). However, the side effects of its used in patients with obesity were starting to be noticed. "*The appetite becomes impaired, the sleep broken, and the heart's action irregular*." If prolonged, the drug would induce a "*serious and obstinate disturbance of the nervous system, and particularly of the nerves controlling the heart, accompanied by palpitation, sweating, weakness, and intense nervousness*." (Foxcroft, 2011) Thus, the use of thyroid hormone for the treatment of obesity was discontinued.

### 1.3.2. Dinitrophenol

During the First World War, French workers that were synthesising 2,4-dinitrophenol (DNP) in munitions factories, were noted to lose weight. "*Workers claim that they have grown thin to a notable extent after several months of work*" (Perkins, 1919). After several studies in animals, DNP was used for the treatment of obesity. However, DNP treatment report, described three deaths, skin rashes, yellow skin colour and peripheral neuritis, stabilising that the drug should be discontinued (Tainter, 1953).

### 1.3.3. Amphetamine: 1932-1968

The rollercoaster medical use of amphetamine is the story of a drug with two personalities. On the pharmacological side it works like a sympathomimetic and is currently use for the treatment of attention deficit/ hyperactive disorder (ADHD) (Greenhill, 2006). The dark side is the abusive consumption of the drug, brought into focus by the recreative use of the highly additive amphetamine-derivates, such as methamphetamine and amphetamine-sulphate (commonly known as "speed").

Amphetamine, from its chemical structure (α-methyl-β-phenethylamine), is a sympathomimetic drug. It was first synthesized in 1887 by the Romanian chemist Edenelau in the University of Berlin. The stimulant properties of this compound were discovered when Gordon Alles tested the drug in himself whilst searching for a nasal decongestant. His blood pressure climbed dramatically and he noted palpitations as well as a "*feeling of well-being*." He was chattier and wittier than normal (Hicks, 2012).

Later on, under the name of Benzedrine (Rasmussen, 2008), amphetamine was tested for people suffering for narcolepsy and then as an agent to *improve mood* (Prinzmetal, 1935; Ulrich & Vidgoff, 1936) and reducing body weight one of the main side effects (Lesses & Myerson, 1938). In 1946 these effects were described as: "*it decreases appetite and helps the patient to follow a diet, producing a sense of well-being. Increasing activity, it promotes proper balance between energy output and caloric intake*" (Bett, 1946).

During the 1930´s in the US amphetamine was largely sold for weight reduction by many small companies without the approval of the FDA (US food and drug administration).

The side effects of the use of amphetamines as anti-obesity were soon clinically described: already in 1938 it was reported that the use of Benzedrine for long periods "*is certainly not without danger, particularly to the circulatory system*" and that "*it felt necessary to take the drug habitually and in ascending doses to produce the desired effect*" (Lesses & Myers, 1994).

In the late 30's in the US and in Europe, amphetamines became a prescription drug for the treatment of narcolepsy, sleeping disorders and ADHD.

Later on, in 1941 the "rainbow pills" started to be commercialised in US (Cohen, Goday & Swann, 2012). They are a "combination of pills (as of amphetamines, laxatives, and thyroid hormones) typically of different colours, formerly taken to curb appetite and promote weight loss" (Cohen, Goday & Swann, 2012). The combination of pills also contained cardiac glycosides, with a very narrow spectrum from therapy to toxicity, as blockers of "nausea". Rainbow Pills often contained barbiturates to subdue amphetamine stimulation. After several deaths were reported, they were banned in the late 1960s. However, the distribution of those pills is still active in illegal online markets (Cohen, Goday & Swann, 2012).

### 1.3.4. Anorectic Sympathomimetic Drugs: 1950-1997

Amphetamine reduces food intake, by activating the sympathetic nervous system, it raises blood pressure and has neurophysiological effects, comprising addiction. Chemists found a way to separate these effects (Samuel and Burland, 1975) reaching the approval of sympathomimetic anorectic drugs, fenfluramine and chlorpentermine, in 1973. Fenfluramine has structural similarities to amphetamine, but, surprisingly, it has a very different mechanisms of action, indeed it blocks the re-uptake of serotonin in the synaptic cleft. The first set-back for this drug occurred in 1981 with 2 cases of pulmonary hypertension (Douglas et al, 1981). In

addition, when co-administrated with phentermine ("Fen-Phen") (Weintraub et al., 1984) it induced a dramatic weight loss, accompanied though by valvular heart disease (Ryan et al., 1999). On September 1997, fenfluramine was removed from the market worldwide.

### 1.3.5. Lorcaserin

The strong weight loss induced by Fenfluramine, a drug acting on serotonin re-uptake, opened the way to the synthetization compounds related to serotonin action that had a role in weight loss. Lorcaserin is a selective serotonin ($5\text{-}HT_{2C}$) receptor agonist (to avoid effects on cardiac valves and pulmonary artery pressures), which reduced food intake in animals and humans. However, during post marketing monitoring, it was found that the point for cancer (skin, prostate, GI, and respiratory) rate ratios were greater than 1.0 in the lorcaserin group when compared to placebo (Sharretts et al., 2020), so the drug was withdrawn from the market in 2020 in the US, while the European Medicines Agency (EMA) had never approved its distribution in Europe.

### 1.3.6. Cannabinoid Receptor Antagonists

Tetrahydrocannabinol is the active ingredient of cannabis, known to stimulate food intake, indeed CB-1 receptors are distributed throughout the brain in the areas related to feeding (Schlicker & Kathmann, 2001). Rimonabant was the first antagonist of the CB-1 receptor and it was shown to inhibit intake of sweet and high-fat diet foods. In addition, pair-feeding experiments showed that loss of weight was not completely due to reduction of food intake (Kirkham, 2005; Pagotto et al., 2006; Bensaid et al., 2003; Juan-Pico et al., 2006), so Rimonabant was approved by the EMA as anti-obesity drug in 2006, but not by the FDA because of concerns about suicidality (King, 2010). Soon after, marketing of rimonabant was abolished by the EMA in 2008.

### 1.3.7. Dopamine D1/D5 Receptor Modulators for Weight Loss

Dopamine has a strong role in eating behaviour, suggesting that the manipulation of dopamine action would be a good strategy for the management of obesity. Five dopamine receptors have been identified (Terry, Gilbert & Cooper, 1995; Hoebel et al., 1989). Agonists to the D1/D5 receptors reduce the duration of feeding by decreasing the frequency. On the other hand, D2 agonists reduce the rate of eating (Hoebel et al., 1989). Although the clinical trials were promising, they were stopped because of psychiatric adverse events in 31% of those receiving the D1/5 agonists.

### 1.3.8. Drugs that modify nutrient´s absorption

One potential approach to reducing body weight involves inhibiting the absorption of ingested nutrients (Liu et al., 2020). Orlistat, a medication approved by both FDA and EMA, operates on this principle by inhibiting intestinal lipases, thereby allowing some triglycerides to remain undigested in the gastrointestinal tract. Another drug, Cetilostat, also targets intestinal lipases. Although it has undergone clinical trials and is approved for clinical use in Japan, two other drugs, cholestyramine and neomycin, have been tested but ultimately discarded as anti-obesity drugs (Kopelman et al., 2007; Kopelman et al., 2009; Dobbins, 1968).

## 2. Anti-diabetic drugs approved for the treatment of obesity

The term "diabesity", coined by Sims et al., back in 1973 emphasises the relationship between the parallel epidemics of diabetes and obesity (Sims et al., 1973, Ng et al., 2021). Addressing obesity involves lifestyle modifications, pharmacotherapy and in more extreme cases bariatric surgery. As we said in the previous sections, obesity increases the risk of suffering several diseases including T2D.
I will now describe the drugs mostly used as both anti-diabetic and anti-obesity and the involvement of tanycytes in their effects.

### 2.1. Metformin

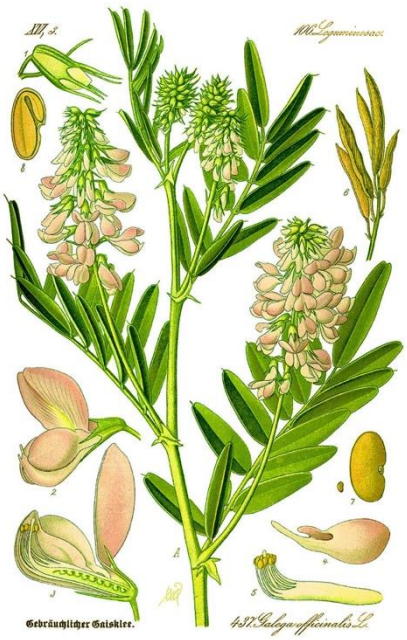



***Figure 13. the Galega officinalis.***
*Botanical illustration of the Galega officinalis, plant long time used in pharmacology and from which metformin active ingredient is isolated (from von Deutschland, 1885).*

Metformin, is a biguanide derivate. It belongs to the family of guanidine, a product from Galega officinalis (Figure 13), a French lilac that has been used against diabetes, hypertension and aging since the Middle Age (Witters, 2001; Bailey, 2017).

With the isolation of galegine by George Tanret in 1914, many biguanides (such as synthelin, metformin, phenformin, and buformin) were produced and explored as antidiabetic medications (Di Magno et al., 2022).

Metformin mechanism of action is still not clear, although we know that it has pleiotropic effects in all body tissues (Figure 14) (Froldi, 2024).

Metformin acts on the liver to lower glucose levels through the reduction of hepatic gluconeogenesis and the improvement of insulin sensitivity and it has been shown to favour weight loss (Rena et al., 2017; Seifarth, Schehler & Schneider, 2013).

The drug is taken up into the hepatocytes by the organic cation transporter 1 (Wang et al., 2002). Because of its chemical positive charge, it is accumulated into the mitochondria and exerts its action by inhibiting the respiratory chain complex 1, blocking ATP synthesis and changing the NAD+: NADH ratio, effects that might have a role in gluconeogenesis fall (Owen et al., 2000; Bridges et al., 2014; El-Mir et al., 2000). AMP high levels also activate the AMP kinase, the metabolic sensor of the cell. The increase of AMP:ATP and ADP:ATP ratios favours the phosphorylation of AMPK and its activation, indeed metformin anti-gluconeogenesis effect is blunted in a model in which AMPK is mutated (Hawley et al., 2010). Recently, it has been shown that metformin, increasing AMP levels can inhibit adenylate cyclase action. The lowering of cyclic AMP (cAMP) levels suppresses the activation of Protein kinase A (PKA). In this way 6- phosphofructo-2-kinase (PFKFB1) is active and in turn activates fructose- 2,6-bisphosphate (F26BP), that blocks the gluconeogenic enzyme fructose-1,6-bisphosphatase, impeding gluconeogenesis mediated by glucagon in hepatocytes (Miller et al., 2013). This ability to suppress gluconeogenesis makes metformin one of the most administered drugs to fight type 2 diabetes, 150 million people around the world are prescribed metformin (An & He, 2016).

Metformin also has a role in the absorption and disposal of glucose in the intestine. Indeed, it decreases the post-prandial glucose response by lowering the SGLT1 expression in enterocytes, reducing the luminal clearance of glucose in rats (Zubiaga et al., 2023). Furthermore, metformin increases ERK-dependent glucose uptake by muscles and adipose tissue stimulated by insulin both in human and mice (Sajan et al.,2010; Adnitt et al., 1973).

Metformin effects on weight loss, in addition to its several other beneficial effects, have also been extensively investigated. It is known that it induces at least 5% of body weight loss (Apolzan et al., 2019; Wang et al., 2013), long term (Apolzan et., 2019), it is effective in patients under antipsychotics and prevents weight gain (according to 25 metanalysis and 12 studies), working for obese patients with and without T2DM (Pu et al., 2020; Chukir et al., 2021).

In obese mice, metformin reduced body weight and enhanced metabolic parameters by inhibiting the differentiation of white adipocytes, thereby influencing fibroblast growth factor 21 (FGF21), a pivotal metabolic hormone that enhances lipolysis in white adipose tissue (WAT) and impedes fat accumulation (Kim et al., 2016). Subsequent well-designed studies have demonstrated that metformin could potentially prevent weight gain in preclinical obesity models by enhancing the metabolic activity of brown adipose tissue (BAT), through mechanisms both dependent and independent of the specific action of UCP1, a molecular marker capable of dissipating chemically bound energy as heat (Breining et al., 2018; Tokubuchu et al., 2017; Liang et al., 2016; Karise et al., 2019).

While the activation of AMPK by metformin is a well-established mechanism (Steinberg et al., 2019), it may not explain all of the drug's actions. There are numerous AMPK-independent pathways that contribute to metformin's effects (Prattichizzo et al., 2018; Steinberg et al., 2019). For instance, metformin has demonstrated anti-adipogenic effects in multipotent C3H10T1/2 mesenchymal stem cells by regulating the activities of osteogenic transcription factor Runt-related transcription factor 2 (Runx2) and adipogenic transcription factor PPAR (Chen et al., 2017). These effects seem unrelated to AMPK activation, instead they involve the suppression of the mammalian target of rapamycin (mTOR)/p70S6K signaling pathway (Chen et al., 2017).

The anti-obesity effects of metformin have attracted considerable attention and have been investigated in human subjects (Li et al., 2013; Chalasani et al., 2018). Studies have demonstrated that metformin can enhance the secretion of GLP1 (Mulherin et al., 2011; Napolitano et al., 2014), as well as the anorectic hormone peptide YY (PYY) (De Fronzo et al., 2016). Another potential mechanism through which metformin may curb appetite, particularly through its action in the gastrointestinal tract, involves modifying bile acid absorption via interaction with the farnesoid X receptor (Napolitano et al., 2014; Lien et al., 2014). This alteration in bile acids is believed to indirectly influence the secretion of appetite-suppressing neuropeptides such as GLP-1 and peptide YY (Kuhre et al., 2018). It has been strongly suggested and confirmed that metformin's anti-obesity effect could be mediated through gut microbiome modulation in both diabetic and non-diabetic human subjects and animals (Inzucchi et al., 1998; Lavrentaki et al., 2018). The improvement of metabolic syndrome by metformin is also linked to reduced levels of low-grade inflammation independent of the gut microbiota (Wu et al., 2017).

Metformin has also been reported to act directly and indirectly on the CNS.

Central-delivered metformin reduces food intake by decreasing expression of the orexigenic NPY in rats (Duan et al., 2013) and by preventing the orexigenic function of exogenous ghrelin by suppressing the mechanistic target of rapamycin (mTOR) (Stevanovic et al., 2012). AMPK, one of the main targets of metformin, is a cellular effector of the orexigenic effects of the NPY-AgRP neurons and its genetic ablation in this cellular population, leads to a lean phenotype (Stark, Ashley, & Andrews, 201; Claret et al., 2007). Strikingly, it has been reported that

metformin blocks the activation of AMPK in cultured NPY neurons, preventing the increases in NPY expression and possibly explaining one of the mechanisms under the drug anorectic action (Chau-Van et al., 2007).

Interestingly, metformin is also permeable to the BBB and it can reach micromolar concentrations in the CSF and several brain regions other than the hypothalamus where it can exert its action (Wilcock et al., 1994, Labuzek et al., 2010). Research indicates its stimulation of intestinal afferents to the NTS through a GLP1R and PKA dependent pathway via AMPK (Duca et al., 2015). Functional MRI investigations have revealed metformin-induced alterations in cerebral metabolism among patients with T2D, leading to reduced metabolism in regions including the parahippocampal gyrus, the ventromedial prefrontal cortex, and the fusiform gyrus (Huang et al., 2014). These brain areas control semantic memory and reward processing and might be modifying the pathophysiological link in between food and reward (de Wit; 2009; Huerta et al., 2014).

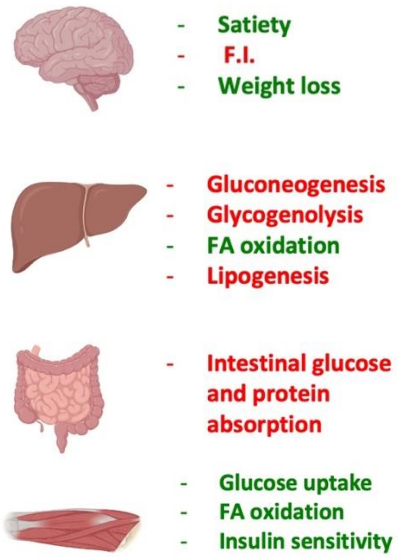



***Figure 14. Graphic summary of metformin metabolic actions. Designed by me with the use of Biorender.***

In the context of my studies, it is very important to note that hypothalamic STAT3 phosphorylation, proxy of a LepR activation (Vaisse et al., 1996), is also induced in obese diabetic rats with metformin administration (Lv et al., 2012), indeed it has been shown that metformin also impacts on leptin receptor expression and it restores leptin sensitivity in obese rats with leptin resistance. (Aubert et al., 2011; Kim et al., 2006).

### 2.2. Alpha-Glucosidase Inhibitors

Alpha-glucosidase inhibitors, which are saccharides, competitively inhibit the alpha-glucosidase enzymes crucial for carbohydrate digestion in the small intestines (30). These inhibitors reversibly impede the enzymatic breakdown of carbohydrates into easily absorbable

sugars. Consequently, they hinder carbohydrate absorption, leading to a decrease in postprandial blood glucose levels by approximately 3 mmol/L (Akmal et al., 2022). Research suggests that alpha-glucosidase inhibitors, particularly acarbose, can effectively reduce body weight by restricting carbohydrate absorption, thereby reducing calorie intake (Lazzaroni et al., 2021). Studies have demonstrated that these drugs significantly decrease body weight compared to placebos, without increasing the risk of hypoglycaemia. However, this weight reduction is accompanied by a higher occurrence of gastrointestinal discomfort (Gao et al., 2018).

### 2.3. SGLT-2 Inhibitors

Sodium-dependent glucose cotransporter proteins-1 (SGLT1) and SGLT-2 (Alicic et al., 2019) are active transporters responsible for glucose reabsorption in the kidney, with SGLT-1 accounting for 10% and SGLT-2 for 90% of this process. SGLT-2 inhibitors are medications that help manage hyperglycaemia in type 2 diabetes patients through an insulin-independent mechanism (De Fronzo et al., 2013). These drugs block approximately 50% of glucose reabsorption. Even in individuals without diabetes, SGLT-2 inhibitors have demonstrated effectiveness in reducing body weight (by eliminating the caloric apport of reabsorbed glucose) and have positive effects in preventing cardiovascular and renal diseases (Butler et al., 2020; Pereira et al., 2019). However, the weight loss achieved is relatively modest due to compensatory mechanisms (Pereira et al., 2019). Consequently, researchers have hypothesized that combining SGLT-2 inhibitors with medications that decrease food intake could be a more effective approach to weight loss therapy (Pereira et al., 2019).

### 2.4. GLP-1 Receptor Agonists

GLP1 degradation by dipeptidylpeptidase 4 (DPP-4) led to the development of GLP1R agonists with an extended half-lives (Ard et al., 2021) exhibit the ability to decelerate stomach emptying, potentiates glucose-stimulate insulin secretion and reduce appetite (Ard et al., 2021). Consequently, they improve glycaemic control and contribute to weight reduction (Ard et al., 2021). Some of these agonists have been approved by American and European drugs agencies for the treatment of type 2 diabetes (T2D) first and obesity afterwards (Drucker et al., 2017).

The discovery of GLP-1 receptor agonists was initiated by the isolation of Exendin-4, a 39 amino acid peptide with 53% similarity to human GLP-1, from the saliva of the Gila monster (Ard et al., 2021; Furman, 2012). In 2005, exenatide, a synthetic peptide derived from Exendin-4, was introduced as the first GLP-1 receptor agonist owing to its exceptional stability (Ard et al., 2021; Furman, 2012). GLP1-Rs are expressed, among other areas, in the hypothalamus and in the brainstem, centres of energy balance regulation (Cork et al., 2015). The therapeutic

potential of GLP-1 analogues is exerted by their ability to reduce food intake an increase in fatty acid oxidation by activating ARH POMC neurons (Dong et al., 2021). In addition, it was proven that, specific GABAergic GLP1-R-expressing neurons in the NTS mediate the anorectic and weight reducing effects of the GLP-1 analog liraglutide (introduced in the next section).
The notable weight loss outcomes achieved with GLP-1 receptor agonists represent a significant breakthrough.

### 2.4.1. Liraglutide

Liraglutide, a drug approved for treating type 2 diabetes (T2D), is an analogue of human GLP-1 modified with the addition of a hexadecanoyl group attached at the amino acid 27 to increase its action (Lear, Amso & Shen, 2019). Its mechanism for inducing weight loss primarily involves reducing appetite centrally (Adams et al., 2018; Beiroa et al., 2014; Fortin et al., 2020; Secher et al., 2014; Sisley et al., 2014) leading to decreased energy intake rather than increasing energy expenditure (van Can et al., 2005). The extent of weight loss with liraglutide correlates with dosage, as evidenced by the SCALE study, where patients experienced weight loss ranging from 6.0 to 8.8 kg with various doses compared to a mean weight loss of 0.2 to 3.0 kg for those on diet and exercise alone (together with placebo) (Wadden et al., 2013). Davies et al. observed significant weight loss in obese patients with T2D treated with two doses of liraglutide over 12 weeks, with mean weight loss percentages of 4.7% and 6.0% compared to 2.2% with placebo (Davies et al., 2015). Early weight loss during liraglutide treatment has been identified as a crucial predictor of later successful response to the medication (Fujioka et al.,2016). Interestingly, in the Obesity and Prediabetes trial (Pi-Sunyer et al., 2015) (overweight or obese patients but without T2D), weight loss was 8% with liraglutide compared with 5.4% in patients with diabetes (Wing et al., 2011).

### 2.4.2. Semaglutide

Semaglutide shares 94% of its sequence with human GLP-1 (Ryan et al., 2021). Compared with liraglutide, semaglutide has a longer fatty acid chain linked to amino acid 26, to increase the binding of the drug to albumin. In addition, the substitution of alanine with 2-aminoisobutyric acid at position 8, makes the peptide more resistant to degradation by peptidases (Lear, Amso & Shen, 2019). One of its strengths, indeed, is definitely the half-life, between 155 and 184 h, allowing for a weekly administration (Chao et al., 2023). Under the name Ozempic® (Novo Nordisk), subcutaneous semaglutide was approved by the FDA and by the European Medicines Agency (EMA) for T2DM (Dhillon, 2018). The FDA and EMA

also authorized Wegovy® as an anti-obesity drug, in 2021 and 2002, respectively, in addition to lifestyle therapies (Singh, Krauthamer & Bjalme-Evans, 2022).

Preclinical studies examining its mechanisms suggest that weight loss is mediated via GLP-1 receptors (GLP-1Rs) in the brain that influence food preference, reduce food intake, and lead to weight loss. Semaglutide does not breach the blood-brain barrier, but it is transported by tanycytes and it directly affects brain regions such as the brainstem, septal nucleus, and hypothalamus by accessing through circumventricular organs (Gabery et al., 2020). Transcriptomic analysis of semaglutide-treated rats brain reveals upregulation of prolactin-releasing hormone and tyrosine hydroxylase in the area postrema, suggesting that semaglutide reduces body weight by directly interacting with various GLP-1R populations (Gabery et al., 2020).

The safety and efficacy of semaglutide have been evaluated in several programs and clinical trials. The SUSTAIN program is the first one and was focused on T2DM patients, with a weekly dose of 0,5 or 1 mg (Arcén et al., 2018; Marso et al., 2016; Pratley et al., 2018; Lingvay et al., 2019; Zinman et al., 2019; Capehorn et al., 2020; Kellerer et al., 2022). It was established that once per week semaglutide administration outperformed other comparators, such as sitagliptin, exenatide, insulin, and placebo, in terms of glycaemic management, BMI, and weight loss (Arcén et al., 2018). In a study contrasting the effects of semaglutide and liraglutide over 3 oral medications, the former drug induced 5,8 kg of body weight loss compared to the 1,9 or the latter. With the exception of gastrointestinal side effects, more present in the semaglutide group, safety profiles were comparable (Capehorn et al., 2020).

The STEP program examined semaglutide safety and efficacy as an anti-obesity drug with a dose of 2,4 mg per week (Wilding et al., 2021; Davies et al.,2021; Wadden et al., 2021), mostly examining patients with a BMI of 30 or 27, and with obesity-associated co-morbidities other than diabetes. These powerful studies show a strong induction of body weight loss in semaglutide-treated patients and, very importantly, we can appreciate also a different efficacy of the drug in T2DM and non-diabetic patients. Indeed, if we compare the percentage of body weight reduction, we can appreciate in the non-diabetic, obese group body a weight loss of 14.9% with respect to the pre-treatment weight, while only a 2.4% weight loss was induced in patients with both obesity and T2D (Wilding et al., 2021). In STEP 4, a withdrawal study, obese patients without diabetes took semaglutide for 68 weeks, and then the group was split, with a part of the participants switching to placebo for the following 48 weeks. Globally, weight loss in the semaglutide group decreased by a 17.4%, compared to a total weight loss of 5% in the placebo group (Rubino et al., 2021).

In addition, the peculiarity of the STEPTEENS study is the focus on 201 obese/overweight adolescents. They received either weekly 2.4 mg of semaglutide or a placebo, together with lifestyle changes (Weghuber et al., 2022). In the semaglutide group, the mean change in BMI at week 68 was a decrease of 16.1% compared to a rise of 0.6% in the placebo group (Weghuber et al., 2022).

In all clinical trials, semaglutide had shown greater effects on weight loss compared to all other treatments.

### 2.4.3. Tirzepatide

The most recent drug approved against T2DM is a synthetic twincretin (a portmanteau for the words two incretins), a dual agonist for both GIP- and GLP-1 receptors (Bradley et al., 2022) named tirzepatide and known under the brand name Mounjaro® (Bradley et al., 2022; Dhirani et al., 2023) given as a subcutaneous injection once per week (Bradley et al., 2022). Tirzepatide is effective in the treatment of T2DM, and overweight and obese patients with or without diabetes (Bradley et al., 2022; Baker, Walley & Levien, 2022).

In the clinical trial known as the SURPASS-1 T2DM patients received either placebo or 5/10/15 mg of tirzepatide, administered subcutaneously once a week during 40 weeks (Rosenstock et al., 2021). Tirzepatide caused an average weight loss of 7.0 and 9.5 kg (Rosenstock et al., 2021). In SURPASS-2, tirzepatide and semaglutide were compared (Frías et al., 2021). T2DM patients on only metformin were randomly assigned to either 1 mg of semaglutide or 5/10/15 mg of tirzepatide (Frías et al., 2021). Tirzepatide outperformed semaglutide, causing average weight losses of 7.6 kg, 9.3 kg, and 11.2 kg, while semaglutide caused a 5.7 kg weight decrease (Frías et al., 2021).

Tirizepatide's anti-obesity properties were highlighted by the SURMOUNT clinical study series (Jastreboff et al., 2022) with patients presenting a BMI of 30 kg/m2 or higher, or a BMI of 27 kg/m2 or higher with related comorbidities, but diabetes. The percentage of participants who lost 5% or more of their body weight was 85% (5mg), 89% (10 mg), 91% (15 mg), and 35% (placebo) (Jastreboff et al., 2022). 10 mg and 15 mg of tirzepatide, induced a body weight reduction of at least 20% of body weight in at least half of the subjects (Jastreboff et al., 2022). The most frequent side effects, particularly during dose escalation, were mild to moderate gastrointestinal problems (Jastreboff et al., 2022).

Despite the encouraging results, more studies are needed to define its long-term effectiveness and safety.

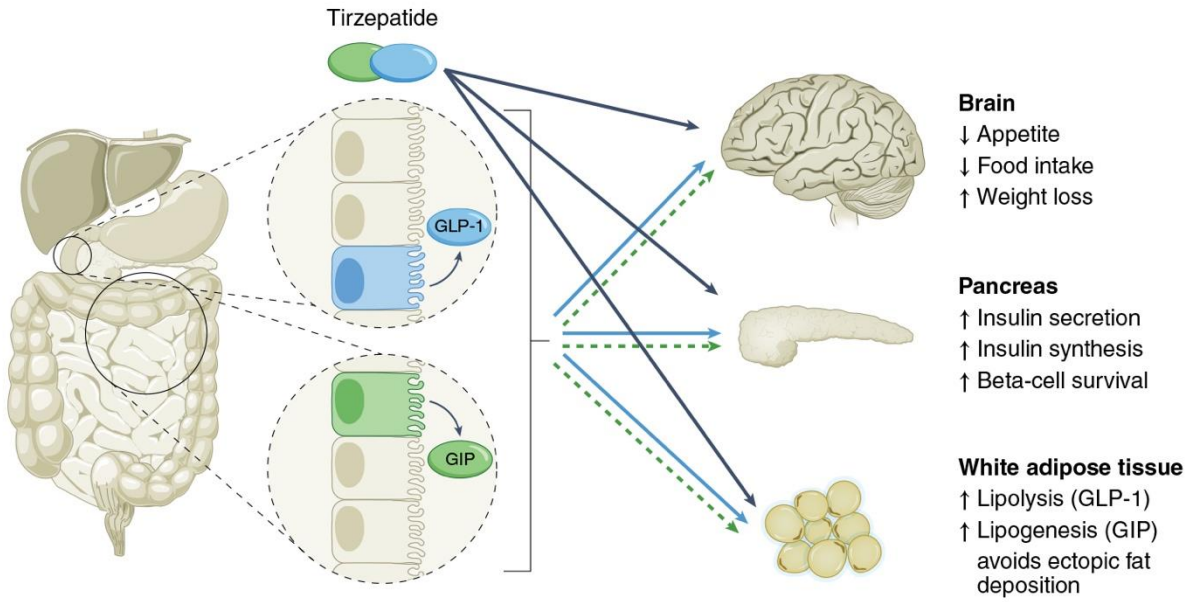



***Figure 15. Metabolic actions of GLP-1 and GIP.***
*The receptors of the two incretins are activated by the new drug against diabetes and obesity: Tirzepatide. (From Moura, Sirica & Ruff, 2022).*

## 3. Tanycytes: novel therapeutic targets for metabolic diseases' treatment

Tanycytes sense and gate to the hypothalamus blood-borne cues (nutrient and hormones), that signs energy status and nutrient availability from peripheral tissues, modulating energy homeostasis. Indeed, in this section, I will review recent pioneer studies that have demonstrated the potential role of median eminence´ tanycytes as novel therapeutic targets to tackle metabolic diseases.

### 3.1. Rescue of tanycytic leptin transport reverses the DIO mice phenotype

For the first time, in Prevot´s laboratory, it was demonstrated that the presence of leptin in MBH explants was significantly reduced in DIO mice (Balland et al., 2014) and the mechanisms that drive this transport were proposed.
In primary cultured rat tanycytes, the inhibition or ERK pathway, blocked the leptin released after being taken up by tanycytes. Thus, it was proposed that ERK pathway may be involved in tanycytic transport of leptin. In DIO mice, the treatment with the epidermal growth factor EGF, a strong activator of ERK pathway by binding its receptor (EGFR), restored leptin central transport of leptin and its signalling in the hypothalamus. In addition, the chronic

administration EGF accelerated weight loss in DIO mice fed with chow diet, an effect that may be related to a restoration of both central leptin transport and sensitivity.
In a following study from Prevot´s lab (Duquenne et al., 2021) we demonstrated that leptin activates the signalling pathway driven by EGFR in primary cultured tanycytes and that the formation of a quaternary complex Leptin–LepR–EGFR–EGF is required for leptin transport. Interestingly, when administering a leptin receptor antagonist (LAN) with no biological activity, it was observed that LAN stayed trapped in the early endosome compartment of tanycytes, due its failure in the activation of the LepR-associated signalling pathway. Strikingly, the discovery that EGF can restore the trafficking of LAN by re-activating the signalling cascade downstream of LepR, add a piece in the mechanism of the LepR-dependent, tanycyte-mediated leptin transport.

These two studies shed a light on the route that leptin follows to enter the brain to modulate energy balance. Tanycytic expression of LepR and the consequent activation of the signalling cascade are essential for the anorexigenic effects of leptin. In mice, diet-induced obesity hampers this signalling mechanism in tanycytes. However, the EGF-mediated reactivation of the leptin transport through the activation of ERK cascade in DIO mice, suggests EGFR as a target for restore leptin sensitivity. Despite the demonstrated effects of EGF in rescuing leptin transport into the hypothalamus ion DIO mice, the possible clinical application cannot be envisioned since EGF is involve in the expression of prooncogenic factors (Jin et al., 2013). Thus, other mechanisms that improve leptin tanycytic transport should be explored.

### 3.2. Endozepines and tanycytic leptin transport

Endozepines are natural counterparts to benzodiazepines and originate from the diazepam-binding inhibitor. They are produced by glial cells in brain regions associated with food intake (Guillebaud et al., 2017). They induce STAT3 phosphorylation in hypothalamic neurons of wild-type mice, yet fail to do so in leptin-deficient mice or in mice with selectively disrupted LepR expression in tanycytes. This suggests that endozepines exert their anti-obesity effects by stimulating tanycytic leptin shuttles by mechanisms that still need to be investigated but that opens a novel possible therapeutic target.

### 3.3. Tanycytes transport Liraglutide

In the study by Imbernon et al., 2022, we showed for the first time that liraglutide transport into the hypothalamus is conveyed by tanycytes and it is essential for its anti-obesity action. Our results indicate that endothelial cells do not express the GLP1R and that liraglutide does not cross the BBB through, but it can freely extravasate fenestrated vessels of the median eminence and reach nearby neurons of the ventromedial ARH. In addition, liraglutide is uptake

by tanycytes after an i.v. administration of liraglutide in order to reach their target neurons in the dorsal ARH, are harbours microvasculature with BBB properties. Interestingly, when we blunt tanycytic transport by knocking-down GLP1R specifically in tanycytes, we show that liraglutide access into the brain and its anti-obesity effects (reduction of food intake, increase in the fatty acid oxidation and weight loss) require tanycytic the activation of GLP1R signalling pathway.

### 3.4. Tanycytes are required for the anti-obesity actions of GLP1R-agonists

In this study by Bakker et al, the metabolic effect of a combined administration of insulin and Exendine 4 (Ex-4) (GLP1R agonist) are shown (Bakker et al., 2022). The significant weight loss observed with Ex-4 administration stems from both reduced feeding and a shift towards peripheral lipid utilization.
ME tanycytes may play an active role in transporting insulin and Ex-4 into the brain's CSF considering the distinct expression patterns of insulin and GLP-1 signalling components in hypothalamic and AP/NTS tanycytes. Peripheral insulin administration, by decreasing glycaemia and more specifically centrally detected glucoprivation, augments the Ex-4 access into hypothalamic/hindbrain structures, with insulin dosage influencing the passage of the GLP-1R agonist. In addition, tanycytes have been well described ad central glucose sensors and they play a role in the regulation of the ME cytoarchitecture, indeed also in this study they show that tanycytic Vegfa partially mediates the metabolic changes induced by acute Ex-4, insulin, and Ex-4 + insulin combined.
Notably, in mice fed for 10 weeks with a high-fat and high-sucrose diet, the beneficial effects on the central transport of Ex-4 together with insulin, are blunted. These results led the authors to hypothesize a defective tanycytic function in response or maybe directly in the sensing of glycaemic drastic changes in a context of obesity and uncontrolled glycaemia. This hypothesis might also explain why in recent clinical trials shown the effectiveness of semaglutide, the weight loss induced by the drug is drastically reduced in obese patients with diabetes when compared to non-diabetic obese patients (Davies et al., 2021; Wilding et al., 2021).

Altogether, these recent studies also encouraged us in the exploration of the role of metformin in a possible therapeutic approach against obesity, as you will read immediately afterwards.

# *Chapter 5: Objectives*

Following the previous work of the host laboratory, during my doctorate studies presented in this manuscript, I aimed to explore the physiological importance of the tanycytic transport of blood-borne molecules into the hypothalamus in energy homeostasis and whether this property would be used as a therapeutic target for the management of obesity and diabetes.

**Objective 1**: To decipher the physiological consequences the specific impairment of tanycytic exocytosis on energy homeostasis on chow and diet-induced obese mice.

**Objective 2**: To determine wether metformin requires tanycyte function to mediate its anti-obesityand anti-diabetic effect.

# *Chapter 5: Materials and methods*

**Animals**

All C57Bl/6J male mice were housed under specific pathogen-free conditions in a temperature-controlled room (21-22°C) with a 12h light/dark cycle, 40% humidity and ad libitum access to food and water. Tg(CAG-BoNT/B,EGFP)U75-56Fwp/J (BoNTB-EGFPloxP-STOP-loxP; JAX Stock No. 018056) mice were generated as previously described (Slezak et al., 2012). Transgenic model mice were bred and genotyped in-house to generate experimental animals. At 8 weeks of age, experimental groups of C57Bl/6J, $LepR^{loxP/loxP}$-$tdTomato^{loxP\text{-}STOP\text{-}loxP}$ and $AMPK^{\alpha1\,flox/\alpha2\,flox}$ mice, animals were fed a high fat diet (60% calories from fat, Research diet Inc. D12492) for 8 weeks. All experiments were performed in 8 to 20 weeks-old mice and with the approval of the Institutional Ethics Committees for the Care and Use of Experimental Animals of the University of Lille and the French Ministry of National Education, Higher Education and Research (APAFIS#2617-2015110517317420 v5), following the guidelines outlined by the European Union Council Directive of September 22, 2010 (2010/63/EU).

**Stereotaxic delivery of TAT-Cre and AAVs**

Tanycyte-selective recombination in:

- BoNTB-EGFPloxP-STOP-loxP mice was performed with Cre/LoxP system in isoflurane-anesthetized 8-week old mice by stereotaxical infusion of a TAT-Cre fusion protein (1.27 mg ml–1) (Peitz et al., 2002), AAV1/2-Dio2::Cre (0.5 x $10^{10}$ genomic particles $\mu l^{-1}$) (Muller-Fielitz et al., 2017) or a 1:1 mix of AAV1/2-Dio2::Cre (2.5 x 109 genomic particles µl–1) and AAV1/2-CMV>EGFP:WPRE (2.5 x 109 genomic particles µl–1) (VectorBuilder, VB010000-9394npt). Both TAT-cre or vehicle were stereotaxically infused into the third ventricle (2 uL; at 0.2 ul/min; anteroposterior, -1.7 mm; midline, 0 mm; dorsoventral, -5.6 mm), 1 week before experiments (Duquenne et al., 2021).
- $LepR^{loxP/loxP}$-$tdTomato^{loxP\text{-}STOP\text{-}loxP}$ mice was performed with Cre/LoxP system in isoflurane-anesthetized 12-week old mice fed with a HFD for 4 weeks by stereotaxical infusion of viral vectors. AAV1/2-Dio2::Cre (0.5 x $10^9$ genomic particles $\mu l^{-1}$) (Muller-Fielitz et al., 2017) or AAV1/2-CMV>EGFP:WPRE (2.5 x 109 genomic particles µl–1) (VectorBuilder, VB010000-9394npt) were administrated.
-$AMPK^{\alpha1\,flox/\alpha2\,flox}$ mice a 1:1 mix of AAV1/2-Dio2::Cre (2.5 x $10^9$ genomic particles $\mu l^{-1}$) and AAV1/2[FLEXon]-CMV>LL:rev(EGFP:Scramble[shRNA]):LL:WPRE (2.5 x 109 genomic particles µl–1) (VectorBuilder, VB220803-1150vgq) or AAV1/2-CMV>EGFP:WPRE (2.5 x $10^9$ genomic particles µl–1) (VectorBuilder, VB010000-9394npt) were administrated.

Both viral vectors containing the genetic information to express Cre or the vehicle were administrated in the lateral ventricle (1 uL; at 0.3 ul/mim; LV; anteroposterior, −0.3 mm; midline, ±1 mm; dorsoventral, −2.5 mm) 4 weeks after the beginning of the HFD and 2 weeks before experiments.

**Pharmacological treatments**

Chronic treatment: Mice were administrated a daily dose of metformin by oral gavage (300 mg/kg) diluted in drinking water during week 7 and 8 of the high fat regimen. The treatment was administrated in one hour before entering into the dark phase.

Acute treatment: to analyse the treatment-induced ERK activation, 45 minutes before the perfusion mice were treated with either metformin (Sigma & Aldrich dissolved in drinking water and administrated by gavage n=4 SD) 300 mg/kg (Coll et al., 2020), water (n=3) and with leptin (3 mg/kg i.p.) 5 minutes before the sacrifice.

**Isolation of hypothalamic tanycytes using fluorescence-activated cell sorting**

Median eminence from TAT-Cre and AAV1/2 Dio2::Cre injected BoNTB-EGFP-loxP-STOP-loxP mice were microdissected, and enzymatically dissociated using Papain Dissociation System (Worthington Papain, Lakewood, NJ) to obtain single-cell suspensions as described (50). FACS experiments were performed using an EPICS ALTA Cell Sorter Cytometer device (Beckman Coulter, Brea, CA, USA). The cell sort decision was based on measurements of EGFP fluorescence (EGFP: excitation: 488 nm; 50 mW; detection: EGFP bandpass 530/30 nm, autofluorescence bandpass 695/40 nm) by comparing cell suspensions from non-infected brain sites (the cortex) and infected brain sites (median eminence). For each animal, in between 3000 and 200 cells EGFP positive and negative cells were sorted directly into 10µL extraction buffer: 0,1% Triton® X-100 (Sigma-Aldrich) and 0,4 U/µl RNaseOUTTM (Thermo Fisher, Waltham, MA, USA).

**Quantitative RT-PCR analyses**

For gene expression analyses of FACS-sorted tanycytes, mRNAs were reverse transcribed using SuperScript® III Reverse Transcriptase (Life Technologies, Carlsbad, CA, USA) and a followed by linear preamplification step using the TaqMan® PreAmp Master Mix Kit protocol (P/N 4366128, Applied Biosystems, Foster City, CA, USA). Real-time PCR was carried out on QuantStudio™ 3 Real-Time PCR System, using exon-boundary-specific TaqMan® Gene Expression Assays: botulinum toxin serotype-B light chain (Custom-made 2169611 A6-APAAJ3N), Vamp1 (Mm00772307_m1), Vamp2 (Mm01325243_m1) and Vamp3 (Mm05693405_s1). Control housekeeping genes: r18S (18S-347 Hs99999901_s1); ACTB (Actb-Mm00607939_s1).

**Indirect calorimetry study**

Mice were individually housed and acclimatized to the cages for 48h before experimental measurements in a 12h light/dark cycle and an ambient temperature of 22 ± 1°C. Experimental group treated with metformin and the respective control groups were housed one week before and for the length of the whole treatment in the metabolis cages. Mice were analyzed for total energy expenditure, oxygen consumption, carbon dioxide production, and food intake using

calorimetric cages (Labmaster, TSE Systems GmbH, Bad Homburg, Germany) (Tschöp et al., 2012). Fat oxidation was calculated as described before (Bruss et al., 2010; Lusk, 1924), using energy expenditure, oxygen consumption, and carbon dioxide production. Mice were monitored daily for body weight and body composition at the beginning and the end of the experiment.

**Hormone measurements**

Serum and microdialysate leptin concentrations were measured by Leptin Elisa Kit (respectively Mouse Leptin DuoSet ELISA Mouse/Rat Leptin ELIOS – Quantikine, MOB00, R&D systems). Serum noradrenaline content was measured using a noradrenaline Elisa kit (BA-E5200, Immusmol). Both measurements were performed following the manufacturer´s instructions. Plasma insulin levels from the glucose tolerance test were measured with a Mouse Insulin Elisa Kit (Mercodia, 10-1247-01),

**Lipids measurements**

Serum cholesterol (Spinreact, no. 1001093), triglycerides (Spinreact, no. 1001310), and non-esterified fatty acid levels (Wako, nos. 436-91995, 434-91795) were measured by colorimetry following kits instructions, and plates were read on a ThermoScientific Multiskan GO spectrophotometer.

**Immunoblot analyses of liver and white adipose tissue**

Tissues were homogenized in a TissueLyser II (Qiagen) in cold RIPA buffer (200 mM Tris/HCl pH 7.4, 130 mM NaCl, 10 (v/v glycerol, 0.1% (v/v) SDS, 1% (v/v) Triton X-100, 10 mM MgCl2) with anti-proteases and anti-phosphatases (Sigma-Aldrich). Lysates were centrifuged for 30 min at 18,000g and 4 °C. Total protein lysates were separated on SDS–polyacrylamide gel electrophoresis (SDS–PAGE), electrotransferred to polyvinylidene difluoride membranes and then probed with the following antibodies: hormone-sensitive lipase/HSL (1 1,000; Abcam, catalog no. ab45422); phospho-HSL (Ser660) (1:1,000; Cell Signaling Technology, catalog no. 4126); Acc (1:1,000; Millipore, catalog no. 04-322); phospho-Acc (Ser79) (1:1,000; Cell Signaling Technology, catalog no. 3661); lipoprotein lipase (1:1,000; H-53, Sant Cruz Biotechnology, catalog no. sc-32885); FAS antibody (1:5,000 Abcam, catalog no. ab128870,); CPT1A: (Abcam, Cambridge, UK, ab128568); and β-actin (1:5,000; Sigma-Aldrich, catalog no. A2228), after incubation of membranes with 5% BSA blocking buffer. Proteins were detected using horseradish peroxidase–conjugated secondary antibodies (Dako, no. PI-2000). Specific immunolabelling was visualized using chemiluminescence following the manufacturer´s instructions (Pierce ECL Western Blotting Substrate, ThermoScientific), and values expressed relative to β-actin.

**Glucose tolerance tests**

After overnight fasting (12 h), the first glycaemia measurement was taken (time 0) and then mice received an intraperitoneal glucose injection (1.5 mg glucose g-1 body weight). Glycaemia was subsequently measured at intervals of 15, 30, 60, 90, 120, and 150 minutes after glucose administration (OneTouch Verio glucometer). Blood samples were collected with capillaries from the tail at times 0, 15, and 30 minutes to detect insulin levels. Following centrifugation, plasma was collected and stored at -80°C until hormone measurement.

**Insulin tolerance test**

Mice were subjected to 6-h fasting after the onset of the light phase, and after basal blood glucose levels were measured (OneTouch Verio glucometer) they received an intraperitoneal injection of insulin (0.75 UI kg–1 body weight). Glycaemia was monitored 15, 30, 45, 60, 120, and 150 minutes after insulin administration.

**Homa-IR**

Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated by using fasting plasma insulin (FPI, μU/L) multiplied by fasting plasma glucose (FPG, mg/dL) divided by a constant (HOMA-IR score = FPI × FPG/405), as previously described (Matthews et al., 1985).

**Mouse pancreatic islet isolation and culture**

Each of the ten mice used for islet isolation was fully anesthetized and sacrificed by cervical dislocation. The mouse was laid with the abdomen facing up and the skin was sterilized with 70% ethanol. An incision was performed around the upper abdomen to expose the pancreas and common bile duct. The pancreas was infused with type V collagenase (1.5mg/ml) via the common bile duct. After perfusion, the pancreas was removed, collected in a 50 ml tube containing 2 ml of enzyme collagenase, and digested in a water bath at 37°C for 8--10 minutes (Annicotte et al., 2009, Bourough et al., 2002). Enzymatic digestion was stopped by adding cold Hanks' Balanced Salt solution containing 1% albumin. A density gradient was then performed with polysucrose 1,132/1,108/1,096/1,069/1,000 (Mediatech) to get a high--purity fraction of islets. Islets showed > 90% purity (endocrine versus exocrine tissue). They were cultured in RPMI--1640 medium (Sigma Aldrich) containing 2 g of glucose, supplemented with 10% FBS and 1% P/S for 18 hours before treatment. For insulin secretion tests, ~30 islets were exposed to either 2.8-- or 20--mM glucose in Krebs—Krebs--Ringer--bicarbonate HEPES buffer containing 0.5% FA--free bovine serum albumin (BSA). Secreted insulin was measured 1 h later using an Ultrasensitive Insulin ELISA kit (Mercodia, 10--1132--01). Insulin content was measured after lysis in a buffer containing 75% of ethanol and 1.5% HCl using mouse insulin ELISA kit. Data are expressed relative to total insulin content.

**RNA extraction, measurement and profiling of pancreatic islets**

Total RNA was extracted from islets using an RNeasy Plus Micro Kit (Qiagen) following the manufacturer's recommendations. mRNA levels were measured after reverse transcription by quantitative PCR with reverse transcription using FastStart SYBR Green master mix (Roche), following the manufacturer's recommendations and with gene-specific oligonucleotides as detailed in Table 1. Expression levels were normalized to cyclophilin mRNA and expressed using the formula 2–ΔCt.

| Gene | Sense Primer | Antisense Primer |
|---|---|---|
| Slc2a2 | AACCGGGATGATTGGCATGT | GGCGAATTTATCCAGCAGCA |
| Gck | GCTCAGTGAACCCCGGTCAGC | TGTGCGCAGCTGCTCTGAGG |
| Kcnj11 | CACAAGCTGGGTTGGGGGCTC | TGCCCCTCAGCTGGGTTCTGC |
| Glp-1r | GTTTCCTCACGGAAGCGCCA | AAGGAACCTGGGGGCCCATC |
| Ins1 | GCCAAACAGCAAAGTCCAGG | GTTGAAACAATGACCTGCTTGC |
| Pcsk1 | TGATGATCGTGTGACGTGGG | GGCAGAGCTGCAGTCATTCT |
| Pdx1 | ATTGTGCGGTGACCTCGGGC | GATGCTGGAGGGCTGTGGCG |
| MafA | TCCGACTGAAACAGAAGCGG | CTCTGGAGCTGGCACTTCTC |
| Nkx2.2 | GTGCAGGGAGTATTGGAGGC | GAAGGGCCAGAGGAGGAGA |
| Hnf1a | GGTGCGTGTCTACAACTGGT | ACCGTACACCGTGGACCTTA |
| Ucn3 | TGATGCCCACCTACTTCCTG | CTGTGTTGAGGCAGCTGAAG |
| NeuroD1 | CTTGGCCAAGAACTACATCTGG | GGAGTAGGGATGCACCGGGAA |
| ATF4 | ATGGCCGGCTATGGATGAT | CGAAGTCAAACTCTTTCAGATCCATT |
| Xbp1t | GAGCAGCAAGTGGTGGATTT | CCGTGAGTTTTCTCCCGTAA |
| Xbp1s | GAGTCCGCAGCAGGTG | GTGTCAGAGTCCATGGGA |

*Table 1. displaying the genes of interest names, sense, and antisense primers sequences as provided by the provider (Thermofisher).*

**Immunofluorescence of pancreatic sections**

Immunofluorescence was performed as described previously (Annicotte et al., 2009). After antigen retrieval using citrate buffer (Sigma), formalin-fixed pancreatic sections (5 μm) were incubated with either anti-insulin (1:1,000; Agilent, catalog no. A0564) or anti-glucagon primary antibodies (1:1,000; Sigma-Aldrich, catalog no. G2654,) and AlexaFluor-594-conjugated goat anti-guinea pig (1:500 Molecular Probes, catalog no. A-11076) and AlexaFluor-488-conjugate goat anti-mouse antibodies (1:500; Thermo Fisher Scientific, catalogue no. A-11001). Images were processed for morphometry using ImageJ software by an observer blind to experimental groups.

**Immunohistochemistry and analysis**

**Objective 1**

Adult mice were sacrificed by decapitation at lights-on in fed state (for pStat3 and MECA32). Brains were harvested before being embedded directly in Tissue Tek (Sakura®) and frozen fresh. Coronal sections 20 µm thick were post-fixed with a 2% paraformaldehyde solution for one hour and processed for immunofluorescence as previously described (Bouret et al., 2012) using primary antibodies directed against pSTAT3 (Tyr705) (1:1000; #9131, Cell Signaling Technology) and against MECA32 (1:500; 550563, BD Parmingen). Double immunofluorescence images were acquired using an Apotome Axio.Z2 microscope (AxioCam MRm camera, Zeiss). Slides were then coded to conceal treatment groups, and pSTAT3 immunoreactive (IR) cells were counted on eight representative sections per animal. MECA-32 immunoreactive vessels are visualized in the superficial plexus of the outer zone of the median eminence, but some MECA-32 immunoreactive vessels form intra-infundibular capillary loops. The total number of fenestrated loops and pSTAT3 immunopositive cells was assessed over the entire rostrocaudal surface of the median eminence and arcuate nucleus in each animal (8 representative median eminence sections per animal). The average number of immunopositive cells or vessels per area was then compared between groups.

**Objective 2**

Mice were anesthetized with an IP injection of ketamine and xylazine (80 mg/kg and 100 mg/kg) (Imbernon et al., 2016). First animals were washed with ice-cold saline solution (0.9% 20 ml) into the heart, then to fix the brain ice-cold 4% paraformaldehyde (PFA) in Borate buffer at pH 9.5 BORAX (Sodium Tetraborate Decahydrate) (20 ml).

Brains were harvested, post fixed overnight and cryoprotected with 30% of sucreose. Brains were then embedded directly in Tissue Tek (Sakura®) and frozen. Coronal sections 40 µm thick were cut and processed for immunofluorescence as previously described (Balland et al., 2014) using primary antibodies directed against pERK (1 Rabbit 1:1000 Cell Signalling 9101). Double immunofluorescence images were acquired using an Apotome Axio.Z2 microscope (AxioCam MRm camera, Zeiss).

**Primary culture**

Primary cultures from median eminences of P10 rats was performed. MEs were collected in (Dulbecco medium without sodium pyruvate + 10% DBS, 1% Penicillin and streptomycin and 1% L-glut) were dissociated mechanically and re-suspended in a T-75 and cells were cultured with the afore mentioned culture medium until 90% confluence was reached, they were then split. All cells were incubated overnight with fasting medium containing DMEM devoid of phenol red, supplemented with L-glutamine, HEPES, insulin, and putrescine (Prevot et al., 2003). Bioactive fluorescent leptin (Cisbio Bioassays) (Vauthier et al., 2013), was used for the

leptin secretion assay. Cells were pre-treated 30' with Metformin 1 mM (Miller et al., 2013) or vehicle (PBS). At the end of the 30', Leptin (10ug/mL (≈5μM)) or vehicle were added to the cells for 15', time necessary for the uptake (Balland et al., 2014). Cells were washed with PBS three times and the medium was changed, to remove the leptin that was not uptaken. 15 minutes after the washes the medium was collected on ice in opaque eppendorf tubes to protect fluorescent leptin spectral integrity in order to detect the secreted
leptin levels, cells were washed three times with PBS and cells were either snap frozen for the WB procedure, or lysed immediately with lysis buffer (25 mM Tris, pH 7.4, 50 mM beta-glycerophosphate, 1% Triton X-100, 1.5 mM EGTA, 0.5 mM EDTA, 1 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 10 μg/ml leupeptin and pepstatin A, 10 μg/ml aprotinin, 100 μg/ml PMSF). Cell lysates were collected on ice in opaque eppendorf tubes. Cells lysates and medium leptin leves were measured in a spectral scanner Thermo Scientific Varioskan® Flash 5250500. Fluorescent intensity was expressed as arbitrary units, and data were analysed as a percentage of the control.

**Western blot**

Frozen cell culture tancytes were thawed, and proteins extracted using fresh lysis buffer, mentioned in the previous section. Proteins were quantified using the Pierce™ BCA Protein Assay Kit (Thermo Scientific™). Aliquots of 20 μL of proteins (10 μg/μL) were charged in a handcast 8% acrylamide gel in electrophoresis chambers filled with running buffer (1x Tris/glycine/SDS buffer in ddH2O). Proteins were run at 130 mv for 1 hour and 30 minutes. Proteins were subsequently transferred on Amersham Hybond P 0.2 (Polyvinylidene difluoride) PVDF blotting membrane (Ge-Lifescience # 10600021) in transfert chambers filled with transfer buffer (1x Tris/Glycine buffer + 20% methanol in dd H2O). The
charge was set at 100mV for 1 hour and 10 minutes. Membranes were then cut according to protein molecular weight and for the blocking step, they were incubated for one hour at RT in 5% non-fat dried milk diluted in TBST 5%. Next membranes were washed and incubated at 4°C O/N with the primary antibodies (Phospho-AMPKα (Thr172) Rabbit,1:1000, Cell Signalling; AMPKα Rabbit 1:1000 Cell Signalling; Anti-GAPDH Rabbit mAb 1:10000 Sigma-Aldrich). The secondary Anti-rabbit IgG, HRP (horseradish peroxidase)-linked Antibody (Cell Signalling, 7074) was used at a 1:2500 concentration for 1 hour at RT. The membranes were then washed 3 times for 5 minutes and revealed in the dark room using a Pierce™ Western Blotting horseradish peroxidase (HRP) substrate for enhanced chemiluminescence (ECL) (Thermofisher) and detected on Amersham Hyperfilm ECL (GE Life Sciences). Films were washed, air-dried and scanned. Densitometric analysis was
performed with Fiji software.

**Leptin sensitivity assay**

Individually housed mice were subjected to fasting for 6h before the onset of the dark phase as previously described (Ryan et al., 2011; Imbernon et al., 2014). Three hours before the refeeding, 2 groups of mice (both BoNTBCtl and BoNTBTan) were challenged with leptin. A group received either murine leptin (3 mg/kg–1; Harbor-UCLA Medical Center) or vehicle (PBS pH 8.0) intraperitoneally. To check the ICV effect of leptin administration, a cannula (Plastic One) was stereotaxically placed in the lateral ventricle (anteroposterior, −0.3 mm; midline, ±1 mm; dorsoventral, −2.5 mm) and leptin (2 μg in 2 μl Harbor-UCLA Medical Center) or vehicle (2 μl of PBS pH 8.0) was administrated to a second group of mice. Body weight and food intake were monitored before,12h, and 24h after leptin administration.

**In vivo microdialysis**

A microdialysis cannula (CMA8 High Cut-off, 100 kDa, 1 mm membrane length; CMA microdialysis) was stereotaxically implanted in the mediobasal hypothalamus (anteroposterior, −1.3 mm; lateral, −0.3 mm; ventral, −6.1 mm) in SD, DIO, DIO-Metformin, $BoNTB^{Ctl}$ and $BoNTB^{Tan}$ isoflurane-anesthetized mice, maintained at 37°C core body temperature using a thermostatically controlled electrical blanket. The cannula was then perfused at 2 μl min–1 with sterile artificial CSF (ACSF; CNS Perfusion Fluid: NaCl 147 mmol l–1, KCl 2.7 mmol l–1, CaCl2 1.2 mmol l–1 and MgCl2 0.85 mmol l–1; CMA) using a microinjection pump (CMA 402, CMA). Following stabilization for 45 min, two basal dialysates of 20 min were collected. Leptin 477 (i.p., 3 mg kg–1 in PBS pH 8.0; Harbor-UCLA Medical Center) was administered to mice and six dialysates of 20 min recovered. Dialysates were placed in a fraction collector (CMA 820) during the experiment and immediately stored at −80 °C until analysis. At the end of the experiment, mice were decapitated and brains were stored immediately in fresh 4% paraformaldehyde. Microtome brain sections (40 μm) were counterstained with DAPI to verify probe location. Only mice in which the probe was positioned between anteroposterior −1.2 and −2.3 were included in analyses.

**Statistics**

Results are given as means ± s.e.m. Samples or animals were excluded when their values were outside ±2 s.d., or when an objective experimental failure was observed; studies were not formally randomized and investigators were not blind to the treatment group, except when mentioned. To test whether populations followed a Gaussian distribution, a normality test was performed (Kolgomorov–Smirnov test for n = 5–7, Shapiro–Wilk test for $n \geq 7$). For normal

distributions, one or two-sided unpaired t-tests were used to compare two populations; for multiple-comparison tests, one or two-way ANOVA followed by Tukey's post hoc multiple-comparison test was used (unless otherwise indicated in the figure legends). For non-Gaussian distributions, Mann–Whitney tests were used to compare two populations, and Kruskal–Wallis followed by Dunn's post hoc test for multiple comparisons. Data analysis was performed using GraphPad Prism Software v.8.0 (version 8.4.2; GraphPad Software, La Jolla, California, USA). The threshold for significance was $P < 0.05$.

# *Chapter 6: Results*

**Objective 1**: To decipher the physiological consequences the specific impairment of tanycytic exocytosis on energy homeostasis on chow and diet-induced obese mice.

Since the mid-20th century, the development of the processed food industry in Western society has created a high-caloric eating culture, which, together with a sedentary lifestyle, resulted in an epidemy of obesity and a plethora of obesity-related disorders such as cardiovascular diseases, type 2 diabetes (T2D), steatohepatitis, cancer, and neurodegenerative disorders (Blüher, 2019). Nutrient sensing mechanisms that maintain glucose and energy homeostasis involve body-brain communication by peripheral humoral and neuronal components, that will inform the brain about peripheral energy status and nutrient availability (Coll, Farooqi & O'Rahilly, 2007; Lustig, 2006; Müller et al., 2022). Impairment of this communication due to both obesogenic and genetic factors can lead to the development of metabolic disorders. In recent decades, advances in neuroendocrinology have enabled the development of new strategies to treat metabolic diseases. These approaches aim to restore communication between the body and the brain that is often disrupted in conditions of energy excess (Mathieu, Martens & Vangoitsenhoven, 2021, Brüning, Fenselau, 2023; Gjermeni et al., 2021). A deeper understanding the elements involve in the communication between the periphery and the brain is essential for developing more effective and long-lasting therapies for metabolic diseases.

Within the tuberal region of the hypothalamus, the median eminence serves as a key central hub for integrating signals related to energy balance by functioning as a circumventricular organ (CVO) (Prevot, Nogueiras & Schwaninger, 2021). The median eminence is the structure closing the ventral aspect of the third ventricle (3V) adjacent to the arcuate nucleus of the hypothalamus (ARH) which is a major site of the metabolic brain containing the melanocortin system (Brüning, Fenselau, 2023). The median eminence houses a dense capillary network with fenestrated endothelium, allowing blood-borne cues about the body's energy status to freely enter the median eminence and the ventromedial ARH (Schaeffer et al., 2013; Mullier et al., 2010). However, the extent of passive molecule diffusion is limited, and the fenestration of capillary loops reaching the ARH is finely regulated according to energy status (Langlet et al., 2013).

Tanycytes are specialized ependymoglial cells that line the 3V floor. They form tight junctions that prevent blood-borne molecules from reaching deeper hypothalamic structures via the cerebrospinal fluid (CSF) (Banks, 2019; García-Cáceres et al., 2019). Fine processes of these cells establish direct contact with fenestrated vessels, controlling the bidirectional exchange of molecules between the bloodstream and the hypothalamus (Prevot et al., 2018; Nampoothiri et al., 2022). This exchange delivers neurohormones into the bloodstream from neurons that regulate the anterior pituitary's activity and permits blood-borne molecules to reach their target neurons in the hypothalamus (Duquenne et al., 2021; Imbernon et al., 2022; Porniece-Kumar et al., 2021; Pena-Leon et al., 2022). Moreover, tanycytes themselves are central to intercellular communication

processes within hypothalamic circuits that regulate energy homeostasis. They sense certain nutrients such as glucose and communicate their concentration to neurons (Lhomme et al., 2021). Selectively manipulating tanycytic activity using the Designer Receptor Exclusively Activated by Designer Drugs (DREADD technology which implies the use of engineered protein receptors selectively activated by ligands to manipulate the activity of cells) or genetically altering their viability has profound consequences on energy homeostasis and circulating metabolic hormone levels (Yu et al., 2023; Rohrbach et al., 2021; Müller-Fielitz et al., 2017). This is particularly relevant given that the conduit of blood-borne metabolic hormones into the hypothalamus by tanycytes is impaired in conditions of energy surplus (Balland et al., 2014; Collden et al., 2015). Our previous research indicates that the disruption of tanycyte shuttles may impair vesicular trafficking within these cells that is crucial for transcytosis processes (Duquenne et al., 2021).

To explore the importance of SNARE-dependent vesicle trafficking in tanycytes for metabolic regulation, we employed Cre recombinase-mediated expression of clostridial botulinum neurotoxin serotype-B light chain (BoNT/B) in tanycytes in adult mice. Our findings demonstrate that the blockade of tanycytic transcytosis alone is sufficient to abrogate the access of blood-borne signals such as leptin into the mediobasal hypothalamus. This manipulation leads to a prediabetic status in mice fed on a standard diet: it promotes intraabdominal fat deposition, glucose intolerance, hyperinsulinemia, and impaired insulin sensitivity, particularly in the context of moderate overweight. Additionally, it heightens sensitivity to obesogenic diets.

**Botulinum neurotoxin type B expression in adult hypothalamic tanycytes promotes body weight gain while increasing carbohydrate consumption.**

To explore the specific function of tanycytes in transporting metabolic hormones from the bloodstream to the brain, we employed a mouse model where we inhibited vesicular transport within tanycytes using botulinum toxin light chain B (BoNT/B). BoNT/B hinders exocytosis in neurons and glial cells by cleaving vesicle-associated membrane proteins 1 and 2 (VAMPs), which facilitate vesicular fusion and transport (Slezak et al., 2012; Schiavo et al., 1992). VAMP3, another target of BoNT/B, is involved both in endocytosis and exocytosis processes (Banerjee et al., 2017; Kasai et al., 2012). For conditional cell-specific BoNT/B expression, we used a transgenic mouse line where BoNT/B and EGFP can be expressed in a Cre-dependent manner (Selzak et al., 2012). To induce Cre expression and thus BoNT/B specifically in tanycytes of these mice, we injected either TAT-Cre into the 3rd ventricle (Duquenne et al., 2021) or of an AAV1/2 vector expressing the Cre recombinase under the control of the tanycytes-specific human deiodinase 2 (DIO2) promoter into the lateral ventricle (Müller-Fielitz et al., 2017). Control littermates were injected with an AAV1/2 vector expressing EGFP. Control- and Cre-injected animals are hereafter referred to as BoNTBCtl and BoNTBTan mice, respectively (Figure 1A). Isolation of tanycytes using fluorescent-activated cell sorting (FACS) from median eminence-ARH microdissected explants showed strong induction of BoNT/B transcripts in BoNTBTan mice in contrast to BoNTBCtl littermates, which did not show any signal (Figure 1B, C). Transcripts for VAMP1-3 were readily detected in FACS-sorted tomato-positive tanycytes isolated from tdTomatoloxP-STOP-loxP mice following TAT-Cre delivery into the third ventricle (Figure 1D). Notably, as expected the presence of BoNT/B did not affect the expression of VAMP1 and VAMP2 genes remained unchanged in targeted tanycytes. However, there was a trend toward downregulation of VAMP3 transcripts compared to tanycytes from tdTomato mice (Figure 1D).

Four weeks after Cre-mediated genetic recombination and intake of a chow diet, mice expressing BoNT/B in tanycytes exhibited increased food intake (Figure 1E) and body weight (Figure 1F) compared to BoNT/BCtl littermates. Surprisingly, indirect calorimetry analyses revealed elevated carbohydrate metabolism at the end of the resting period, as indicated by a higher respiratory exchange ratio (RER) closer to the absolute value of 1 rather than 0,7 as for lipids consumption (Figure 1G), along with increased energy expenditure and ambulatory activity (Supplementary Figure 1A-B). At 12 weeks post recombination, the body weight gap between BoNTBTan and control littermates continued to widen (Figure 1F) with increased accumulation of visceral fat (Figure 1H) and higher circulating levels of leptin (Figure 1I).

When animals were placed on a high-fat diet (HFD) four weeks after genetic recombination, body weight gain markedly accelerated in BoNT/BTan mice, with significant changes occurring already within two weeks after initiating this diet (Figure 1J). As observed with the chow diet (Figure 1G, 1H), indirect calorimetry at four weeks on the HFD showed that

BoNT/BTan mice exhibited increased food intake (Figure 1K), accompanied by increased use of carbohydrates as the primary energy source during both resting and active periods (Figure 1L). However, no marked differences were observed in energy expenditure or ambulatory activity (Supplementary Figure 1C-D). Altogether these data show that impairing SNARE-dependent vesicle release from tanycytes enhances food intake and body weight. These changes were possibly caused by disruption of the feedback loops between peripheral tissues and the brain, leading to miscommunication regarding energy balance.

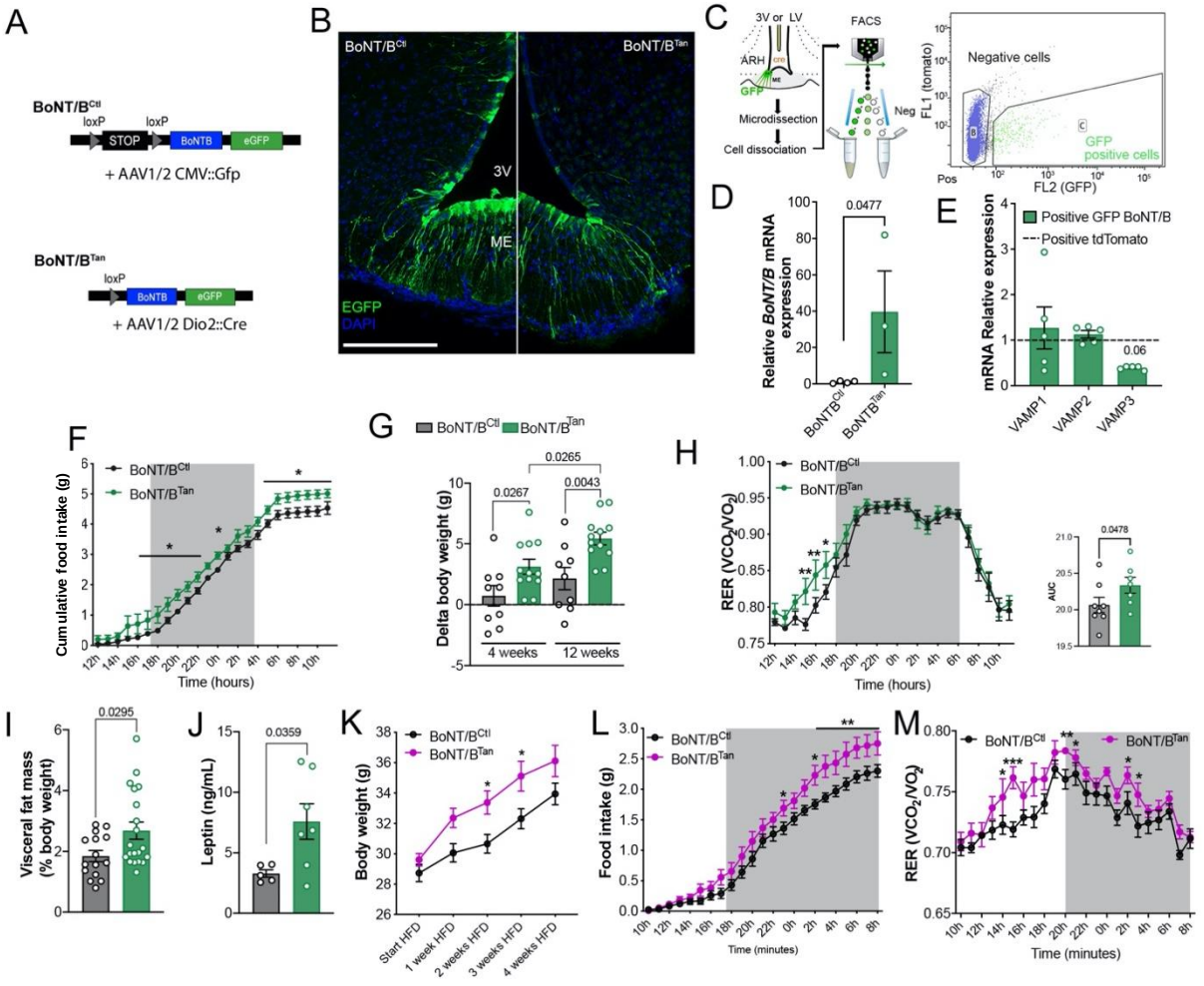



**Figure 1. Alteration of vesicular trafficking in median eminence tanycytes promotes body weight gain.**

(**A**) Schematics for the Cre-dependent induction of BoNT/B in tanycytes after the injection of recombinant TAT-Cre or vehicle into the third ventricle, or AAV1/2-Dio2-Cre or vehicle in the lateral ventricle of BoNTB-EGFP$^{loxP\text{-}STOP\text{-}loxP}$ mice. (**B**) Representative photomicrographs of EGFP immunopositive median eminence tanycytes in BoNTB$^{Ctl}$ and BoNTB$^{Tan}$ mice 4 weeks after Cre-induced recombination. Scale bar, 200 µm. (**C**) Diagram and gating strategy for the sorting of EGFP-positive putative tanycytes following AAV1/2-Dio2-Cre infusion into the lateral ventricle (LV) of BoNTB-EGFP$^{loxP\text{-}STOP\text{-}loxP}$ mice. (**D**) mRNA levels of Botulinum neurotoxin light chain B mRNA in EGFP-positive FACS-sorted cells from ME and ARH BoNTBTan and BoNTB$^{Ctl}$ explants 4 weeks after Cre-induced genetic recombination. One-tailed unpaired *t*-test; n = 4 and 3 mice. BoNTB$^{Tan}$ and BoNTB$^{Ctl}$ EGFP-negative FACS-sorted cells Botulinum neurotoxin light chain B mRNA levels were undetectable. (**E**) Expression of VAMP1, VAMP2 and VAMP3 mRNA in EGFP-positive FACS-sorted cells of BoNTB$^{Tan}$ mice (n = 5), 4 weeks after Cre-induced recombination, normalized over the same mRNA expression (Value =1) in Tomato-positive FACS-sorted cells of TdTomato$^{Tan}$ mice (n = 5) after Cre-induced recombination. Two-tailed unpaired t-test, *VAMP1* p = 0.6162; VAMP2 p = 0.4231; VAMP3 p = 0.0606. (**F**) Curves representing 24h of cumulative food intake in BoNTB$^{Ctl}$ (n = 7) and BoNTB$^{Tan}$ (n = 8) mice, 4 weeks after the Cre-induced genetic recombination. Two-way ANOVA, Interaction p = 0.6123, Subjects p = 0.0266. LSD post-hoc test, 16.00h p = 0.0411; 17.00h p = 0.0414; 18.00h p = 0.0164; 19.00h p = 0.0159; 20.00h p = 0.0112; 21.00h p = 0.0324; 22.00h p = 0.0357; 4.00h p = 0.041; 5.00h p = 0.0361; 6.00h p = 0.013; 7.00h p = 0.0154; 8.00h p = 0.0103; 9.00h p =

0.0108; 10.00h p = 0.0095 and 11.00h p= 0.0275. (**G**) Body weight change of $BoNTB^{Ctl}$ (n = 9) and $BoNTB^{Tan}$ (n = 12) mice 4 and 12 weeks after Cre-induced genetic recombination. Values are expressed in grams (gr). Two-way ANOVA, Time p= 0.0008; Šídák's multiple comparisons test, $BoNTB^{Ctl}$ 4 weeks versus $BoNTB^{Ctl}$ 12 weeks p = 0,254. (**H**) Curves representing 24h of respiratory exchange ratio (RER) in $BoNTB^{Ctl}$ (n = 7) and $BoNTB^{Tan}$ (n = 8) mice 4 weeks after the Cre-induced recombination. Two-way ANOVA, Interaction p = 0,5424; Time p = <0.0001; Subjects p = 0.0927. LSD post-hoc test, 15.00h p = 0.0039; 16.00h p = 0.0081; 17.00h p = 0.0179. The area under the curve (AUC), one-tailed unpaired t-test. (**I**) Visceral fat mass expressed as a percentage of the body weight in $BoNTB^{Ctl}$ (n = 14) and $BoNTB^{Tan}$ (n = 12) 12 weeks after the Cre-induced genetic recombination. Two-tailed Mann-Whitney test. Values are expressed as percentages calculated over the body weight. (**J**) Circulating leptin levels of $BoNTB^{Ctl}$ (n = 5) and $BoNTB^{Tan}$ (n = 7) mice 12 weeks after the Cre-induced genetic recombination. Two-tailed unpaired t-test. (**K**) Body weight follow-up in $BoNTB^{Ctl}$ (n = 6) and $BoNTB^{Tan}$ (n = 5) mice fed with a high-fat diet (HFD) for 4 weeks, 4 weeks after Cre-induced recombination. Two-way ANOVA, Interaction p = 0.0821; Time p < 0.0001; Subjects p = 0.0228. The two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, at the start of the HFD, p = 0.2245; 1-week HFD p = 0.0153; 2-week HFD p = 0.0121; 3-week HFD p = 0.0292 and 4-week HFD p = 0.0994. (**L**) Curves representing 24h cumulative food intake in $BoNTB^{Ctl}$ (n = 6) and $BoNTB^{Tan}$ (n = 6) mice 8 weeks after the Cre-induced recombination and 4 weeks of HFD. Two-way ANOVA, Interaction p < 0.0001; Time p < 0.0001; Subject p = 0.0672. LSD post-hoc test, 23.00h p = 0.0452; 1.00h p = 0.0276; 2.00h p = 0.0042; 3.00h p = 0.0022; 4.00h p = 0.0052; 5.00h p = 0.0034; 6.00h p = 0.0042; 7.00h p = 0.0064; 8.00h p = 0.0066; 9.00h p = 0.0111. (**M**) Curves representing 24h RER in $BoNTB^{Ctl}$ (n = 6) and $BoNTB^{Tan}$ (n = 6) mice 8 weeks after the Cre-induced recombination and 4 weeks of HFD. Two-way ANOVA, Interaction p = 0.5955; Time p < 0.0001; Subject p = 0.0169. LSD post-hoc test, 14.00h p = 0.0442; 15.00h p = 0.0002; 17.00h p = 0.0067; 20.00h p = 0.0379; 2.00h p = 0.0432 and 3.00h p = 0.0229.
Data are expressed as means ± SEM. p < 0.05 values are indicated in the figures.

**BoNT/B expression in tanycytes blocks blood-borne leptin transport into the hypothalamus**

Increased food intake (Figure 1E) concomitant with elevated adiposity and circulating levels of leptin in BonT/BTan mice (Figure 1H and 1I) raises the possibility that these animals may be developing hypothalamic resistance to circulating leptin. This could be caused by defective leptin transport across the blood-brain barrier into the CSF via tanycytes, as seen in various animal models at early stages of diet-induced obesity (Duquenne et al., 2021; Balland et al., 2014; Chmielewski et al., 2019). First, to test whether this phenomenon occurs under physiological conditions, we assessed endogenous STAT3 activation in the ARH at the specific time of the day when lights are switched on, after overnight feeding, when circulating leptin levels are at their highest (Sukumaran et al., 2010). This paradigm has previously been shown to be linked to endogenous LepR activation (Langlet al al., 2013; Balland et al., 2019). Four weeks after Cre-induced recombination, the number of P-STAT-3 immunoreactive cells in the ARH was diminished by about 30% in BoNT/BTan mice when compared to BoNT/BCtl littermates (Figure 2B). In contrast, an increased number of P-STAT3-immunoreactive cells was visualized in the median eminence in BoNT/BTan mice compared to control littermates (Figure 2C). This suggests that in BoNT/BTan mice, circulating leptin extravasates from the fenestrated capillaries of the median eminence but remains trapped within the median eminence parenchyma. There, it may activate local LepR-expressing cells without being transported into the hypothalamus. In support of this, examination of the fenestral diaphragms of median eminence endothelial cells using antibodies to MECA-32 showed that the number of fenestrated capillary loops in the median eminence and the vmARH were comparable in BoNT/BTan and control littermates (Figure 2A, 2D and 2E). Finally, twelve weeks after Cre-induced recombination, we subjected BoNT/BTan mice as well as their control littermates to intraperitoneal (i.p.) or intracerebroventricular (i.c.v.) injection of exogenous leptin and measured food intake 24h and 12h later, respectively (Figure 2F). While exogenous leptin injected i.c.v. directly into the lateral ventricle reduced feeding in both groups of mice efficiently (Figure 2G), exogenous leptin injected intraperitoneally showed this effect only in BoNT/BCtl mice, but not in BoNT/BTan mice (Figure 2H). This confirmed that BoNT/BTan mice are sensitive to central leptin but resistant to peripheral leptin suggesting a block of the tanycytic transport of blood-borne leptin into the hypothalamus. To further explore the impact of BoNT/B expression in tanycytes on blood-borne leptin transport into the ARH, we stereotaxically implanted microdialysis probes into the mediobasal hypothalamus of both BoNTBCtl and BoNTBTan mice (Figure 2I). Leptin levels in the hypothalamus were monitored in dialysates over time. Forty minutes after administering an intraperitoneal bolus of leptin, we observed leptin transport into the mediobasal hypothalamus in BoNTBCtl mice, following a kinetic pattern that was not observed in BoNTBTan littermates. In the latter, leptin levels remained close to the detection limit of the approach (Figure 2J). Altogether, these

results unequivocally demonstrate that tanycytic transcytosis is required for the transport of blood-borne leptin into the hypothalamus.

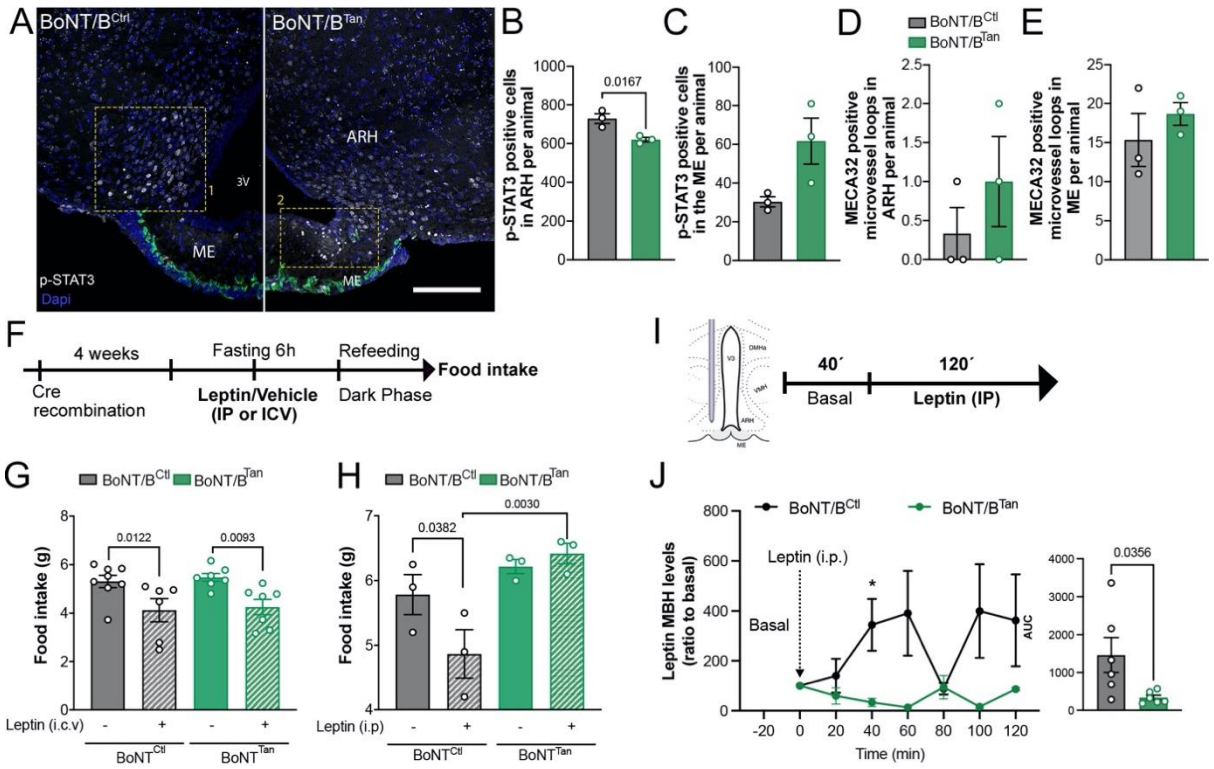



**Figure 2. Blockade of exocytosis in ME tanycytes impairs leptin sensitivity and its transport into the medio basal hypothalamus**

(**A**) Representative photomicrographs of phosphoSTAT3 immunopositivity (white dots) in median eminence and medio basal hypothalamus cells and MECA32 immunopositive microvessel loops (green) in BoNTB$^{Ctl}$ and BoNTB$^{Tan}$ mice 4 weeks after Cre-induced genetic recombination. Yellow dotted insets one and two delimitate the ARH and ME respectively. Scale bar, 200 µm. (**B**,**C**) Quantification of phosphoSTAT3 immunopositive cells in the ARH (**B**) and ME (**C**) of BoNTB$^{Ctl}$ (n = 3) and BoNTB$^{Tan}$ (n = 3) mice 4 weeks after Cre-induced genetic recombination. Two-tailed unpaired t-test. In ARH (**B**); ME (**C**), p = 0.0617. (**D**,**E**) Quantification of the number of MECA32 immunopositive microvessel loops in the ARH (**D**) and ME (**E**) in in BoNTB$^{Ctl}$ (n = 3) and BoNTB$^{Tan}$ (n = 3) mice 4 weeks after Cre-induced genetic recombination. Two-tailed unpaired t-test. In ARH (**D**), p = 0.3739; ME (**E**), p = 0.4165. (**F**) Protocol of leptin tolerance test (i.p. 3 mg/kg or i.c.v. 1 mg/kg) in BoNTB$^{Ctl}$ and BoNTB$^{Tan}$ mice 12 weeks after Cre-induced genetic recombination. (**G**) Cumulative food intake 24 hours after leptin or vehicle injection in BoNTB$^{Ctl}$ (n = 7-8) and BoNTB$^{Tan}$ (n = 3) mice. Two-way ANOVA, Interaction p = 0.0653; Treatment p = 0.2079; Subject p = 0.0053. LSD post hoc test, BoNTB$^{Ctl}{}_{Veh}$ vs. BoNTB$^{Tan}{}_{Veh}$ p = 0.2751 and BoNTB$^{Tan}{}_{Veh}$ vs. BoNTB$^{Tan}{}_{Leptin}$ p = 0.6034. (**H**) Cumulative food intake 12 hours after leptin or vehicle injection in BoNTB$^{Ctl}$ (n = 3) and BoNTB$^{Tan}$ (n = 3). Two-way ANOVA, Interaction p = 0.9514; Treatment p = 0.0006; Subject p = 0.6308. LSD post hoc test, BoNTB$^{Ctl}{}_{Veh}$ vs. BoNTB$^{Tan}{}_{Veh}$ p = 0.6908 and BoNTB$^{Ctl}{}_{Leptin}$ vs. BoNTB$^{Tan}{}_{Leptin}$ p = 0.7740. (**I**) Schematic diagram of the stereotactic implantation of the microdialysis probe in the mediobasal hypothalamus (MBH) and the protocol of the same experiment, that investigates exogenous leptin central transport in BoNTB$^{Ctl}$ and BoNTB$^{Tan}$ mice 4 weeks after Cre-induced genetic recombination. (**J**) Leptin concentrations in the MBH extracellular fluid collected by microdialysis every 20 min following intraperitoneal leptin (t, 0 min) injection (3 mg/kg) in BoNTB$^{Ctl}$ (n = 6) and BoNTB$^{Tan}$ mice (n = 6). Mixed-effects analysis, Time p = 0.3901; Subject p = 0.0423; Interaction p = 0.1708. LSD post hoc test, 40min p = 0.0387. AUC, two-tailed unpaired t-test.

Data are expressed as means ± SEM. $p < 0.05$ values are indicated in the figures.

**BonT/B expression in hypothalamic tanycytes alters peripheral lipid homeostasis**

Despite the higher visceral fat mass and leptinemia observed in BoNT/BTan mice compared to their BoNT/BCtl littermates (Figure 1E-F), no significant differences were found in either fatty acid oxidation (Figure 3A) or serum levels of triglycerides (Figure 3B), cholesterol (Figure 3C), and non-esterified free fatty acids (NEFAS) between the two groups of mice (Figure 3D). To delve deeper into lipid metabolism, we examined the lipolysis and lipogenesis pathways in both the liver in visceral adipose tissue of BoNT/BCtl and BoNT/BTan littermates using Western blot analyses.

In the liver, BoNT/B expression in tanycytes did not affect markers of de novo lipogenesis, including the total protein levels and phosphorylation of acetyl-CoA carboxylase (ACCa) and fatty acid synthase (FAS) (Figure 3E, H). Also, protein expression of carnitine palmitoyl transferase 1-A (CPT1-A), an indicator of fatty acid oxidation, remained unchanged (Figure 3F, H). However, there was a notable reduction in lipoprotein lipase protein (LPL) levels (Figure 3G, 3H), suggesting either a decrease in hepatic lipid uptake, or a decreased triglyceride breakdown in favour of storage.

In the epididymal white adipose tissue, BoNT/BTan mice showed higher ratio of phosphorylated ACCa (pACC)/ACCa, indicating a decrease in fatty acid synthesis. No differences were observed in the protein levels of the fatty acid oxidation marker CPT1A or in the lipid uptake marker LPL. However, BoNT/BTan mice exhibited reduced phosphorylation of hormone-sensitive lipase (HSL) compared to control mice (Figure 3M, 3L), suggesting a decrease in triglyceride mobilization, possibly due to lower noradrenaline levels, an activator of HSL-mediated lipolysis (Figure 3N) (Kolditz et al., 2010; Bartness et al., 2014). Overall, these results suggest that the blockade of tanycyte transcytosis in BoNT/BTan mice reduces both lipid synthesis and mobilization in the white adipose tissue. This could be due to the incapacity of leptin to reach the ARH and promote peripheral lipid mobilization (Buettner et al., 2008), as well as hindered signaling to the sympathetic nervous system via the melanocortin system (Sohn et al., 2013).

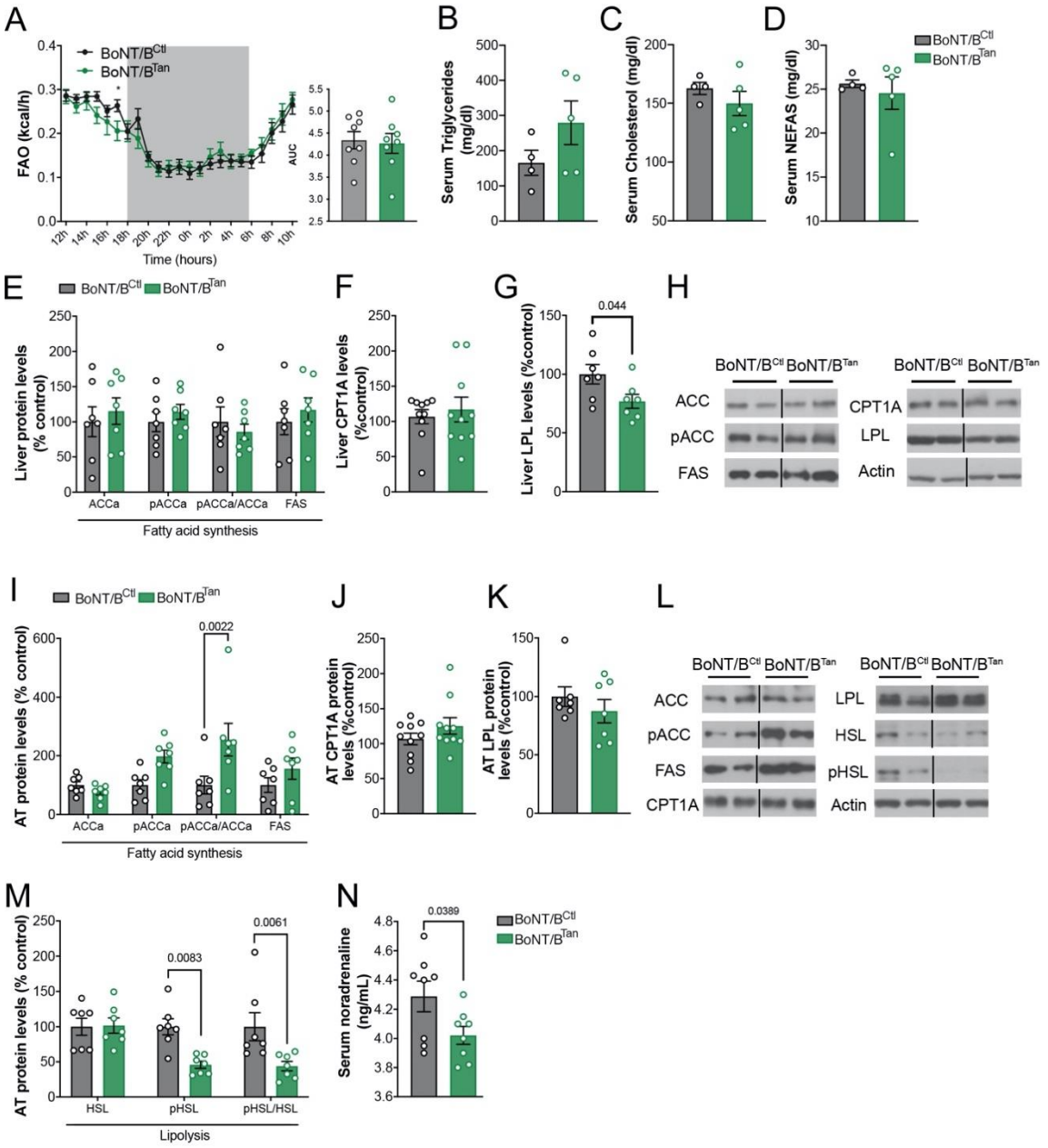



**Figure 3. BoNTB expression in tanycytes reduces lipid mobilisation in adipose tissue.**

(**A**) Curves representing 24h fatty acid oxidation (FAO) in BoNTB$^{Ctl}$ (n = 7) and BoNTB$^{Tan}$ (n = 8) mice, 4 weeks after the Cre-induced genetic recombination. Two-way ANOVA, Interaction p = 0.3634; Time p < 0.0001; Subjects p = 0.8217. LSD post-hoc test, 17h p = 0.0483). AUC, one-tailed unpaired t-test, p = 0.406. (**B**-**D**) Circulating levels of triglycerides (**B**), cholesterol (**C**) and non-esterified fatty acids (NEFAs, **D**) of BoNTB$^{Ctl}$ (n = 4) and BoNTB$^{Tan}$ (n = 5) mice 12 weeks after the Cre-induced genetic recombination. Two-tailed unpaired t-test; triglycerides, p = 0.1832; cholesterol, p = 0.3396, t-test; NEFAs, p = 0.626. (**E**-**G**) Production levels of selected proteins involved in fatty acid (FA) synthesis in liver (**E**), carnitine-palmitoyl transferase A (CPT1A) (**F**) and lipoprotein lipapse (LPL) (**G**), from BoNTB$^{Ctl}$ (n = 7) and BoNTB$^{Tan}$ (n = 10) mice 12 weeks after the Cre-induced genetic recombination. In **E**, one-way anova, p = 0.0005. Šídák's multiple comparisons test ACCa BoNTB$^{Ctl}$ vs. ACCa BoNTB$^{Tan}$ p = 0.9638, t = 0.5811; pACCa BoNTBCtl vs. pACCa BoNTB$^{Tan}$ p = 0.9721 t = 0.5405; pACCa/ACCa BoNTBCtl vs. pACCa/ACCa BoNTB$^{Tan}$ p = 0.9227 t = 0.7239 and FAS BoNTB$^{Ctl}$ vs. FAS BoNTB$^{Tan}$ p = 0.9456 t = 0.6528. In **F**, two-tailed unpaired t-test, p = 0.6178. In **G**, two-tailed unpaired t-test. (**H**) Representative immunoblots of proteins from panels **E**-**G**. (**I**-**M**) Expression levels of selected proteins involved in FA synthesis in adipose tissue (**I**), CPT1A (**J**), LPL (**K**) and proteins involved in lipolysis (**M**), from

BoNTB$^{Ctl}$ (n=7) and BoNTB$^{Tan}$ (n=10) mice 12 weeks after the Cre-induced genetic recombination. In **I**, one-way anova, p = 0.0007, F = 4.431. Šídák's multiple comparisons test ACCa BoNTB$^{Ctl}$ vs. ACCa BoNTB$^{Tan}$, p = 0.9646; pACCa BoNTB$^{Ctl}$ vs. pACCa BoNTB$^{Tan}$ p = 0.0927; pACCa/ACCa BoNTB$^{Ctl}$ vs. pACCa/ACCa BoNTB$^{Tan}$; and FAS BoNTB$^{Ctl}$ vs. FAS BoNTB$^{Tan}$ p = 0.5632. In **J**, two-tailed unpaired p = 0.2124, t test. In **K**, two-tailed Mann Whitney test, p = 0.535. In **M**, one-way anova, p = 0.0005. Šídák's multiple comparisons test, HSL BoNTB$^{Ctl}$ vs. HSL BoNTB$^{Tan}$ p = 0.9995; pHSL BoNTB$^{Ctl}$ vs. pHSL BoNTB$^{Tan}$ and pHSL/HSL BoNTB$^{Ctl}$ vs. pHSL/HSL BoNTB$^{Tan}$. (**L**) Representative immunoblots of proteins in (**I**-**K**, **M**). (**N**) Circulating noradrenaline levels of BoNTB$^{Ctl}$ (n=8) and BoNTB$^{Tan}$ (n=8) mice 12 weeks after the Cre-induced genetic recombination. One-tailed Mann Whitney test.

Data are expressed as means ± SEM. $p < 0.05$ values are indicated in the figures.

**Expression of BoNT/B in tanycytes promotes glucose intolerance and insulin resistance associated with pancreatic beta cell compensation**

At 12 weeks after BoNT/B induction in tanycytes, BoNT/BTan mice exhibited impaired tolerance to exogenous glucose (Figure 4A). Monitoring of glucose-stimulated insulin release monitoring revealed heightened insulin secretion in BoNT/BTan mice compared to control BoNT/BCtl littermates, suggesting a beginning of insulin resistance (Figure 4B). An insulin tolerance test further confirmed this observation (Figure 4C). Additionally, insulin sensitivity and the homeostatic model assessment of insulin resistance (HOMA-IR) were found to be elevated in BoNT/BTan mice compared to control mice (Figure 4D), indicating a prediabetic, insulin-resistant state in BoNT/BTan overweight mice. To assess potential changes in pancreatic function in mice expressing BoNT/B in tanycytes, we examined glucose-stimulated insulin secretion (GSIS) in pancreatic islets isolated from both BoNT/BTan and BoNT/BCtl littermates 12 weeks after Cre-mediated recombination. Consistent with the prediabetic, insulin-resistant phenotype observed in BoNT/BTan mice, isolated islets demonstrated heightened insulin secretion in response to 20 mM glucose, but similar insulin content compared to those from control BoNT/BCtl littermates (Figure 4E, F), suggesting the activation of compensatory mechanisms in β cells following insulin resistance in these animals (Weir & Bohner-Weir, 2004). Gene expression profiling of β-cells from BoNTBCtl and BoNTBTan islets revealed notable transcriptional alterations in markers of β-cell function and endoplasmic reticulum (ER) stress. In β-cells from BoNTBTan mice compared to controls, the heightened GSIS was accompanied by upregulation of mRNA levels in genes implicated in glucose sensing and insulin secretion, including Slc2a2 and Glp-1r. Conversely, there was a decrease in expression of the proinsulin processing gene Pcsk1 (Figure 4G). Additionally, differences were noted in the expression of genes associated with pancreatic islet identity and function, such as reduced expression of Pdx1 involved in β-cell maturation and survival (Johnson et al., 2003), and elevated levels of Hnf1a, MafA and NeuroD1 genes, all contributing to β-cell identity (Figure 3G). Notably, β cells from BoNTBTan mice exhibited elevated expression of the early onset ER-stress marker Atf4 (Figure H), suggesting that the increased insulin secretion observed in these mice following insulin resistance may trigger an ER unfolded protein response and ER stress. This response is typically associated with impaired β-cell function under chronic high glucose (Back & Kaufman, 2012). Despite this, no differences were observed yet in surface area (Figure 4I), size (Figure 4J), or morphology (Figure 4K) of pancreatic islets from BoNTBTan compared to those from BoNTBCtl mice.

Overall, these findings suggest that blocking tanycyte transcytosis alters pancreatic function, resulting in glucose intolerance, insulin resistance, and potential disruptions in β-cell function and identity, as well as affecting the ER stress response, without altering pancreatic islet morphology.

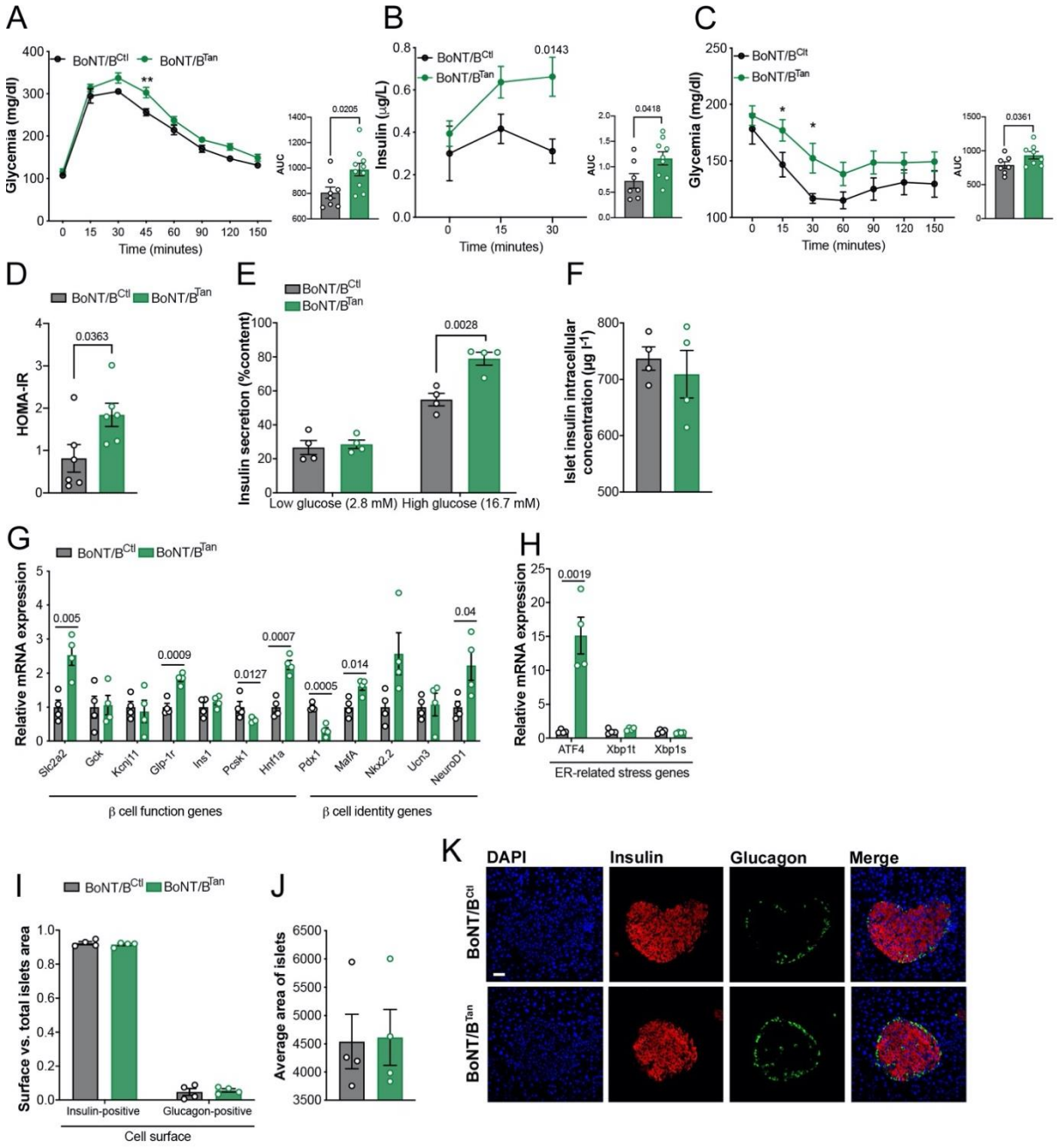
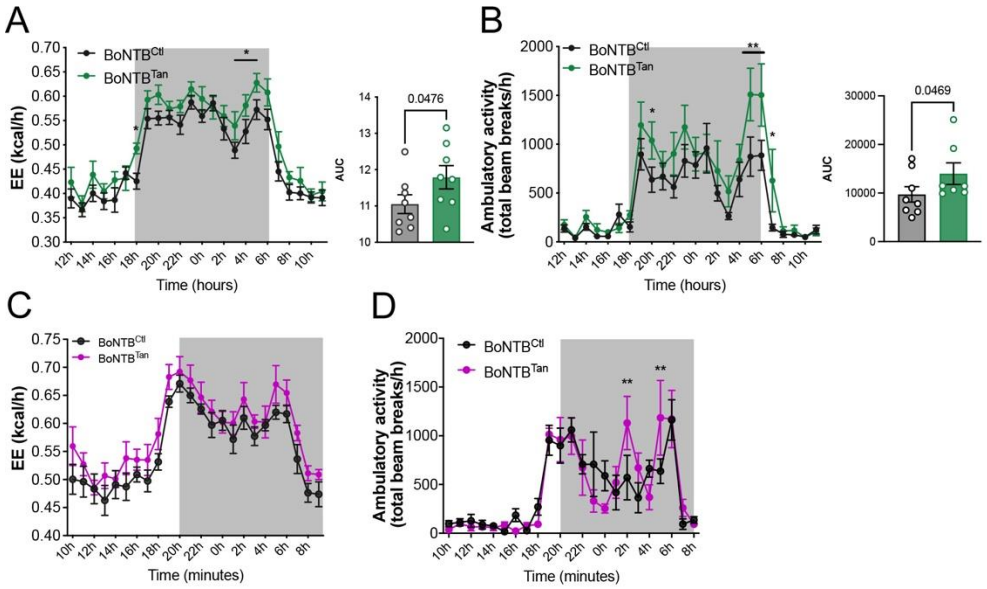



**Figure 4. Expression of BoNT/B in tanycytes impairs glucose metabolism triggering compensatory mechanisms in pancreatic beta cells**.

(**A**) Curve representing glycaemia during a glucose tolerance test in BoNTB$^{Ctl}$ (n = 7) and BoNTB$^{Tan}$ (n = 8) mice 12 weeks after the Cre-induced genetic recombination. Two-way ANOVA, Interaction p = 0.2853; Time p = <0.0001; Treatment p = 0,0167. Bonferroni's multiple comparisons test, 45' p = 0,0006, 120' p = 0,0376. AUC, two-tailed unpaired t-test. (**B**) Serum insulin levels 0, 15 and 30 minutes after intraperitoneal delivery of glucose (2g/kg) during the glucose tolerance test in **A**. Two-way ANOVA, Interaction p = 0.1142; Time p = 0.0138; Treatment p = 0.0364. Šídák's multiple comparisons test, 0min p = 0.8174; 15min p = 0.1925. AUC, two-tailed unpaired t-test. (**C**) Glycaemia during an insulin tolerance test in BoNTB$^{Ctl}$ (n = 7) and BoNTB$^{Tan}$ (n = 8) mice 12 weeks after the Cre-induced genetic recombination. Two-way ANOVA; Time p = <0.0001; Subject p = 0.0864,. Uncorrected Fisher's LSD, 15min p = 0.0377; 30min p = 0.0150. AUC, one-tailed unpaired t-test. (**D**) Homa-IR, two-tailed unpaired t-test. (**E**) Insulin secretion in a static incubation of pancreatic islets isolated from BoNTBCtl (n = 4) and BoNTB$^{Tan}$ (n = 4) mice, 12 weeks after the Cre-induced genetic recombination. Two-way ANOVA,

Interaction p = 0.0091; Glucose concentration p = <0.0001; Subject p = 0,0034. Šídák's multiple comparisons test, Low glucose $_{(2,8\ mM)}$:BoNTB$^{Ctl}$ vs. Low glucose $_{(2,8\ mM)}$:BoNTB$^{Tan}$ p = 0.9995; Low glucose $_{(2,8\ mM)}$:BoNTB$^{Ctl}$ vs. High glucose $_{(16,7\ mM)}$:BoNTB$^{Ctl}$ p = 0.0007; Low glucose $_{(2,8\ mM)}$:BoNTB$^{Tan}$ vs. High glucose $_{(16,7\ mM)}$:BoNTB$^{Tan}$ p = <0.0001. (**F**) Intracellular insulin content of pancreatic islets isolated from BoNTB$^{Ctl}$ (n = 4) and BoNTB$^{Tan}$ (n = 4) mice 12 weeks after the Cre-induced genetic recombination. Two-tailed unpaired t-test, p = 0.5756. (**G**,**H**) Relative mRNA expression levels of β-cell function and identity (**G**) and endoplasmic reticulum (ER) stress markers (**H**) in pancreatic islets isolated from BoNTB$^{Ctl}$ (n= 4) and BoNTB$^{Tan}$ (n= 4) mice 12 weeks after the Cre-induced genetic recombination. Unpaired t test, two-stage step-up (Benjamini, Krieger, and Yekutieli). In **G**, *Gck* p = 0.8919; *Kcnj11* p = 0.7457; *Ins1* p = 0.3688; *Pcsk1* p = 0.07637; *Nkx2.2* p = 0.0555 and *Ucn3* p = 0.8395. In **H**, *Xbp1t* p = 0.1444; *Xbp1s* p = 0.2788. (**I**) Ratio between insulin-positive and glucagon-positive area to total islet surface area in BoNTB$^{Ctl}$ (n = 4) and BoNTB$^{Tan}$ (n = 4) mice 12 weeks after the Cre-induced genetic recombination. Two-way ANOVA, Interaction p = 0,516; Insulin/Glucagon p = < 0.0001; Subjects p = 0.9949. Šídák's multiple comparisons test, Insulin-positive:BoNTB$^{Ctl}$ vs. Insulin-positive:BoNTB$^{Tan}$ p = 0.9609 and Glucagon-positive:BoNTB$^{Ctl}$ vs. Glucagon-positive :BoNTB$^{Tan}$ p = 0.9588. (**J**) Average surface area of pancreatic islets from **I**. Two-tailed unpaired t-test, p = 0.9182. (**K**) Representative photomicrographs representing nuclei (blue), insulin (red) and glucagon (green) in isolated pancreatic islets from BoNTB$^{Ctl}$ and BoNTB$^{Tan}$ mice 4 weeks after Cre-induced recombination in **I** and **J**. Scale bar, 50 μm.

Data are expressed as means ± SEM. p < 0.05 values are indicated in the figures.




**Supplemenatry figure 1**

(**A**-**B**) Curves representing 24h energy expenditure **A** and ambulatory activity **B** in BoNTB$^{Ctl}$ (n = 7) and BoNTB$^{Tan}$ (n = 8) mice 4 weeks after the Cre-induced genetic recombination, two-way ANOVA. In **A**, Interaction p =0.6239; Time p < 0.0001; Subject p = 0.1029. LSD post hoc, 18h p =0.0164; 5h p = 0.0486 and 6h p = 0.0475. AUC, one-tailed Mann Whitney. In **B**, Interaction p = 0.0868; Time p < 0.0001; Subject p = 0.1383. LSD post-hoc test, 20h p = 0.0481; 5h p = 0.0018; 6h p = 0.0025 and 7h p = 0.0182. AUC, one-tailed Mann Whitney. (**C**) Curves representing 24h EE in BoNTB$^{Ctl}$ (n = 6) and BoNTB$^{Tan}$ (n = 6) mice 8 weeks after the Cre-induced genetic recombination and 4 weeks of HFD. Two-way ANOVA, Interaction p = 0.9293; Time p < 0.0001; Subject p = 0.2078. LSD post-hoc test. (**D**) Curves representing 24h ambulatory activity in BoNTB$^{Ctl}$ (n = 6) and BoNTB$^{Tan}$ (n = 6) mice 8 weeks after the Cre-induced genetic recombination and 4 weeks of HFD. Two-way ANOVA, Interaction p = 0.2022; Time p < 0.0001; Subject p = 0.8224. LSD post-hoc test, 2.00 p = 0.0064, 5.00 p = 0.0075. Data are expressed as means ± SEM. p < 0.05 values are indicated in the figures.

**Objective 2**: To determine whether metformin requires tanycyte function to mediate its anti-obesity and anti-diabetic effect.

Tanycytes endocytose leptin in a leptin- and EGF- receptor dependent manner, involving vesicular trafficking and extracellular signal-regulated kinases (ERK) signalling. After being uptake by tanycytes, the hormone is released into the CSF in the third ventricle from where it reaches hypothalamic nuclei through volume-transmission mechanims (Balland et al., 2014 and Duquenne 2021).

Individuals with a normal BMI, show proportional leptin levels in the CSF to circulating one, however, this ratio is impaired in obese individuals (Caro et al., 1996). Actually, in patients with obesity leptinaemia is extremely high, but the hormone fails to exert its anorectic action due to its inability to reach the CSF first and the hypothalamus then (Caro et al., 1996; Schwartz et al., 1996). Although it has been described that central access of leptin is impaired in mice fed with HFD for 8 weeks (Balland et al., 2014), hypothalamic neurons still respond to leptin after its central administration under the same conditions (Van Heek et al., 1997). Importantly, central leptin administration starts to lose its effectiveness in mice after 15 weeks on HFD (El-Haschimi et al., 2000). These data suggest that the onset of diet-induce obesity is characterized by a loss leptin transport into the brain first, and a loss of central leptin sensitivity after. The idea of the alteration of leptin transport into the brain may precede any metabolic dysfunction is strengthened by studies in minipigs (Chmielewski et al., 2019). Alterations in the shuttling of circulating leptin into the CSF was detected as soon as the mini-pigs started on a HFD become overweight but maintaining a healthy metabolically phenotype at after 10 weeks on a HFD (Chmielewski et al., 2019).

The saturability of leptin transport into the central nervous system beyond a certain limit, is confirmed since in DIO mice, leptin accumulates in median eminence tanycytes and does not reach the MBH (Balland., et al 2014).

Interestingly, the blockade of ERK activation in a tanycytic primary culture during a leptin transport assay, dramatically blunts the secretion of leptin previously taken up by tanycytes (Balland et al., 2014), suggesting that boosting ERK phosphorylation in DIO mice, could restore leptin central transport.

When the epidermal growth factor (EGF), a potent activator of the ERK signalling pathway, was administrated in mice fed with HFD for 8 weeks, EGF re-establishes tanycytic leptin transport. Importantly, one-week EGF treatment accelerated DIO mice weight loss as soon as they returned to a standard diet (Balland et al., 2014). Although we have recently shown that the heterodimer formed by EGFR/LEPR is functional for tanycytes to mediate leptin transcytosis (Duquenne et al., 2021), EGF cannot be utilised as a treatment, since it is known to have prooncogenic effects (Jin et al., 2013).

Metformin, a known anti-diabetic drug that mainly acts through an indirect activation of the AMP-activated protein kinase (AMPK) (Hawley et al., 2010), increases leptin sensitivity in rats fed a high fat diet (Kim et al., 2006). Specifically, the co-treatment of metformin and leptin increased the anorectic effects of the latter, together with an activation of the leptin-signalling pathway reflected by the phosphorylation of the signal-transducer and activator of transcription-3 (STAT3) in the hypothalamus (Kim et al., 2006). In addition, metformin also induces the upregulation of muscles glucose transport dependant on ERK phosphorylation (Sajan et al., 2010), which activation has been associated to a rescue of central leptin transport in HFD-fed mice (Balland et al., 2014). The characteristics of this molecule led us to hypothesize and test its action on the amelioration of tanycytic leptin transport in DIO mice.

**Metformin induces body weight loss by decreasing food intake**

To test the effect of metformin in the metabolic profile of DIO C57Bl6/j male mice, they were daily treated with either 300 mg/kg of metformin or vehicle (drinking water) by gavage during the last 2-weeks of their 8-week HFD and challenged in a calorimetric system (Figure 1A). Metformin treatment reduced body weight from the day 3 of administration (Figure 1B) as previously described (Coll et al., 2020). At the end of the treatment, Metformin treated mice, showed a decreased percentage of body fat mass and an increased percentage of the lean mass when compare to vehicle treated ones (Figure 1C-E). As expected, due to the loss of fat mass induce by metformin, it was observed a decrease in leptinaemia (Figure 1D). Importantly metformin treatment reduces food intake, that is reflected in a reduction in glucose consumption as shown in the resting respiratory ratio (RER) and energy expenditure (Figure 1F-H).

To test whether the actions of metformin in weight-loss were either due to the reduction of food intake or maybe due to an effect in nutritional partitioning, (Cho et al., 2015), we performed a pair-feeding experiment. In this experiment, the pair-fed group was treated with vehicle but food restricted with the hourly amount of food consumed by the metformin-treated group (Figure 1J).

Both metformin-treated mice and pair-fed once, showed a similar significant reduction of body and RER in comparison to vehicle treated mice (Figure 1K,L). So far, these data indicate that the metabolic actions of metformin treatment in DIO mice, are a consequence of its effects over feeding behaviour.

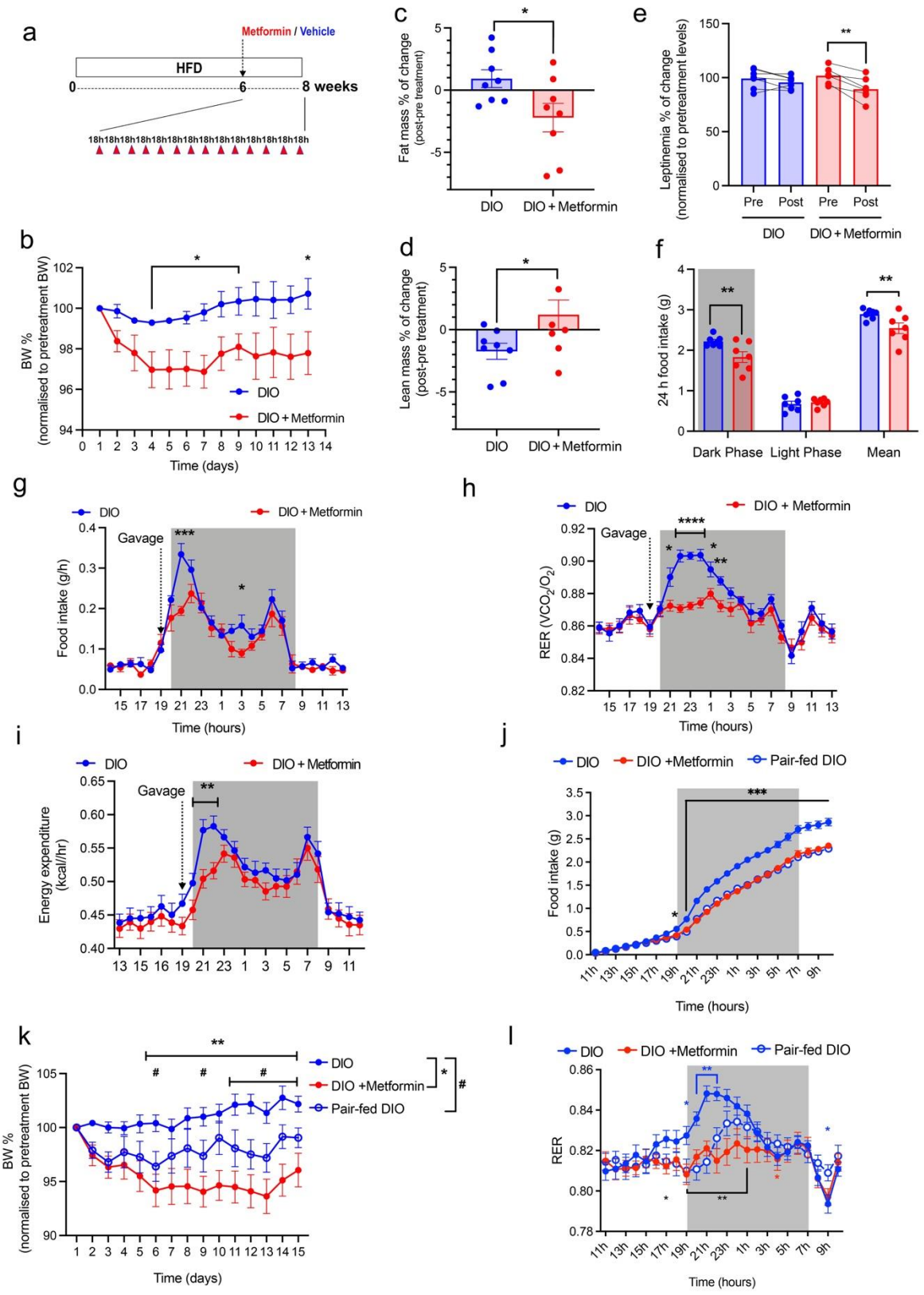

a
Metformin / Vehicle
HFD
0
6
8 weeks
18h18h18h18h18h18h18h18h18h18h18h18h18h18h
b
BW %
(normalised to pretreatment BW)
DIO
DIO + Metformin
Time (days)
c
Fat mass % of change
(post-pre treatment)
DIO
DIO + Metformin
d
Lean mass % of change
(post-pre treatment)
DIO
DIO + Metformin
e
Leptinemia % of change
(normalised to pretreatment levels)
Pre
Post
Pre
Post
DIO
DIO + Metformin
f
24 h food intake (g)
Dark Phase
Light Phase
Mean
g
DIO
DIO + Metformin
Gavage
Food intake (g/h)
Time (hours)
h
DIO
DIO + Metformin
Gavage
RER (VCO2/O2)
Time (hours)
i
DIO
DIO + Metformin
Gavage
Energy expenditure
(kcal//hr)
j
DIO
DIO +Metformin
Pair-fed DIO
Food intake (g)
Time (hours)
k
DIO
DIO +Metformin
Pair-fed DIO
BW %
(normalised to pretreatment BW)
Time (days)
l
DIO
DIO +Metformin
Pair-fed DIO
RER
Time (hours)

**Figure 1. Metformin induces body weight loss by decreasing food intake**

(**A**) Schematic representation of the experimental design: 8-week-old male mice underwent a 8-week HFD. During the last 2 weeks of diet, they received a daily gavage at 18h00 of either water or metformin (300 mg/kg in water). (**B**) Curve representing the evolution of body weight in percentage normalized to the weight before treatment. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM. (n=7) (**C**) Delta between lean mass percentage measured after and before vehicle or metformin treatment. One-sided unpaired t-test. Values shown are means ± SEM. Each dot represents one mouse (n=5). (**D**) Delta between lean mass percentage measured after and before vehicle or metformin treatment. One-sided unpaired t-test. Values shown are means ± SEM. Each dot represents one mouse (n=5). (**E**) Circulating leptin levels in vehicle treated and metformin treated mice. Values are expressed in percentage and normalized to pretreatment serum leptin levels. A paired two-sided t-test was applied for comparisons before the same group before and after treatment. Each dot represents one mouse (n=5). (**F**) Cumulative food intake in the dark/light phase and the mean of the two, as an average calculated over the 14 days of vehicle and metformin treatment. One-sided unpaired t-test. Values shown are means ± SEM. Each dot represents one mouse (n=7). (**G**) Curve representing a 24-hour period hour-by-hour food intake (expressed as grams eaten per hour) calculated as the average values of the 14-day vehicle or metformin treatment. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM (n=7) (**H**) Curve representing a 24-hour period hour-by-hour respiratory exchange ratio (expressed as the ratio in between CO2 volume over O2 volume) calculated as the average values of the 14-day vehicle or metformin treatment. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM (n=7). (**J**) Curve representing a 24-hour period hour-by-hour energy expenditure (expressed as kcal burnt per hour) calculated as the average values of the 14-day vehicle or metformin treatment. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM (n=7). (**J**) Curve representing a 24-hour period hour-by-hour cumulative food intake (expressed as grams) calculated as the average values of the 14-day vehicle treatment, metformin treatment or pair-feeding. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM (n=12,12 and 9 respectively). (**K**) Curve representing the evolution of body weight in percentage normalized to the weight before treatment of vehicle treated, metformin treated and pair-fed vehicle treated mice. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM. (n=12,12 and 9 respectively). (**L**) Curve representing a 24-hour period hour-by-hour respiratory exchange ratio (expressed as the ratio in between CO2 volume over O2 volume) calculated as the average values of the 14-day vehicle treatment, metformin treatment or pair-feeding. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM (n=7).

**Metformin chronic treatment restores leptin transport into the MBH of DIO mice**

As mentioned before, mice that underwent HFD for 8 weeks, shown a dramatic impairment in the blood-borne leptin transport to the MBH (Balland et al., 2014). Considering the metabolic profile of metformin treated animals, we wondered whether the reduction of both body weight and food intake could be due to a restoration of leptin central transport by metformin.

Thus, to monitor and detect leptin content in the hypothalamus, microdialysis probes were stereotaxically implanted into the MBH parenchyma in anesthetized mice fed with either standard diet (SD) or HFD after the treatment with metformin or vehicle (Figure 2A, B). After the probes implantation, the system allowed us to collect dialysate fractions of MBH every 20 minutes (Figure 2A).

Twenty minutes after the intraperitoneal administration of leptin, SD-fed mice dialysates displayed a significant rise in leptin content over basal levels, showing then a second raise at 80 minutes. Both peaks of leptin content were blunted in dialysate fractions from HFD-fed mice treated with vehicle (Figure 2C). However, in DIO mice treated with metformin for 14 day their dialysates showed leptin content at 20 and 80 minutes, rescuing the central transport kinetics as of the SD mice (Figure 2D). Given the importance of glycaemia in the central transport of molecules in the brain (Langlet et al., 2013; Bakker et al., 2022), we also monitored blood-glucose levels that could have been affected by the treatment or anaesthesia.

To excluding a possible extravasation of leptin due to hypoglycaemia-induced fenestration of the ARH vessels (CITE), glycaemia was monitored during the microdialysis, showing no significant differences (Figure 2E). This experiment demonstrates that a 14-day metformin treatment can rescue leptin central transport in DIO mice fed with HFD for 8 weeks.

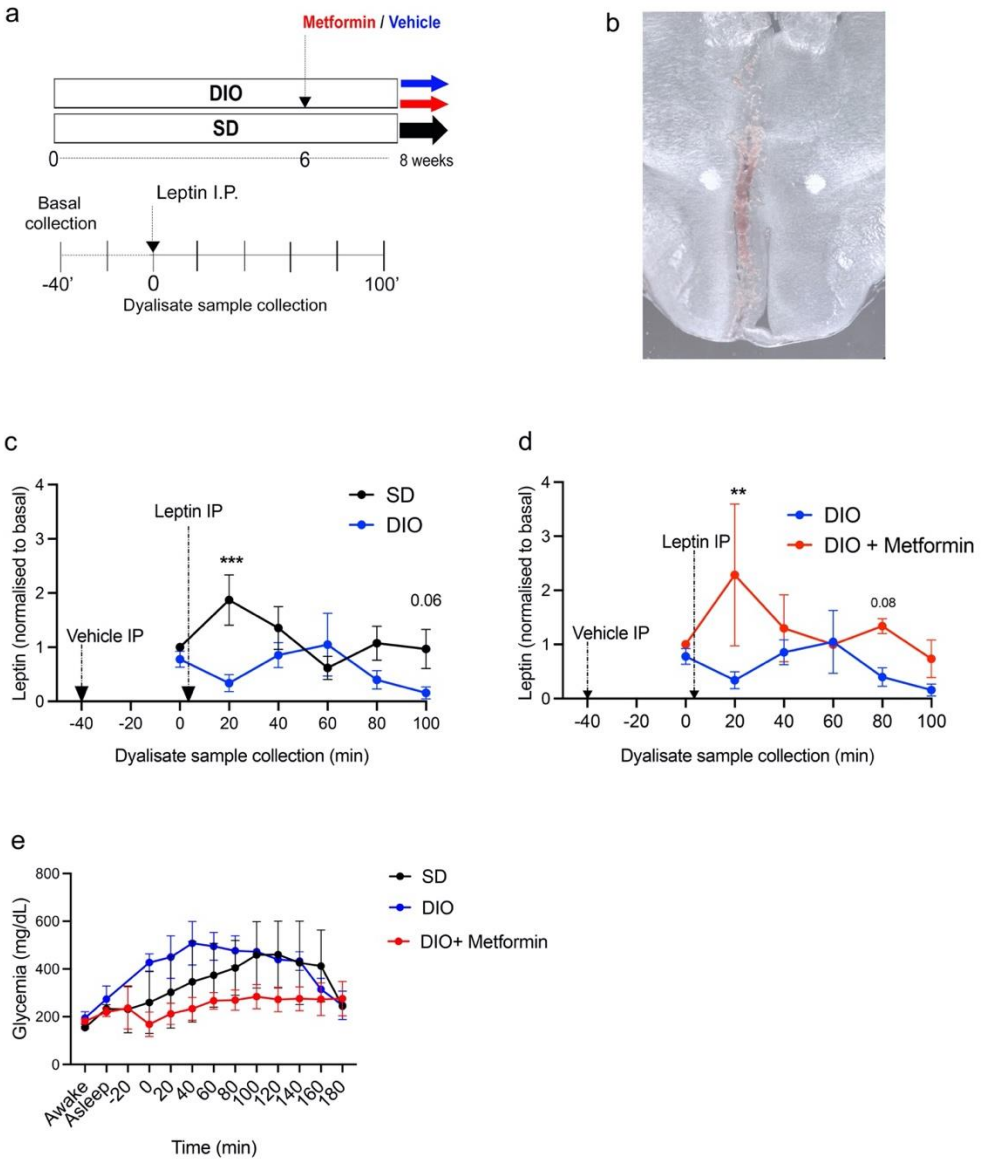



**Figure 2. Metformin chronic treatments restore leptin transport into the MBH of DIO mice.**

(**A**) Schematic representation of the experimental design: 8-week-old male mice were either fed a standard diet, either a HFD for 8 weeks. The cohort of HFD-fed mice was further subdivided in two groups treated with a daily gavage at 18h00 of either vehicle or metformin for 14 days. After the treatments the animals were anesthetized and microdialysis probes were stereotaxically implanted in the mediobasal hypothalamus of anaesthetise mice. Dialysates samples were collected every 20'. (**B**) Representative picture at the loop of a coronal brain section of showing the canula implantation placement. (**C**) Curve representing leptin concentrations in the MBH extracellular liquid after an i.p. injection, normalized over leptin MBH basal levels in SD and DIO mice. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM. (n=5). (**D**) Curve representing leptin concentrations in the MBH extracellular liquid after an i.p. injection, normalized over leptin MBH basal levels in DIO mice treated with vehicle or metformin. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM. (n=5) (**e**) Glycaemia of SD-fed mice gavaged for two weeks with water and and HFD-fed mice after a daily gavage of either water or metformin (300 mg/kg in water) for two weeks under anaesthesia during the microdialysis experiment.

**Metformin anti-obesity action requires LepR expression in tanycytes**

Given the pivotal role of tanycytes in the central transport of leptin (Balland et al., 2014; Duquenne et al., 2021) we hypothesized that the rescue of leptin central transport induced by metformin in DIO mice, was mediated by tanycytes.

To test our hypothesis, we induced the selective ablation of leptin receptor in tanycytes of 4-weeks- HFD-fed LepR$^{loxp/loxp}$-TdTomato mice by the stereotactic administration of an AAV1/2-Dio2-Cre vector or an AAV1/2-CMV-GFP control vector in lateral ventricle. Cre- and control administrated mice are hereafter reffered as LepR$^{tanKO}$ and LepR$^{loxp/loxp}$ respectively. Then, 2 weeks after the viral delivery, both experimental groups were treated with metformin or vehicle during the last 2 weeks of their 8-week total HFD (Figure 3A-C). Remarkably, while metformin treatment significantly decreased body weight in HFD-fed LepR$^{loxp/loxp}$ mice, it fails to induce body weight loss in HFD-fed LepR$^{tanKO}$ littermates (Figure 3F-H). Importantly, metformin cannot reduce food intake HFD-fed LepR$^{tanKO}$ as in their controls (Figure 3H, I), suggesting that the ablation of LepR in tanaytes impedes metformin to improve leptin passage to the MBH. According to the food intake, energy expenditure levels in HFD-fed LEepR$^{loxp/loxp}$ mice were decreased by metformin, while there were no effects on LepR$^{tanKO}$ littermates (Figure 3J, K). Unexpectedly metformin had no effect on the RER of both experimental groups (Figure 3L, M). These results indicate that for metformin to reduce food intake and weight loss, the tanycytic LepR must be intact.

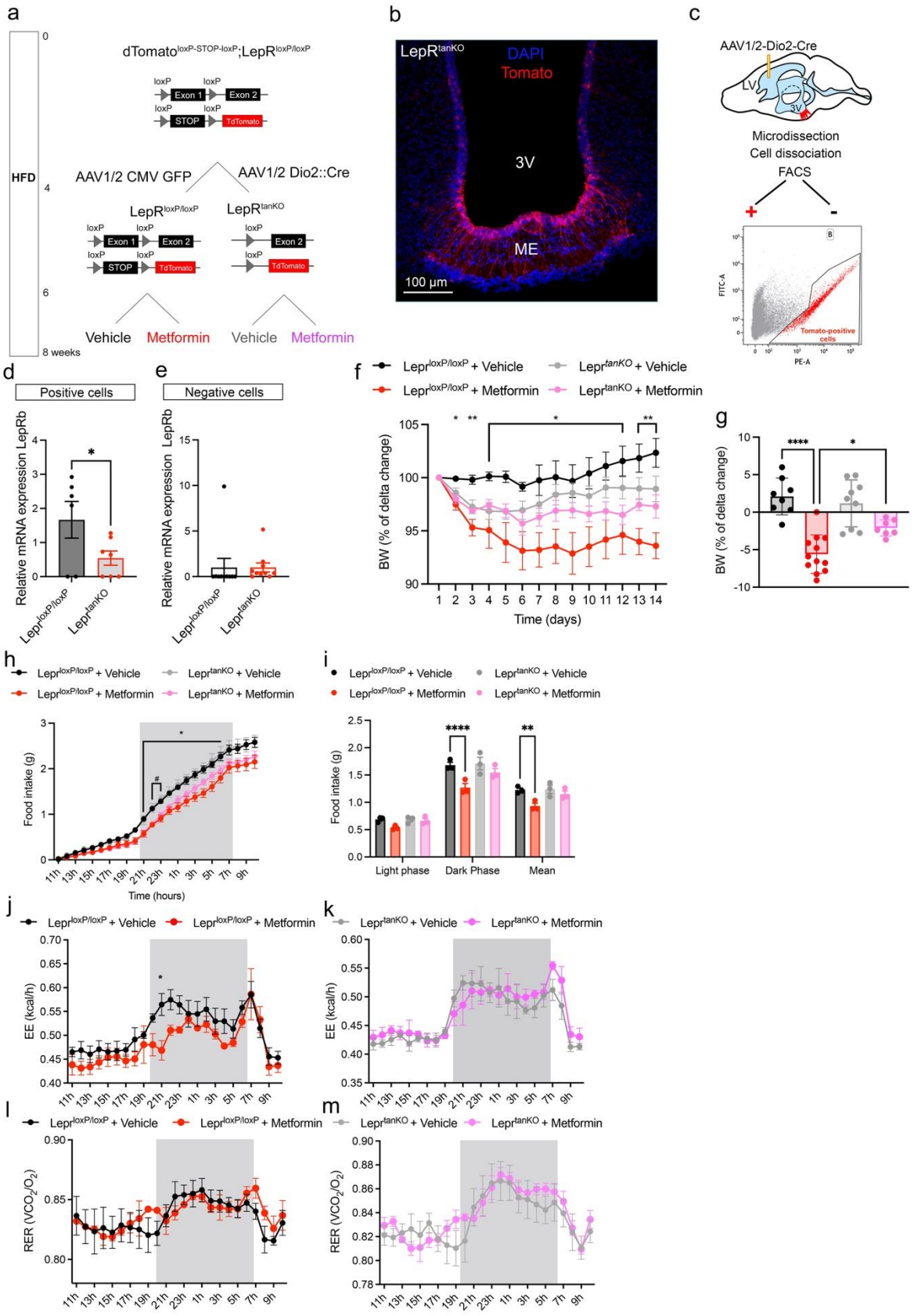



**Figure 3. Metformin anti-obesity action requires LepR expression in tanycytes**

(**A**) Schematic representation for the Cre-dependent induction of LepR knock-out in tanycytes of *LepR*$^{loxP/loxP}$;*tdTomato*$^{loxP\text{-}STOP\text{-}loxP}$ mice fed a HFD for 4 weeks, following the injection of AAV1/2:Dio2:Cre or

AAV1/2:Dio2 in the lateral ventricle. Subsequently *LepR*$^{TanKO}$ and *LepR*$^{loxP/loxP}$ at week 6 of HFD were treated with a daily gavage of either water or metformin (300 mg/kg in drinking water) for two weeks, until the end of the 8-week HFD. **(B**) Representative photomicrographs of Tomato immunopositive median eminence tanycytes in Lepr$^{TanKO}$ mice 4 weeks after Cre-induced recombination. Scale bar, 100 µm. (**C**) Diagram and gating strategy for the sorting of Tomato-positive putative tanycytes following AAV1/2-Dio2-Cre infusion into the lateral ventricle (LV) of dTomato$_{loxP\text{-}STOP\text{-}loxP}$;LepR$_{loxP/loxP}$ mice. (**D, E**) mRNA levels of LeprB mRNA in Tomato-positive (**D**) and negative (**E**) FACS-sorted cells from ME and ARH *LepR*$^{loxP/loxP}$ and *LepR*$^{TanKO}$ explants 4 weeks after Cre-induced genetic recombination. One-tailed unpaired *t*-test; n = 6 and 7 mice. (**F**) Curve representing the evolution of body weight in percentage normalized to the weight before treatment in *LepR*$^{loxP/loxP}$ and *LepR*$^{TanKO}$ mice. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM. (n=9) (**G**) Percentage of delta body weight change (after treatment – before treatment) after the 14-day vehicle or metformin treatment in *LepR*$^{loxP/loxP}$ and *LepR*$^{TanKO}$. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM. Each dot represents one mouse. (**H**) Curve representing a 24-hour period hour-by-hour cumulative food intake (expressed as grams) calculated as the average values of the 14-day vehicle or metformin treatment in *LepR*$^{loxP/loxP}$ and *LepR*$^{TanKO}$. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM (n=3). (**I**) Food intake rate (expressed as grams eaten per hour) in the dark/light phase and the mean of the two, as an average calculated over the 14 days of vehicle or metformin treatment in *LepR*$^{loxP/loxP}$ and *LepR*$^{TanKO}$. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM. Each dot represents one mouse (n=3). (**J, K**) Curve representing a 24-hour period hour-by-hour energy expenditure (expressed as kcal burnt per hour) calculated as the average values of the 14-day vehicle or metformin treatment in *LepR*$^{loxP/loxP}$ (**J**) and *LepR*$^{TanKO}$ (**K**). Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM (n=4). (**L, M**) Curve representing a 24-hour period hour-by-hour respiratory exchange ratio (expressed as the ratio in between CO2 volume over O2 volume) calculated as the average values of the 14-day vehicle or metformin treatment in *LepR*$^{loxP/loxP}$ (**L**) and *LepR*$^{TanKO}$ (**M**). Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM (n=4).

**Co-administration of metformin and leptin activates ERK pathway and AMPK-pathway in tanycytes does not mediate metformin actions on body weight.**

To assess whether metformin boosts leptin tancytic transport tanycytes, we perfomed a leptin-transport assay in rat primary cultured tanycytes (Figure 4A). Cells pre-treated with metformin for 30 minutes before including leptin in the medium, significantly released more leptin in the medium after its uptake when compare with PBS treated cells (Figure 4B), suggesting that metformin can boost the signalling pathway involve in the tanycytic leptin transport.
In order explore the pathway involved in this process, we questioned the invoment of metfonin in both AMPK and ERK pathways.
As mentioned, EGFR-ERK-pathway activation is sufficient to rescue of leptin transport into the MBH (Balland et al., 2014) and t has been published that metformin may activates this pathway too (Sajan et al, 2010). To observed whether metformin is involved in ERK-phosphorilation (pERK), a single gavage bous of metformin (300 mg/kg,) or vehicle together with an exogenous administration of leptin or its vehicle (3 mg/kg i.p.) in lean mice were, 45 and 5 minutes before perfusion respectively. After performing immunohistochemistry of pERK in median eminence brain sections, we observed ERK-phosphorilation when metformin and leptin are co-administrated with metformin and leptin are immunopositive (Figure 4C), suggesting a role of the kinase in the metformin-induced restoration of leptin central transport.
It is wide known that AMPK is one of the most studied metformin targets (Zhou et al., 2001)
Indeed, metformin treatment in primary culture of tanycytes, induces AMPK-phosphorilation (Figure 4D, E). So as to dig into this putative molecular mechanism of action of metformin, we tested the effects of the drug in a mouse model in which the catalytic subunits of AMPK were selectively ablated in tanycytes.
Thus, 4-week HFD-fed AMPK$^{\alpha 1\text{-}2loxp/loxp}$ mice were stereotactically administratated with either a 1:1 cocktail of AAV1/2-Dio2-Cre and AAV1/2[FLEXon] -CMV> LL:rev(EGFP : Scramble [shRNA]) :LL :WPRE vectors or an AAV1/2-CMV-GFP control vector in the lateral ventricle. Cre- and control administrated mice are hereafter reffered as AMPK$^{tanKO}$ and AMPK$^{loxp/loxp}$ respectively. Furthermote both experimental groups were treated with metformin or vehicle during the last 2 weeks of their 8-week HFD (Figure 3G-I).
Unexpectedly, metformin seems to induce weight loss both in AMPK$^{loxp/loxp}$ and in AMPK$^{tanKO}$ mice (Figure 3J, K) suggesting that for its anti-obesity effect, the activation of the kinase AMPK is dispensable.

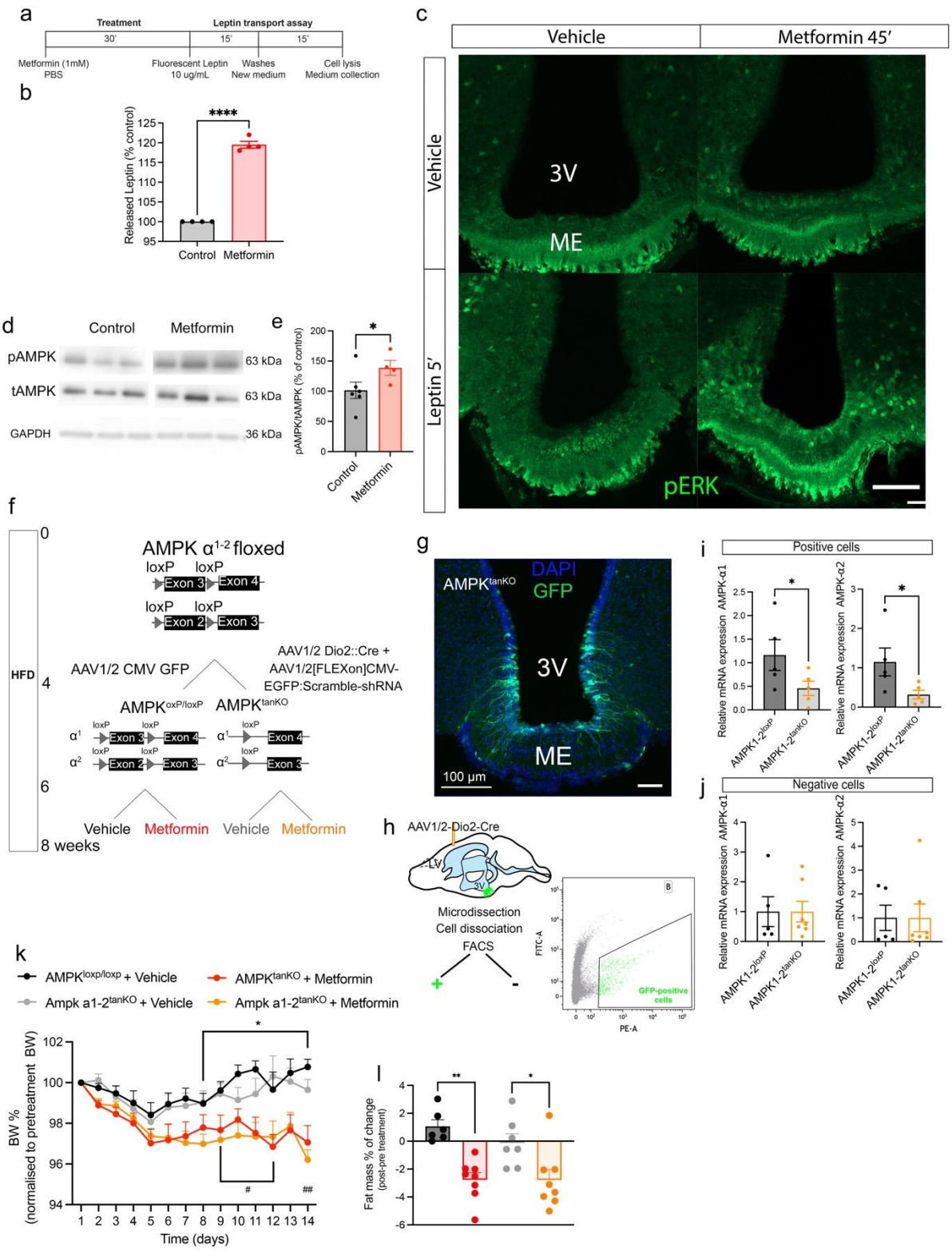

a
Treatment
Leptin transport assay
30'
15'
15'
Metformin (1mM)
PBS
Fluorescent Leptin
10 ug/mL
Washes
New medium
Cell lysis
Medium collection
b
****
Released Leptin (% control)
Control
Metformin
c
Vehicle
Metformin 45'
Vehicle
Leptin 5'
3V
ME
pERK
d
Control
Metformin
pAMPK
tAMPK
GAPDH
63 kDa
63 kDa
36 kDa
e
*
pAMPK/tAMPK (% of control)
Control
Metformin
f
HFD
0
4
6
8 weeks
AMPK α1-2 floxed
loxP
Exon 3
Exon 4
Exon 2
Exon 3
AAV1/2 CMV GFP
AAV1/2 Dio2::Cre +
AAV1/2[FLEXon]CMV-
EGFP:Scramble-shRNA
AMPKoxP/loxP
AMPKtanKO
Vehicle
Metformin
Vehicle
Metformin
g
AMPKtanKO
DAPI
GFP
3V
ME
100 µm
h
AAV1/2-Dio2-Cre
3V
Microdissection
Cell dissociation
FACS
FITC-A
PE-A
GFP-positive cells
i
Positive cells
Relative mRNA expression AMPK-α1
Relative mRNA expression AMPK-α2
AMPK1-2loxP
AMPK1-2tanKO
j
Negative cells
Relative mRNA expression AMPK-α1
Relative mRNA expression AMPK-α2
AMPK1-2loxP
AMPK1-2tanKO
k
AMPKloxp/loxp + Vehicle
AMPKtanKO + Metformin
Ampk a1-2tanKO + Vehicle
Ampk a1-2tanKO + Metformin
BW %
(normalised to pretreatment BW)
Time (days)
*
#
##
l
Fat mass % of change
(post-pre treatment)
**
*

**Figure 4. Co-administration of metformin and leptin activates ERK pathway and AMPK-pathway in tanycytes does not mediate metformin actions on body weight.**

(**A**) Schematic representation of the experimental design of the leptin transport assay. Primary culture of tanycytes was treated with either Metformin (1 mM) or PBS for 30 minutes. Fluorescent bioactive leptin (10 ug/mL) was added for 15 minutes. The cells were washed and the medium was changed for a fresh one. 15 minutes after, the medium was collected and the cells are lysated. (**B**) Levels of fluorescent bioactive leptin in the medium of cells untreated (Control) and treated with metformin (Metformin) as explained in (**A**), expressed as percentage to the control. Two-tailed t-test. Each dot represents one well (n=4). (**C**) Immunofluorescence pictures of mice showing the presence of pERK (green) in tanycytes due to co-administration of metformin (300 mg/kg) and leptin (3 mg/kg). (**D**) Western blot showing the phosphorylation of AMPK at the endpoint of the protocol explained in figure (a). (**E**) Quantification of the western blot shown in figure (c). Two-tailed t-test. Each dot represents one well (n=4). (**F**) Schematic representation for the Cre-dependent induction of AMPK alpha 1 and alpha 2 catalitic subunits knock-out in tanycytes of $AMPK^{\alpha 1\text{-}2loxp/loxp}$ mice fed a HFD for 4 weeks, following the injection of a 1:1 cocktail of AAV1/2-Dio2-Cre and AAV1/2[FLEXon] -CMV> LL:rev(EGFP : Scramble [shRNA]) :LL :WPRE vectors or AAV1/2-CMV-GFP in the lateral ventricle. Subsequently $AMPK^{loxP/loxP}$ and $AMPK^{TanKO}$ at week 6 of HFD were treated with a daily gavage of either water or metformin (300 mg/kg in water) for two weeks, until the end of the 8-week HFD. (**G**) Representative photomicrographs of GFP immunopositive median eminence tanycytes in $AMPK^{TanKO}$ mice 4 weeks after Cre-induced recombination. Scale bar, 100 µm. (**H**) Diagram and gating strategy for the sorting of GFP-positive putative tanycytes following AAV1/2-Dio2-Cre and AAV1/2[FLEXon] -CMV> LL:rev(EGFP : Scramble [shRNA]) :LL :WPRE infusion into the lateral ventricle (LV) of $AMPK^{\alpha 1\text{-}2loxp/loxp}$ mice. (**i-j**) mRNA levels of AMPKα1 (**I**) and AMPKα2 (**J**) in GFP-positive and negative FACS-sorted cells from ME and ARH $AMPK^{loxP/loxP}$ and $AMPK^{TanKO}$ explants 4 weeks after Cre-induced genetic recombination. One-tailed unpaired *t*-test. Values shown are means ± SEM. (n = 5 and 7 mice). (**K**) Curve representing the evolution of body weight in percentage normalized to the weight before treatment respectively in $AMPK^{loxP/loxP}$ and $AMPK^{TanKO}$ mice. Two-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM. (n=5). (**L**) Percentage of delta body weight change (after treatment – before treatment) after the 14-day vehicle or metformin treatment in $AMPK^{loxP/loxP}$ and $AMPK^{TanKO}$. Two-way ANOVA. Values shown are means ± SEM. Each dot represents one mouse (n=4).

**Acute, but not chronic metformin treatment action ameliorates glucose tolerance and insulin sensitivity in 8-week HFD fed mice.**

Metformin is known to be the first-line drug used against type 2 diabetes for its gluconeogenesis lowering effect in liver (Cao et al., 2014) and for increasing peripheral insulin sensitivity (Wiernsperger & Bailey; 1999). Here, glucose metabolism was assessed in 8-week HFD-fed mice after a 2-week daily metformin treatment.

In patients with T2D that undergo metformin treatment, the drug is taken once or twice daily in order to observe the efficiency of metformin action over glucose metabolism (Pala & Rotella, 2014). Thus, in order to observe the actions of a 2-week metformin treatment in DIO mice over glucose tolerance, I performed the experiments 12 and 6 hours after the last administration of metformin.

When the last dose of metformin was administrated 12 hours before performing an oral glucose tolerance test (oGTT) (Figure 5A), metformin did not improve glucose tolerance nor lowered insulin secretion (Figure 5B, C) in HFD fed mice. However, metformin prevented higher HOMA-IR induce by HFD (Figure 5D).

In order to better asses the action of metformin in HFD-fed mice after 2-week treatment, glucose metabolism was studied 6 hours after the last dose as previously described (Coll et al., 2020). Metformin delays the peak of glucose after the OGTT (Figure 5E) compared with the other 2 groups and it ameliorates glucose tolerance (Figure 5E). It also prevents an HDF-induced higher insulin secretion after an oral glucose bolus (Figure 5F, G).

Metformin amelioration in insulin sensitivity impaired by HFD, is also supported by the HOMA index (Figure 5H) and by the insulin tolerance test, were metformin treated HFD-fed mice showed same glucose as SD-fed mice (Figure 5I).

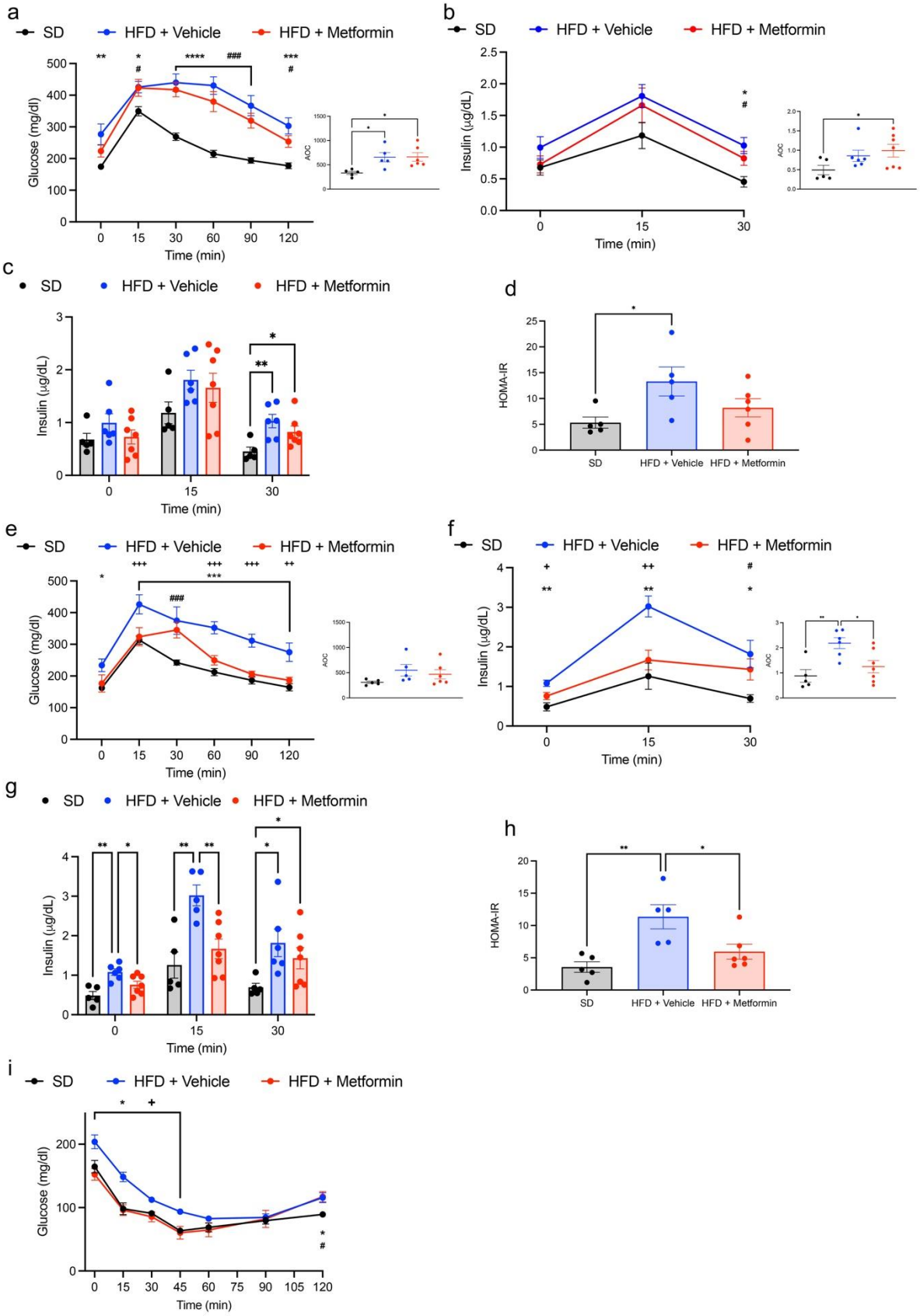

a
SD
HFD + Vehicle
HFD + Metformin
Glucose (mg/dl)
Time (min)
AOC
b
Insulin (μg/dL)
c
d
HOMA-IR
e
f
g
h
i

**Figure 5. Glucose metabolism and insulin secretion 12 and 6 hours after last dose of metformin in HFD fed mice treated for 2 weeks.**

(**A**) Glycaemia and area of the curve after an oral glucose tolerance test (2g/kg) in SD-fed mice gavaged for two weeks with water and and HFD-fed mice after a daily gavage of either water or metformin (300 mg/kg in water) for two weeks. Last dose of metformin is administrated 12 hours before. Two-way ANOVA followed by Fisher's LSD post hoc test. * = differences between SD and HFD Vehicle, # = differences between SD and HFD Metformin. One-way ANOVA followed by Fisher's LSD post hoc test. (SD n=5, HFD vehicle n=5, HFD metformin n=6). Values shown are means ± SEM. (**B-C**) Curve and columns representing serum concentration of insulin during the first 30 min of the glucose tolerance test shown in figure a. Two-way ANOVA followed by Fisher's LSD post hoc test. * = differences between SD and HFD Vehicle, # = differences between SD and HFD Metformin, + = differences between HFD Vehicle and HFD Metformin. Graph is representing the area of the curve. One-way ANOVA followed by Fisher's LSD post hoc test. (SD n=5, HFD vehicle n=5, HFD metformin n=6). Values shown are means ± SEM. (**D**) HOMA-IR calculated on values represented on graphs a and b-c. One-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM. (SD n=5, HFD vehicle n=5, HFD metformin n=6). (**E**) Glycaemia and area of the curve after an oral glucose tolerance test (2g/kg) in SD-fed mice gavaged for two weeks with water and and HFD-fed mice after a daily gavage of either water or metformin (300 mg/kg in water) for two weeks. Last dose of metformin is administrated 6 hours before. Two-way ANOVA followed by Fisher's LSD post hoc test. * = differences between SD and HFD Vehicle, # = differences between SD and HFD Metformin. One-way ANOVA followed by Fisher's LSD post hoc test. (SD n=5, HFD vehicle n=5, HFD metformin n=6). Values shown are means ± SEM. (**F-G**) Curve and columns representing serum concentration of insulin during the first 30 min of the glucose tolerance test shown in figure d. Two-way ANOVA followed by Fisher's LSD post hoc test. * = differences between SD and HFD Vehicle, # = differences between SD and HFD Metformin, + = differences between HFD Vehicle and HFD Metformin. Graph is representing the area of the curve. One-way ANOVA followed by Fisher's LSD post hoc test. (SD n=5, HFD vehicle n=5, HFD metformin n=6). Values shown are means ± SEM. (**H**) HOMA-IR calculated on values represented on graphs e and f-g. One-way ANOVA followed by Fisher's LSD post hoc test. Values shown are means ± SEM. (SD n=5, HFD vehicle n=5, HFD metformin n=6). (**I**) Glycaemia after an insulin tolerance test (0,75 U/kg) in SD-fed mice gavaged for two weeks with water and HFD-fed mice after a daily gavage of either water or metformin (300 mg/kg in water) for two weeks. Last dose of metformin is administrated 6 hours before. Mice were fasted for 6 hours. Two-way ANOVA followed by Fisher's LSD post hoc test. * = differences between SD and HFD Vehicle, # = differences between SD and HFD Metformin. Values shown are means ± SEM. (SD n=5, HFD vehicle n=5, HFD metformin n=6).

# *Chapter 7: Discussion and conclusion*

## Objective 1: Inhibition of transcytosis in tanycytes induces feeding, adiposity and glucose intolerance

Over the last decade, tanycytes have been proposed as the conduit for peripheral information into the hypothalamus, aiding in the regulation of body homeostasis. To achieve this, they actively transport blood-borne signals across the blood-cerebrospinal fluid (CSF) barrier they form (Balland et al., 2024). Our study provides compelling support for this hypothesis. By selectively expressing BoNT/B in tanycytes to inhibit vesicular trafficking, we demonstrate that tanycytic transcytosis activity is an essential requirement for this process.

Previous studies have demonstrated that conditional ablation or impaired function of median eminence tanycytes results in a phenotype similar to that observed in our model, characterized by increased adiposity, insulin resistance, elevated caloric intake, and leptin resistance (Rohrbach et al.,2021; Yoo et al., 2020). However, these studies also revealed compromised barrier properties in the median eminence, allowing peripheral cues to bypass the tanycytic barrier and tanycyte-mediated changes in barrier architecture induced by energy status (Langlet et al., 2013) or circadian rhythms (Rodríguez-Cotrés et al., 2022). Through a more targeted approach, we can now elucidate the specific role of tanycytic transport in modulating energy balance. By expressing BoNTB in tanycytes, we impede the transport of blood-borne molecules transported via tanycytic transcytosis into the ARH, and deprive BBB-protected ARH neurons from peripheral cues. Our observations indicate that, in addition to promoting overweight, BoNTB expression in hypothalamic tanycytes induces insulin resistance and glucose intolerance, underscoring the involvement of tanycytes and their transcytotic activity in the pathogenesis of type 2 diabetes.

Our study highlights the crucial role of transcytotic vesicular trafficking in transporting peptide hormones, such as leptin, from the pituitary portal blood circulation to the hypothalamus for maintaining energy homeostasis. However, it is essential to recognize that blocking vesicular transcytotic activity with our current approach may disrupt the transport of various other blood-borne metabolic hormones released by peripheral tissues, which are known to exert an action in the hypothalamus. Therefore, the metabolic phenotype observed in BoNTBTan mice likely results from a global impairment of accessing multiple metabolic signals to the brain rather than a specific disruption of a single feedback loop. In addition, BoNTB may also impair the release of tanycyte-derived signals that play a role in these processes. For example, the impaired regulation of glucose homeostasis in BoNTBTan mice could be attributed to the combined alteration of both leptin and insulin transports into the ARH. Notably, when insulin transport into the ARH was impaired by ablating insulin receptors in tanycytes, mice exhibited insulin resistance without changes in glucose tolerance (Porniece-Kumar et al., 2021).

Conversely, mice with abrogated leptin transport due to selective knockout of LepR in tanycytes showed in early stages increased insulin secretion despite maintaining normal glucose and insulin tolerance (Duquenne et al., 2021). Remarkably, the absence of both insulin and leptin receptors in ARH POMC neurons, one of the two key neuronal targets of the metabolic hormones transported by tanycytes, resulted in glucose intolerance, insulin resistance, and heightened glucose-stimulated insulin secretion from pancreatic islet (Hill et al., 2010), a phenotype similar to that observed in BoNTBTan mice.

Environmental and age-related changes in tanycyte function could significantly impact healthy aging. For instance, obese individuals appear to exhibit impaired transport of leptin from the bloodstream into the cerebrospinal fluid (Schwatrz et al., 1996; Caro et al., 1996), a condition observed in diet-induced obesity in minipigs (Chmielewski et al., 2019) and mice (Balland et al., 2014), as well as in mice with selective knockout of leptin receptors in tanycytes (Duquenne et al., 2021). This points to tanycytes as a prime target of aging processes. Whether this impairment extends to other peptide metabolic hormones remains to be explored. Nevertheless, it is well-established that midlife obesity and diabetes are risk factors for Alzheimer's disease and other forms of dementia later in life (Nianogo et al., 2022). The BoNTBTan mouse model offers a unique opportunity to explore whether tanycyte dysfunction, implicated in type 2 diabetes and overweight, also contributes to dementia and other hormonally controlled pathologic changes. Their aging may disrupt communication between the brain and peripheral organs, thus compromising overall body homeostasis and healthy aging. This possibility is especially relevant considering that the bridge formed by tanycytes between the CSF and blood seems to be compromised in Alzheimer's patients (Sauvé et al., 2022).

Recent studies underscore the pivotal role of the central nervous system in the mechanisms of action of emerging anti-obesity and anti-diabetic medications. In line with this, our research has recently revealed that hindering tanycytic transcytosis via botulinum neurotoxin expression not only impedes liraglutide transport into the brain but also mitigates its anti-obesity effects on parameters such as food intake, body weight, fat mass, and fatty acid oxidation (Imbernon et al., 2022). Additionally, the novel drugs combining GLP-1R and glucose-dependent insulinotropic polypeptide (GIP) co-agonism exert their regulatory effects on body weight and food intake through central nervous system pathways (Zhang et al., 2021; Liskiexicz et al., 2023). Consequently, the BoNTBTan mouse model presents as a valuable asset for elucidating the mechanisms of action of emerging pharmaceuticals and elucidating their impact on the intricate body-brain communication.

## Objetive 2: Metformin anti-obesity effect is dependent on tanycyte-mediated leptin transport

Within the second objective we observed that metformin actions over food intake and body weight reduction in DIO mice fed HFD for 8 weeks, is due its ability to improve leptin passage into the MBH, in a process that requires Lepr expression in tanycytes, and independently of metformin effects over AMPK.

In order to observed the effects of metformin in energy homeostasis in obese mice, we daily administrated metformin by gavage for 2 weeks in DIO mice as previously (Coll et al., 2020). Giving our hypothesis that metformin may ameliorate leptin transport and to act over it target neurons in the hypothalamus, we used mice fed for 8-weeks with HDF, where their ability to transport leptin into the brain was impaired by not the central leptin sensitivity (cite the papers)

To have a controlled dose of metformin administration to every single animal in the home-cage, metformin or vehicle (300 mg/kg, drinking water) was daily administrated by gavage, taking into consideration the daily variation of the body weight due to the treatment.

The option of providing metformin in the drinking water was discarded because of the mice aversion to the bitter taste of metformin (Hermann et al., 1992).

So far, we demonstrated that the actions of metformin in 8-week HFD fed mice over body weight loss is due its anorexigenic effects, corroborated by the pair-fed experiments. However, it can be observed that in matched-fed mice with the metformin treated group, they seem to lose less weight that the metformin group, as well as a tendency of using more glucose as energy source reflected in the RER. Thus, to corroborate that the anti-obesity actions of metformin are exclusively due to its action in feeding behaviour, it could be interesting to repeat the experience by changing the pair-fed settings of the metabolic cages.

In a previous study by the laboratory, it was shown that a chronic treatment of EGF in the last week of an 8-week-long HFD could accelerate weight loss once HFD-fed mice were replaced to a chow diet (Balland et al., 2014). But here we observed that, a 2-week long metformin treatment induces a significant weight loss already at day 4 of the treatment, while animals are still fed with an HFD, highlighting the efficiency of metformin in weight loss.

Is the effect of metformin on weight loss and leptin central transport mediated by tanycytes?

We don't know whether metformin might firstly act peripherally or centrally, knowing that it can cross the BBB (Cao et al., 2022), to induce weight loss and then, due to an amelioration of metabolism, also restore tanycytic leptin transcytosis. In order to prove this, we exploited the mouse model in which we can abolish the expression of LepR selectively in tanycytes. Using a mouse model of both HFD and $LepR^{TanKO}$. We observed that metformin fails to reduce food

intake and weight loss, unveiling that metformin may improve the passage of leptin into the MBH. Importantly, these animals are DIO-hyperleptinemia, but still central-sensitive to leptin signalling. This may explain the efficiency of metformin, allowing the endogenous leptin to boost anorexigenic feeding behaviour.

We know that the metabolic phenotype of our double model is only due to the HFD, because a metabolic effect of the LepR knock out starts to be visible 4 weeks after the recombination (Duquenne et al., 2021), here we only wait for 2 weeks, in order for the recombination to happen. When we treat the animals, the loss weight and anorectic action of metformin is lost, showing that metformin action is mediated by restoration of HFD-disrupted tanycytic leptin transport.

In contrast, a recent study by Harrison et al., (2018) confirms tanycytic leptin uptake, but shows intact leptin central transport in a fluorescent blood–brain barrier tracing HFD fed mice model. This research however is achieved studying mice that underwent at least 20 weeks of HFD, whereas our results focused on the early phase of obesity onset, when only resistance of peripheral leptin is ongoing. In the progression of the disease morphological changes in the cytoarchitecture of the median eminence vessels and tanycytes might lead to the passage of leptin to MBH, similar to what was shown by Langlet et al., (2013), but in a contest of central leptin resistance, when also centrally administrated leptin is not perceived by neurons.

During my doctorate studies, we implemented a very powerful technic that allows us to measure the transport of leptin in the MBH on living, anesthetized animals. The stereotaxic implantation of microdialysis probes allowed us to show for the first time the kinetic of leptin brain entrance in SD-fed mice (Duquenne et al., 2021) which we confirmed in my study. At the same time, we show that leptin transport is actually damaged in HFD-fed mice, showing the same kinetic of leptin entrance in the brain as LeprKO mice, result which strongly suggests the implication of tanycytes in the impaired leptin central transport caused by HFD feeding. Intriguingly, the metformin treatment restores the transport. Here it is important to state that this is a chronic effect of metformin. Indeed, the last metformin dose was provided 12 hours before the experiment and we know that metformin half-life is estimated at 6 hours (Scheen et al., 1996). Therefore, we can discard possible, acute metformin-induced lowering of glycaemia with the consequent fenestration of vessels and possible extravasation and diffusion of leptin in the MBH (Langlet et al., 2013). In addition, one of the side effects, of the anaesthesia is the high increase in glycaemia, which is not associated to an increase in ME vessels fenestration.

In a previous study, it was shown that metformin anti-obesity action is mediated by the liver-produced GDF15 (Coll et al., 2020). To know whether the metformin-induced amelioration in

tanycytic leptin transport is the result of a direct pharmacological action of metformin on tanycytes and not due by a secondary metabolic effect/metabolite, we tested the effect of metformin on leptin transport assay in a primary culture of tanycytes. The metformin treatment increases the release of leptin that was previously taken up by tanycytes, suggesting a direct effect of the drug on the ependymoglial cells, although further studies need to be performed in order to exclude the involvement of further mechanisms. Indeed, we cannot discard that part of the actions of metformin may involve GDF15, since in our models we did not measure GDF15 neither we explored yet the expression of its receptor in tanycytes

Interestingly, Metformin was shown to induce muscle glucose uptake dependent on ERK phosphorylation (Sajan et al., 2010). Balland and colleagues (2014) displayed the EGF-induced Erk activation role in the rescue of leptin transport to the MBH, hence we reasoned that the metformin-mediated tanycytic leptin passage increase might have been exerted through ERK activation. We tested an acute administration of metformin together with exogenous leptin in mice, co-administration that was proven to induce a sensitization of leptin in obese rats (Kim et al., 2006), we were able to appreciate the activation of the ERK kinase in tanycytes.
How could ERK induce reactivation of the vesicle trafficking? It was shown (Wang et al., 2015) that ERK can remove the auto-inhibiton of Rab8, a molecule necessary for exocytosis. This molecular mechanism is still to be investigated and it should be implemented studying its link with intracellular calcium waves, that have been proven to be activated in tanycytes by leptin (Duquenne et al., 2021) and to be essential for VAMP-mediated exocytosis

It is well known that metformin activates AMPK (Hawley et al., 2010). After showing that one hour of metformin administration induces AMPK activation in tanycytes in vitro, we tested the role of AMPK in metformin-ameliorated leptin central transport by administering the drug in mice in which AMPK catalytic subunits were selectively ablated in in tanycytes (Viollet et al., 2003). Intriguingly, the weight loss effect of metformin was still present in these mice, suggesting that AMPK is dispensable for the metformin-mediated amelioration of leptin transport.

Knowing that metformin has a powerful effect glycaemia, we tested the action of the drug on the circulating glucose levels of our model. Metformin delays the peak of glucose after the OGTT (Figure 5, e) compared with the other 2 groups, probably due to metformin effect over intestinal glucose absorption (Zubiaga et al., 2023). Interestingly, the effect of metformin was proven to be acute, and cannot be, at the state of our knowledge, linked to the chronic amelioration of tanycyte-mediate leptin central transport that we observe. All the experiments

were conducted on male mice, limiting the results only to one sex was necessary in order to exclude the reported oestrous cycle-induced alterations in metabolic regulation (Mauvais-Jarvis, 2015). Parallel studies in a female population would be interesting in order to have a wider insight on female neuroendocrine control of metabolism in physiology and in the obesity onset, and in order to actually test a candidate anti-obesity drug in female subjects.

Overall, this thesis describes the positive effect of metformin in the transport of leptin from the periphery to the brain when animals are in the early phase of obesity onset (Chmiewleski et al., 2019). So far This time-window is crucial for the importance of the metformin-mediated amelioration of leptin central transport. At this moment of the HFD indeed, central leptin resistance is not present yet and bypassing the barrier that blocks leptin at the level of the ME is sufficient for leptin to exert its action (Van Heek et al., 1997). However, the administration of central leptin starts to lose its effectiveness at week 15 of HFD (El Haschimi et al., 2000), suggesting that the therapeutic action of metformin should be exploited by administrating the drug in the early phase of obesity to prevent its progression and linked comorbidities, thanks to the restoration in the access of leptin to its target neurons.
test a candidate anti-obesity drug in female subjects.

Metformin-induced weight loss cannot reach the 15%- 20% weight loss reached by patients treated with semaglutide or tirzepatide respectively (Rubino et al., 2021 & Jastreboff et al., 2022), so the study of this drug could seem anachronistic.
However, we need to take into consideration that metformin-induced weight loss is slower and milder, which can avoid the side effects as reduced lean mass and reduced resting metabolic rate, induced by an abrupt weight loss (Ashtary-Larky et al., 2020) caused by the new generation anti-obesity drugs like Semagltuide and Tirzepatide (le Roux et al., 2023). In addition, in every Tirzepatide leaflet consultable online, the dose-dependent induction of thyroid cancer in rats by the drug is reported. Nowadays we do not know wether these new generation drugs could have longterm side effects, while metformin has been used for almost a century now and its side effects are very mild.
Ramarkably, Tirzepatide or semaglutide are both prescribed to adults with obesity or overweight together with at least one weight related condition (high pressure, cholesterol, diabetes) according to the FDA and EMA. Our results on the other side, point out metformin as a drug that can be administrated in a very specific window of time to prevent the further onset of obesity and its related comorbidities. We show here that the therapeutic effect of metformin is mediated by the amelioration of tanycytic leptin transport in the MBH of 8-week HFD fed mice, when mice still do not show a central resistance to leptin (Van Heek et al., 1997). Our results suggest us that metformin treatment could be ineffective in mice that

underwent 15 weeks of HFD, because an eventual amelioration of leptin central transport would be useless, considering the onset of central leptin resistance (El Haschimi et al., 2000). The utilization of the cheap and well-tolerated metformin in the prevention of the onset of obesity, stands out as an alternative to the new Glp-1 receptor agonist drug family. These new generation drugs represent a big expense for the public health system, although their effectiveness in the fight against obesity and the correlated comorbidities clearly defines this expense as a good investment. However, the price of these drugs raises the question of whether they could actually be distributed equally also in developing countries, where the levels of obesity are rising. In addition, the use of these drugs against obesity has recently caused shortages announced by national and international drug administration agencies, putting the life of people suffering of type II diabetes at danger and crying out for alternative treatments.

Therefore, the early intervention through a metformin treatment targeted to the reestablishment of the lost leptin central transport mechanism, could avoid the necessity to use very powerful drugs now used against obesity (e.g., semaglutide), which long term effects are still not completely undisclosed.

## Conclusion

In the present work, we tackled the debated issue of leptin central transport and a possible metformin action, in the restoration of it in a mouse model of diet-induced obesity. Collectively, our data support a significant role of tanycytes in transport of blood-borne leptin in the area of the hypothalamus that control energy homeostasis. This particular leptin transport was already described in the literature, but it was lacking a mechanistic explanation. By blocking exocytosis specifically in tanycytes, we showed that this mechanism is necessary for leptin to reach the brain and that it is crucial for the preservation of a healthy metabolism. Furthermore, metformin anti-obesity action was proven to be mediated by the tanycytic leptin transport, adding an important piece in the understanding of a drug that has been used for decades and shows a plethora of different effects. Altogether, these observations represent the first description of the exocytosis of leptin by tanycytes and pointing metformin as a safe way to treat obesity in early onset of the disease.

# *Bibliography*

Abbott, N. J., Patabendige, A. A., Dolman, D. E., Yusof, S. R., & Begley, D. J. (2010). Structure and function of the blood-brain barrier. *Neurobiology of disease*, *37*(1), 13–25. https://doi.org/10.1016/j.nbd.2009.07.030

Adair, L., & Dahly, D. (2005). Developmental determinants of blood pressure in adults. *Annual review of nutrition*, *25*, 407–434. https://doi.org/10.1146/annurev.nutr.25.050304.092538

Adams, J. M., Pei, H., Sandoval, D. A., Seeley, R. J., Chang, R. B., Liberles, S. D., & Olson, D. P. (2018). Liraglutide Modulates Appetite and Body Weight Through Glucagon-Like Peptide 1 Receptor-Expressing Glutamatergic Neurons. *Diabetes*, *67*(8), 1538–1548. https://doi.org/10.2337/db17-1385

Adeva, M. M., Calviño, J., Souto, G., & Donapetry, C. (2012). Insulin resistance and the metabolism of branched-chain amino acids in humans. *Amino acids*, *43*(1), 171–181. https://doi.org/10.1007/s00726-011-1088-7

Adnitt, P. I., Frayn, K. N., & Turner, P. (1973). Effects of metformin on glucose uptake by isolated human skeletal muscle. *British journal of pharmacology*, *47*(3), 617P–618P.

Adriaenssens, A., Broichhagen, J., de Bray, A., Ast, J., Hasib, A., Jones, B., Tomas, A., Burgos, N. F., Woodward, O., Lewis, J., O'Flaherty, E., El, K., Cui, C., Harada, N., Inagaki, N., Campbell, J., Brierley, D., Hodson, D. J., Samms, R., Gribble, F., … Reimann, F. (2023). Hypothalamic and brainstem glucose-dependent insulinotropic polypeptide receptor neurons employ distinct mechanisms to affect feeding. *JCI insight*, *8*(10), e164921. https://doi.org/10.1172/jci.insight.164921

Aguilera Vasquez, N., & Nielsen, D. E. (2022). The Endocannabinoid System and Eating Behaviours: a Review of the Current State of the Evidence. *Current nutrition reports*, *11*(4), 665–674. https://doi.org/10.1007/s13668-022-00436-x

Ahima, R. S., & Flier, J. S. (2000). Adipose tissue as an endocrine organ. *Trends in endocrinology and metabolism: TEM*, *11*(8), 327–332. https://doi.org/10.1016/s1043-2760(00)00301-5

Ahima, R. S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E., & Flier, J. S. (1996). Role of leptin in the neuroendocrine response to fasting. *Nature*, *382*(6588), 250–252. https://doi.org/10.1038/382250a0

Ahrén, B., Atkin, S. L., Charpentier, G., Warren, M. L., Wilding, J. P. H., Birch, S., Holst, A. G., & Leiter, L. A. (2018). Semaglutide induces weight loss in subjects with type 2 diabetes regardless of baseline BMI or gastrointestinal adverse events in the SUSTAIN 1 to 5 trials. *Diabetes, obesity & metabolism*, *20*(9), 2210–2219. https://doi.org/10.1111/dom.13353

Akiyoshi, K., Kobayashi, S., Shichibe, S., Mix, D., Baudys, M., Kim, S. W., & Sunamoto, J. (1998). Self-assembled hydrogel nanoparticle of cholesterol-bearing pullulan as a carrier of protein drugs: complexation and stabilization of insulin. *Journal of controlled release : official journal of the Controlled Release Society*, *54*(3), 313–320. https://doi.org/10.1016/s0168-3659(98)00017-0

Akmal M, Wadhwa R. Alpha Glucosidase Inhibitors.(2022). *StatPearls. Treasure Island (FL): StatPearls Publishing.* https://www.ncbi.nlm.nih.gov/books/NBK557848/

Akmayev, I. G., Fidelina, O. V., Kabolova, Z. A., Popov, A. P., & Schitkova, T. A. (1973). Morphological aspects of the hypothalamic-hypophyseal system. IV. Medial basal hypothalamus. An experimental morphological study. *Zeitschrift fur Zellforschung und mikroskopische Anatomie (Vienna, Austria : 1948)*, *137*(4), 493–512. https://doi.org/10.1007/BF00307226

Alhadeff, A. L., Rupprecht, L. E., & Hayes, M. R. (2012). GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake. *Endocrinology*, *153*(2), 647–658. https://doi.org/10.1210/en.2011-1443

Alicic, R. Z., Neumiller, J. J., Johnson, E. J., Dieter, B., & Tuttle, K. R. (2019). Sodium-Glucose Cotransporter 2 Inhibition and Diabetic Kidney Disease. *Diabetes*, *68*(2), 248–257. https://doi.org/10.2337/dbi18-0007

Allison, M. B., & Myers, M. G., Jr (2014). 20 years of leptin: connecting leptin signaling to biological function. *The Journal of endocrinology*, *223*(1), T25–T35. https://doi.org/10.1530/JOE-14-0404

An, H., & He, L. (2016). Current understanding of metformin effect on the control of hyperglycemia in diabetes. *The Journal of endocrinology*, *228*(3), R97–R106. https://doi.org/10.1530/JOE-15-0447

Anisimov V. N. (2013). Metformin: do we finally have an anti-aging drug?. *Cell cycle (Georgetown, Tex.)*, *12*(22), 3483–3489. https://doi.org/10.4161/cc.26928

Annicotte, J. S., Blanchet, E., Chavey, C., Iankova, I., Costes, S., Assou, S., Teyssier, J., Dalle, S., Sardet, C., & Fajas, L. (2009). The CDK4-pRB-E2F1 pathway controls insulin secretion. *Nature cell biology*, *11*(8), 1017–1023. https://doi.org/10.1038/ncb1915

Apolzan, J. W., Venditti, E. M., Edelstein, S. L., Knowler, W. C., Dabelea, D., Boyko, E. J., Pi-Sunyer, X., Kalyani, R. R., Franks, P. W., Srikanthan, P., Gadde, K. M., & Diabetes Prevention Program Research Group (2019). Long-Term Weight Loss With Metformin or Lifestyle Intervention in the Diabetes Prevention Program Outcomes Study. *Annals of internal medicine*, *170*(10), 682–690. https://doi.org/10.7326/M18-1605

Aponte, Y., Atasoy, D., & Sternson, S. M. (2011). AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. *Nature neuroscience*, *14*(3), 351–355. https://doi.org/10.1038/nn.2739

Ard, J., Fitch, A., Fruh, S., & Herman, L. (2021). Weight Loss and Maintenance Related to the Mechanism of Action of Glucagon-Like Peptide 1 Receptor Agonists. *Advances in therapy*, *38*(6), 2821–2839. https://doi.org/10.1007/s12325-021-01710-0

Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S., Shirakami, G., Usui, T., Shimatsu, A., Doi, K., Hosoda, H., Kojima, M., Kangawa, K., & Nakao, K. (2001). Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *The Journal of clinical endocrinology and metabolism*, *86*(10), 4753–4758. https://doi.org/10.1210/jcem.86.10.7885

Ashtary-Larky, D., Bagheri, R., Abbasnezhad, A., Tinsley, G. M., Alipour, M., & Wong, A. (2020). Effects of gradual weight loss *v*. rapid weight loss on body composition and RMR: a systematic review and meta-analysis. *The British journal of nutrition*, *124*(11), 1121–1132. https://doi.org/10.1017/S000711452000224X

Atasoy, D., Betley, J. N., Su, H. H., & Sternson, S. M. (2012). Deconstruction of a neural circuit for hunger. *Nature*, *488*(7410), 172–177. https://doi.org/10.1038/nature11270

Aubert, G., Mansuy, V., Voirol, M. J., Pellerin, L., & Pralong, F. P. (2011). The anorexigenic effects of metformin involve increases in hypothalamic leptin receptor expression. *Metabolism: clinical and experimental*, *60*(3), 327–334. https://doi.org/10.1016/j.metabol.2010.02.007

Avena, N. (2015) Hedonic Eating: How the Pleasure of Food Affects Our Brains and Behavior; *Oxford University Press*.

Avena, N. M., Rada, P., & Hoebel, B. G. (2008). Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neuroscience and biobehavioral reviews*, *32*(1), 20–39. https://doi.org/10.1016/j.neubiorev.2007.04.019

Back, S. H., & Kaufman, R. J. (2012). Endoplasmic reticulum stress and type 2 diabetes. *Annual review of biochemistry*, *81*, 767–793. https://doi.org/10.1146/annurev-biochem-072909-095555

Bado, A., Levasseur, S., Attoub, S., Kermorgant, S., Laigneau, J. P., Bortoluzzi, M. N., Moizo, L., Lehy, T., Guerre-Millo, M., Le Marchand-Brustel, Y., & Lewin, M. J. (1998). The stomach is a source of leptin. *Nature*, *394*(6695), 790–793. https://doi.org/10.1038/29547

Bagnasco, M., Tulipano, G., Melis, M. R., Argiolas, A., Cocchi, D., & Muller, E. E. (2003). Endogenous ghrelin is an orexigenic peptide acting in the arcuate nucleus in response to fasting. *Regulatory peptides*, *111*(1-3), 161–167. https://doi.org/10.1016/s0167-0115(02)00283-5

Bailey C. J. (2017). Metformin: historical overview. *Diabetologia*, *60*(9), 1566–1576. https://doi.org/10.1007/s00125-017-4318-z

Baker, D. E., Walley, K., & Levien, T. L. (2023). Tirzepatide. *Hospital pharmacy*, *58*(3), 227–243. https://doi.org/10.1177/00185787221125724

Baker, R. A., & Herkenham, M. (1995). Arcuate nucleus neurons that project to the hypothalamic paraventricular nucleus: neuropeptidergic identity and consequences of adrenalectomy on mRNA levels in the rat. *The Journal of comparative neurology*, *358*(4), 518–530. https://doi.org/10.1002/cne.903580405

Bakker, W., Imbernon, M., Salinas, C. G., Moro Chao, D. H., Hassouna, R., Morel, C., Martin, C., Leger, C., Denis, R. G. P., Castel, J., Peter, A., Heni, M., Maetzler, W., Nielsen, H. S., Duquenne, M., Schwaninger, M., Lundh, S., Johan Hogendorf, W. F., Gangarossa, G., Secher, A., … Luquet, S. (2022). Acute changes in systemic glycemia gate access and action of GLP-1R agonist on brain structures controlling energy homeostasis. *Cell reports*, *41*(8), 111698. https://doi.org/10.1016/j.celrep.2022.111698

Balland, E., Chen, W., Tiganis, T., & Cowley, M. A. (2019). Persistent Leptin Signaling in the Arcuate Nucleus Impairs Hypothalamic Insulin Signaling and Glucose Homeostasis in Obese Mice. *Neuroendocrinology*, *109*(4), 374–390. https://doi.org/10.1159/000500201

Balland, E., Dam, J., Langlet, F., Caron, E., Steculorum, S., Messina, A., Rasika, S., Falluel-Morel, A., Anouar, Y., Dehouck, B., Trinquet, E., Jockers, R., Bouret, S. G., & Prévot, V. (2014). Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain. *Cell metabolism*, *19*(2), 293–301. https://doi.org/10.1016/j.cmet.2013.12.015

Baluska, F., Volkmann, D., & Barlow, P. W. (2004). Eukaryotic cells and their cell bodies: Cell Theory revised. *Annals of botany*, *94*(1), 9–32. https://doi.org/10.1093/aob/mch109

Bamshad, M., Song, C. K., & Bartness, T. J. (1999). CNS origins of the sympathetic nervous system outflow to brown adipose tissue. *The American journal of physiology*, *276*(6), R1569–R1578. https://doi.org/10.1152/ajpregu.1999.276.6.R1569

Banerjee, M., Joshi, S., Zhang, J., Moncman, C. L., Yadav, S., Bouchard, B. A., Storrie, B., & Whiteheart, S. W. (2017). Cellubrevin/vesicle-associated membrane protein-3-mediated endocytosis and trafficking regulate platelet functions. *Blood*, *130*(26), 2872–2883. https://doi.org/10.1182/blood-2017-02-768176

Banerjee, R. R., Rangwala, S. M., Shapiro, J. S., Rich, A. S., Rhoades, B., Qi, Y., Wang, J., Rajala, M. W., Pocai, A., Scherer, P. E., Steppan, C. M., Ahima, R. S., Obici, S., Rossetti, L., & Lazar, M. A. (2004). Regulation of fasted blood glucose by resistin. *Science (New York, N.Y.)*, *303*(5661), 1195–1198. https://doi.org/10.1126/science.1092341

Banks W. A. (2004). The many lives of leptin. *Peptides*, *25*(3), 331–338. https://doi.org/10.1016/j.peptides.2004.02.014

Banks W. A. (2019). The blood-brain barrier as an endocrine tissue. *Nature reviews. Endocrinology*, *15*(8), 444–455. https://doi.org/10.1038/s41574-019-0213-7

Banks, A. S., Davis, S. M., Bates, S. H., & Myers, M. G., Jr (2000). Activation of downstream signals by the long form of the leptin receptor. *The Journal of biological chemistry*, *275*(19), 14563–14572. https://doi.org/10.1074/jbc.275.19.14563

Banks, W. A., Kastin, A. J., Huang, W., Jaspan, J. B., & Maness, L. M. (1996). Leptin enters the brain by a saturable system independent of insulin. *Peptides*, *17*(2), 305–311. https://doi.org/10.1016/0196-9781(96)00025-3

Barahona, M. J., Ferrada, L., Vera, M., & Nualart, F. (2024). Tanycytes release glucose using the glucose-6-phosphatase system during hypoglycemia to control hypothalamic energy balance. *Molecular metabolism*, 101940. Advance online publication. https://doi.org/10.1016/j.molmet.2024.101940

Barford, K., Keeler, A., McMahon, L., McDaniel, K., Yap, C. C., Deppmann, C. D., & Winckler, B. (2018). Transcytosis of TrkA leads to diversification of dendritic signaling endosomes. *Scientific reports*, *8*(1), 4715. https://doi.org/10.1038/s41598-018-23036-8

Barker D. J. (1990). The fetal and infant origins of adult disease. *BMJ (Clinical research ed.)*, *301*(6761), 1111. https://doi.org/10.1136/bmj.301.6761.1111

Barragán, J. M., Rodríguez, R. E., & Blázquez, E. (1994). Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7-36) amide in rats. *The American journal of physiology*, *266*(3 Pt 1), E459–E466. https://doi.org/10.1152/ajpendo.1994.266.3.E459

Barrett, P., Ivanova, E., Graham, E. S., Ross, A. W., Wilson, D., Plé, H., Mercer, J. G., Ebling, F. J., Schuhler, S., Dupré, S. M., Loudon, A., & Morgan, P. J. (2006). Photoperiodic regulation of cellular retinol binding protein, CRBP1 [corrected] and nestin in tanycytes of the third ventricle ependymal layer of the Siberian hamster. *The Journal of endocrinology*, *191*(3), 687–698. https://doi.org/10.1677/joe.1.06929

Bartness, T. J., Kay Song, C., Shi, H., Bowers, R. R., & Foster, M. T. (2005). Brain-adipose tissue cross talk. *The Proceedings of the Nutrition Society*, *64*(1), 53–64. https://doi.org/10.1079/pns2004409

Bartness, T. J., Liu, Y., Shrestha, Y. B., & Ryu, V. (2014). Neural innervation of white adipose tissue and the control of lipolysis. *Frontiers in neuroendocrinology*, *35*(4), 473–493. https://doi.org/10.1016/j.yfrne.2014.04.001

Bartness, T. J., Vaughan, C. H., & Song, C. K. (2010). Sympathetic and sensory innervation of brown adipose tissue. *International journal of obesity (2005)*, *34 Suppl 1*(0 1), S36–S42. https://doi.org/10.1038/ijo.2010.182

Baskin, D. G., Figlewicz Lattemann, D., Seeley, R. J., Woods, S. C., Porte, D., Jr, & Schwartz, M. W. (1999). Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. *Brain research*, *848*(1-2), 114–123. https://doi.org/10.1016/s0006-8993(99)01974-5

Bechtold, D. A., Sidibe, A., Saer, B. R., Li, J., Hand, L. E., Ivanova, E. A., Darras, V. M., Dam, J., Jockers, R., Luckman, S. M., & Loudon, A. S. (2012). A role for the melatonin-related receptor GPR50 in leptin signaling, adaptive thermogenesis, and torpor. *Current biology : CB*, *22*(1), 70–77. https://doi.org/10.1016/j.cub.2011.11.043

Begley, D. J., & Brightman, M. W. (2003). Structural and functional aspects of the blood-brain barrier. *Progress in drug research. Fortschritte der Arzneimittelforschung. Progres des recherches pharmaceutiques*, *61*, 39–78. https://doi.org/10.1007/978-3-0348-8049-7_2

Beiroa, D., Imbernon, M., Gallego, R., Senra, A., Herranz, D., Villarroya, F., Serrano, M., Fernø, J., Salvador, J., Escalada, J., Dieguez, C., Lopez, M., Frühbeck, G., & Nogueiras, R. (2014). GLP-1 agonism stimulates brown

adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes*, *63*(10), 3346–3358. https://doi.org/10.2337/db14-0302

Beiroa, D., Imbernon, M., Gallego, R., Senra, A., Herranz, D., Villarroya, F., Serrano, M., Fernø, J., Salvador, J., Escalada, J., Dieguez, C., Lopez, M., Frühbeck, G., & Nogueiras, R. (2014). GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes*, *63*(10), 3346–3358. https://doi.org/10.2337/db14-0302

Bence, K. K., Delibegovic, M., Xue, B., Gorgun, C. Z., Hotamisligil, G. S., Neel, B. G., & Kahn, B. B. (2006). Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nature medicine*, *12*(8), 917–924. https://doi.org/10.1038/nm1435

Benevento, M., Alpár, A., Gundacker, A., Afjehi, L., Balueva, K., Hevesi, Z., Hanics, J., Rehman, S., Pollak, D. D., Lubec, G., Wulff, P., Prevot, V., Horvath, T. L., & Harkany, T. (2024). A brainstem-hypothalamus neuronal circuit reduces feeding upon heat exposure. *Nature*, *628*(8009), 826–834. https://doi.org/10.1038/s41586-024-07232-3

Benford, H., Bolborea, M., Pollatzek, E., Lossow, K., Hermans-Borgmeyer, I., Liu, B., Meyerhof, W., Kasparov, S., & Dale, N. (2017). A sweet taste receptor-dependent mechanism of glucosensing in hypothalamic tanycytes. *Glia*, *65*(5), 773–789. https://doi.org/10.1002/glia.23125

Benjannet, S., Rondeau, N., Day, R., Chrétien, M., & Seidah, N. G. (1991). PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(9), 3564–3568. https://doi.org/10.1073/pnas.88.9.3564

Benoit, S. C., Air, E. L., Coolen, L. M., Strauss, R., Jackman, A., Clegg, D. J., Seeley, R. J., & Woods, S. C. (2002). The catabolic action of insulin in the brain is mediated by melanocortins. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *22*(20), 9048–9052. https://doi.org/10.1523/JNEUROSCI.22-20-09048.2002

Benomar, Y., Amine, H., Crépin, D., Al Rifai, S., Riffault, L., Gertler, A., & Taouis, M. (2016). Central Resistin/TLR4 Impairs Adiponectin Signaling, Contributing to Insulin and FGF21 Resistance. Diabetes, 65(4), 913–926. https://doi.org/10.2337/db15-1029

Bensaid, M., Gary-Bobo, M., Esclangon, A., Maffrand, J. P., Le Fur, G., Oury-Donat, F., & Soubrié, P. (2003). The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Molecular pharmacology*, *63*(4), 908–914. https://doi.org/10.1124/mol.63.4.908

Berglund, E. D., Liu, T., Kong, X., Sohn, J. W., Vong, L., Deng, Z., Lee, C. E., Lee, S., Williams, K. W., Olson, D. P., Scherer, P. E., Lowell, B. B., & Elmquist, J. K. (2014). Melanocortin 4 receptors in autonomic neurons regulate thermogenesis and glycemia. *Nature neuroscience*, *17*(7), 911–913. https://doi.org/10.1038/nn.3737

Bernard, C., Della Zuana, O., & Ktorza, A. (2013). Interaction entre facteurs d'environnement et fonds génétique dans le diabète de type 2 - L'apport des modèles animaux [Interaction between environment and genetic background in type 2 diabetes: lessons from animal models]. *Medecine sciences : M/S*, *29*(8-9), 791–799. https://doi.org/10.1051/medsci/2013298020

Bernardis, L. L., Luboshitzky, R., & Bellinger, L. L. (1981). Long-term effects of insulin in weanling rats with dorsomedial hypothalamic hypophagia: food intake, efficiency of food utilization, body weight and composition. *Physiology & behavior*, *27*(3), 469–474. https://doi.org/10.1016/0031-9384(81)90333-4

Berndt, S. I., Gustafsson, S., Mägi, R., Ganna, A., Wheeler, E., Feitosa, M. F., Justice, A. E., Monda, K. L., Croteau-Chonka, D. C., Day, F. R., Esko, T., Fall, T., Ferreira, T., Gentilini, D., Jackson, A. U., Luan, J., Randall, J. C., Vedantam, S., Willer, C. J., Winkler, T. W., … Ingelsson, E. (2013). Genome-wide meta-analysis identifies 11 new loci for anthropometric traits and provides insights into genetic architecture. *Nature genetics*, *45*(5), 501–512. https://doi.org/10.1038/ng.2606

Berridge, K. C., & Kringelbach, M. L. (2015). Pleasure systems in the brain. Neuron, 86(3), 646–664. https://doi.org/10.1016/j.neuron.2015.02.018

Berthoud H. R. (2002). Multiple neural systems controlling food intake and body weight. *Neuroscience and biobehavioral reviews*, *26*(4), 393–428. https://doi.org/10.1016/s0149-7634(02)00014-3

Berthoud, H. R., & Jeanrenaud, B. (1979). Acute hyperinsulinemia and its reversal by vagotomy after lesions of the ventromedial hypothalamus in anesthetized rats. *Endocrinology*, *105*(1), 146–151. https://doi.org/10.1210/endo-105-1-146

Bestetti G, Rossi GL. (1983) Hypothalamic lesions associated with streptozotocin diabetes, rat. *In: Endocrine System. Berlin: Springer*, p. 331–338.

Betley, J. N., Cao, Z. F., Ritola, K. D., & Sternson, S. M. (2013). Parallel, redundant circuit organization for homeostatic control of feeding behavior. *Cell*, *155*(6), 1337–1350. https://doi.org/10.1016/j.cell.2013.11.002

BETT W. R. (1946). Benzedrine sulphate in clinical medicine; a survey of the literature. *Postgraduate medical journal*, *22*(250), 205–218. https://doi.org/10.1136/pgmj.22.250.205

Bewick, G. A., Gardiner, J. V., Dhillo, W. S., Kent, A. S., White, N. E., Webster, Z., Ghatei, M. A., & Bloom, S. R. (2005). Post-embryonic ablation of AgRP neurons in mice leads to a lean, hypophagic phenotype. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 19(12), 1680–1682. https://doi.org/10.1096/fj.04-3434fje

Bi, S., Robinson, B. M., & Moran, T. H. (2003). Acute food deprivation and chronic food restriction differentially affect hypothalamic NPY mRNA expression. *American journal of physiology. Regulatory, integrative and comparative physiology*, *285*(5), R1030–R1036. https://doi.org/10.1152/ajpregu.00734.2002

Bjørbaek, C., Elmquist, J. K., Frantz, J. D., Shoelson, S. E., & Flier, J. S. (1998). Identification of SOCS-3 as a potential mediator of central leptin resistance. *Molecular cell*, *1*(4), 619–625. https://doi.org/10.1016/s1097-2765(00)80062-3

Bjørbaek, C., Elmquist, J. K., Michl, P., Ahima, R. S., van Bueren, A., McCall, A. L., & Flier, J. S. (1998). Expression of leptin receptor isoforms in rat brain microvessels. *Endocrinology*, *139*(8), 3485–3491. https://doi.org/10.1210/endo.139.8.6154

Björntorp, P., & Sjöström, L. (1978). Carbohydrate storage in man: speculations and some quantitative considerations. *Metabolism: clinical and experimental*, *27*(12 Suppl 2), 1853–1865. https://doi.org/10.1016/s0026-0495(78)80004-3

Blüher M. (2019). Obesity: global epidemiology and pathogenesis. *Nature reviews. Endocrinology*, *15*(5), 288–298. https://doi.org/10.1038/s41574-019-0176-8

Blüher, S., Shah, S., & Mantzoros, C. S. (2009). Leptin deficiency: clinical implications and opportunities for therapeutic interventions. *Journal of investigative medicine : the official publication of the American Federation for Clinical Research*, *57*(7), 784–788. https://doi.org/10.2310/JIM.0b013e3181b9163d

Bolborea, M., Pollatzek, E., Benford, H., Sotelo-Hitschfeld, T., & Dale, N. (2020). Hypothalamic tanycytes generate acute hyperphagia through activation of the arcuate neuronal network. *Proceedings of the National*

*Academy of Sciences of the United States of America*, *117*(25), 14473–14481. https://doi.org/10.1073/pnas.1919887117

Bonet T. A guide to the Practical Physician: Shewing from the most approved authors, both ancient and modern, the truest and safest way of curing all diseases, internal and external, whether by medicine, surgery, or diet 1684, *London: Thomas Flesher*.

Boudaba, N., Marion, A., Huet, C., Pierre, R., Viollet, B., & Foretz, M. (2018). AMPK Re-Activation Suppresses Hepatic Steatosis but its Downregulation Does Not Promote Fatty Liver Development. *EBioMedicine*, *28*, 194–209. https://doi.org/10.1016/j.ebiom.2018.01.008

Boulant, J. A., & Hardy, J. D. (1974). The effect of spinal and skin temperatures on the firing rate and thermosensitivity of preoptic neurones. *The Journal of physiology*, *240*(3), 639–660. https://doi.org/10.1113/jphysiol.1974.sp010627

Bouret S. G. (2012). Nutritional programming of hypothalamic development: critical periods and windows of opportunity. *International journal of obesity supplements*, *2*(Suppl 2), S19–S24. https://doi.org/10.1038/ijosup.2012.17

Bouret, S. G. (2017). Development of Hypothalamic Circuits That Control Food Intake and Energy Balance. In R. B. S. Harris (Ed.), *Appetite and Food Intake: Central Control*. (2nd ed., pp. 135–154). CRC Press/Taylor & Francis.

Bouret, S. G., Draper, S. J., & Simerly, R. B. (2004). Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science (New York, N.Y.)*, *304*(5667), 108–110. https://doi.org/10.1126/science.1095004

Bourouh, C., Courty, E., Rolland, L., Pasquetti, G., Gromada, X., Rabhi, N., Carney, C., Moreno, M., Boutry, R., Caron, E., Benfodda, Z., Meffre, P., Kerr-Conte, J., Pattou, F., Froguel, P., Bonnefond, A., Oger, F., & Annicotte, J. S. (2022). The transcription factor E2F1 controls the GLP-1 receptor pathway in pancreatic β cells. *Cell reports*, *40*(6), 111170. https://doi.org/10.1016/j.celrep.2022.111170

Bradley, C. L., McMillin, S. M., Hwang, A. Y., & Sherrill, C. H. (2023). Tirzepatide, the Newest Medication for Type 2 Diabetes: A Review of the Literature and Implications for Clinical Practice. *The Annals of pharmacotherapy*, *57*(7), 822–836. https://doi.org/10.1177/10600280221134127

Bram I. Therapeutic hyperthryroidism, in international clinics; a quarterly of clinical lectures. *J.B.*

Bray G. A. (1991). Treatment for obesity: a nutrient balance/nutrient partition approach. *Nutrition reviews*, *49*(2), 33–45. https://doi.org/10.1111/j.1753-4887.1991.tb02990.x

Breining, P., Jensen, J. B., Sundelin, E. I., Gormsen, L. C., Jakobsen, S., Busk, M., Rolighed, L., Bross, P., Fernandez-Guerra, P., Markussen, L. K., Rasmussen, N. E., Hansen, J. B., Pedersen, S. B., Richelsen, B., & Jessen, N. (2018). Metformin targets brown adipose tissue in vivo and reduces oxygen consumption in vitro. *Diabetes, obesity & metabolism*, *20*(9), 2264–2273. https://doi.org/10.1111/dom.13362

Bridges, H. R., Jones, A. J., Pollak, M. N., & Hirst, J. (2014). Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. *The Biochemical journal*, *462*(3), 475–487. https://doi.org/10.1042/BJ20140620

Brierley, D. I., Holt, M. K., Singh, A., de Araujo, A., McDougle, M., Vergara, M., Afaghani, M. H., Lee, S. J., Scott, K., Maske, C., Langhans, W., Krause, E., de Kloet, A., Gribble, F. M., Reimann, F., Rinaman, L., de Lartigue, G., & Trapp, S. (2021). Central and peripheral GLP-1 systems independently suppress eating. *Nature metabolism*, *3*(2), 258–273. https://doi.org/10.1038/s42255-021-00344-4

Brinker, T., Stopa, E., Morrison, J., & Klinge, P. (2014). A new look at cerebrospinal fluid circulation. *Fluids and barriers of the CNS*, *11*, 10. https://doi.org/10.1186/2045-8118-11-10

Brito, M. N., Brito, N. A., Baro, D. J., Song, C. K., & Bartness, T. J. (2007). Differential activation of the sympathetic innervation of adipose tissues by melanocortin receptor stimulation. *Endocrinology*, *148*(11), 5339–5347. https://doi.org/10.1210/en.2007-0621

Brobeck, J. R., Tepperman, J., & Long, C. N. (1943). Experimental Hypothalamic Hyperphagia in the Albino Rat. *The Yale journal of biology and medicine*, *15*(6), 831–853.

Broberger, C., & Hökfelt, T. (2001). Hypothalamic and vagal neuropeptide circuitries regulating food intake. *Physiology & behavior*, *74*(4-5), 669–682. https://doi-org.proxy.insermbiblio.inist.fr/10.1016/s0031-9384(01)00611-4

Broberger, C., Johansen, J., Brismar, H., Johansson, C., Schalling, M., & Hökfelt, T. (1999). Changes in neuropeptide Y receptors and pro-opiomelanocortin in the anorexia (anx/anx) mouse hypothalamus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *19*(16), 7130–7139. https://doi.org/10.1523/JNEUROSCI.19-16-07130.1999

Broberger, C., Johansen, J., Johansson, C., Schalling, M., & Hökfelt, T. (1998). The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(25), 15043–15048. https://doi.org/10.1073/pnas.95.25.15043

Broberger, C., Visser, T. J., Kuhar, M. J., & Hökfelt, T. (1999). Neuropeptide Y innervation and neuropeptide-Y-Y1-receptor-expressing neurons in the paraventricular hypothalamic nucleus of the mouse. *Neuroendocrinology*, *70*(5), 295–305. https://doi.org/10.1159/000054490

Brown, P. D., Davies, S. L., Speake, T., & Millar, I. D. (2004). Molecular mechanisms of cerebrospinal fluid production. *Neuroscience*, *129*(4), 957–970. https://doi.org/10.1016/j.neuroscience.2004.07.003

Brüning, J. C., & Fenselau, H. (2023). Integrative neurocircuits that control metabolism and food intake. *Science (New York, N.Y.)*, *381*(6665), eabl7398. https://doi.org/10.1126/science.abl7398

Brüning, J. C., Gautam, D., Burks, D. J., Gillette, J., Schubert, M., Orban, P. C., Klein, R., Krone, W., Müller-Wieland, D., & Kahn, C. R. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science (New York, N.Y.)*, *289*(5487), 2122–2125. https://doi.org/10.1126/science.289.5487.2122

Brüning, J. C., Gautam, D., Burks, D. J., Gillette, J., Schubert, M., Orban, P. C., Klein, R., Krone, W., Müller-Wieland, D., & Kahn, C. R. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science (New York, N.Y.)*, *289*(5487), 2122–2125. https://doi.org/10.1126/science.289.5487.2122

Bruss, M. D., Khambatta, C. F., Ruby, M. A., Aggarwal, I., & Hellerstein, M. K. (2010). Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. *American journal of physiology. Endocrinology and metabolism*, *298*(1), E108–E116. https://doi.org/10.1152/ajpendo.00524.2009

Buettner, C., Muse, E. D., Cheng, A., Chen, L., Scherer, T., Pocai, A., Su, K., Cheng, B., Li, X., Harvey-White, J., Schwartz, G. J., Kunos, G., Rossetti, L., & Buettner, C. (2008). Leptin controls adipose tissue lipogenesis via central, STAT3-independent mechanisms. *Nature medicine*, *14*(6), 667–675. https://doi.org/10.1038/nm1775

Buijs, R. M., Swaab, D. F., Dogterom, J., & van Leeuwen, F. W. (1978). Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. *Cell and tissue research*, *186*(3), 423–433. https://doi.org/10.1007/BF00224932

Burcelin, R., Uldry, M., Foretz, M., Perrin, C., Dacosta, A., Nenniger-Tosato, M., Seydoux, J., Cotecchia, S., & Thorens, B. (2004). Impaired glucose homeostasis in mice lacking the alpha1b-adrenergic receptor subtype. *The Journal of biological chemistry*, *279*(2), 1108–1115. https://doi.org/10.1074/jbc.M307788200

Burguera, B., Couce, M. E., Long, J., Lamsam, J., Laakso, K., Jensen, M. D., Parisi, J. E., & Lloyd, R. V. (2000). The long form of the leptin receptor (OB-Rb) is widely expressed in the human brain. *Neuroendocrinology*, *71*(3), 187–195. https://doi.org/10.1159/000054536

Butler, J., Handelsman, Y., Bakris, G., & Verma, S. (2020). Use of sodium-glucose co-transporter-2 inhibitors in patients with and without type 2 diabetes: implications for incident and prevalent heart failure. *European journal of heart failure*, *22*(4), 604–617. https://doi.org/10.1002/ejhf.1708

Butt, A. M., Jones, H. C., & Abbott, N. J. (1990). Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study. *The Journal of physiology*, *429*, 47–62. https://doi.org/10.1113/jphysiol.1990.sp018243

Cajal, S.R. (1909-1911). Histologie dù Système Nerveux de l´homme et des vertébrés. Maloine, París.

Campbell, J. N., Macosko, E. Z., Fenselau, H., Pers, T. H., Lyubetskaya, A., Tenen, D., Goldman, M., Verstegen, A. M., Resch, J. M., McCarroll, S. A., Rosen, E. D., Lowell, B. B., & Tsai, L. T. (2017). A molecular census of arcuate hypothalamus and median eminence cell types. *Nature neuroscience*, *20*(3), 484–496. https://doi.org/10.1038/nn.4495

Campfield, L. A., Smith, F. J., Guisez, Y., Devos, R., & Burn, P. (1995). Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science (New York, N.Y.)*, *269*(5223), 546–549. https://doi.org/10.1126/science.7624778

Campos, A., Port, J. D., & Acosta, A. (2022). Integrative Hedonic and Homeostatic Food Intake Regulation by the Central Nervous System: Insights from Neuroimaging. *Brain sciences*, *12*(4), 431. https://doi.org/10.3390/brainsci12040431

CANNON W. B. (1945). Adaptaciones de la homeostasis [Homeostasis adaptations]. *Anales de medicina. Ateneo Ramon y Cajal, Mexico*, *3*, 1–9.

Cannon, B., & Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiological reviews*, *84*(1), 277–359. https://doi.org/10.1152/physrev.00015.2003

Cao, G., Gong, T., Du, Y., Wang, Y., Ge, T., & Liu, J. (2022). Mechanism of metformin regulation in central nervous system: Progression and future perspectives. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 156, 113686. https://doi.org/10.1016/j.biopha.2022.113686

Cao, W. H., & Morrison, S. F. (2006). Glutamate receptors in the raphe pallidus mediate brown adipose tissue thermogenesis evoked by activation of dorsomedial hypothalamic neurons. *Neuropharmacology*, *51*(3), 426–437. https://doi.org/10.1016/j.neuropharm.2006.03.031

Cao, W., Medvedev, A. V., Daniel, K. W., & Collins, S. (2001). beta-Adrenergic activation of p38 MAP kinase in adipocytes: cAMP induction of the uncoupling protein 1 (UCP1) gene requires p38 MAP kinase. *The Journal of biological chemistry*, *276*(29), 27077–27082. https://doi.org/10.1074/jbc.M101049200

Capehorn, M. S., Catarig, A. M., Furberg, J. K., Janez, A., Price, H. C., Tadayon, S., Vergès, B., & Marre, M. (2020). Efficacy and safety of once-weekly semaglutide 1.0mg vs once-daily liraglutide 1.2mg as add-on to 1-3 oral antidiabetic drugs in subjects with type 2 diabetes (SUSTAIN 10). *Diabetes & metabolism*, *46*(2), 100–109. https://doi.org/10.1016/j.diabet.2019.101117

Caquineau, C., Leng, G., Guan, X. M., Jiang, M., Van der Ploeg, L., & Douglas, A. J. (2006). Effects of alpha-melanocyte-stimulating hormone on magnocellular oxytocin neurones and their activation at intromission in male rats. Journal of neuroendocrinology, 18(9), 685–691. https://doi.org/10.1111/j.1365-2826.2006.01465.x

Caro, J. F., Kolaczynski, J. W., Nyce, M. R., Ohannesian, J. P., Opentanova, I., Goldman, W. H., Lynn, R. B., Zhang, P. L., Sinha, M. K., & Considine, R. V. (1996). Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet (London, England)*, *348*(9021), 159–161. https://doi.org/10.1016/s0140-6736(96)03173-x

Caro, J. F., Kolaczynski, J. W., Nyce, M. R., Ohannesian, J. P., Opentanova, I., Goldman, W. H., Lynn, R. B., Zhang, P. L., Sinha, M. K., & Considine, R. V. (1996). Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet (London, England)*, *348*(9021), 159–161. https://doi.org/10.1016/s0140-6736(96)03173-x

Caron, E., Sachot, C., Prevot, V., & Bouret, S. G. (2010). Distribution of leptin-sensitive cells in the postnatal and adult mouse brain. *The Journal of comparative neurology*, *518*(4), 459–476. https://doi.org/10.1002/cne.22219

Carreño, F. R., & Seelaender, M. C. (2004). Liver denervation affects hepatocyte mitochondrial fatty acid transport capacity. *Cell biochemistry and function*, *22*(1), 9–17. https://doi.org/10.1002/cbf.1047

Cason, A. M., Smith, R. J., Tahsili-Fahadan, P., Moorman, D. E., Sartor, G. C., & Aston-Jones, G. (2010). Role of orexin/hypocretin in reward-seeking and addiction: implications for obesity. *Physiology & behavior*, *100*(5), 419–428. https://doi.org/10.1016/j.physbeh.2010.03.009

Cassell, M. D., Freedman, L. J., & Shi, C. (1999). The intrinsic organization of the central extended amygdala. *Annals of the New York Academy of Sciences*, *877*, 217–241. https://doi.org/10.1111/j.1749-6632.1999.tb09270.x

Castiglioni A. (1941) A History of Medicine., *New York: A. A. Knopf*.

Castro, D. C., & Berridge, K. C. (2014). Opioid hedonic hotspot in nucleus accumbens shell: mu, delta, and kappa maps for enhancement of sweetness "liking" and "wanting". *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *34*(12), 4239–4250. https://doi.org/10.1523/JNEUROSCI.4458-13.2014

Cefalu W. T. (2009). Inflammation, insulin resistance, and type 2 diabetes: back to the future?. *Diabetes*, *58*(2), 307–308. https://doi.org/10.2337/db08-1656

Chalasani, N., Vuppalanchi, R., Rinella, M., Middleton, M. S., Siddiqui, M. S., Barritt, A. S., 4th, Kolterman, O., Flores, O., Alonso, C., Iruarrizaga-Lejarreta, M., Gil-Redondo, R., Sirlin, C. B., & Zemel, M. B. (2018). Randomised clinical trial: a leucine-metformin-sildenafil combination (NS-0200) vs placebo in patients with non-alcoholic fatty liver disease. *Alimentary pharmacology & therapeutics*, *47*(12), 1639–1651. https://doi.org/10.1111/apt.14674

Chambers, A. P., Sorrell, J. E., Haller, A., Roelofs, K., Hutch, C. R., Kim, K. S., Gutierrez-Aguilar, R., Li, B., Drucker, D. J., D'Alessio, D. A., Seeley, R. J., & Sandoval, D. A. (2017). The Role of Pancreatic Preproglucagon in Glucose Homeostasis in Mice. *Cell metabolism*, *25*(4), 927–934.e3. https://doi.org/10.1016/j.cmet.2017.02.008

Chao, A. M., Tronieri, J. S., Amaro, A., & Wadden, T. A. (2023). Semaglutide for the treatment of obesity. *Trends in cardiovascular medicine*, *33*(3), 159–166. https://doi.org/10.1016/j.tcm.2021.12.008

Chao, A. M., Wadden, T. A., Ashare, R. L., Loughead, J., & Schmidt, H. D. (2019). Tobacco Smoking, Eating Behaviors, and Body Weight: A Review. *Current addiction reports*, *6*, 191–199. https://doi.org/10.1007/s40429-019-00253-3

Chau-Van, C., Gamba, M., Salvi, R., Gaillard, R. C., & Pralong, F. P. (2007). Metformin inhibits adenosine 5'-monophosphate-activated kinase activation and prevents increases in neuropeptide Y expression in cultured hypothalamic neurons. *Endocrinology*, *148*(2), 507–511. https://doi.org/10.1210/en.2006-1237

Chaudhary, N., Gomez, G. A., Howes, M. T., Lo, H. P., McMahon, K. A., Rae, J. A., Schieber, N. L., Hill, M. M., Gaus, K., Yap, A. S., & Parton, R. G. (2014). Endocytic crosstalk: cavins, caveolins, and caveolae regulate clathrin-independent endocytosis. *PLoS biology*, *12*(4), e1001832. https://doi.org/10.1371/journal.pbio.1001832

Chen, H., Charlat, O., Tartaglia, L. A., Woolf, E. A., Weng, X., Ellis, S. J., Lakey, N. D., Culpepper, J., Moore, K. J., Breitbart, R. E., Duyk, G. M., Tepper, R. I., & Morgenstern, J. P. (1996). Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell*, *84*(3), 491–495. https://doi.org/10.1016/s0092-8674(00)81294-5

Chen, I. C., Kuo, J., Ko, W. J., Shih, H. C., & Kuo, C. D. (2016). Increased flow resistance and decreased flow rate in patients with acute respiratory distress syndrome: The role of autonomic nervous modulation. *Journal of the Chinese Medical Association : JCMA*, *79*(1), 17–24. https://doi.org/10.1016/j.jcma.2015.10.001

Chen, S. C., Brooks, R., Houskeeper, J., Bremner, S. K., Dunlop, J., Viollet, B., Logan, P. J., Salt, I. P., Ahmed, S. F., & Yarwood, S. J. (2017). Metformin suppresses adipogenesis through both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms. *Molecular and cellular endocrinology*, *440*, 57–68. https://doi.org/10.1016/j.mce.2016.11.011

Chen, Y., Lin, Y. C., Kuo, T. W., & Knight, Z. A. (2015). Sensory detection of food rapidly modulates arcuate feeding circuits. *Cell*, *160*(5), 829–841. https://doi.org/10.1016/j.cell.2015.01.033

Chmielewski, A., Hubert, T., Descamps, A., Mazur, D., Daoudi, M., Ciofi, P., Fontaine, C., Caiazzo, R., Pattou, F., Prevot, V., & Pigeyre, M. (2019). Preclinical Assessment of Leptin Transport into the Cerebrospinal Fluid in Diet-Induced Obese Minipigs. *Obesity (Silver Spring, Md.)*, *27*(6), 950–956. https://doi.org/10.1002/oby.22465

Chmielewski, A., Hubert, T., Descamps, A., Mazur, D., Daoudi, M., Ciofi, P., Fontaine, C., Caiazzo, R., Pattou, F., Prevot, V., & Pigeyre, M. (2019). Preclinical Assessment of Leptin Transport into the Cerebrospinal Fluid in Diet-Induced Obese Minipigs. *Obesity (Silver Spring, Md.)*, *27*(6), 950–956. https://doi.org/10.1002/oby.22465

Christensen N. J. (1991). The biochemical assessment of sympathoadrenal activity in man. *Clinical autonomic research : official journal of the Clinical Autonomic Research Society*, *1*(2), 167–172. https://doi.org/10.1007/BF01826215

Chua, S. C., Jr, Liu, S. M., Li, Q., Sun, A., DeNino, W. F., Heymsfield, S. B., & Guo, X. E. (2004). Transgenic complementation of leptin receptor deficiency. II. Increased leptin receptor transgene dose effects on obesity/diabetes and fertility/lactation in lepr-db/db mice. *American journal of physiology. Endocrinology and metabolism*, *286*(3), E384–E392. https://doi.org/10.1152/ajpendo.00349.2003

Chukir, T., Mandel, L., Tchang, B. G., Al-Mulla, N. A., Igel, L. I., Kumar, R. B., Waitman, J., Aronne, L. J., & Shukla, A. P. (2021). Metformin-induced weight loss in patients with or without type 2 diabetes/prediabetes: A retrospective cohort study. *Obesity research & clinical practice*, *15*(1), 64–68. https://doi.org/10.1016/j.orcp.2020.12.005

Ciofi P. (2011). The arcuate nucleus as a circumventricular organ in the mouse. *Neuroscience letters*, *487*(2), 187–190. https://doi.org/10.1016/j.neulet.2010.10.01

Ciriello, J., McMurray, J. C., Babic, T., & de Oliveira, C. V. (2003). Collateral axonal projections from hypothalamic hypocretin neurons to cardiovascular sites in nucleus ambiguus and nucleus tractus solitarius. *Brain research*, *991*(1-2), 133–141. https://doi.org/10.1016/j.brainres.2003.08.016

Claret, M., Smith, M. A., Batterham, R. L., Selman, C., Choudhury, A. I., Fryer, L. G., Clements, M., Al-Qassab, H., Heffron, H., Xu, A. W., Speakman, J. R., Barsh, G. S., Viollet, B., Vaulont, S., Ashford, M. L., Carling, D., & Withers, D. J. (2007). AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. *The Journal of clinical investigation*, *117*(8), 2325–2336. https://doi.org/10.1172/JCI31516

Clark D. E. (2003). In silico prediction of blood-brain barrier permeation. *Drug discovery today*, *8*(20), 927–933. https://doi.org/10.1016/s1359-6446(03)02827-7

Clasadonte, J., & Prevot, V. (2018). The special relationship: glia-neuron interactions in the neuroendocrine hypothalamus. Nature reviews. Endocrinology, 14(1), 25–44. https://doi.org/10.1038/nrendo.2017.124

Clasadonte, J., Scemes, E., Wang, Z., Boison, D., & Haydon, P. G. (2017). Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle. *Neuron*, *95*(6), 1365–1380.e5. https://doi.org/10.1016/j.neuron.2017.08.022

Clément, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M., Dina, C., Chambaz, J., Lacorte, J. M., Basdevant, A., Bougnères, P., Lebouc, Y., Froguel, P., & Guy-Grand, B. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*, *392*(6674), 398–401. https://doi.org/10.1038/32911

Cohen, P. A., Goday, A., & Swann, J. P. (2012). The return of rainbow diet pills. *American journal of public health*, *102*(9), 1676–1686. https://doi.org/10.2105/AJPH.2012.300655

Coleman D. L. (1973). Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia*, *9*(4), 294–298. https://doi.org/10.1007/BF01221857

Coll, A. P., Chen, M., Taskar, P., Rimmington, D., Patel, S., Tadross, J. A., Cimino, I., Yang, M., Welsh, P., Virtue, S., Goldspink, D. A., Miedzybrodzka, E. L., Konopka, A. R., Esponda, R. R., Huang, J. T., Tung, Y. C. L., Rodriguez-Cuenca, S., Tomaz, R. A., Harding, H. P., Melvin, A., … O'Rahilly, S. (2020). GDF15 mediates the effects of metformin on body weight and energy balance. *Nature*, *578*(7795), 444–448. https://doi.org/10.1038/s41586-019-1911-y

Coll, A. P., Farooqi, I. S., & O'Rahilly, S. (2007). The hormonal control of food intake. *Cell*, *129*(2), 251–262. https://doi.org/10.1016/j.cell.2007.04.001

Collden, G., Balland, E., Parkash, J., Caron, E., Langlet, F., Prevot, V., & Bouret, S. G. (2014). Neonatal overnutrition causes early alterations in the central response to peripheral ghrelin. *Molecular metabolism*, *4*(1), 15–24. https://doi.org/10.1016/j.molmet.2014.10.003

Collden, G., Balland, E., Parkash, J., Caron, E., Langlet, F., Prevot, V., & Bouret, S. G. (2014). Neonatal overnutrition causes early alterations in the central response to peripheral ghrelin. *Molecular metabolism*, *4*(1), 15–24. https://doi.org/10.1016/j.molmet.2014.10.003

Conard N. J. (2009). A female figurine from the basal Aurignacian of Hohle Fels Cave in southwestern Germany. *Nature*, *459*(7244), 248–252. https://doi.org/10.1038/nature07995

Cone R. D. (2005). Anatomy and regulation of the central melanocortin system. *Nature neuroscience*, *8*(5), 571–578. https://doi.org/10.1038/nn1455

Cork, S. C., Richards, J. E., Holt, M. K., Gribble, F. M., Reimann, F., & Trapp, S. (2015). Distribution and characterisation of Glucagon-like peptide-1 receptor expressing cells in the mouse brain. *Molecular metabolism*, *4*(10), 718–731. https://doi.org/10.1016/j.molmet.2015.07.008

Cota, D., Proulx, K., Smith, K. A., Kozma, S. C., Thomas, G., Woods, S. C., & Seeley, R. J. (2006). Hypothalamic mTOR signaling regulates food intake. *Science (New York, N.Y.)*, *312*(5775), 927–930. https://doi.org/10.1126/science.1124147

Cowley, M. A., Smart, J. L., Rubinstein, M., Cerdán, M. G., Diano, S., Horvath, T. L., Cone, R. D., & Low, M. J. (2001). Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*, *411*(6836), 480–484. https://doi.org/10.1038/35078085

Dagon, Y., Hur, E., Zheng, B., Wellenstein, K., Cantley, L. C., & Kahn, B. B. (2012). p70S6 kinase phosphorylates AMPK on serine 491 to mediate leptin's effect on food intake. *Cell metabolism*, *16*(1), 104–112. https://doi.org/10.1016/j.cmet.2012.05.010

Dallman, M. F., Pecoraro, N. C., & la Fleur, S. E. (2005). Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain, behavior, and immunity*, *19*(4), 275–280. https://doi.org/10.1016/j.bbi.2004.11.004

Date, Y., Murakami, N., Toshinai, K., Matsukura, S., Niijima, A., Matsuo, H., Kangawa, K., & Nakazato, M. (2002). The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology*, *123*(4), 1120–1128. https://doi.org/10.1053/gast.2002.35954

Davies, M. J., Bergenstal, R., Bode, B., Kushner, R. F., Lewin, A., Skjøth, T. V., Andreasen, A. H., Jensen, C. B., DeFronzo, R. A., & NN8022-1922 Study Group (2015). Efficacy of Liraglutide for Weight Loss Among Patients With Type 2 Diabetes: The SCALE Diabetes Randomized Clinical Trial. *JAMA*, *314*(7), 687–699. https://doi.org/10.1001/jama.2015.9676

Davies, M., Færch, L., Jeppesen, O. K., Pakseresht, A., Pedersen, S. D., Perreault, L., Rosenstock, J., Shimomura, I., Viljoen, A., Wadden, T. A., Lingvay, I., & STEP 2 Study Group (2021). Semaglutide 2·4 mg once a week in adults with overweight or obesity, and type 2 diabetes (STEP 2): a randomised, double-blind, double-dummy, placebo-controlled, phase 3 trial. *Lancet (London, England)*, *397*(10278), 971–984. https://doi.org/10.1016/S0140-6736(21)00213-0

Davis, J. F., Choi, D. L., Shurdak, J. D., Krause, E. G., Fitzgerald, M. F., Lipton, J. W., Sakai, R. R., & Benoit, S. C. (2011). Central melanocortins modulate mesocorticolimbic activity and food seeking behavior in the rat. *Physiology & behavior*, *102*(5), 491–495. https://doi.org/10.1016/j.physbeh.2010.12.017

Davson H. (1956). Physiology of the ocular and cerebrospinal fluid. London: Churchill.

de Araujo, I. E., Schatzker, M., & Small, D. M. (2020). Rethinking Food Reward. Annual review of psychology, 71, 139–164. https://doi.org/10.1146/annurev-psych-122216-011643

de Luca, C., Kowalski, T. J., Zhang, Y., Elmquist, J. K., Lee, C., Kilimann, M. W., Ludwig, T., Liu, S. M., & Chua, S. C., Jr (2005). Complete rescue of obesity, diabetes, and infertility in db/db mice by neuron-specific LEPR-B transgenes. *The Journal of clinical investigation*, *115*(12), 3484–3493. https://doi.org/10.1172/JCI24059

De Solis, A. J., Del Río-Martín, A., Radermacher, J., Chen, W., Steuernagel, L., Bauder, C. A., Eggersmann, F. R., Morgan, D. A., Cremer, A. L., Sué, M., Germer, M., Kukat, C., Vollmar, S., Backes, H., Rahmouni, K., Kloppenburg, P., & Brüning, J. C. (2024). Reciprocal activity of AgRP and POMC neurons governs coordinated control of feeding and metabolism. *Nature metabolism*, *6*(3), 473–493. https://doi.org/10.1038/s42255-024-00987-z

De Souza, C. T., Araujo, E. P., Bordin, S., Ashimine, R., Zollner, R. L., Boschero, A. C., Saad, M. J., & Velloso, L. A. (2005). Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology*, *146*(10), 4192–4199. https://doi.org/10.1210/en.2004-1520

de Wit H. (2009). Impulsivity as a determinant and consequence of drug use: a review of underlying processes. *Addiction biology*, *14*(1), 22–31. https://doi.org/10.1111/j.1369-1600.2008.00129.x

De Fronzo, R. A., Hompesch, M., Kasichayanula, S., Liu, X., Hong, Y., Pfister, M., Morrow, L. A., Leslie, B. R., Boulton, D. W., Ching, A., LaCreta, F. P., & Griffen, S. C. (2013). Characterization of renal glucose reabsorption in response to dapagliflozin in healthy subjects and subjects with type 2 diabetes. *Diabetes care*, *36*(10), 3169–3176. https://doi.org/10.2337/dc13-0387

De Fronzo, R., Fleming, G. A., Chen, K., & Bicsak, T. A. (2016). Metformin-associated lactic acidosis: Current perspectives on causes and risk. *Metabolism: clinical and experimental*, *65*(2), 20–29. https://doi.org/10.1016/j.metabol.2015.10.014

Denis, R. G., Joly-Amado, A., Cansell, C., Castel, J., Martinez, S., Delbes, A. S., & Luquet, S. (2014). Central orchestration of peripheral nutrient partitioning and substrate utilization: implications for the metabolic syndrome. *Diabetes & metabolism*, *40*(3), 191–197. https://doi-org.proxy.insermbiblio.inist.fr/10.1016/j.diabet.2013.11.002

Denver, R. J., Bonett, R. M., & Boorse, G. C. (2011). Evolution of leptin structure and function. *Neuroendocrinology*, *94*(1), 21–38. https://doi.org/10.1159/000328435

Dhillon S. (2018). Semaglutide: First Global Approval. *Drugs*, *78*(2), 275–284. https://doi.org/10.1007/s40265-018-0871-0

Dhillon, H., Zigman, J. M., Ye, C., Lee, C. E., McGovern, R. A., Tang, V., Kenny, C. D., Christiansen, L. M., White, R. D., Edelstein, E. A., Coppari, R., Balthasar, N., Cowley, M. A., Chua, S., Jr, Elmquist, J. K., & Lowell, B. B. (2006). Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron*, *49*(2), 191–203. https://doi.org/10.1016/j.neuron.2005.12.021

Dhirani, D., Shahid, A., & Mumtaz, H. (2023). A new kind of diabetes medication approved by the FDA: is there hope for obesity?. *International journal of surgery (London, England)*, *109*(2), 81–82. https://doi.org/10.1097/JS9.0000000000000044

Di Magno, L., Di Pastena, F., Bordone, R., Coni, S., & Canettieri, G. (2022). The Mechanism of Action of Biguanides: New Answers to a Complex Question. *Cancers*, *14*(13), 3220. https://doi.org/10.3390/cancers14133220

Di Spiezio, A., Sandin, E. S., Dore, R., Müller-Fielitz, H., Storck, S. E., Bernau, M., Mier, W., Oster, H., Jöhren, O., Pietrzik, C. U., Lehnert, H., & Schwaninger, M. (2018). The LepR-mediated leptin transport across brain barriers controls food reward. *Molecular metabolism*, *8*, 13–22. https://doi.org/10.1016/j.molmet.2017.12.001

Diano, S., Leonard, J. L., Meli, R., Esposito, E., & Schiavo, L. (2003). Hypothalamic type II iodothyronine deiodinase: a light and electron microscopic study. *Brain research*, *976*(1), 130–134. https://doi.org/10.1016/s0006-8993(03)02692-1

Dicken, M. S., Tooker, R. E., & Hentges, S. T. (2012). Regulation of GABA and glutamate release from proopiomelanocortin neuron terminals in intact hypothalamic networks. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *32*(12), 4042–4048. https://doi.org/10.1523/JNEUROSCI.6032-11.2012

Dickson, S. L., Shirazi, R. H., Hansson, C., Bergquist, F., Nissbrandt, H., & Skibicka, K. P. (2012). The glucagon-like peptide 1 (GLP-1) analogue, exendin-4, decreases the rewarding value of food: a new role for mesolimbic GLP-1 receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *32*(14), 4812–4820. https://doi.org/10.1523/JNEUROSCI.6326-11.2012

Dobbins, W. O., 3rd, Herrero, B. A., & Mansbach, C. M. (1968). Morphologic alterations associated with neomycin induced malabsorption. *The American journal of the medical sciences*, *255*, 63–77. https://doi.org/10.1097/00000441-196801000-00011

Dong, Y., Carty, J., Goldstein, N., He, Z., Hwang, E., Chau, D., Wallace, B., Kabahizi, A., Lieu, L., Peng, Y., Gao, Y., Hu, L., Betley, J. N., & Williams, K. W. (2021). Time and metabolic state-dependent effects of GLP-1R agonists on NPY/AgRP and POMC neuronal activity in vivo. *Molecular metabolism*, *54*, 101352. https://doi.org/10.1016/j.molmet.2021.101352

Dorje G, Parfionovich Y. (1992) Tibetan Medical Paintings: Illustrations to the Blue Beryl Treatise of Sangye Gyamtso (1653-1705). *New York: H.N. Abrams, Inc.*

Douglas, J. G., Munro, J. F., Kitchin, A. H., Muir, A. L., & Proudfoot, A. T. (1981). Pulmonary hypertension and fenfluramine. *British medical journal (Clinical research ed.)*, *283*(6296), 881–883. https://doi.org/10.1136/bmj.283.6296.881

Duan, Y., Zhang, R., Zhang, M., Sun, L., Dong, S., Wang, G., Zhang, J., & Zhao, Z. (2013). Metformin inhibits food intake and neuropeptide Y gene expression in the hypothalamus. *Neural regeneration research*, *8*(25), 2379–2388. https://doi.org/10.3969/j.issn.1673-5374.2013.25.009

Duca, F. A., Côté, C. D., Rasmussen, B. A., Zadeh-Tahmasebi, M., Rutter, G. A., Filippi, B. M., & Lam, T. K. (2015). Metformin activates a duodenal Ampk-dependent pathway to lower hepatic glucose production in rats. *Nature medicine*, *21*(5), 506–511. https://doi.org/10.1038/nm.3787

Dunn J, Grider MH. Physiology, Adenosine Triphosphate. [Updated 2023 Feb 13]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553175/

Dunn, S. L., Björnholm, M., Bates, S. H., Chen, Z., Seifert, M., & Myers, M. G., Jr (2005). Feedback inhibition of leptin receptor/Jak2 signaling via Tyr1138 of the leptin receptor and suppressor of cytokine signaling 3. *Molecular endocrinology (Baltimore, Md.)*, *19*(4), 925–938. https://doi.org/10.1210/me.2004-0353

Duquenne, M., Folgueira, C., Bourouh, C., Millet, M., Silva, A., Clasadonte, J., Imbernon, M., Fernandois, D., Martinez-Corral, I., Kusumakshi, S., Caron, E., Rasika, S., Deliglia, E., Jouy, N., Oishi, A., Mazzone, M., Trinquet, E., Tavernier, J., Kim, Y. B., Ory, S., … Prévot, V. (2021). Leptin brain entry via a tanycytic LepR-EGFR shuttle controls lipid metabolism and pancreas function. *Nature metabolism*, *3*(8), 1071–1090. https://doi.org/10.1038/s42255-021-00432-5

Duvernoy, H. M., & Risold, P. Y. (2007). The circumventricular organs: an atlas of comparative anatomy and vascularization. *Brain research reviews*, *56*(1), 119–147. https://doi.org/10.1016/j.brainresrev.2007.06.002

Dyrna, F., Hanske, S., Krueger, M., & Bechmann, I. (2013). The blood-brain barrier. *Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology*, *8*(4), 763–773. https://doi.org/10.1007/s11481-013-9473-5

Ehrlich P. Das Sauerstoff-Bedürfniss des Organismus, eine farbenanalytische Studie. Berlin: August Hirschwald; 1885.

El-Haschimi, K., Pierroz, D. D., Hileman, S. M., Bjørbaek, C., & Flier, J. S. (2000). Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *The Journal of clinical investigation*, *105*(12), 1827–1832. https://doi.org/10.1172/JCI9842

El-Mir, M. Y., Nogueira, V., Fontaine, E., Avéret, N., Rigoulet, M., & Leverve, X. (2000). Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. *The Journal of biological chemistry*, *275*(1), 223–228. https://doi.org/10.1074/jbc.275.1.223

el-Salhy, M., Stenling, R., & Grimelius, L. (1993). Peptidergic innervation and endocrine cells in the human liver. *Scandinavian journal of gastroenterology*, *28*(9), 809–815. https://doi.org/10.3109/00365529309104014

Elias, C. F., Lee, C., Kelly, J., Aschkenasi, C., Ahima, R. S., Couceyro, P. R., Kuhar, M. J., Saper, C. B., & Elmquist, J. K. (1998). Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron*, *21*(6), 1375–1385. https://doi.org/10.1016/s0896-6273(00)80656-x

Elizondo-Vega, R., Cortes-Campos, C., Barahona, M. J., Oyarce, K. A., Carril, C. A., & García-Robles, M. A. (2015). The role of tanycytes in hypothalamic glucosensing. *Journal of cellular and molecular medicine*, *19*(7), 1471–1482. https://doi.org/10.1111/jcmm.12590

Enriori, P. J., Evans, A. E., Sinnayah, P., Jobst, E. E., Tonelli-Lemos, L., Billes, S. K., Glavas, M. M., Grayson, B. E., Perello, M., Nillni, E. A., Grove, K. L., & Cowley, M. A. (2007). Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. *Cell metabolism*, *5*(3), 181–194. https://doi.org/10.1016/j.cmet.2007.02.004

Eriksson, J. G., Forsén, T., Tuomilehto, J., Jaddoe, V. W., Osmond, C., & Barker, D. J. (2002). Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. *Diabetologia*, *45*(3), 342–348. https://doi.org/10.1007/s00125-001-0757-6

Faber, C. L., Deem, J. D., Phan, B. A., Doan, T. P., Ogimoto, K., Mirzadeh, Z., Schwartz, M. W., & Morton, G. J. (2021). Leptin receptor neurons in the dorsomedial hypothalamus regulate diurnal patterns of feeding, locomotion, and metabolism. *eLife*, *10*, e63671. https://doi.org/10.7554/eLife.63671

Fadel, J., & Deutch, A. Y. (2002). Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. *Neuroscience*, *111*(2), 379–387. https://doi.org/10.1016/s0306-4522(02)00017-9

Faouzi, M., Leshan, R., Björnholm, M., Hennessey, T., Jones, J., & Münzberg, H. (2007). Differential accessibility of circulating leptin to individual hypothalamic sites. *Endocrinology*, *148*(11), 5414–5423. https://doi.org/10.1210/en.2007-0655

Farooqi, I. S., & O'Rahilly, S. (2005). Monogenic obesity in humans. *Annual review of medicine*, *56*, 443–458. https://doi.org/10.1146/annurev.med.56.062904.144924

Farooqi, I. S., Matarese, G., Lord, G. M., Keogh, J. M., Lawrence, E., Agwu, C., Sanna, V., Jebb, S. A., Perna, F., Fontana, S., Lechler, R. I., DePaoli, A. M., & O'Rahilly, S. (2002). Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *The Journal of clinical investigation*, *110*(8), 1093–1103. https://doi.org/10.1172/JCI15693

Farr, S. A., Banks, W. A., & Morley, J. E. (2006). Effects of leptin on memory processing. *Peptides*, *27*(6), 1420–1425. https://doi.org/10.1016/j.peptides.2005.10.006

Faulconbridge, L. F., Cummings, D. E., Kaplan, J. M., & Grill, H. J. (2003). Hyperphagic effects of brainstem ghrelin administration. *Diabetes*, *52*(9), 2260–2265. https://doi.org/10.2337/diabetes.52.9.2260

Fekete, C., & Lechan, R. M. (2014). Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions. *Endocrine reviews*, *35*(2), 159–194. https://doi.org/10.1210/er.2013-1087

Fekete, C., Mihály, E., Luo, L. G., Kelly, J., Clausen, J. T., Mao, Q., Rand, W. M., Moss, L. G., Kuhar, M., Emerson, C. H., Jackson, I. M., & Lechan, R. M. (2000). Association of cocaine- and amphetamine-regulated

transcript-immunoreactive elements with thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus and its role in the regulation of the hypothalamic-pituitary-thyroid axis during fasting. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *20*(24), 9224–9234. https://doi.org/10.1523/JNEUROSCI.20-24-09224.2000

Fenselau, H., Campbell, J. N., Verstegen, A. M., Madara, J. C., Xu, J., Shah, B. P., Resch, J. M., Yang, Z., Mandelblat-Cerf, Y., Livneh, Y., & Lowell, B. B. (2017). A rapidly acting glutamatergic ARC→PVH satiety circuit postsynaptically regulated by α-MSH. *Nature neuroscience*, *20*(1), 42–51. https://doi.org/10.1038/nn.4442

Fernández-Chacón, R., Königstorfer, A., Gerber, S. H., García, J., Matos, M. F., Stevens, C. F., Brose, N., Rizo, J., Rosenmund, C., & Südhof, T. C. (2001). Synaptotagmin I functions as a calcium regulator of release probability. *Nature*, *410*(6824), 41–49. https://doi.org/10.1038/35065004

Figlewicz, D. P., Evans, S. B., Murphy, J., Hoen, M., & Baskin, D. G. (2003). Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain research*, *964*(1), 107–115. https://doi.org/10.1016/s0006-8993(02)04087-8

Finan, B., Yang, B., Ottaway, N., Smiley, D. L., Ma, T., Clemmensen, C., Chabenne, J., Zhang, L., Habegger, K. M., Fischer, K., Campbell, J. E., Sandoval, D., Seeley, R. J., Bleicher, K., Uhles, S., Riboulet, W., Funk, J., Hertel, C., Belli, S., Sebokova, E., … Tschöp, M. H. (2015). A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. *Nature medicine*, *21*(1), 27–36. https://doi.org/10.1038/nm.3761

Fioramonti, X., Contié, S., Song, Z., Routh, V. H., Lorsignol, A., & Pénicaud, L. (2007). Characterization of glucosensing neuron subpopulations in the arcuate nucleus: integration in neuropeptide Y and pro-opio melanocortin networks?. *Diabetes*, *56*(5), 1219–1227. https://doi.org/10.2337/db06-0567

Fitzpatrick, A. L., Kuller, L. H., Lopez, O. L., Diehr, P., O'Meara, E. S., Longstreth, W. T., Jr, & Luchsinger, J. A. (2009). Midlife and late-life obesity and the risk of dementia: cardiovascular health study. Archives of neurology, 66(3), 336–342. https://doi.org/10.1001/archneurol.2008.582

Flamment, M., Hajduch, E., Ferré, P., & Foufelle, F. (2012). New insights into ER stress-induced insulin resistance. Trends in endocrinology and metabolism: TEM, 23(8), 381–390. https://doi.org/10.1016/j.tem.2012.06.003

Flatt, J. P., Ravussin, E., Acheson, K. J., & Jéquier, E. (1985). Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *The Journal of clinical investigation*, *76*(3), 1019–1024. https://doi.org/10.1172/JCI112054

Flemyng M. (1760) A Discourse on the Nature, Causes, and Cure of Corpulency. Illustrated by a Remarkable Case, Read Before the Royal Society, November 1757. And Now First Published, by Malcolm Flemyng, M.D.: L. Davis and C. Reymers.

Fortin, S. M., Lipsky, R. K., Lhamo, R., Chen, J., Kim, E., Borner, T., Schmidt, H. D., & Hayes, M. R. (2020). GABA neurons in the nucleus tractus solitarius express GLP-1 receptors and mediate anorectic effects of liraglutide in rats. *Science translational medicine*, *12*(533), eaay8071. https://doi.org/10.1126/scitranslmed.aay8071

Fortin, S. M., Lipsky, R. K., Lhamo, R., Chen, J., Kim, E., Borner, T., Schmidt, H. D., & Hayes, M. R. (2020). GABA neurons in the nucleus tractus solitarius express GLP-1 receptors and mediate anorectic effects of liraglutide in rats. *Science translational medicine*, *12*(533), eaay8071. https://doi.org/10.1126/scitranslmed.aay8071

Frayling, C., Britton, R., & Dale, N. (2011). ATP-mediated glucosensing by hypothalamic tanycytes. *The Journal of physiology*, *589*(Pt 9), 2275–2286. https://doi.org/10.1113/jphysiol.2010.202051

Frayn KN. *Metabolic Regulation: A Human Perspective*. Wiley; 2013.

Frederich, R. C., Hamann, A., Anderson, S., Löllmann, B., Lowell, B. B., & Flier, J. S. (1995). Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nature medicine*, *1*(12), 1311–1314. https://doi.org/10.1038/nm1295-1311

Frías, J. P., Davies, M. J., Rosenstock, J., Pérez Manghi, F. C., Fernández Landó, L., Bergman, B. K., Liu, B., Cui, X., Brown, K., & SURPASS-2 Investigators (2021). Tirzepatide versus Semaglutide Once Weekly in Patients with Type 2 Diabetes. *The New England journal of medicine*, *385*(6), 503–515. https://doi.org/10.1056/NEJMoa2107519

Friedman, J. M., & Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature*, *395*(6704), 763–770. https://doi.org/10.1038/27376

Froldi G. (2024). View on Metformin: Antidiabetic and Pleiotropic Effects, Pharmacokinetics, Side Effects, and Sex-Related Differences. *Pharmaceuticals (Basel, Switzerland)*, *17*(4), 478. https://doi.org/10.3390/ph17040478

Frühbeck, G., Méndez-Giménez, L., Fernández-Formoso, J. A., Fernández, S., & Rodríguez, A. (2014). Regulation of adipocyte lipolysis. *Nutrition research reviews*, *27*(1), 63–93. https://doi.org/10.1017/S095442241400002X

Fujioka, K., O'Neil, P. M., Davies, M., Greenway, F., C W Lau, D., Claudius, B., Skjøth, T. V., Bjørn Jensen, C., & P H Wilding, J. (2016). Early Weight Loss with Liraglutide 3.0 mg Predicts 1-Year Weight Loss and is Associated with Improvements in Clinical Markers. *Obesity (Silver Spring, Md.)*, *24*(11), 2278–2288. https://doi.org/10.1002/oby.21629

Fulton, S., Pissios, P., Manchon, R. P., Stiles, L., Frank, L., Pothos, E. N., Maratos-Flier, E., & Flier, J. S. (2006). Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron*, *51*(6), 811–822. https://doi.org/10.1016/j.neuron.2006.09.006

Furman B. L. (2012). The development of Byetta (exenatide) from the venom of the Gila monster as an anti-diabetic agent. *Toxicon : official journal of the International Society on Toxinology*, *59*(4), 464–471. https://doi.org/10.1016/j.toxicon.2010.12.016

Gabery, S., Salinas, C. G., Paulsen, S. J., Ahnfelt-Rønne, J., Alanentalo, T., Baquero, A. F., Buckley, S. T., Farkas, E., Fekete, C., Frederiksen, K. S., Helms, H. C. C., Jeppesen, J. F., John, L. M., Pyke, C., Nøhr, J., Lu, T. T., Polex-Wolf, J., Prevot, V., Raun, K., Simonsen, L., … Hogendorf, W. F. J. (2020). Semaglutide lowers body weight in rodents via distributed neural pathways. *JCI insight*, *5*(6), e133429. https://doi.org/10.1172/jci.insight.133429

Galgani, J., & Ravussin, E. (2008). Energy metabolism, fuel selection and body weight regulation. *International journal of obesity (2005)*, *32 Suppl 7*(Suppl 7), S109–S119. https://doi.org/10.1038/ijo.2008.246

Galic, S., Sachithanandan, N., Kay, T. W., & Steinberg, G. R. (2014). Suppressor of cytokine signalling (SOCS) proteins as guardians of inflammatory responses critical for regulating insulin sensitivity. The Biochemical journal, 461(2), 177–188. https://doi.org/10.1042/BJ20140143

Gao, X., Cai, X., Yang, W., Chen, Y., Han, X., & Ji, L. (2018). Meta-analysis and critical review on the efficacy and safety of alpha-glucosidase inhibitors in Asian and non-Asian populations. *Journal of diabetes investigation*, *9*(2), 321–331. https://doi.org/10.1111/jdi.12711

Gao, Y., Vidal-Itriago, A., Kalsbeek, M. J., Layritz, C., García-Cáceres, C., Tom, R. Z., Eichmann, T. O., Vaz, F. M., Houtkooper, R. H., van der Wel, N., Verhoeven, A. J., Yan, J., Kalsbeek, A., Eckel, R. H., Hofmann, S. M.,

& Yi, C. X. (2017). Lipoprotein Lipase Maintains Microglial Innate Immunity in Obesity. *Cell reports*, *20*(13), 3034–3042. https://doi.org/10.1016/j.celrep.2017.09.008

García-Cáceres, C., Balland, E., Prevot, V., Luquet, S., Woods, S. C., Koch, M., Horvath, T. L., Yi, C. X., Chowen, J. A., Verkhratsky, A., Araque, A., Bechmann, I., & Tschöp, M. H. (2019). Role of astrocytes, microglia, and tanycytes in brain control of systemic metabolism. *Nature neuroscience*, *22*(1), 7–14. https://doi.org/10.1038/s41593-018-0286-y

García, M., Millán, C., Balmaceda-Aguilera, C., Castro, T., Pastor, P., Montecinos, H., Reinicke, K., Zúñiga, F., Vera, J. C., Oñate, S. A., & Nualart, F. (2003). Hypothalamic ependymal-glial cells express the glucose transporter GLUT2, a protein involved in glucose sensing. *Journal of neurochemistry*, *86*(3), 709–724. https://doi.org/10.1046/j.1471-4159.2003.01892.x

Gehlert, D. R., Chronwall, B. M., Schafer, M. P., & O'Donohue, T. L. (1987). Localization of neuropeptide Y messenger ribonucleic acid in rat and mouse brain by in situ hybridization. *Synapse (New York, N.Y.)*, *1*(1), 25–31. https://doi.org/10.1002/syn.890010106

Geller, S., Arribat, Y., Netzahualcoyotzi, C., Lagarrigue, S., Carneiro, L., Zhang, L., Amati, F., Lopez-Mejia, I. C., & Pellerin, L. (2019). Tanycytes Regulate Lipid Homeostasis by Sensing Free Fatty Acids and Signaling to Key Hypothalamic Neuronal Populations via FGF21 Secretion. *Cell metabolism*, *30*(4), 833–844.e7. https://doi.org/10.1016/j.cmet.2019.08.004

Georgescu, T., Lyons, D., Doslikova, B., Garcia, A. P., Marston, O., Burke, L. K., Chianese, R., Lam, B. Y. H., Yeo, G. S. H., Rochford, J. J., Garfield, A. S., & Heisler, L. K. (2020). Neurochemical Characterization of Brainstem Pro-Opiomelanocortin Cells. *Endocrinology*, *161*(4), bqaa032. https://doi.org/10.1210/endocr/bqaa032

Gerich, J., Davis, J., Lorenzi, M., Rizza, R., Bohannon, N., Karam, J., Lewis, S., Kaplan, R., Schultz, T., & Cryer, P. (1979). Hormonal mechanisms of recovery from insulin-induced hypoglycemia in man. *The American journal of physiology*, *236*(4), E380–E385. https://doi.org/10.1152/ajpendo.1979.236.4.E380

Ghilardi, N., Ziegler, S., Wiestner, A., Stoffel, R., Heim, M. H., & Skoda, R. C. (1996). Defective STAT signaling by the leptin receptor in diabetic mice. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(13), 6231–6235. https://doi.org/10.1073/pnas.93.13.6231

Gielkens, H. A., Verkijk, M., Lam, W. F., Lamers, C. B., & Masclee, A. A. (1998). Effects of hyperglycemia and hyperinsulinemia on satiety in humans. *Metabolism: clinical and experimental*, *47*(3), 321–324. https://doi.org/10.1016/s0026-0495(98)90264-5

Gjermeni, E., Kirstein, A. S., Kolbig, F., Kirchhof, M., Bundalian, L., Katzmann, J. L., Laufs, U., Blüher, M., Garten, A., & Le Duc, D. (2021). Obesity-An Update on the Basic Pathophysiology and Review of Recent Therapeutic Advances. *Biomolecules*, *11*(10), 1426. https://doi.org/10.3390/biom11101426

Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019. Results. Institute for Health Metrics and Evaluation. 2020

Goldmann EE. Die äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der "vitalen Färbung". Beitr Klin Chir. 1909;64:192–265.

Gonul, E., Duz, B., Kahraman, S., Kayali, H., Kubar, A., & Timurkaynak, E. (2002). Early pericyte response to brain hypoxia in cats: an ultrastructural study. *Microvascular research*, *64*(1), 116–119. https://doi.org/10.1006/mvre.2002.2413

Greenhill L. L. (2006). The science of stimulant abuse. *Pediatric annals*, *35*(8), 552–556. https://doi.org/10.3928/0090-4481-20060801-07

Gropp, E., Shanabrough, M., Borok, E., Xu, A. W., Janoschek, R., Buch, T., Plum, L., Balthasar, N., Hampel, B., Waisman, A., Barsh, G. S., Horvath, T. L., & Brüning, J. C. (2005). Agouti-related peptide-expressing neurons are mandatory for feeding. Nature neuroscience, 8(10), 1289–1291. https://doi.org/10.1038/nn1548

Grosshans, B. L., Ortiz, D., & Novick, P. (2006). Rabs and their effectors: achieving specificity in membrane traffic. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(32), 11821–11827. https://doi.org/10.1073/pnas.0601617103

Gruner OC. (1930) A Treatise on the Canon of Medicine of Avicenna, Incorporating a Translation of the First Book., *London: Luzac & Co*.

Guadaño-Ferraz, A., Obregón, M. J., St Germain, D. L., & Bernal, J. (1997). The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(19), 10391–10396. https://doi.org/10.1073/pnas.94.19.10391

Guan, X. M., Yu, H., Trumbauer, M., Frazier, E., Van der Ploeg, L. H., & Chen, H. (1998). Induction of neuropeptide Y expression in dorsomedial hypothalamus of diet-induced obese mice. *Neuroreport*, *9*(15), 3415–3419. https://doi.org/10.1097/00001756-199810260-00015

Guettet, C., Rostaqui, N., Mathé, D., Lecuyer, B., Navarro, N., & Jacotot, B. (1991). Effect of chronic glucagon administration on lipoprotein composition in normally fed, fasted and cholesterol-fed rats. *Lipids*, *26*(6), 451–458. https://doi.org/10.1007/BF02536072

Guillebaud, F., Girardet, C., Abysique, A., Gaigé, S., Barbouche, R., Verneuil, J., Jean, A., Leprince, J., Tonon, M. C., Dallaporta, M., Lebrun, B., & Troadec, J. D. (2017). Glial Endozepines Inhibit Feeding-Related Autonomic Functions by Acting at the Brainstem Level. *Frontiers in neuroscience*, *11*, 308. https://doi.org/10.3389/fnins.2017.00308

Güldner, F. H., & Wolff, J. R. (1973). Neurono-glial synaptoid contacts in the median eminence of the rat: ultrastructure, staining properties and distribution on tanycytes. *Brain research*, *61*, 217–234. https://doi.org/10.1016/0006-8993(73)90529-5

Guo, F., Bakal, K., Minokoshi, Y., & Hollenberg, A. N. (2004). Leptin signaling targets the thyrotropin-releasing hormone gene promoter in vivo. *Endocrinology*, *145*(5), 2221–2227. https://doi.org/10.1210/en.2003-1312

Haan, N., Goodman, T., Najdi-Samiei, A., Stratford, C. M., Rice, R., El Agha, E., Bellusci, S., & Hajihosseini, M. K. (2013). Fgf10-expressing tanycytes add new neurons to the appetite/energy-balance regulating centers of the postnatal and adult hypothalamus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *33*(14), 6170–6180. https://doi.org/10.1523/JNEUROSCI.2437-12.2013

Hagan, M. M., Havel, P. J., Seeley, R. J., Woods, S. C., Ekhator, N. N., Baker, D. G., Hill, K. K., Wortman, M. D., Miller, A. H., Gingerich, R. L., & Geracioti, T. D. (1999). Cerebrospinal fluid and plasma leptin measurements: covariability with dopamine and cortisol in fasting humans. *The Journal of clinical endocrinology and metabolism*, *84*(10), 3579–3585. https://doi.org/10.1210/jcem.84.10.6034

Hâkansson, M. L., Brown, H., Ghilardi, N., Skoda, R. C., & Meister, B. (1998). Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *18*(1), 559–572. https://doi.org/10.1523/JNEUROSCI.18-01-00559.1998

Halaas, J. L., Boozer, C., Blair-West, J., Fidahusein, N., Denton, D. A., & Friedman, J. M. (1997). Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(16), 8878–8883. https://doi.org/10.1073/pnas.94.16.8878

Hansen M. B. (2003). The enteric nervous system I: organisation and classification. *Pharmacology & toxicology*, *92*(3), 105–113. https://doi.org/10.1034/j.1600-0773.2003.t01-1-920301.x

Hardie, D. G., Ross, F. A., & Hawley, S. A. (2012). AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nature reviews. Molecular cell biology*, *13*(4), 251–262. https://doi.org/10.1038/nrm3311

Harington C. R. (1926). Chemistry of Thyroxine: Constitution and Synthesis of Desiodo-Thyroxine. *The Biochemical journal*, *20*(2), 300–313. https://doi.org/10.1042/bj0200300

Harris, M., Aschkenasi, C., Elias, C. F., Chandrankunnel, A., Nillni, E. A., Bjøorbaek, C., Elmquist, J. K., Flier, J. S., & Hollenberg, A. N. (2001). Transcriptional regulation of the thyrotropin-releasing hormone gene by leptin and melanocortin signaling. *The Journal of clinical investigation*, *107*(1), 111–120. https://doi.org/10.1172/JCI10741

Haskell-Luevano, C., & Monck, E. K. (2001). Agouti-related protein functions as an inverse agonist at a constitutively active brain melanocortin-4 receptor. *Regulatory peptides*, *99*(1), 1–7. https://doi.org/10.1016/s0167-0115(01)00234-8

Havrankova, J., Roth, J., & Brownstein, M. (1978). Insulin receptors are widely distributed in the central nervous system of the rat. *Nature*, *272*(5656), 827–829. https://doi.org/10.1038/272827a0

Hawke, Z., Ivanov, T. R., Bechtold, D. A., Dhillon, H., Lowell, B. B., & Luckman, S. M. (2009). PACAP neurons in the hypothalamic ventromedial nucleus are targets of central leptin signaling. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(47), 14828–14835. https://doi.org/10.1523/JNEUROSCI.1526-09.2009

Hawley, S. A., Ross, F. A., Chevtzoff, C., Green, K. A., Evans, A., Fogarty, S., Towler, M. C., Brown, L. J., Ogunbayo, O. A., Evans, A. M., & Hardie, D. G. (2010). Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell metabolism*, *11*(6), 554–565. https://doi.org/10.1016/j.cmet.2010.04.001

Hegyi, K., Fülöp, K., Kovács, K., Tóth, S., & Falus, A. (2004). Leptin-induced signal transduction pathways. *Cell biology international*, *28*(3), 159–169. https://doi.org/10.1016/j.cellbi.2003.12.003

Heller, S. R., & Cryer, P. E. (1991). Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. *Diabetes*, *40*(2), 223–226. https://doi.org/10.2337/diab.40.2.223

Hentges, S. T., Otero-Corchon, V., Pennock, R. L., King, C. M., & Low, M. J. (2009). Proopiomelanocortin expression in both GABA and glutamate neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(43), 13684–13690. https://doi.org/10.1523/JNEUROSCI.3770-09.2009

Hermann LS, Melender A. New York: Wiley; 1992. Biguanides: basic aspects and clinical use; pp. 292–7

Hicks J. (2012) Fast Times: The Life, Death, and Rebirth of Amphetamine, in *Science History Institute*..

High Level Panel of Experts on Food Security and Nutrition. (2023). Reducing inequalities for food security and nutrition. *Rome, CFS HLPE-FSN*. www.fao.org/cfs/cfs-hlpe

Higuchi, S., Irie, K., Yamaguchi, R., Katsuki, M., Araki, M., Ohji, M., Hayakawa, K., Mishima, S., Akitake, Y., Matsuyama, K., Mishima, K., Mishima, K., Iwasaki, K., & Fujiwara, M. (2012). Hypothalamic 2-arachidonoylglycerol regulates multistage process of high-fat diet preferences. *PloS one*, *7*(6), e38609. https://doi.org/10.1371/journal.pone.0038609

Hill, J. W., Elias, C. F., Fukuda, M., Williams, K. W., Berglund, E. D., Holland, W. L., Cho, Y. R., Chuang, J. C., Xu, Y., Choi, M., Lauzon, D., Lee, C. E., Coppari, R., Richardson, J. A., Zigman, J. M., Chua, S., Scherer, P. E., Lowell, B. B., Brüning, J. C., & Elmquist, J. K. (2010). Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. *Cell metabolism*, *11*(4), 286–297. https://doi.org/10.1016/j.cmet.2010.03.002

Hill, J. W., Elias, C. F., Fukuda, M., Williams, K. W., Berglund, E. D., Holland, W. L., Cho, Y. R., Chuang, J. C., Xu, Y., Choi, M., Lauzon, D., Lee, C. E., Coppari, R., Richardson, J. A., Zigman, J. M., Chua, S., Scherer, P. E., Lowell, B. B., Brüning, J. C., & Elmquist, J. K. (2010). Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. *Cell metabolism*, *11*(4), 286–297. https://doi.org/10.1016/j.cmet.2010.03.002

Hoebel, B. G., Hernandez, L., Schwartz, D. H., Mark, G. P., & Hunter, G. A. (1989). Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior. Theoretical and clinical implications. *Annals of the New York Academy of Sciences*, *575*, 171–193. https://doi.org/10.1111/j.1749-6632.1989.tb53242.x

*Hofer H (1958). "Zur Morphologie der circumventriculären Organe des Zwischenhirns der Säugetiere". Verhandlungen der Deutschen Zoologischen Gesellschaft.* ***55****: 202–251.*

Hofmann, K., Lamberz, C., Piotrowitz, K., Offermann, N., But, D., Scheller, A., Al-Amoudi, A., & Kuerschner, L. (2017). Tanycytes and a differential fatty acid metabolism in the hypothalamus. *Glia*, *65*(2), 231–249. https://doi.org/10.1002/glia.23088

Holst J. J. (2004). On the physiology of GIP and GLP-1. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme*, *36*(11-12), 747–754. https://doi.org/10.1055/s-2004-826158

Holt, M. K., Richards, J. E., Cook, D. R., Brierley, D. I., Williams, D. L., Reimann, F., Gribble, F. M., & Trapp, S. (2019). Preproglucagon Neurons in the Nucleus of the Solitary Tract Are the Main Source of Brain GLP-1, Mediate Stress-Induced Hypophagia, and Limit Unusually Large Intakes of Food. *Diabetes*, *68*(1), 21–33. https://doi.org/10.2337/db18-0729

Holzer P. (1998). Neural injury, repair, and adaptation in the GI tract. II. The elusive action of capsaicin on the vagus nerve. *The American journal of physiology*, *275*(1), G8–G13. https://doi.org/10.1152/ajpgi.1998.275.1.G8

Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z. W., Gao, X. B., Thurmon, J. J., Marinelli, M., & DiLeone, R. J. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron*, *51*(6), 801–810. https://doi.org/10.1016/j.neuron.2006.08.023

Hoosein, N. M., & Gurd, R. S. (1984). Identification of glucagon receptors in rat brain. *Proceedings of the National Academy of Sciences of the United States of America*, *81*(14), 4368–4372. https://doi.org/10.1073/pnas.81.14.4368

Horowitz, M., & Nauck, M. A. (2006). To be or not to be--an incretin or enterogastrone?. *Gut*, *55*(2), 148–150. https://doi.org/10.1136/gut.2005.071787

Horstmann E. (1954). Die Faserglia des Selachiergehirns [The fiber glia of selacean brain]. *Zeitschrift fur Zellforschung und mikroskopische Anatomie (Vienna, Austria : 1948)*, *39*(6), 588–617.

Horvath, T. L., Bechmann, I., Naftolin, F., Kalra, S. P., & Leranth, C. (1997). Heterogeneity in the neuropeptide Y-containing neurons of the rat arcuate nucleus: GABAergic and non-GABAergic subpopulations. *Brain research*, *756*(1-2), 283–286. https://doi.org/10.1016/s0006-8993(97)00184-4

Horvath, T. L., Diano, S., & van den Pol, A. N. (1999). Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *19*(3), 1072–1087. https://doi.org/10.1523/JNEUROSCI.19-03-01072.1999

Horvath, T. L., Peyron, C., Diano, S., Ivanov, A., Aston-Jones, G., Kilduff, T. S., & van Den Pol, A. N. (1999). Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. *The Journal of comparative neurology*, *415*(2), 145–159.

Horvath, T. L., Sarman, B., García-Cáceres, C., Enriori, P. J., Sotonyi, P., Shanabrough, M., Borok, E., Argente, J., Chowen, J. A., Perez-Tilve, D., Pfluger, P. T., Brönneke, H. S., Levin, B. E., Diano, S., Cowley, M. A., & Tschöp, M. H. (2010). Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(33), 14875–14880. https://doi.org/10.1073/pnas.1004282107

Hosogai, N., Fukuhara, A., Oshima, K., Miyata, Y., Tanaka, S., Segawa, K., Furukawa, S., Tochino, Y., Komuro, R., Matsuda, M., & Shimomura, I. (2007). Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes*, *56*(4), 901–911. https://doi.org/10.2337/db06-0911

Hosoi, T., Sasaki, M., Miyahara, T., Hashimoto, C., Matsuo, S., Yoshii, M., & Ozawa, K. (2008). Endoplasmic reticulum stress induces leptin resistance. *Molecular pharmacology*, *74*(6), 1610–1619. https://doi.org/10.1124/mol.108.050070

Hryhorczuk, C., Sheng, Z., Décarie-Spain, L., Giguère, N., Ducrot, C., Trudeau, L. É., Routh, V. H., Alquier, T., & Fulton, S. (2018). Oleic Acid in the Ventral Tegmental Area Inhibits Feeding, Food Reward, and Dopamine Tone. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *43*(3), 607–616. https://doi.org/10.1038/npp.2017.203

https://www.who.int/news-room/fact-sheets/detail/diabetes

https://www.who.int/news-room/fact-sheets/detail/diabetes

Huang, Y. C., Hsu, C. C., Lin, W. C., Yin, T. K., Huang, C. W., Wang, P. W., Chang, H. H., & Chiu, N. T. (2014). Effects of metformin on the cerebral metabolic changes in type 2 diabetic patients. *TheScientificWorldJournal*, *2014*, 694326. https://doi.org/10.1155/2014/694326

Huerta, C. I., Sarkar, P. R., Duong, T. Q., Laird, A. R., & Fox, P. T. (2014). Neural bases of food perception: coordinate-based meta-analyses of neuroimaging studies in multiple modalities. *Obesity (Silver Spring, Md.)*, *22*(6), 1439–1446. https://doi.org/10.1002/oby.20659

Hummel, K. P., Dickie, M. M., & Coleman, D. L. (1966). Diabetes, a new mutation in the mouse. *Science (New York, N.Y.)*, *153*(3740), 1127–1128. https://doi.org/10.1126/science.153.3740.1127

Huszar, D., Lynch, C. A., Fairchild-Huntress, V., Dunmore, J. H., Fang, Q., Berkemeier, L. R., Gu, W., Kesterson, R. A., Boston, B. A., Cone, R. D., Smith, F. J., Campfield, L. A., Burn, P., & Lee, F. (1997). Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell*, *88*(1), 131–141. https://doi.org/10.1016/s0092-8674(00)81865-6

Huxley, R., Owen, C. G., Whincup, P. H., Cook, D. G., Rich-Edwards, J., Smith, G. D., & Collins, R. (2007). Is birth weight a risk factor for ischemic heart disease in later life?. *The American journal of clinical nutrition*, *85*(5), 1244–1250. https://doi.org/10.1093/ajcn/85.5.1244

Hwa, J. J., Ghibaudi, L., Gao, J., & Parker, E. M. (2001). Central melanocortin system modulates energy intake and expenditure of obese and lean Zucker rats. *American journal of physiology. Regulatory, integrative and comparative physiology*, *281*(2), R444–R451. https://doi.org/10.1152/ajpregu.2001.281.2.R444

Ibrahim, N., Bosch, M. A., Smart, J. L., Qiu, J., Rubinstein, M., Rønnekleiv, O. K., Low, M. J., & Kelly, M. J. (2003). Hypothalamic proopiomelanocortin neurons are glucose responsive and express K(ATP) channels. *Endocrinology*, *144*(4), 1331–1340. https://doi.org/10.1210/en.2002-221033

Ichimura, A., Hara, T., & Hirasawa, A. (2014). Regulation of Energy Homeostasis via GPR120. *Frontiers in endocrinology*, *5*, 111. https://doi.org/10.3389/fendo.2014.00111

Imbernon, M., Beiroa, D., Vázquez, M. J., Morgan, D. A., Veyrat-Durebex, C., Porteiro, B., Díaz-Arteaga, A., Senra, A., Busquets, S., Velásquez, D. A., Al-Massadi, O., Varela, L., Gándara, M., López-Soriano, F. J., Gallego, R., Seoane, L. M., Argiles, J. M., López, M., Davis, R. J., Sabio, G., … Nogueiras, R. (2013). Central melanin-concentrating hormone influences liver and adipose metabolism via specific hypothalamic nuclei and efferent autonomic/JNK1 pathways. *Gastroenterology*, *144*(3), 636–649.e6. https://doi.org/10.1053/j.gastro.2012.10.051

Imbernon, M., Sanchez-Rebordelo, E., Gallego, R., Gandara, M., Lear, P., Lopez, M., Dieguez, C., & Nogueiras, R. (2014). Hypothalamic KLF4 mediates leptin's effects on food intake via AgRP. *Molecular metabolism*, *3*(4), 441–451. https://doi.org/10.1016/j.molmet.2014.04.001

Inokuchi, A., Oomura, Y., & Nishimura, H. (1984). Effect of intracerebroventricularly infused glucagon on feeding behavior. *Physiology & behavior*, *33*(3), 397–400. https://doi.org/10.1016/0031-9384(84)90160-4

Inzucchi, S. E., Maggs, D. G., Spollett, G. R., Page, S. L., Rife, F. S., Walton, V., & Shulman, G. I. (1998). Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. *The New England journal of medicine*, *338*(13), 867–872. https://doi.org/10.1056/NEJM199803263381303

Ionescu, E., Rohner-Jeanrenaud, F., Berthoud, H. R., & Jeanrenaud, B. (1983). Increases in plasma insulin levels in response to electrical stimulation of the dorsal motor nucleus of the vagus nerve. *Endocrinology*, *112*(3), 904–910. https://doi.org/10.1210/endo-112-3-904

Ivanova, E. A., Bechtold, D. A., Dupré, S. M., Brennand, J., Barrett, P., Luckman, S. M., & Loudon, A. S. (2008). Altered metabolism in the melatonin-related receptor (GPR50) knockout mouse. *American journal of physiology. Endocrinology and metabolism*, *294*(1), E176–E182. https://doi.org/10.1152/ajpendo.00199.2007

Jais, A., & Brüning, J. C. (2017). Hypothalamic inflammation in obesity and metabolic disease. *The Journal of clinical investigation*, *127*(1), 24–32. https://doi.org/10.1172/JCI88878

Jang, P. G., Namkoong, C., Kang, G. M., Hur, M. W., Kim, S. W., Kim, G. H., Kang, Y., Jeon, M. J., Kim, E. H., Lee, M. S., Karin, M., Baik, J. H., Park, J. Y., Lee, K. U., Kim, Y. B., & Kim, M. S. (2010). NF-kappaB activation in hypothalamic pro-opiomelanocortin neurons is essential in illness- and leptin-induced anorexia. *The Journal of biological chemistry*, *285*(13), 9706–9715. https://doi.org/10.1074/jbc.M109.070706

Jansen, A. S., Hoffman, J. L., & Loewy, A. D. (1997). CNS sites involved in sympathetic and parasympathetic control of the pancreas: a viral tracing study. *Brain research*, *766*(1-2), 29–38. https://doi.org/10.1016/s0006-8993(97)00532-5

Janzer, R. C., & Raff, M. C. (1987). Astrocytes induce blood-brain barrier properties in endothelial cells. *Nature*, *325*(6101), 253–257. https://doi.org/10.1038/325253a0

Jastreboff, A. M., Aronne, L. J., Ahmad, N. N., Wharton, S., Connery, L., Alves, B., Kiyosue, A., Zhang, S., Liu, B., Bunck, M. C., Stefanski, A., & SURMOUNT-1 Investigators (2022). Tirzepatide Once Weekly for the

Treatment of Obesity. *The New England journal of medicine*, *387*(3), 205–216. https://doi.org/10.1056/NEJMoa2206038

Jastreboff, A. M., Sinha, R., Lacadie, C., Small, D. M., Sherwin, R. S., & Potenza, M. N. (2013). Neural correlates of stress- and food cue-induced food craving in obesity: association with insulin levels. *Diabetes care*, *36*(2), 394–402. https://doi.org/10.2337/dc12-1112

Jégou, S., Boutelet, I., & Vaudry, H. (2000). Melanocortin-3 receptor mRNA expression in pro-opiomelanocortin neurones of the rat arcuate nucleus. *Journal of neuroendocrinology*, *12*(6), 501–505. https://doi.org/10.1046/j.1365-2826.2000.00477.x

Jerlhag, E., Egecioglu, E., Dickson, S. L., Douhan, A., Svensson, L., & Engel, J. A. (2007). Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addiction biology*, *12*(1), 6–16. https://doi.org/10.1111/j.1369-1600.2006.00041.x

Jessen, N. A., Munk, A. S., Lundgaard, I., & Nedergaard, M. (2015). The Glymphatic System: A Beginner's Guide. *Neurochemical research*, *40*(12), 2583–2599. https://doi.org/10.1007/s11064-015-1581-6

Ji, L., Li, H., Guo, X., Li, Y., Hu, R., & Zhu, Z. (2013). Impact of baseline BMI on glycemic control and weight change with metformin monotherapy in Chinese type 2 diabetes patients: phase IV open-label trial. *PloS one*, *8*(2), e57222. https://doi.org/10.1371/journal.pone.0057222

Jin, L., Wessely, O., Marcusson, E. G., Ivan, C., Calin, G. A., & Alahari, S. K. (2013). Prooncogenic factors miR-23b and miR-27b are regulated by Her2/Neu, EGF, and TNF-α in breast cancer. *Cancer research*, *73*(9), 2884–2896. https://doi.org/10.1158/0008-5472.CAN-12-2162

Jin, L., Zhang, S., Burguera, B. G., Couce, M. E., Osamura, R. Y., Kulig, E., & Lloyd, R. V. (2000). Leptin and leptin receptor expression in rat and mouse pituitary cells. *Endocrinology*, *141*(1), 333–339. https://doi.org/10.1210/endo.141.1.7260

Johnson, J. D., Ahmed, N. T., Luciani, D. S., Han, Z., Tran, H., Fujita, J., Misler, S., Edlund, H., & Polonsky, K. S. (2003). Increased islet apoptosis in Pdx1+/- mice. *The Journal of clinical investigation*, *111*(8), 1147–1160. https://doi.org/10.1172/JCI16537

Joly-Amado, A., Denis, R. G., Castel, J., Lacombe, A., Cansell, C., Rouch, C., Kassis, N., Dairou, J., Cani, P. D., Ventura-Clapier, R., Prola, A., Flamment, M., Foufelle, F., Magnan, C., & Luquet, S. (2012). Hypothalamic AgRP-neurons control peripheral substrate utilization and nutrient partitioning. *The EMBO journal*, *31*(22), 4276–4288. https://doi.org/10.1038/emboj.2012.250

Juan-Picó, P., Fuentes, E., Bermúdez-Silva, F. J., Javier Díaz-Molina, F., Ripoll, C., Rodríguez de Fonseca, F., & Nadal, A. (2006). Cannabinoid receptors regulate Ca(2+) signals and insulin secretion in pancreatic beta-cell. *Cell calcium*, *39*(2), 155–162. https://doi.org/10.1016/j.ceca.2005.10.005

Jurek, B., & Neumann, I. D. (2018). The Oxytocin Receptor: From Intracellular Signaling to Behavior. *Physiological reviews*, *98*(3), 1805–1908. https://doi.org/10.1152/physrev.00031.2017

Karemaker J. M. (2017). An introduction into autonomic nervous function. *Physiological measurement*, *38*(5), R89–R118. https://doi.org/10.1088/1361-6579/aa6782

Karise, I., Bargut, T. C., Del Sol, M., Aguila, M. B., & Mandarim-de-Lacerda, C. A. (2019). Metformin enhances mitochondrial biogenesis and thermogenesis in brown adipocytes of mice. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *111*, 1156–1165. https://doi.org/10.1016/j.biopha.2019.01.021

Kasai, H., Takahashi, N., & Tokumaru, H. (2012). Distinct initial SNARE configurations underlying the diversity of exocytosis. *Physiological reviews*, *92*(4), 1915–1964. https://doi.org/10.1152/physrev.00007.2012

Kaushik, S., Arias, E., Kwon, H., Lopez, N. M., Athonvarangkul, D., Sahu, S., Schwartz, G. J., Pessin, J. E., & Singh, R. (2012). Loss of autophagy in hypothalamic POMC neurons impairs lipolysis. *EMBO reports*, *13*(3), 258–265. https://doi.org/10.1038/embor.2011.260

Kellerer, M., Kaltoft, M. S., Lawson, J., Nielsen, L. L., Strojek, K., Tabak, Ö., & Jacob, S. (2022). Effect of once-weekly semaglutide versus thrice-daily insulin aspart, both as add-on to metformin and optimized insulin glargine treatment in participants with type 2 diabetes (SUSTAIN 11): A randomized, open-label, multinational, phase 3b trial. *Diabetes, obesity & metabolism*, *24*(9), 1788–1799. https://doi.org/10.1111/dom.14765

Kelley, D. E., Goodpaster, B., Wing, R. R., & Simoneau, J. A. (1999). Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *The American journal of physiology*, *277*(6), E1130–E1141. https://doi.org/10.1152/ajpendo.1999.277.6.E1130

KENNEDY G. C. (1953). The role of depot fat in the hypothalamic control of food intake in the rat. *Proceedings of the Royal Society of London. Series B, Biological sciences*, *140*(901), 578–596. https://doi.org/10.1098/rspb.1953.0009

Kern, W., Benedict, C., Schultes, B., Plohr, F., Moser, A., Born, J., Fehm, H. L., & Hallschmid, M. (2006). Low cerebrospinal fluid insulin levels in obese humans. *Diabetologia*, *49*(11), 2790–2792. https://doi.org/10.1007/s00125-006-0409-y

Kettner, N. M., Mayo, S. A., Hua, J., Lee, C., Moore, D. D., & Fu, L. (2015). Circadian Dysfunction Induces Leptin Resistance in Mice. *Cell metabolism*, *22*(3), 448–459. https://doi.org/10.1016/j.cmet.2015.06.005

Kievit, P., Howard, J. K., Badman, M. K., Balthasar, N., Coppari, R., Mori, H., Lee, C. E., Elmquist, J. K., Yoshimura, A., & Flier, J. S. (2006). Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. *Cell metabolism*, *4*(2), 123–132. https://doi.org/10.1016/j.cmet.2006.06.010

Killion, E. A., Lu, S. C., Fort, M., Yamada, Y., Véniant, M. M., & Lloyd, D. J. (2020). Glucose-Dependent Insulinotropic Polypeptide Receptor Therapies for the Treatment of Obesity, Do Agonists = Antagonists?. *Endocrine reviews*, *41*(1), bnz002. https://doi.org/10.1210/endrev/bnz002

Kim, E. K., Lee, S. H., Jhun, J. Y., Byun, J. K., Jeong, J. H., Lee, S. Y., Kim, J. K., Choi, J. Y., & Cho, M. L. (2016). Metformin Prevents Fatty Liver and Improves Balance of White/Brown Adipose in an Obesity Mouse Model by Inducing FGF21. *Mediators of inflammation*, *2016*, 5813030. https://doi.org/10.1155/2016/5813030

Kim, K. W., Zhao, L., Donato, J., Jr, Kohno, D., Xu, Y., Elias, C. F., Lee, C., Parker, K. L., & Elmquist, J. K. (2011). Steroidogenic factor 1 directs programs regulating diet-induced thermogenesis and leptin action in the ventral medial hypothalamic nucleus. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(26), 10673–10678. https://doi.org/10.1073/pnas.1102364108

Kim, K. W., Zhao, L., Donato, J., Jr, Kohno, D., Xu, Y., Elias, C. F., Lee, C., Parker, K. L., & Elmquist, J. K. (2011). Steroidogenic factor 1 directs programs regulating diet-induced thermogenesis and leptin action in the ventral medial hypothalamic nucleus. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(26), 10673–10678. https://doi.org/10.1073/pnas.1102364108

Kim, M. S., Small, C. J., Stanley, S. A., Morgan, D. G., Seal, L. J., Kong, W. M., Edwards, C. M., Abusnana, S., Sunter, D., Ghatei, M. A., & Bloom, S. R. (2000). The central melanocortin system affects the hypothalamo-pituitary thyroid axis and may mediate the effect of leptin. *The Journal of clinical investigation*, *105*(7), 1005–1011. https://doi.org/10.1172/JCI8857

Kim, Y. W., Kim, J. Y., Park, Y. H., Park, S. Y., Won, K. C., Choi, K. H., Huh, J. Y., & Moon, K. H. (2006). Metformin restores leptin sensitivity in high-fat-fed obese rats with leptin resistance. *Diabetes*, *55*(3), 716–724. https://doi.org/10.2337/diabetes.55.03.06.db05-0917

King A. (2010). Prevention: Neuropsychiatric adverse effects signal the end of the line for rimonabant. *Nature reviews. Cardiology*, *7*(11), 602. https://doi.org/10.1038/nrcardio.2010.148

Kirchgessner, A. L., & Sclafani, A. (1988). PVN-hindbrain pathway involved in the hypothalamic hyperphagia-obesity syndrome. *Physiology & behavior*, *42*(6), 517–528. https://doi.org/10.1016/0031-9384(88)90153-9

Kirkham T. C. (2005). Endocannabinoids in the regulation of appetite and body weight. *Behavioural pharmacology*, *16*(5-6), 297–313. https://doi.org/10.1097/00008877-200509000-00004

Kishi, T., Aschkenasi, C. J., Lee, C. E., Mountjoy, K. G., Saper, C. B., & Elmquist, J. K. (2003). Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. *The Journal of comparative neurology*, *457*(3), 213–235. https://doi.org/10.1002/cne.10454

Kittner, H., Franke, H., Harsch, J. I., El-Ashmawy, I. M., Seidel, B., Krügel, U., & Illes, P. (2006). Enhanced food intake after stimulation of hypothalamic P2Y1 receptors in rats: modulation of feeding behaviour by extracellular nucleotides. *The European journal of neuroscience*, *24*(7), 2049–2056. https://doi.org/10.1111/j.1460-9568.2006.05071.x

Knauf, C., Cani, P. D., Perrin, C., Iglesias, M. A., Maury, J. F., Bernard, E., Benhamed, F., Grémeaux, T., Drucker, D. J., Kahn, C. R., Girard, J., Tanti, J. F., Delzenne, N. M., Postic, C., & Burcelin, R. (2005). Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. *The Journal of clinical investigation*, *115*(12), 3554–3563. https://doi.org/10.1172/JCI25764

Koch, M., Varela, L., Kim, J. G., Kim, J. D., Hernández-Nuño, F., Simonds, S. E., Castorena, C. M., Vianna, C. R., Elmquist, J. K., Morozov, Y. M., Rakic, P., Bechmann, I., Cowley, M. A., Szigeti-Buck, K., Dietrich, M. O., Gao, X. B., Diano, S., & Horvath, T. L. (2015). Hypothalamic POMC neurons promote cannabinoid-induced feeding. *Nature*, *519*(7541), 45–50. https://doi.org/10.1038/nature14260

Kojima, M., & Kangawa, K. (2005). Ghrelin: structure and function. *Physiological reviews*, *85*(2), 495–522. https://doi.org/10.1152/physrev.00012.2004

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, *402*(6762), 656–660. https://doi.org/10.1038/45230

Kolditz, C. I., & Langin, D. (2010). Adipose tissue lipolysis. *Current opinion in clinical nutrition and metabolic care*, *13*(4), 377–381. https://doi.org/10.1097/MCO.0b013e32833bed6a

Kong, D., Tong, Q., Ye, C., Koda, S., Fuller, P. M., Krashes, M. J., Vong, L., Ray, R. S., Olson, D. P., & Lowell, B. B. (2012). GABAergic RIP-Cre neurons in the arcuate nucleus selectively regulate energy expenditure. *Cell*, *151*(3), 645–657. https://doi.org/10.1016/j.cell.2012.09.020

Kopelman, P., Bryson, A., Hickling, R., Rissanen, A., Rossner, S., Toubro, S., & Valensi, P. (2007). Cetilistat (ATL-962), a novel lipase inhibitor: a 12-week randomized, placebo-controlled study of weight reduction in obese patients. *International journal of obesity (2005)*, *31*(3), 494–499. https://doi.org/10.1038/sj.ijo.0803446

Kopelman, P., Groot, G.deH., Rissanen, A., Rossner, S., Toubro, S., Palmer, R., Hallam, R., Bryson, A., & Hickling, R. I. (2010). Weight loss, HbA1c reduction, and tolerability of cetilistat in a randomized, placebo-controlled phase 2 trial in obese diabetics: comparison with orlistat (Xenical). *Obesity (Silver Spring, Md.)*, *18*(1), 108–115. https://doi.org/10.1038/oby.2009.155

Krashes, M. J., Koda, S., Ye, C., Rogan, S. C., Adams, A. C., Cusher, D. S., Maratos-Flier, E., Roth, B. L., & Lowell, B. B. (2011). Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. *The Journal of clinical investigation*, *121*(4), 1424–1428. https://doi.org/10.1172/JCI46229

Krashes, M. J., Shah, B. P., Koda, S., & Lowell, B. B. (2013). Rapid versus delayed stimulation of feeding by the endogenously released AgRP neuron mediators GABA, NPY, and AgRP. *Cell metabolism*, *18*(4), 588–595. https://doi.org/10.1016/j.cmet.2013.09.009

Kreymann, B., Williams, G., Ghatei, M. A., & Bloom, S. R. (1987). Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet (London, England), 2(8571), 1300–1304. https://doi.org/10.1016/s0140-6736(87)91194-9

Krisch, B., Leonhardt, H., & Buchheim, W. (1978). The functional and structural border between the CSF- and blood-milieu in the circumventricular organs (organum vasculosum laminae terminalis, subfornical organ, area postrema) of the rat. *Cell and tissue research*, *195*(3), 485–497. https://doi.org/10.1007/BF00233891

Kristensen, P., Judge, M. E., Thim, L., Ribel, U., Christjansen, K. N., Wulff, B. S., Clausen, J. T., Jensen, P. B., Madsen, O. D., Vrang, N., Larsen, P. J., & Hastrup, S. (1998). Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature*, *393*(6680), 72–76. https://doi.org/10.1038/29993

Kryger M. H. (1983). Sleep apnea. From the needles of Dionysius to continuous positive airway pressure. *Archives of internal medicine*, *143*(12), 2301–2303. https://doi.org/10.1001/archinte.143.12.2301

Kubota, N., Yano, W., Kubota, T., Yamauchi, T., Itoh, S., Kumagai, H., Kozono, H., Takamoto, I., Okamoto, S., Shiuchi, T., Suzuki, R., Satoh, H., Tsuchida, A., Moroi, M., Sugi, K., Noda, T., Ebinuma, H., Ueta, Y., Kondo, T., Araki, E., … Kadowaki, T. (2007). Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell metabolism*, *6*(1), 55–68. https://doi.org/10.1016/j.cmet.2007.06.003

Kuhre, R. E., Wewer Albrechtsen, N. J., Larsen, O., Jepsen, S. L., Balk-Møller, E., Andersen, D. B., Deacon, C. F., Schoonjans, K., Reimann, F., Gribble, F. M., Albrechtsen, R., Hartmann, B., Rosenkilde, M. M., & Holst, J. J. (2018). Bile acids are important direct and indirect regulators of the secretion of appetite- and metabolism-regulating hormones from the gut and pancreas. *Molecular metabolism*, *11*, 84–95. https://doi.org/10.1016/j.molmet.2018.03.007

Kurtcuoglu, V., Soellinger, M., Summers, P., Poulikakos, D., & Boesiger, P. (2007). Mixing and modes of mass transfer in the third cerebral ventricle: a computational analysis. *Journal of biomechanical engineering*, *129*(5), 695–702. https://doi.org/10.1115/1.2768376

Kuzawa, C. W., & Adair, L. S. (2003). Lipid profiles in adolescent Filipinos: relation to birth weight and maternal energy status during pregnancy. *The American journal of clinical nutrition*, *77*(4), 960–966. https://doi.org/10.1093/ajcn/77.4.960

Kwon, E., Joung, H. Y., Liu, S. M., Chua, S. C., Jr, Schwartz, G. J., & Jo, Y. H. (2020). Optogenetic stimulation of the liver-projecting melanocortinergic pathway promotes hepatic glucose production. Nature communications, 11(1), 6295. https://doi.org/10.1038/s41467-020-20160-w

Kwon, O., Kim, K. W., & Kim, M. S. (2016). Leptin signalling pathways in hypothalamic neurons. *Cellular and molecular life sciences : CMLS*, *73*(7), 1457–1477. https://doi.org/10.1007/s00018-016-2133-1

la Fleur, S. E., Kalsbeek, A., Wortel, J., & Buijs, R. M. (2000). Polysynaptic neural pathways between the hypothalamus, including the suprachiasmatic nucleus, and the liver. *Brain research*, *871*(1), 50–56. https://doi.org/10.1016/s0006-8993(00)02423-9

Łabuzek, K., Suchy, D., Gabryel, B., Bielecka, A., Liber, S., & Okopień, B. (2010). Quantification of metformin by the HPLC method in brain regions, cerebrospinal fluid and plasma of rats treated with lipopolysaccharide. *Pharmacological reports : PR*, *62*(5), 956–965. https://doi.org/10.1016/s1734-1140(10)70357-1

Lafont, F., Verkade, P., Galli, T., Wimmer, C., Louvard, D., & Simons, K. (1999). Raft association of SNAP receptors acting in apical trafficking in Madin-Darby canine kidney cells. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(7), 3734–3738. https://doi.org/10.1073/pnas.96.7.3734

Lam, B. Y. H., Cimino, I., Polex-Wolf, J., Nicole Kohnke, S., Rimmington, D., Iyemere, V., Heeley, N., Cossetti, C., Schulte, R., Saraiva, L. R., Logan, D. W., Blouet, C., O'Rahilly, S., Coll, A. P., & Yeo, G. S. H. (2017). Heterogeneity of hypothalamic pro-opiomelanocortin-expressing neurons revealed by single-cell RNA sequencing. *Molecular metabolism*, *6*(5), 383–392. https://doi.org/10.1016/j.molmet.2017.02.007

Lam, B. Y. H., Cimino, I., Polex-Wolf, J., Nicole Kohnke, S., Rimmington, D., Iyemere, V., Heeley, N., Cossetti, C., Schulte, R., Saraiva, L. R., Logan, D. W., Blouet, C., O'Rahilly, S., Coll, A. P., & Yeo, G. S. H. (2017). Heterogeneity of hypothalamic pro-opiomelanocortin-expressing neurons revealed by single-cell RNA sequencing. *Molecular metabolism*, *6*(5), 383–392. https://doi.org/10.1016/j.molmet.2017.02.007

Lam, T. K., Gutierrez-Juarez, R., Pocai, A., Bhanot, S., Tso, P., Schwartz, G. J., & Rossetti, L. (2007). Brain glucose metabolism controls the hepatic secretion of triglyceride-rich lipoproteins. *Nature medicine*, *13*(2), 171–180. https://doi.org/10.1038/nm1540

Langlet, F., Levin, B. E., Luquet, S., Mazzone, M., Messina, A., Dunn-Meynell, A. A., Balland, E., Lacombe, A., Mazur, D., Carmeliet, P., Bouret, S. G., Prevot, V., & Dehouck, B. (2013). Tanycytic VEGF-A boosts blood-hypothalamus barrier plasticity and access of metabolic signals to the arcuate nucleus in response to fasting. *Cell metabolism*, *17*(4), 607–617. https://doi.org/10.1016/j.cmet.2013.03.004

Langlet, F., Mullier, A., Bouret, S. G., Prevot, V., & Dehouck, B. (2013). Tanycyte-like cells form a blood-cerebrospinal fluid barrier in the circumventricular organs of the mouse brain. *The Journal of comparative neurology*, *521*(15), 3389–3405. https://doi.org/10.1002/cne.23355

Lavrentaki, A., Ali, A., Cooper, B. G., & Tahrani, A. A. (2019). MECHANISMS OF ENDOCRINOLOGY: Mechanisms of disease: the endocrinology of obstructive sleep apnoea. *European journal of endocrinology*, *180*(3), R91–R125. https://doi.org/10.1530/EJE-18-0411

Lazutkaite, G., Soldà, A., Lossow, K., Meyerhof, W., & Dale, N. (2017). Amino acid sensing in hypothalamic tanycytes via umami taste receptors. *Molecular metabolism*, *6*(11), 1480–1492. https://doi.org/10.1016/j.molmet.2017.08.015

Lazzaroni, E., Ben Nasr, M., Loretelli, C., Pastore, I., Plebani, L., Lunati, M. E., Vallone, L., Bolla, A. M., Rossi, A., Montefusco, L., Ippolito, E., Berra, C., D'Addio, F., Zuccotti, G. V., & Fiorina, P. (2021). Anti-diabetic drugs and weight loss in patients with type 2 diabetes. *Pharmacological research*, *171*, 105782. https://doi.org/10.1016/j.phrs.2021.105782

Le Foll, C., Irani, B. G., Magnan, C., Dunn-Meynell, A. A., & Levin, B. E. (2009). Characteristics and mechanisms of hypothalamic neuronal fatty acid sensing. *American journal of physiology. Regulatory, integrative and comparative physiology*, *297*(3), R655–R664. https://doi.org/10.1152/ajpregu.00223.2009

Lear, S., Amso, Z., & Shen, W. (2019). Engineering PEG-fatty acid stapled, long-acting peptide agonists for G protein-coupled receptors. *Methods in enzymology*, *622*, 183–200. https://doi.org/10.1016/bs.mie.2019.02.008

Leckstrom, A., Lew, P. S., Poritsanos, N. J., & Mizuno, T. M. (2011). Central melanocortin receptor agonist reduces hepatic lipogenic gene expression in streptozotocin-induced diabetic mice. *Life sciences*, *88*(15-16), 664–669. https://doi.org/10.1016/j.lfs.2011.01.026

Lee, G. H., Proenca, R., Montez, J. M., Carroll, K. M., Darvishzadeh, J. G., Lee, J. I., & Friedman, J. M. (1996). Abnormal splicing of the leptin receptor in diabetic mice. *Nature*, *379*(6566), 632–635. https://doi.org/10.1038/379632a0

Leinninger, G. M., Jo, Y. H., Leshan, R. L., Louis, G. W., Yang, H., Barrera, J. G., Wilson, H., Opland, D. M., Faouzi, M. A., Gong, Y., Jones, J. C., Rhodes, C. J., Chua, S., Jr, Diano, S., Horvath, T. L., Seeley, R. J., Becker, J. B., Münzberg, H., & Myers, M. G., Jr (2009). Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell metabolism*, *10*(2), 89–98. https://doi.org/10.1016/j.cmet.2009.06.011

Leinninger, G. M., Opland, D. M., Jo, Y. H., Faouzi, M., Christensen, L., Cappellucci, L. A., Rhodes, C. J., Gnegy, M. E., Becker, J. B., Pothos, E. N., Seasholtz, A. F., Thompson, R. C., & Myers, M. G., Jr (2011). Leptin action via neurotensin neurons controls orexin, the mesolimbic dopamine system and energy balance. *Cell metabolism*, *14*(3), 313–323. https://doi.org/10.1016/j.cmet.2011.06.016

Lenoir, M., Serre, F., Cantin, L., & Ahmed, S. H. (2007). Intense sweetness surpasses cocaine reward. *PloS one*, *2*(8), e698. https://doi.org/10.1371/journal.pone.0000698

Leon, D. A., Lithell, H. O., Vâgerö, D., Koupilová, I., Mohsen, R., Berglund, L., Lithell, U. B., & McKeigue, P. M. (1998). Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915-29. *BMJ (Clinical research ed.)*, *317*(7153), 241–245. https://doi.org/10.1136/bmj.317.7153.241

Leon, S., Simon, V., Lee, T. H., Steuernagel, L., Clark, S., Biglari, N., Lesté-Lasserre, T., Dupuy, N., Cannich, A., Bellocchio, L., Zizzari, P., Allard, C., Gonzales, D., Le Feuvre, Y., Lhuillier, E., Brochard, A., Nicolas, J. C., Teillon, J., Nikolski, M., Marsicano, G., … Quarta, C. (2024). Single cell tracing of Pomc neurons reveals recruitment of 'Ghost' subtypes with atypical identity in a mouse model of obesity. *Nature communications*, *15*(1), 3443. https://doi.org/10.1038/s41467-024-47877-2

le Roux, C. W., Hankosky, E. R., Wang, D., Malik, R., Yu, M., Hickey, A., Kan, H., Bunck, M. C., Stefanski, A., Garcia-Perez, L. E., & Wharton, S. (2023). Tirzepatide 10 and 15 mg compared with semaglutide 2.4 mg for the treatment of obesity: An indirect treatment comparison. *Diabetes, obesity & metabolism*, *25*(9), 2626–2633. https://doi.org/10.1111/dom.15148

Lesses MF, Myerson A. (1938) Human Autonomic Pharmacology. *New England Journal of Medicine.*

Lesses, M. F., & Myerson, A. (1994). Human autonomic pharmacology. XVI. Benzedrine sulfate as an aid in the treatment of obesity. 1938. *Obesity research*, *2*(3), 286–292. https://doi.org/10.1002/j.1550-8528.1994.tb00061.x

Lhomme, T., Clasadonte, J., Imbernon, M., Fernandois, D., Sauve, F., Caron, E., da Silva Lima, N., Heras, V., Martinez-Corral, I., Mueller-Fielitz, H., Rasika, S., Schwaninger, M., Nogueiras, R., & Prevot, V. (2021). Tanycytic networks mediate energy balance by feeding lactate to glucose-insensitive POMC neurons. The Journal of clinical investigation, 131(18), e140521. https://doi.org/10.1172/JCI140521

Li, C. S., Cho, Y. K., & Smith, D. V. (2005). Modulation of parabrachial taste neurons by electrical and chemical stimulation of the lateral hypothalamus and amygdala. *Journal of neurophysiology*, *93*(3), 1183–1196. https://doi.org/10.1152/jn.00828.2004

Li, H., & Yu, X. (2013). Emerging role of JNK in insulin resistance. Current diabetes reviews, 9(5), 422–428. https://doi.org/10.2174/15733998113099990074

Li, H., Matheny, M., Tümer, N., & Scarpace, P. J. (1998). Aging and fasting regulation of leptin and hypothalamic neuropeptide Y gene expression. *The American journal of physiology*, *275*(3), E405–E411. https://doi.org/10.1152/ajpendo.1998.275.3.E405

Li, X. L., Aou, S., Oomura, Y., Hori, N., Fukunaga, K., & Hori, T. (2002). Impairment of long-term potentiation and spatial memory in leptin receptor-deficient rodents. *Neuroscience*, *113*(3), 607–615. https://doi.org/10.1016/s0306-4522(02)00162-8

Liang, X., Yang, Q., Zhang, L., Maricelli, J. W., Rodgers, B. D., Zhu, M. J., & Du, M. (2016). Maternal high-fat diet during lactation impairs thermogenic function of brown adipose tissue in offspring mice. *Scientific reports*, *6*, 34345. https://doi.org/10.1038/srep34345

Liao, P. Y., Chiu, Y. M., Yu, J. H., & Chen, S. K. (2020). Mapping Central Projection of Oxytocin Neurons in Unmated Mice Using Cre and Alkaline Phosphatase Reporter. *Frontiers in neuroanatomy*, *14*, 559402. https://doi.org/10.3389/fnana.2020.559402

Lien, F., Berthier, A., Bouchaert, E., Gheeraert, C., Alexandre, J., Porez, G., Prawitt, J., Dehondt, H., Ploton, M., Colin, S., Lucas, A., Patrice, A., Pattou, F., Diemer, H., Van Dorsselaer, A., Rachez, C., Kamilic, J., Groen, A. K., Staels, B., & Lefebvre, P. (2014). Metformin interferes with bile acid homeostasis through AMPK-FXR crosstalk. *The Journal of clinical investigation*, *124*(3), 1037–1051. https://doi.org/10.1172/JCI68815

Lin, S., Thomas, T. C., Storlien, L. H., & Huang, X. F. (2000). Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity*, *24*(5), 639–646. https://doi.org/10.1038/sj.ijo.0801209

Lindberg, D., Chen, P., & Li, C. (2013). Conditional viral tracing reveals that steroidogenic factor 1-positive neurons of the dorsomedial subdivision of the ventromedial hypothalamus project to autonomic centers of the hypothalamus and hindbrain. *The Journal of comparative neurology*, *521*(14), 3167–3190. https://doi.org/10.1002/cne.23338

Lingvay, I., Catarig, A. M., Frias, J. P., Kumar, H., Lausvig, N. L., le Roux, C. W., Thielke, D., Viljoen, A., & McCrimmon, R. J. (2019). Efficacy and safety of once-weekly semaglutide versus daily canagliflozin as add-on to metformin in patients with type 2 diabetes (SUSTAIN 8): a double-blind, phase 3b, randomised controlled trial. *The lancet. Diabetes & endocrinology*, *7*(11), 834–844. https://doi.org/10.1016/S2213-8587(19)30311-0

*Lippincott.: Philadelphia.* p. 48-61.

Liu, T. T., Liu, X. T., Chen, Q. X., & Shi, Y. (2020). Lipase Inhibitors for Obesity: A Review. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *128*, 110314. https://doi.org/10.1016/j.biopha.2020.110314

Liu, T., Kong, D., Shah, B. P., Ye, C., Koda, S., Saunders, A., Ding, J. B., Yang, Z., Sabatini, B. L., & Lowell, B. B. (2012). Fasting activation of AgRP neurons requires NMDA receptors and involves spinogenesis and increased excitatory tone. *Neuron*, *73*(3), 511–522. https://doi.org/10.1016/j.neuron.2011.11.027

Lockie, S. H., Heppner, K. M., Chaudhary, N., Chabenne, J. R., Morgan, D. A., Veyrat-Durebex, C., Ananthakrishnan, G., Rohner-Jeanrenaud, F., Drucker, D. J., DiMarchi, R., Rahmouni, K., Oldfield, B. J., Tschöp, M. H., & Perez-Tilve, D. (2012). Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. *Diabetes*, *61*(11), 2753–2762. https://doi.org/10.2337/db11-1556

Loos, R. J. F., & Yeo, G. S. H. (2022). The genetics of obesity: from discovery to biology. *Nature reviews. Genetics*, *23*(2), 120–133. https://doi.org/10.1038/s41576-021-00414-z

López, M., Alvarez, C. V., Nogueiras, R., & Diéguez, C. (2013). Energy balance regulation by thyroid hormones at central level. *Trends in molecular medicine*, *19*(7), 418–427. https://doi.org/10.1016/j.molmed.2013.04.004

López, M., Lage, R., Saha, A. K., Pérez-Tilve, D., Vázquez, M. J., Varela, L., Sangiao-Alvarellos, S., Tovar, S., Raghay, K., Rodríguez-Cuenca, S., Deoliveira, R. M., Castañeda, T., Datta, R., Dong, J. Z., Culler, M., Sleeman,

M. W., Alvarez, C. V., Gallego, R., Lelliott, C. J., Carling, D., … Vidal-Puig, A. (2008). Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. *Cell metabolism*, *7*(5), 389–399. https://doi.org/10.1016/j.cmet.2008.03.006

López, M., Nogueiras, R., Tena-Sempere, M., & Diéguez, C. (2016). Hypothalamic AMPK: a canonical regulator of whole-body energy balance. *Nature reviews. Endocrinology*, *12*(7), 421–432. https://doi.org/10.1038/nrendo.2016.67

Low, S. H., Chapin, S. J., Wimmer, C., Whiteheart, S. W., Kömüves, L. G., Mostov, K. E., & Weimbs, T. (1998). The SNARE machinery is involved in apical plasma membrane trafficking in MDCK cells. *The Journal of cell biology*, *141*(7), 1503–1513. https://doi.org/10.1083/jcb.141.7.1503

Lu, X. Y., Barsh, G. S., Akil, H., & Watson, S. J. (2003). Interaction between alpha-melanocyte-stimulating hormone and corticotropin-releasing hormone in the regulation of feeding and hypothalamo-pituitary-adrenal responses. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *23*(21), 7863–7872. https://doi.org/10.1523/JNEUROSCI.23-21-07863.2003

Luquet, S., Perez, F. A., Hnasko, T. S., & Palmiter, R. D. (2005). NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science (New York, N.Y.)*, *310*(5748), 683–685. https://doi.org/10.1126/science.1115524

Lusk G. (1924) Animal calorimetry, twenty-fourth paper: analysis of the oxidation of mixtures of carbohydrate and fat. *J Biol Chem.* 59(1):41–42. https://doi:10.1016/S0021-9258(18)85293-0.

Lustig R. H. (2006). Childhood obesity: behavioral aberration or biochemical drive? Reinterpreting the First Law of Thermodynamics. *Nature clinical practice. Endocrinology & metabolism*, *2*(8), 447–458. https://doi.org/10.1038/ncpendmet022

Lutter, M., & Nestler, E. J. (2009). Homeostatic and hedonic signals interact in the regulation of food intake. *The Journal of nutrition*, *139*(3), 629–632. https://doi.org/10.3945/jn.108.097618

Lv, W. S., Wen, J. P., Li, L., Sun, R. X., Wang, J., Xian, Y. X., Cao, C. X., Wang, Y. L., & Gao, Y. Y. (2012). The effect of metformin on food intake and its potential role in hypothalamic regulation in obese diabetic rats. *Brain research*, *1444*, 11–19. https://doi.org/10.1016/j.brainres.2012.01.028

Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H., Wihler, C., Koliatsos, V. E., & Tessarollo, L. (1999). Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(26), 15239–15244. https://doi.org/10.1073/pnas.96.26.15239

Lyu, X., Lyu, T., Wang, X., Zhu, H., Pan, H., Wang, L., Yang, H., & Gong, F. (2021). The Antiobesity Effect of GLP-1 Receptor Agonists Alone or in Combination with Metformin in Overweight /Obese Women with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis. *International journal of endocrinology*, *2021*, 6616693. https://doi.org/10.1155/2021/6616693

Maejima, Y., Yokota, S., Nishimori, K., & Shimomura, K. (2018). The Anorexigenic Neural Pathways of Oxytocin and Their Clinical Implication. *Neuroendocrinology*, *107*(1), 91–104. https://doi.org/10.1159/000489263

Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Kim, S., Lallone, R., & Ranganathan, S. (1995). Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature medicine*, *1*(11), 1155–1161. https://doi.org/10.1038/nm1195-1155

Majdic, G., Young, M., Gomez-Sanchez, E., Anderson, P., Szczepaniak, L. S., Dobbins, R. L., McGarry, J. D., & Parker, K. L. (2002). Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity. *Endocrinology*, *143*(2), 607–614. https://doi.org/10.1210/endo.143.2.8652

Major RH. A History of Medicine. (1954). *Springfield, IL: Charles C Thomas.*

Malmström, R., Taskinen, M. R., Karonen, S. L., & Yki-Järvinen, H. (1996). Insulin increases plasma leptin concentrations in normal subjects and patients with NIDDM. *Diabetologia*, *39*(8), 993–996. https://doi.org/10.1007/BF00403921

Manceau, R., Majeur, D., & Alquier, T. (2020). Neuronal control of peripheral nutrient partitioning. *Diabetologia*, *63*(4), 673–682. https://doi-org.proxy.insermbiblio.inist.fr/10.1007/s00125-020-05104-9

Mantzoros, C. S., Qu, D., Frederich, R. C., Susulic, V. S., Lowell, B. B., Maratos-Flier, E., & Flier, J. S. (1996). Activation of beta(3) adrenergic receptors suppresses leptin expression and mediates a leptin-independent inhibition of food intake in mice. *Diabetes*, *45*(7), 909–914. https://doi.org/10.2337/diab.45.7.909

Markan, K. R., Jurczak, M. J., & Brady, M. J. (2010). Stranger in a strange land: roles of glycogen turnover in adipose tissue metabolism. *Molecular and cellular endocrinology*, *318*(1-2), 54–60. https://doi.org/10.1016/j.mce.2009.08.013

Marks, J. L., Porte, D., Jr, Stahl, W. L., & Baskin, D. G. (1990). Localization of insulin receptor mRNA in rat brain by in situ hybridization. *Endocrinology*, *127*(6), 3234–3236. https://doi.org/10.1210/endo-127-6-3234

Marso, S. P., Bain, S. C., Consoli, A., Eliaschewitz, F. G., Jódar, E., Leiter, L. A., Lingvay, I., Rosenstock, J., Seufert, J., Warren, M. L., Woo, V., Hansen, O., Holst, A. G., Pettersson, J., Vilsbøll, T., & SUSTAIN-6 Investigators (2016). Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *The New England journal of medicine*, *375*(19), 1834–1844. https://doi.org/10.1056/NEJMoa1607141

Martínez-Sánchez, N., Moreno-Navarrete, J. M., Contreras, C., Rial-Pensado, E., Fernø, J., Nogueiras, R., Diéguez, C., Fernández-Real, J. M., & López, M. (2017). Thyroid hormones induce browning of white fat. *The Journal of endocrinology*, *232*(2), 351–362. https://doi.org/10.1530/JOE-16-0425

Martínez, F., Cifuentes, M., Tapia, J. C., & Nualart, F. (2019). The median eminence as the hypothalamic area involved in rapid transfer of glucose to the brain: functional and cellular mechanisms. *Journal of molecular medicine (Berlin, Germany)*, *97*(8), 1085–1097. https://doi.org/10.1007/s00109-019-01799-5

Masuda, K., Koizumi, A., Nakajima, K., Tanaka, T., Abe, K., Misaka, T., & Ishiguro, M. (2012). Characterization of the modes of binding between human sweet taste receptor and low-molecular-weight sweet compounds. *PloS one*, *7*(4), e35380. https://doi.org/10.1371/journal.pone.0035380

Masuzaki, H., Ogawa, Y., Sagawa, N., Hosoda, K., Matsumoto, T., Mise, H., Nishimura, H., Yoshimasa, Y., Tanaka, I., Mori, T., & Nakao, K. (1997). Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nature medicine*, *3*(9), 1029–1033. https://doi.org/10.1038/nm0997-1029

Mathieu, C., Martens, P. J., & Vangoitsenhoven, R. (2021). One hundred years of insulin therapy. *Nature reviews. Endocrinology*, *17*(12), 715–725. https://doi.org/10.1038/s41574-021-00542-w

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, *28*(7), 412–419. https://doi.org/10.1007/BF00280883

Mauvais-Jarvis, F., Clegg, D. J., & Hevener, A. L. (2013). The role of estrogens in control of energy balance and glucose homeostasis. *Endocrine reviews*, *34*(3), 309–338. https://doi.org/10.1210/er.2012-1055

McClean, P. L., Irwin, N., Cassidy, R. S., Holst, J. J., Gault, V. A., & Flatt, P. R. (2007). GIP receptor antagonism reverses obesity, insulin resistance, and associated metabolic disturbances induced in mice by prolonged consumption of high-fat diet. *American journal of physiology. Endocrinology and metabolism*, *293*(6), E1746–E1755. https://doi.org/10.1152/ajpendo.00460.2007

McClellan, K. M., Parker, K. L., & Tobet, S. (2006). Development of the ventromedial nucleus of the hypothalamus. *Frontiers in neuroendocrinology*, *27*(2), 193–209. https://doi.org/10.1016/j.yfrne.2006.02.002

McCowen, K. C., Maykel, J. A., Bistrian, B. R., & Ling, P. R. (2002). Circulating ghrelin concentrations are lowered by intravenous glucose or hyperinsulinemic euglycemic conditions in rodents. *The Journal of endocrinology*, *175*(2), R7–R11. https://doi.org/10.1677/joe.0.175r007

McCutcheon, J. E., & Williams, D. L. (2021). Introduction to the special issue: Homeostatic vs. Hedonic feeding. *Physiology & behavior*, *236*, 113415. https://doi.org/10.1016/j.physbeh.2021.113415

Merino, B., Díez-Fernández, C., Ruiz-Gayo, M., & Somoza, B. (2006). Choroid plexus epithelial cells co-express the long and short form of the leptin receptor. *Neuroscience letters*, *393*(2-3), 269–272. https://doi.org/10.1016/j.neulet.2005.10.003

Merrill, G. F., Kurth, E. J., Hardie, D. G., & Winder, W. W. (1997). AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *The American journal of physiology*, *273*(6), E1107–E1112. https://doi.org/10.1152/ajpendo.1997.273.6.E1107

Milanski, M., Degasperi, G., Coope, A., Morari, J., Denis, R., Cintra, D. E., Tsukumo, D. M., Anhe, G., Amaral, M. E., Takahashi, H. K., Curi, R., Oliveira, H. C., Carvalheira, J. B., Bordin, S., Saad, M. J., & Velloso, L. A. (2009). Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(2), 359–370. https://doi.org/10.1523/JNEUROSCI.2760-08.2009

Millán, C., Martínez, F., Cortés-Campos, C., Lizama, I., Yañez, M. J., Llanos, P., Reinicke, K., Rodríguez, F., Peruzzo, B., Nualart, F., & García, M. A. (2010). Glial glucokinase expression in adult and post-natal development of the hypothalamic region. *ASN neuro*, *2*(3), e00035. https://doi.org/10.1042/AN20090059

Miller, R. A., Chu, Q., Xie, J., Foretz, M., Viollet, B., & Birnbaum, M. J. (2013). Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature*, *494*(7436), 256–260. https://doi.org/10.1038/nature11808

Million, M., & Larauche, M. (2016). Stress, sex, and the enteric nervous system. *Neurogastroenterology and motility*, *28*(9), 1283–1289. https://doi.org/10.1111/nmo.12937

Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y. B., Lee, A., Xue, B., Mu, J., Foufelle, F., Ferré, P., Birnbaum, M. J., Stuck, B. J., & Kahn, B. B. (2004). AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature*, *428*(6982), 569–574. https://doi.org/10.1038/nature02440

Minokoshi, Y., Haque, M. S., & Shimazu, T. (1999). Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. *Diabetes*, *48*(2), 287–291. https://doi.org/10.2337/diabetes.48.2.287

Minokoshi, Y., Kim, Y. B., Peroni, O. D., Fryer, L. G., Müller, C., Carling, D., & Kahn, B. B. (2002). Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, *415*(6869), 339–343. https://doi.org/10.1038/415339a

Minokoshi, Y., Kim, Y. B., Peroni, O. D., Fryer, L. G., Müller, C., Carling, D., & Kahn, B. B. (2002). Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, *415*(6869), 339–343. https://doi.org/10.1038/415339a

Mirzadeh, Z., Kusne, Y., Duran-Moreno, M., Cabrales, E., Gil-Perotin, S., Ortiz, C., Chen, B., Garcia-Verdugo, J. M., Sanai, N., & Alvarez-Buylla, A. (2017). Bi- and uniciliated ependymal cells define continuous floor-plate-derived tanycytic territories. *Nature communications*, *8*, 13759. https://doi.org/10.1038/ncomms13759

Miselis, R. R., & Epstein, A. N. (1975). Feeding induced by intracerebroventricular 2-deoxy-D-glucose in the rat. *The American journal of physiology*, *229*(5), 1438–1447. https://doi.org/10.1152/ajplegacy.1975.229.5.1438

Montague, C. T., Farooqi, I. S., Whitehead, J. P., Soos, M. A., Rau, H., Wareham, N. J., Sewter, C. P., Digby, J. E., Mohammed, S. N., Hurst, J. A., Cheetham, C. H., Earley, A. R., Barnett, A. H., Prins, J. B., & O'Rahilly, S. (1997). Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*, *387*(6636), 903–908. https://doi.org/10.1038/43185

Monteleone, A. M., Piscitelli, F., Dalle Grave, R., El Ghoch, M., Di Marzo, V., Maj, M., & Monteleone, P. (2017). Peripheral Endocannabinoid Responses to Hedonic Eating in Binge-Eating Disorder. *Nutrients*, *9*(12), 1377. https://doi.org/10.3390/nu9121377

Moon, H. S., Dalamaga, M., Kim, S. Y., Polyzos, S. A., Hamnvik, O. P., Magkos, F., Paruthi, J., & Mantzoros, C. S. (2013). Leptin's role in lipodystrophic and nonlipodystrophic insulin-resistant and diabetic individuals. *Endocrine reviews*, *34*(3), 377–412. https://doi.org/10.1210/er.2012-1053

Morita, S., Furube, E., Mannari, T., Okuda, H., Tatsumi, K., Wanaka, A., & Miyata, S. (2016). Heterogeneous vascular permeability and alternative diffusion barrier in sensory circumventricular organs of adult mouse brain. *Cell and tissue research*, *363*(2), 497–511. https://doi.org/10.1007/s00441-015-2207-7

Morrison C. D. (2009). Leptin signaling in brain: A link between nutrition and cognition?. Biochimica et biophysica acta, 1792(5), 401–408. https://doi.org/10.1016/j.bbadis.2008.12.004

Morrison, S. F., Madden, C. J., & Tupone, D. (2014). Central neural regulation of brown adipose tissue thermogenesis and energy expenditure. *Cell metabolism*, *19*(5), 741–756. https://doi.org/10.1016/j.cmet.2014.02.007

Morton, G. J., & Schwartz, M. W. (2001). The NPY/AgRP neuron and energy homeostasis. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity*, *25 Suppl 5*, S56–S62. https://doi.org/10.1038/sj.ijo.0801915

Mountjoy, K. G., Robbins, L. S., Mortrud, M. T., & Cone, R. D. (1992). The cloning of a family of genes that encode the melanocortin receptors. *Science (New York, N.Y.)*, *257*(5074), 1248–1251. https://doi.org/10.1126/science.1325670

Moura, F. A., Scirica, B. M., & Ruff, C. T. (2022). Tirzepatide for diabetes: on track to SURPASS current therapy. *Nature medicine*, *28*(3), 450–451. https://doi.org/10.1038/s41591-022-01733-2

Mroz, P. A., Finan, B., Gelfanov, V., Yang, B., Tschöp, M. H., DiMarchi, R. D., & Perez-Tilve, D. (2019). Optimized GIP analogs promote body weight lowering in mice through GIPR agonism not antagonism. *Molecular metabolism*, *20*, 51–62. https://doi.org/10.1016/j.molmet.2018.12.001

Mulherin, A. J., Oh, A. H., Kim, H., Grieco, A., Lauffer, L. M., & Brubaker, P. L. (2011). Mechanisms underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell. *Endocrinology*, *152*(12), 4610–4619. https://doi.org/10.1210/en.2011-1485

Müller-Fielitz, H., Stahr, M., Bernau, M., Richter, M., Abele, S., Krajka, V., Benzin, A., Wenzel, J., Kalies, K., Mittag, J., Heuer, H., Offermanns, S., & Schwaninger, M. (2017). Tanycytes control the hormonal output of the hypothalamic-pituitary-thyroid axis. *Nature communications*, *8*(1), 484. https://doi.org/10.1038/s41467-017-00604-6

Müller, T. D., Blüher, M., Tschöp, M. H., & DiMarchi, R. D. (2022). Anti-obesity drug discovery: advances and challenges. *Nature reviews. Drug discovery*, *21*(3), 201–223. https://doi.org/10.1038/s41573-021-00337-8

Mullier, A., Bouret, S. G., Prevot, V., & Dehouck, B. (2010). Differential distribution of tight junction proteins suggests a role for tanycytes in blood-hypothalamus barrier regulation in the adult mouse brain. *The Journal of comparative neurology*, *518*(7), 943–962. https://doi.org/10.1002/cne.22273

Mullier, A., Bouret, S. G., Prevot, V., & Dehouck, B. (2010). Differential distribution of tight junction proteins suggests a role for tanycytes in blood-hypothalamus barrier regulation in the adult mouse brain. *The Journal of comparative neurology*, *518*(7), 943–962. https://doi.org/10.1002/cne.22273

Münzberg, H., Flier, J. S., & Bjørbaek, C. (2004). Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology*, *145*(11), 4880–4889. https://doi.org/10.1210/en.2004-0726

Myers M. G., Jr (2004). Leptin receptor signaling and the regulation of mammalian physiology. *Recent progress in hormone research*, *59*, 287–304. https://doi.org/10.1210/rp.59.1.287

Myers, M. G., & Leibel, R. L. (2015). Lessons From Rodent Models of Obesity. In K. R. Feingold (Eds.) et. al., *Endotext*. MDText.com, Inc.

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., & Matsukura, S. (2001). A role for ghrelin in the central regulation of feeding. *Nature*, *409*(6817), 194–198. https://doi.org/10.1038/35051587

NamKoong, C., Kim, M. S., Jang, B. T., Lee, Y. H., Cho, Y. M., & Choi, H. J. (2017). Central administration of GLP-1 and GIP decreases feeding in mice. *Biochemical and biophysical research communications*, *490*(2), 247–252. https://doi.org/10.1016/j.bbrc.2017.06.031

Nampoothiri, S., Nogueiras, R., Schwaninger, M., & Prevot, V. (2022). Glial cells as integrators of peripheral and central signals in the regulation of energy homeostasis. *Nature metabolism*, *4*(7), 813–825. https://doi.org/10.1038/s42255-022-00610-z

Nance, D. M., & Sanders, V. M. (2007). Autonomic innervation and regulation of the immune system (1987-2007). *Brain, behavior, and immunity*, *21*(6), 736–745. https://doi.org/10.1016/j.bbi.2007.03.008

Napolitano, A., Miller, S., Nicholls, A. W., Baker, D., Van Horn, S., Thomas, E., Rajpal, D., Spivak, A., Brown, J. R., & Nunez, D. J. (2014). Novel gut-based pharmacology of metformin in patients with type 2 diabetes mellitus. *PloS one*, *9*(7), e100778. https://doi.org/10.1371/journal.pone.0100778

Nedergaard, J., Bengtsson, T., & Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *American journal of physiology. Endocrinology and metabolism*, *293*(2), E444–E452. https://doi.org/10.1152/ajpendo.00691.2006

Neel J. V. (1962). Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"?. *American journal of human genetics*, *14*(4), 353–362.

Ng, A. C. T., Delgado, V., Borlaug, B. A., & Bax, J. J. (2021). Diabesity: the combined burden of obesity and diabetes on heart disease and the role of imaging. *Nature reviews. Cardiology*, *18*(4), 291–304. https://doi.org/10.1038/s41569-020-00465-5

Nguyen, A. D., Mitchell, N. F., Lin, S., Macia, L., Yulyaningsih, E., Baldock, P. A., Enriquez, R. F., Zhang, L., Shi, Y. C., Zolotukhin, S., Herzog, H., & Sainsbury, A. (2012). Y1 and Y5 receptors are both required for the regulation of food intake and energy homeostasis in mice. *PloS one*, *7*(6), e40191. https://doi.org/10.1371/journal.pone.0040191

Nianogo, R. A., Rosenwohl-Mack, A., Yaffe, K., Carrasco, A., Hoffmann, C. M., & Barnes, D. E. (2022). Risk Factors Associated With Alzheimer Disease and Related Dementias by Sex and Race and Ethnicity in the US. *JAMA neurology*, *79*(6), 584–591. https://doi.org/10.1001/jamaneurol.2022.0976

Nieto-Vazquez, I., Fernández-Veledo, S., Krämer, D. K., Vila-Bedmar, R., Garcia-Guerra, L., & Lorenzo, M. (2008). Insulin resistance associated to obesity: the link TNF-alpha. *Archives of physiology and biochemistry*, *114*(3), 183–194. https://doi.org/10.1080/13813450802181047

Nijenhuis, W. A., Oosterom, J., & Adan, R. A. (2001). AgRP(83-132) acts as an inverse agonist on the human-melanocortin-4 receptor. *Molecular endocrinology (Baltimore, Md.)*, *15*(1), 164–171. https://doi.org/10.1210/mend.15.1.0578

Nitta, T., Hata, M., Gotoh, S., Seo, Y., Sasaki, H., Hashimoto, N., Furuse, M., & Tsukita, S. (2003). Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. *The Journal of cell biology*, *161*(3), 653–660. https://doi.org/10.1083/jcb.200302070

Nogueiras, R., Gallego, R., Gualillo, O., Caminos, J. E., García-Caballero, T., Casanueva, F. F., & Diéguez, C. (2003). Resistin is expressed in different rat tissues and is regulated in a tissue- and gender-specific manner. *FEBS letters*, *548*(1-3), 21–27. https://doi.org/10.1016/s0014-5793(03)00708-7

Nogueiras, R., López, M., & Diéguez, C. (2010). Regulation of lipid metabolism by energy availability: a role for the central nervous system. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, *11*(3), 185–201. https://doi.org/10.1111/j.1467-789X.2009.00669.x

Nogueiras, R., Pérez-Tilve, D., Veyrat-Durebex, C., Morgan, D. A., Varela, L., Haynes, W. G., Patterson, J. T., Disse, E., Pfluger, P. T., López, M., Woods, S. C., DiMarchi, R., Diéguez, C., Rahmouni, K., Rohner-Jeanrenaud, F., & Tschöp, M. H. (2009). Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted in diet-induced obesity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(18), 5916–5925. https://doi.org/10.1523/JNEUROSCI.5977-08.2009

Nogueiras, R., Wiedmer, P., Perez-Tilve, D., Veyrat-Durebex, C., Keogh, J. M., Sutton, G. M., Pfluger, P. T., Castaneda, T. R., Neschen, S., Hofmann, S. M., Howles, P. N., Morgan, D. A., Benoit, S. C., Szanto, I., Schrott, B., Schürmann, A., Joost, H. G., Hammond, C., Hui, D. Y., Woods, S. C., … Tschöp, M. H. (2007). The central melanocortin system directly controls peripheral lipid metabolism. *The Journal of clinical investigation*, *117*(11), 3475–3488. https://doi.org/10.1172/JCI31743

Nonogaki K. (2000). New insights into sympathetic regulation of glucose and fat metabolism. *Diabetologia*, *43*(5), 533–549. https://doi.org/10.1007/s001250051341

Nutrition Classics. The Anatomical Record, Volume 78, 1940: Hypothalamic lesions and adiposity in the rat. (1983). *Nutrition reviews*, *41*(4), 124–127. https://doi.org/10.1111/j.1753-4887.1983.tb07169.x

Obici, S., Feng, Z., Karkanias, G., Baskin, D. G., & Rossetti, L. (2002). Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nature neuroscience*, *5*(6), 566–572. https://doi.org/10.1038/nn0602-861

Obici, S., Feng, Z., Tan, J., Liu, L., Karkanias, G., & Rossetti, L. (2001). Central melanocortin receptors regulate insulin action. *The Journal of clinical investigation*, *108*(7), 1079–1085. https://doi.org/10.1172/JCI12954

Ogus, S., Ke, Y., Qiu, J., Wang, B., & Chehab, F. F. (2003). Hyperleptinemia precipitates diet-induced obesity in transgenic mice overexpressing leptin. *Endocrinology*, *144*(7), 2865–2869. https://doi.org/10.1210/en.2002-0178

Oken, E., & Gillman, M. W. (2003). Fetal origins of obesity. *Obesity research*, *11*(4), 496–506. https://doi.org/10.1038/oby.2003.69

Ollmann, M. M., Wilson, B. D., Yang, Y. K., Kerns, J. A., Chen, Y., Gantz, I., & Barsh, G. S. (1997). Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science (New York, N.Y.)*, *278*(5335), 135–138. https://doi.org/10.1126/science.278.5335.135

Ord W M. Report of a committee of the Clinical Society of London nominated December 14, 1883, to investigate the subject of myxoedema. *Trans Clin Soc Lond* 188821 (supp)

Orellana, J. A., Sáez, P. J., Cortés-Campos, C., Elizondo, R. J., Shoji, K. F., Contreras-Duarte, S., Figueroa, V., Velarde, V., Jiang, J. X., Nualart, F., Sáez, J. C., & García, M. A. (2012). Glucose increases intracellular free Ca(2+) in tanycytes via ATP released through connexin 43 hemichannels. *Glia*, *60*(1), 53–68. https://doi.org/10.1002/glia.21246

Ouchi, N., Kihara, S., Funahashi, T., Matsuzawa, Y., & Walsh, K. (2003). Obesity, adiponectin and vascular inflammatory disease. *Current opinion in lipidology*, *14*(6), 561–566. https://doi.org/10.1097/00041433-200312000-00003

Oveisi, F., Gaetani, S., Eng, K. T., & Piomelli, D. (2004). Oleoylethanolamide inhibits food intake in free-feeding rats after oral administration. *Pharmacological research*, *49*(5), 461–466. https://doi.org/10.1016/j.phrs.2003.12.006

Owen, M. R., Doran, E., & Halestrap, A. P. (2000). Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *The Biochemical journal*, *348 Pt 3*(Pt 3), 607–614.

Ozcan, L., Ergin, A. S., Lu, A., Chung, J., Sarkar, S., Nie, D., Myers, M. G., Jr, & Ozcan, U. (2009). Endoplasmic reticulum stress plays a central role in development of leptin resistance. *Cell metabolism*, *9*(1), 35–51. https://doi.org/10.1016/j.cmet.2008.12.004

Pagotto, U., Marsicano, G., Cota, D., Lutz, B., & Pasquali, R. (2006). The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocrine reviews*, *27*(1), 73–100. https://doi.org/10.1210/er.2005-0009

Pala, L., & Rotella, C. M. (2014). The "slower" the better. *Journal of endocrinological investigation*, *37*(5), 497–498. https://doi.org/10.1007/s40618-014-0065-x

Palkovits, M., Mezey, E., & Eskay, R. L. (1987). Pro-opiomelanocortin-derived peptides (ACTH/beta-endorphin/alpha-MSH) in brainstem baroreceptor areas of the rat. *Brain research*, *436*(2), 323–338. https://doi.org/10.1016/0006-8993(87)91676-3

Pannacciulli, N., Le, D. S., Salbe, A. D., Chen, K., Reiman, E. M., Tataranni, P. A., & Krakoff, J. (2007). Postprandial glucagon-like peptide-1 (GLP-1) response is positively associated with changes in neuronal activity of brain areas implicated in satiety and food intake regulation in humans. NeuroImage, 35(2), 511–517. https://doi.org/10.1016/j.neuroimage.2006.12.035

Papavramidou, N., & Christopoulou-Aletra, H. (2008). Management of obesity in the writings of Soranus of Ephesus and Caelius Aurelianus. *Obesity surgery*, *18*(6), 763–765. https://doi.org/10.1007/s11695-007-9362-1

Pappas, T. N., Debas, H. T., Goto, Y., & Taylor, I. L. (1985). Peptide YY inhibits meal-stimulated pancreatic and gastric secretion. *The American journal of physiology*, *248*(1 Pt 1), G118–G123. https://doi.org/10.1152/ajpgi.1985.248.1.G118

Paranjape, S. A., Chan, O., Zhu, W., Horblitt, A. M., Grillo, C. A., Wilson, S., Reagan, L., & Sherwin, R. S. (2011). Chronic reduction of insulin receptors in the ventromedial hypothalamus produces glucose intolerance and islet dysfunction in the absence of weight gain. *American journal of physiology. Endocrinology and metabolism*, *301*(5), E978–E983. https://doi.org/10.1152/ajpendo.00304.2011

Paranjape, S. A., Chan, O., Zhu, W., Horblitt, A. M., McNay, E. C., Cresswell, J. A., Bogan, J. S., McCrimmon, R. J., & Sherwin, R. S. (2010). Influence of insulin in the ventromedial hypothalamus on pancreatic glucagon secretion in vivo. *Diabetes*, *59*(6), 1521–1527. https://doi.org/10.2337/db10-0014

Parton, L. E., Ye, C. P., Coppari, R., Enriori, P. J., Choi, B., Zhang, C. Y., Xu, C., Vianna, C. R., Balthasar, N., Lee, C. E., Elmquist, J. K., Cowley, M. A., & Lowell, B. B. (2007). Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. *Nature*, *449*(7159), 228–232. https://doi.org/10.1038/nature06098

Pastor-Villaescusa, B., Cañete, M. D., Caballero-Villarraso, J., Hoyos, R., Latorre, M., Vázquez-Cobela, R., Plaza-Díaz, J., Maldonado, J., Bueno, G., Leis, R., Gil, Á., Cañete, R., & Aguilera, C. M. (2017). Metformin for Obesity in Prepubertal and Pubertal Children: A Randomized Controlled Trial. *Pediatrics*, *140*(1), e20164285. https://doi.org/10.1542/peds.2016-4285

Peitz, M., Pfannkuche, K., Rajewsky, K., & Edenhofer, F. (2002). Ability of the hydrophobic FGF and basic TAT peptides to promote cellular uptake of recombinant Cre recombinase: a tool for efficient genetic engineering of mammalian genomes. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(7), 4489–4494. https://doi.org/10.1073/pnas.032068699

Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T., & Collins, F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science (New York, N.Y.)*, *269*(5223), 540–543. https://doi.org/10.1126/science.7624776

Pena-Leon, V., Folgueira, C., Barja-Fernández, S., Pérez-Lois, R., Da Silva Lima, N., Martin, M., Heras, V., Martinez-Martinez, S., Valero, P., Iglesias, C., Duquenne, M., Al-Massadi, O., Beiroa, D., Souto, Y., Fidalgo, M., Sowmyalakshmi, R., Guallar, D., Cunarro, J., Castelao, C., Senra, A., … Seoane, L. M. (2022). Prolonged breastfeeding protects from obesity by hypothalamic action of hepatic FGF21. *Nature metabolism*, *4*(7), 901–917. https://doi.org/10.1038/s42255-022-00602-z

Penhos, J. C., Wu, C. H., Daunas, J., Reitman, M., & Levine, R. (1966). Effect of glucagon on the metabolism of lipids and on urea formation by the perfused rat liver. *Diabetes*, *15*(10), 740–748. https://doi.org/10.2337/diab.15.10.740

Peppiatt, C. M., Howarth, C., Mobbs, P., & Attwell, D. (2006). Bidirectional control of CNS capillary diameter by pericytes. *Nature*, *443*(7112), 700–704. https://doi.org/10.1038/nature05193

Pereira, M. J., & Eriksson, J. W. (2019). Emerging Role of SGLT-2 Inhibitors for the Treatment of Obesity. *Drugs*, *79*(3), 219–230. https://doi.org/10.1007/s40265-019-1057-0

Peress, N. S., & Tompkins, D. (1981). Effect of molecular charge on choroid-plexus permeability: Tracer studies with cationized ferritins. *Cell and tissue research*, *219*(2), 425–431. https://doi.org/10.1007/BF00210160

Perkins RG. (1919) A Study of the Munitions intoxications in France. Public Health Rep. (1896-1970);34:2335. doi: 10.2307/4575357.

Peruzzo, B., Pastor, F. E., Blázquez, J. L., Amat, P., & Rodríguez, E. M. (2004). Polarized endocytosis and transcytosis in the hypothalamic tanycytes of the rat. *Cell and tissue research*, *317*(2), 147–164. https://doi.org/10.1007/s00441-004-0899-1

Petrov, T., Howarth, A. G., Krukoff, T. L., & Stevenson, B. R. (1994). Distribution of the tight junction-associated protein ZO-1 in circumventricular organs of the CNS. *Brain research. Molecular brain research*, *21*(3-4), 235–246. https://doi.org/10.1016/0169-328x(94)90254-2

Pi-Sunyer, X., Astrup, A., Fujioka, K., Greenway, F., Halpern, A., Krempf, M., Lau, D. C., le Roux, C. W., Violante Ortiz, R., Jensen, C. B., Wilding, J. P., & SCALE Obesity and Prediabetes NN8022-1839 Study Group (2015). A Randomized, Controlled Trial of 3.0 mg of Liraglutide in Weight Management. *The New England journal of medicine*, *373*(1), 11–22. https://doi.org/10.1056/NEJMoa1411892

Piper, M. L., Unger, E. K., Myers, M. G., Jr, & Xu, A. W. (2008). Specific physiological roles for signal transducer and activator of transcription 3 in leptin receptor-expressing neurons. *Molecular endocrinology (Baltimore, Md.)*, *22*(3), 751–759. https://doi.org/10.1210/me.2007-0389

Poggioli, R., Vergoni, A. V., & Bertolini, A. (1986). ACTH-(1-24) and alpha-MSH antagonize feeding behavior stimulated by kappa opiate agonists. *Peptides*, *7*(5), 843–848. https://doi.org/10.1016/0196-9781(86)90104-x

Pontzer, H., Raichlen, D. A., Wood, B. M., Mabulla, A. Z., Racette, S. B., & Marlowe, F. W. (2012). Hunter-gatherer energetics and human obesity. *PloS one*, *7*(7), e40503. https://doi.org/10.1371/journal.pone.0040503

Porniece Kumar, M., Cremer, A. L., Klemm, P., Steuernagel, L., Sundaram, S., Jais, A., Hausen, A. C., Tao, J., Secher, A., Pedersen, T. Å., Schwaninger, M., Wunderlich, F. T., Lowell, B. B., Backes, H., & Brüning, J. C. (2021). Insulin signalling in tanycytes gates hypothalamic insulin uptake and regulation of AgRP neuron activity. *Nature metabolism*, *3*(12), 1662–1679. https://doi.org/10.1038/s42255-021-00499-0

Porniece Kumar, M., Cremer, A. L., Klemm, P., Steuernagel, L., Sundaram, S., Jais, A., Hausen, A. C., Tao, J., Secher, A., Pedersen, T. Å., Schwaninger, M., Wunderlich, F. T., Lowell, B. B., Backes, H., & Brüning, J. C. (2021). Insulin signalling in tanycytes gates hypothalamic insulin uptake and regulation of AgRP neuron activity. *Nature metabolism*, *3*(12), 1662–1679. https://doi.org/10.1038/s42255-021-00499-0

Porteiro, B., Díaz-Ruíz, A., Martínez, G., Senra, A., Vidal, A., Serrano, M., Gualillo, O., López, M., Malagón, M. M., Diéguez, C., & Nogueiras, R. (2013). Ghrelin requires p53 to stimulate lipid storage in fat and liver. *Endocrinology*, *154*(10), 3671–3679. https://doi.org/10.1210/en.2013-1176

Powis, J. E., Bains, J. S., & Ferguson, A. V. (1998). Leptin depolarizes rat hypothalamic paraventricular nucleus neurons. *The American journal of physiology*, *274*(5), R1468–R1472. https://doi.org/10.1152/ajpregu.1998.274.5.R1468

Pratley, R. E., Aroda, V. R., Lingvay, I., Lüdemann, J., Andreassen, C., Navarria, A., Viljoen, A., & SUSTAIN 7 investigators (2018). Semaglutide versus dulaglutide once weekly in patients with type 2 diabetes (SUSTAIN 7): a randomised, open-label, phase 3b trial. *The lancet. Diabetes & endocrinology*, *6*(4), 275–286. https://doi.org/10.1016/S2213-8587(18)30024-X

Prattichizzo, F., Giuliani, A., Mensà, E., Sabbatinelli, J., De Nigris, V., Rippo, M. R., La Sala, L., Procopio, A. D., Olivieri, F., & Ceriello, A. (2018). Pleiotropic effects of metformin: Shaping the microbiome to manage type 2 diabetes and postpone ageing. *Ageing research reviews*, *48*, 87–98. https://doi.org/10.1016/j.arr.2018.10.003

Prevot, V., Dehouck, B., Sharif, A., Ciofi, P., Giacobini, P., & Clasadonte, J. (2018). The Versatile Tanycyte: A Hypothalamic Integrator of Reproduction and Energy Metabolism. *Endocrine reviews*, *39*(3), 333–368. https://doi.org/10.1210/er.2017-00235

Prevot, V., Nogueiras, R., & Schwaninger, M. (2021). Tanycytes in the infundibular nucleus and median eminence and their role in the blood-brain barrier. *Handbook of clinical neurology*, *180*, 253–273. https://doi.org/10.1016/B978-0-12-820107-7.00016-1

Prillaman M. (2023). Why BMI is flawed - and how to redefine obesity. *Nature*, *622*(7982), 232–233. https://doi.org/10.1038/d41586-023-03143-x

Prinzmetal M. (1935) The Use of Benzedrine for the Treatment of Narcolepsy. Journal of the American Medical Association.;105(25):2051-2054. DOI: .10.1001/jama.1935.02760510023006

Pu, R., Shi, D., Gan, T., Ren, X., Ba, Y., Huo, Y., Bai, Y., Zheng, T., & Cheng, N. (2020). Effects of metformin in obesity treatment in different populations: a meta-analysis. *Therapeutic advances in endocrinology and metabolism*, *11*, 2042018820926000. https://doi.org/10.1177/2042018820926000

Qiu, J., Wagner, E. J., Rønnekleiv, O. K., & Kelly, M. J. (2018). Insulin and leptin excite anorexigenic pro-opiomelanocortin neurones via activation of TRPC5 channels. *Journal of neuroendocrinology*, *30*(2), 10.1111/jne.12501. https://doi.org/10.1111/jne.12501

Quarta, C., Claret, M., Zeltser, L. M., Williams, K. W., Yeo, G. S. H., Tschöp, M. H., Diano, S., Brüning, J. C., & Cota, D. (2021). POMC neuronal heterogeneity in energy balance and beyond: an integrated view. *Nature metabolism*, *3*(3), 299–308. https://doi.org/10.1038/s42255-021-00345-3

Quesada, I., Tudurí, E., Ripoll, C., & Nadal, A. (2008). Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and diabetes. *The Journal of endocrinology*, *199*(1), 5–19. https://doi.org/10.1677/JOE-08-0290

Quiñones, M., Al-Massadi, O., Gallego, R., Fernø, J., Diéguez, C., López, M., & Nogueiras, R. (2015). Hypothalamic CaMKKβ mediates glucagon anorectic effect and its diet-induced resistance. *Molecular metabolism*, *4*(12), 961–970. https://doi.org/10.1016/j.molmet.2015.09.014

Rahmouni, K., Sigmund, C. D., Haynes, W. G., & Mark, A. L. (2009). Hypothalamic ERK mediates the anorectic and thermogenic sympathetic effects of leptin. *Diabetes*, *58*(3), 536–542. https://doi.org/10.2337/db08-0822

Raposinho, P. D., White, R. B., & Aubert, M. L. (2003). The melanocortin agonist Melanotan-II reduces the orexigenic and adipogenic effects of neuropeptide Y (NPY) but does not affect the NPY-driven suppressive effects on the gonadotropic and somatotropic axes in the male rat. *Journal of neuroendocrinology*, *15*(2), 173–181. https://doi.org/10.1046/j.1365-2826.2003.00962.x

Rasmussen N. (2008). America's first amphetamine epidemic 1929-1971: a quantitative and qualitative retrospective with implications for the present. *American journal of public health*, *98*(6), 974–985. https://doi.org/10.2105/AJPH.2007.110593

Rasmussen, D. D., Boldt, B. M., Wilkinson, C. W., Yellon, S. M., & Matsumoto, A. M. (1999). Daily melatonin administration at middle age suppresses male rat visceral fat, plasma leptin, and plasma insulin to youthful levels. *Endocrinology*, *140*(2), 1009–1012. https://doi.org/10.1210/endo.140.2.6674

Ravussin, E., Lillioja, S., Anderson, T. E., Christin, L., & Bogardus, C. (1986). Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *The Journal of clinical investigation*, *78*(6), 1568–1578. https://doi.org/10.1172/JCI112749

Recabal, A., Caprile, T., & García-Robles, M. L. A. (2017). Hypothalamic Neurogenesis as an Adaptive Metabolic Mechanism. *Frontiers in neuroscience*, *11*, 190. https://doi.org/10.3389/fnins.2017.00190

Reese, T. S., & Karnovsky, M. J. (1967). Fine structural localization of a blood-brain barrier to exogenous peroxidase. *The Journal of cell biology*, *34*(1), 207–217. https://doi.org/10.1083/jcb.34.1.207

Reilly, S., Grigson, P. S., & Norgren, R. (1993). Parabrachial nucleus lesions and conditioned taste aversion: evidence supporting an associative deficit. *Behavioral neuroscience*, *107*(6), 1005–1017. https://doi.org/10.1037//0735-7044.107.6.1005

Rena, G., Hardie, D. G., & Pearson, E. R. (2017). The mechanisms of action of metformin. *Diabetologia*, *60*(9), 1577–1585. https://doi.org/10.1007/s00125-017-4342-z

Rezai-Zadeh, K., Yu, S., Jiang, Y., Laque, A., Schwartzenburg, C., Morrison, C. D., Derbenev, A. V., Zsombok, A., & Münzberg, H. (2014). Leptin receptor neurons in the dorsomedial hypothalamus are key regulators of energy expenditure and body weight, but not food intake. *Molecular metabolism*, *3*(7), 681–693. https://doi.org/10.1016/j.molmet.2014.07.008

Richard, D., Monge-Roffarello, B., Chechi, K., Labbé, S. M., & Turcotte, E. E. (2012). Control and physiological determinants of sympathetically mediated brown adipose tissue thermogenesis. *Frontiers in endocrinology*, *3*, 36. https://doi.org/10.3389/fendo.2012.00036

Ritter, S., Dinh, T. T., & Zhang, Y. (2000). Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. *Brain research*, *856*(1-2), 37–47. https://doi.org/10.1016/s0006-8993(99)02327-6

Rizzoti, K., & Lovell-Badge, R. (2017). Pivotal role of median eminence tanycytes for hypothalamic function and neurogenesis. *Molecular and cellular endocrinology*, *445*, 7–13. https://doi.org/10.1016/j.mce.2016.08.020

Robins, S. C., Stewart, I., McNay, D. E., Taylor, V., Giachino, C., Goetz, M., Ninkovic, J., Briancon, N., Maratos-Flier, E., Flier, J. S., Kokoeva, M. V., & Placzek, M. (2013). α-Tanycytes of the adult hypothalamic third ventricle include distinct populations of FGF-responsive neural progenitors. *Nature communications*, *4*, 2049. https://doi.org/10.1038/ncomms3049

Rodríguez-Cortés, B., Hurtado-Alvarado, G., Martínez-Gómez, R., León-Mercado, L. A., Prager-Khoutorsky, M., & Buijs, R. M. (2022). Suprachiasmatic nucleus-mediated glucose entry into the arcuate nucleus determines the daily rhythm in blood glycemia. *Current biology : CB*, *32*(4), 796–805.e4. https://doi.org/10.1016/j.cub.2021.12.039

Rodriguez-Diaz, R., & Caicedo, A. (2014). Neural control of the endocrine pancreas. *Best practice & research. Clinical endocrinology & metabolism*, *28*(5), 745–756. https://doi.org/10.1016/j.beem.2014.05.002

Rodríguez, E., Guerra, M., Peruzzo, B., & Blázquez, J. L. (2019). Tanycytes: A rich morphological history to underpin future molecular and physiological investigations. *Journal of neuroendocrinology*, *31*(3), e12690. https://doi.org/10.1111/jne.12690

Rohrbach, A., Caron, E., Dali, R., Brunner, M., Pasquettaz, R., Kolotuev, I., Santoni, F., Thorens, B., & Langlet, F. (2021). Ablation of glucokinase-expressing tanycytes impacts energy balance and increases adiposity in mice. *Molecular metabolism*, *53*, 101311. https://doi.org/10.1016/j.molmet.2021.101311

Rolland-Cachera, M. F., Deheeger, M., Maillot, M., & Bellisle, F. (2006). Early adiposity rebound: causes and consequences for obesity in children and adults. *International journal of obesity (2005)*, *30 Suppl 4*, S11–S17. https://doi.org/10.1038/sj.ijo.0803514

Rolls E. T. (2007). Sensory processing in the brain related to the control of food intake. *The Proceedings of the Nutrition Society*, *66*(1), 96–112. https://doi.org/10.1017/S0029665107005332

Rosenstock, J., Wysham, C., Frías, J. P., Kaneko, S., Lee, C. J., Fernández Landó, L., Mao, H., Cui, X., Karanikas, C. A., & Thieu, V. T. (2021). Efficacy and safety of a novel dual GIP and GLP-1 receptor agonist tirzepatide in patients with type 2 diabetes (SURPASS-1): a double-blind, randomised, phase 3 trial. *Lancet (London, England)*, *398*(10295), 143–155. https://doi.org/10.1016/S0140-6736(21)01324-6

Rosenthal, R., Günzel, D., Theune, D., Czichos, C., Schulzke, J. D., & Fromm, M. (2017). Water channels and barriers formed by claudins. *Annals of the New York Academy of Sciences*, *1397*(1), 100–109. https://doi.org/10.1111/nyas.13383

Rossi, M. A., & Stuber, G. D. (2018). Overlapping Brain Circuits for Homeostatic and Hedonic Feeding. *Cell metabolism*, *27*(1), 42–56. https://doi.org/10.1016/j.cmet.2017.09.021

Rossi, M. A., & Stuber, G. D. (2018). Overlapping Brain Circuits for Homeostatic and Hedonic Feeding. *Cell metabolism*, *27*(1), 42–56. https://doi.org/10.1016/j.cmet.2017.09.021

Ruan, H. B., Dietrich, M. O., Liu, Z. W., Zimmer, M. R., Li, M. D., Singh, J. P., Zhang, K., Yin, R., Wu, J., Horvath, T. L., & Yang, X. (2014). O-GlcNAc transferase enables AgRP neurons to suppress browning of white fat. *Cell*, *159*(2), 306–317. https://doi.org/10.1016/j.cell.2014.09.010

Rubino, D., Abrahamsson, N., Davies, M., Hesse, D., Greenway, F. L., Jensen, C., Lingvay, I., Mosenzon, O., Rosenstock, J., Rubio, M. A., Rudofsky, G., Tadayon, S., Wadden, T. A., Dicker, D., & STEP 4 Investigators (2021). Effect of Continued Weekly Subcutaneous Semaglutide vs Placebo on Weight Loss Maintenance in Adults With Overweight or Obesity: The STEP 4 Randomized Clinical Trial. *JAMA*, *325*(14), 1414–1425. https://doi.org/10.1001/jama.2021.3224

Rui L. (2013). Brain regulation of energy balance and body weight. *Reviews in endocrine & metabolic disorders*, *14*(4), 387–407. https://doi.org/10.1007/s11154-013-9261-9

Russell, A. W., Horowitz, M., Ritz, M., MacIntosh, C., Fraser, R., & Chapman, I. M. (2001). The effect of acute hyperglycaemia on appetite and food intake in Type 1 diabetes mellitus. *Diabetic medicine : a journal of the British Diabetic Association*, *18*(9), 718–725. https://doi.org/10.1046/j.1464-5491.2001.00545.x

Ryan D. H. (2021). Next Generation Antiobesity Medications: Setmelanotide, Semaglutide, Tirzepatide and Bimagrumab: What do They Mean for Clinical Practice?. *Journal of obesity & metabolic syndrome*, *30*(3), 196–208. https://doi.org/10.7570/jomes21033

Ryan, D. H., Bray, G. A., Helmcke, F., Sander, G., Volaufova, J., Greenway, F., Subramaniam, P., & Glancy, D. L. (1999). Serial echocardiographic and clinical evaluation of valvular regurgitation before, during, and after treatment with fenfluramine or dexfenfluramine and mazindol or phentermine. *Obesity research*, *7*(4), 313–322. https://doi.org/10.1002/j.1550-8528.1999.tb00414.x

Ryan, K. K., Li, B., Grayson, B. E., Matter, E. K., Woods, S. C., & Seeley, R. J. (2011). A role for central nervous system PPAR-γ in the regulation of energy balance. *Nature medicine*, *17*(5), 623–626. https://doi.org/10.1038/nm.2349

Ryo, M., Nakamura, T., Kihara, S., Kumada, M., Shibazaki, S., Takahashi, M., Nagai, M., Matsuzawa, Y., & Funahashi, T. (2004). Adiponectin as a biomarker of the metabolic syndrome. *Circulation journal : official journal of the Japanese Circulation Society*, *68*(11), 975–981. https://doi.org/10.1253/circj.68.975

Sajan, M. P., Bandyopadhyay, G., Miura, A., Standaert, M. L., Nimal, S., Longnus, S. L., Van Obberghen, E., Hainault, I., Foufelle, F., Kahn, R., Braun, U., Leitges, M., & Farese, R. V. (2010). AICAR and metformin, but not exercise, increase muscle glucose transport through AMPK-, ERK-, and PDK1-dependent activation of atypical PKC. *American journal of physiology. Endocrinology and metabolism*, *298*(2), E179–E192. https://doi.org/10.1152/ajpendo.00392.2009

Saltiel, A. R., & Kahn, C. R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*, *414*(6865), 799–806. https://doi.org/10.1038/414799a

Samuel PD, Burland WL. (1975) Drug Treatment of Obesity, in Obesity in perspective : a conference., *U.S. Govt. Print. Off.: Washington.*

Sánchez, E., Vargas, M. A., Singru, P. S., Pascual, I., Romero, F., Fekete, C., Charli, J. L., & Lechan, R. M. (2009). Tanycyte pyroglutamyl peptidase II contributes to regulation of the hypothalamic-pituitary-thyroid axis

through glial-axonal associations in the median eminence. *Endocrinology*, *150*(5), 2283–2291. https://doi.org/10.1210/en.2008-1643

Sanders, N. M., Dunn-Meynell, A. A., & Levin, B. E. (2004). Third ventricular alloxan reversibly impairs glucose counterregulatory responses. *Diabetes*, *53*(5), 1230–1236. https://doi.org/10.2337/diabetes.53.5.1230

Sanders, N. M., Dunn-Meynell, A. A., & Levin, B. E. (2004). Third ventricular alloxan reversibly impairs glucose counterregulatory responses. *Diabetes*, *53*(5), 1230–1236. https://doi.org/10.2337/diabetes.53.5.1230

Sanders, N. M., Dunn-Meynell, A. A., & Levin, B. E. (2004). Third ventricular alloxan reversibly impairs glucose counterregulatory responses. *Diabetes*, *53*(5), 1230–1236. https://doi.org/10.2337/diabetes.53.5.1230

Sarraf, P., Frederich, R. C., Turner, E. M., Ma, G., Jaskowiak, N. T., Rivet, D. J., 3rd, Flier, J. S., Lowell, B. B., Fraker, D. L., & Alexander, H. R. (1997). Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *The Journal of experimental medicine*, *185*(1), 171–175. https://doi.org/10.1084/jem.185.1.171

Sarruf, D. A., Yu, F., Nguyen, H. T., Williams, D. L., Printz, R. L., Niswender, K. D., & Schwartz, M. W. (2009). Expression of peroxisome proliferator-activated receptor-gamma in key neuronal subsets regulating glucose metabolism and energy homeostasis. *Endocrinology*, *150*(2), 707–712. https://doi.org/10.1210/en.2008-0899

Sarton G. (1962) Sarton on the History of Science. Essays by George Sarton., *Cambridge: Harvard University Press.*

Sarwar, N., Gao, P., Seshasai, S. R., Gobin, R., Kaptoge, S., Di Angelantonio, E., Ingelsson, E., Lawlor, D. A., Selvin, E., Stampfer, M., Stehouwer, C. D., Lewington, S., Pennells, L., Thompson, A., Sattar, N., White, I. R., Ray, K. K., & Danesh, J. (2010). Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet (London, England)*, *375*(9733), 2215–2222. https://doi.org/10.1016/S0140-6736(10)60484-9

Saucisse, N., Mazier, W., Simon, V., Binder, E., Catania, C., Bellocchio, L., Romanov, R. A., Léon, S., Matias, I., Zizzari, P., Quarta, C., Cannich, A., Meece, K., Gonzales, D., Clark, S., Becker, J. M., Yeo, G. S. H., Fioramonti, X., Merkle, F. T., Wardlaw, S. L., … Cota, D. (2021). Functional heterogeneity of POMC neurons relies on mTORC1 signaling. *Cell reports*, *37*(2), 109800. https://doi.org/10.1016/j.celrep.2021.109800

Sauvé F., Ternier G., Dewisme J., Lebouvier T., Dupré E., Danis C., Rasika S., Kim Y.B., Ciofi P., Giacobini P., Buée L., Landrieu I., Pasquier F., Maurage C.A., Nogueiras R., Schwaninger M., Prevot V. (2022) Tanycytes are degraded in Alzheimer's Disease, disrupting the brain-to-blood efflux of Tau. *medRxiv*, https://doi.org/10.1101/2022.1105.1104.22274181

Schaeffer, M., Langlet, F., Lafont, C., Molino, F., Hodson, D. J., Roux, T., Lamarque, L., Verdié, P., Bourrier, E., Dehouck, B., Banères, J. L., Martinez, J., Méry, P. F., Marie, J., Trinquet, E., Fehrentz, J. A., Prévot, V., & Mollard, P. (2013). Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(4), 1512–1517. https://doi.org/10.1073/pnas.1212137110

Schaeffer, M., Langlet, F., Lafont, C., Molino, F., Hodson, D. J., Roux, T., Lamarque, L., Verdié, P., Bourrier, E., Dehouck, B., Banères, J. L., Martinez, J., Méry, P. F., Marie, J., Trinquet, E., Fehrentz, J. A., Prévot, V., & Mollard, P. (2013). Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(4), 1512–1517. https://doi.org/10.1073/pnas.1212137110

Scheen A. J. (1996). Clinical pharmacokinetics of metformin. Clinical pharmacokinetics, 30(5), 359–371. https://doi.org/10.2165/00003088-199630050-00003

Scherer, T., O'Hare, J., Diggs-Andrews, K., Schweiger, M., Cheng, B., Lindtner, C., Zielinski, E., Vempati, P., Su, K., Dighe, S., Milsom, T., Puchowicz, M., Scheja, L., Zechner, R., Fisher, S. J., Previs, S. F., & Buettner, C. (2011). Brain insulin controls adipose tissue lipolysis and lipogenesis. *Cell metabolism*, *13*(2), 183–194. https://doi.org/10.1016/j.cmet.2011.01.008

Schiavo, G., Benfenati, F., Poulain, B., Rossetto, O., Polverino de Laureto, P., DasGupta, B. R., & Montecucco, C. (1992). Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature*, *359*(6398), 832–835. https://doi.org/10.1038/359832a0

Schiavo, G., Stenbeck, G., Rothman, J. E., & Söllner, T. H. (1997). Binding of the synaptic vesicle v-SNARE, synaptotagmin, to the plasma membrane t-SNARE, SNAP-25, can explain docked vesicles at neurotoxin-treated synapses. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(3), 997–1001. https://doi.org/10.1073/pnas.94.3.997

Schlicker, E., & Kathmann, M. (2001). Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends in pharmacological sciences*, *22*(11), 565–572. https://doi-org.proxy.insermbiblio.inist.fr/10.1016/s0165-6147(00)01805-8

Schmidt, W. E., Siegel, E. G., & Creutzfeldt, W. (1985). Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. *Diabetologia*, *28*(9), 704–707. https://doi.org/10.1007/BF00291980

Schneeberger, M., Gomis, R., & Claret, M. (2014). Hypothalamic and brainstem neuronal circuits controlling homeostatic energy balance. *The Journal of endocrinology*, *220*(2), T25–T46. https://doi.org/10.1530/JOE-13-0398

Schutz, Y., Flatt, J. P., & Jéquier, E. (1989). Failure of dietary fat intake to promote fat oxidation: a factor favoring the development of obesity. *The American journal of clinical nutrition*, *50*(2), 307–314. https://doi.org/10.1093/ajcn/50.2.307

Schwartz, M. W., Peskind, E., Raskind, M., Boyko, E. J., & Porte, D., Jr (1996). Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nature medicine*, *2*(5), 589–593. https://doi.org/10.1038/nm0596-589

Schwartz, M. W., Seeley, R. J., Campfield, L. A., Burn, P., & Baskin, D. G. (1996). Identification of targets of leptin action in rat hypothalamus. *The Journal of clinical investigation*, *98*(5), 1101–1106. https://doi.org/10.1172/JCI118891

Schwartz, M. W., Sipols, A. J., Marks, J. L., Sanacora, G., White, J. D., Scheurink, A., Kahn, S. E., Baskin, D. G., Woods, S. C., & Figlewicz, D. P. (1992). Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology*, *130*(6), 3608–3616. https://doi.org/10.1210/endo.130.6.1597158

Secher, A., Jelsing, J., Baquero, A. F., Hecksher-Sørensen, J., Cowley, M. A., Dalbøge, L. S., Hansen, G., Grove, K. L., Pyke, C., Raun, K., Schäffer, L., Tang-Christensen, M., Verma, S., Witgen, B. M., Vrang, N., & Bjerre Knudsen, L. (2014). The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *The Journal of clinical investigation*, *124*(10), 4473–4488. https://doi.org/10.1172/JCI75276

Seifarth, C., Schehler, B., & Schneider, H. J. (2013). Effectiveness of metformin on weight loss in non-diabetic individuals with obesity. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association*, *121*(1), 27–31. https://doi.org/10.1055/s-0032-1327734

Shankar, S. S., Shankar, R. R., Mixson, L. A., Miller, D. L., Pramanik, B., O'Dowd, A. K., Williams, D. M., Frederick, C. B., Beals, C. R., Stoch, S. A., Steinberg, H. O., & Kelley, D. E. (2018). Native Oxyntomodulin Has

Significant Glucoregulatory Effects Independent of Weight Loss in Obese Humans With and Without Type 2 Diabetes. *Diabetes*, *67*(6), 1105–1112. https://doi.org/10.2337/db17-1331

Shanley, L. J., Irving, A. J., & Harvey, J. (2001). Leptin enhances NMDA receptor function and modulates hippocampal synaptic plasticity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *21*(24), RC186. https://doi.org/10.1523/JNEUROSCI.21-24-j0001.2001

Sharretts, J., Galescu, O., Gomatam, S., Andraca-Carrera, E., Hampp, C., & Yanoff, L. (2020). Cancer Risk Associated with Lorcaserin - The FDA's Review of the CAMELLIA-TIMI 61 Trial. *The New England journal of medicine*, *383*(11), 1000–1002. https://doi.org/10.1056/NEJMp2003873

Shen, J., Tanida, M., Yao, J. F., Niijima, A., & Nagai, K. (2008). Biphasic effects of orexin-A on autonomic nerve activity and lipolysis. *Neuroscience letters*, *444*(2), 166–171. https://doi.org/10.1016/j.neulet.2008.08.031

Shepro, D., & Morel, N. M. (1993). Pericyte physiology. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, *7*(11), 1031–1038. https://doi.org/10.1096/fasebj.7.11.8370472

Shi, X., Wang, X., Li, Q., Su, M., Chew, E., Wong, E. T., Lacza, Z., Radda, G. K., Tergaonkar, V., & Han, W. (2013). Nuclear factor κB (NF-κB) suppresses food intake and energy expenditure in mice by directly activating the Pomc promoter. *Diabetologia*, *56*(4), 925–936. https://doi.org/10.1007/s00125-013-2831-2

Shi, Y. C., Lau, J., Lin, Z., Zhang, H., Zhai, L., Sperk, G., Heilbronn, R., Mietzsch, M., Weger, S., Huang, X. F., Enriquez, R. F., Baldock, P. A., Zhang, L., Sainsbury, A., Herzog, H., & Lin, S. (2013). Arcuate NPY controls sympathetic output and BAT function via a relay of tyrosine hydroxylase neurons in the PVN. *Cell metabolism*, *17*(2), 236–248. https://doi.org/10.1016/j.cmet.2013.01.006

Shi, Z., Pelletier, N. E., Wong, J., Li, B., Sdrulla, A. D., Madden, C. J., Marks, D. L., & Brooks, V. L. (2020). Leptin increases sympathetic nerve activity via induction of its own receptor in the paraventricular nucleus. *eLife*, *9*, e55357. https://doi.org/10.7554/eLife.55357

Shimazu T. (1979). Nervous control of peripheral metabolism. *Acta physiologica Polonica*, *30*(18 Suppl), 1–18.

Shimazu, T., & Fujimoto, T. (1971). Regulation of glycogen metabolism in liver by the autonomic nervous system. IV. Neural control of glycogen biosynthesis. *Biochimica et biophysica acta*, *252*(1), 18–27. https://doi.org/10.1016/0304-4165(71)90088-2

Shin, S., You, I. J., Jeong, M., Bae, Y., Wang, X. Y., Cawley, M. L., Han, A., & Lim, B. K. (2023). Early adversity promotes binge-like eating habits by remodeling a leptin-responsive lateral hypothalamus-brainstem pathway. *Nature neuroscience*, *26*(1), 79–91. https://doi.org/10.1038/s41593-022-01208-0

Shirazi, R., Palsdottir, V., Collander, J., Anesten, F., Vogel, H., Langlet, F., Jaschke, A., Schürmann, A., Prévot, V., Shao, R., Jansson, J. O., & Skibicka, K. P. (2013). Glucagon-like peptide 1 receptor induced suppression of food intake, and body weight is mediated by central IL-1 and IL-6. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(40), 16199–16204. https://doi.org/10.1073/pnas.1306799110

Shiuchi, T., Haque, M. S., Okamoto, S., Inoue, T., Kageyama, H., Lee, S., Toda, C., Suzuki, A., Bachman, E. S., Kim, Y. B., Sakurai, T., Yanagisawa, M., Shioda, S., Imoto, K., & Minokoshi, Y. (2009). Hypothalamic orexin stimulates feeding-associated glucose utilization in skeletal muscle via sympathetic nervous system. *Cell metabolism*, *10*(6), 466–480. https://doi.org/10.1016/j.cmet.2009.09.013

Short T. (1727) A Discourse Concerning the Causes and Effects of Corpulency: Together with the Method for its Prevention and Cure., *London: Oxford Arms in Warwick Lane*.

Sidibe, A., Mullier, A., Chen, P., Baroncini, M., Boutin, J. A., Delagrange, P., Prevot, V., & Jockers, R. (2010). Expression of the orphan GPR50 protein in rodent and human dorsomedial hypothalamus, tanycytes and median eminence. *Journal of pineal research*, *48*(3), 263–269. https://doi.org/10.1111/j.1600-079X.2010.00750.x

Siéssere, S., Vitti, M., Sousa, L. G., Semprini, M., Iyomasa, M. M., & Regalo, S. C. (2008). Anatomic variation of cranial parasympathetic ganglia. *Brazilian oral research*, *22*(2), 101–105. https://doi.org/10.1590/s1806-83242008000200002

Sigerist HE. (1961) A History of Medicine. Vol. 1. Primitive and Archaic Medicine. Vol. 2. Early Greek, Hindu, and Persian Medicine. *Publication / Historical Library, Yale Medical Library., New York,: Oxford University Press.*

Silva J. E. (2003). The thermogenic effect of thyroid hormone and its clinical implications. *Annals of internal medicine*, *139*(3), 205–213.

Silver, I. A., & Erecińska, M. (1998). Glucose-induced intracellular ion changes in sugar-sensitive hypothalamic neurons. *Journal of neurophysiology*, *79*(4), 1733–1745. https://doi.org/10.1152/jn.1998.79.4.1733

Sims, E. A., Danforth, E., Jr, Horton, E. S., Bray, G. A., Glennon, J. A., & Salans, L. B. (1973). Endocrine and metabolic effects of experimental obesity in man. *Recent progress in hormone research*, *29*, 457–496. https://doi.org/10.1016/b978-0-12-571129-6.50016-6

Singh, G., Krauthamer, M., & Bjalme-Evans, M. (2022). Wegovy (semaglutide): a new weight loss drug for chronic weight management. *Journal of investigative medicine : the official publication of the American Federation for Clinical Research*, *70*(1), 5–13. https://doi.org/10.1136/jim-2021-001952

Sipols, A. J., Baskin, D. G., & Schwartz, M. W. (1995). Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes*, *44*(2), 147–151. https://doi.org/10.2337/diab.44.2.147

Sisley, S., Gutierrez-Aguilar, R., Scott, M., D'Alessio, D. A., Sandoval, D. A., & Seeley, R. J. (2014). Neuronal GLP1R mediates liraglutide's anorectic but not glucose-lowering effect. *The Journal of clinical investigation*, *124*(6), 2456–2463. https://doi.org/10.1172/JCI72434

Sisó, S., Jeffrey, M., & González, L. (2010). Sensory circumventricular organs in health and disease. *Acta neuropathologica*, *120*(6), 689–705. https://doi.org/10.1007/s00401-010-0743-5

Slezak, M., Grosche, A., Niemiec, A., Tanimoto, N., Pannicke, T., Münch, T. A., Crocker, B., Isope, P., Härtig, W., Beck, S. C., Huber, G., Ferracci, G., Perraut, M., Reber, M., Miehe, M., Demais, V., Lévêque, C., Metzger, D., Szklarczyk, K., Przewlocki, R., … Pfrieger, F. W. (2012). Relevance of exocytotic glutamate release from retinal glia. *Neuron*, *74*(3), 504–516. https://doi.org/10.1016/j.neuron.2012.03.027

Smith, G. P., & Epstein, A. N. (1969). Increased feeding in response to decreased glucose utilization in the rat and monkey. *The American journal of physiology*, *217*(4), 1083–1087. https://doi.org/10.1152/ajplegacy.1969.217.4.1083

Smith, M. A., Hisadome, K., Al-Qassab, H., Heffron, H., Withers, D. J., & Ashford, M. L. (2007). Melanocortins and agouti-related protein modulate the excitability of two arcuate nucleus neuron populations by alteration of resting potassium conductances. *The Journal of physiology*, *578*(Pt 2), 425–438. https://doi.org/10.1113/jphysiol.2006.119479

Sofroniew M. V. (1980). Projections from vasopressin, oxytocin, and neurophysin neurons to neural targets in the rat and human. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*, *28*(5), 475–478. https://doi.org/10.1177/28.5.7381192

Sohn, J. W., Harris, L. E., Berglund, E. D., Liu, T., Vong, L., Lowell, B. B., Balthasar, N., Williams, K. W., & Elmquist, J. K. (2013). Melanocortin 4 receptors reciprocally regulate sympathetic and parasympathetic preganglionic neurons. *Cell*, *152*(3), 612–619. https://doi.org/10.1016/j.cell.2012.12.022

Sohn, J. W., Xu, Y., Jones, J. E., Wickman, K., Williams, K. W., & Elmquist, J. K. (2011). Serotonin 2C receptor activates a distinct population of arcuate pro-opiomelanocortin neurons via TRPC channels. *Neuron*, *71*(3), 488–497. https://doi.org/10.1016/j.neuron.2011.06.012

Söllner, T., Bennett, M. K., Whiteheart, S. W., Scheller, R. H., & Rothman, J. E. (1993). A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. *Cell*, *75*(3), 409–418. https://doi.org/10.1016/0092-8674(93)90376-2

Söllner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., & Rothman, J. E. (1993). SNAP receptors implicated in vesicle targeting and fusion. *Nature*, *362*(6418), 318–324. https://doi.org/10.1038/362318a0

Song, C. K., Jackson, R. M., Harris, R. B., Richard, D., & Bartness, T. J. (2005). Melanocortin-4 receptor mRNA is expressed in sympathetic nervous system outflow neurons to white adipose tissue. *American journal of physiology. Regulatory, integrative and comparative physiology*, *289*(5), R1467–R1476. https://doi.org/10.1152/ajpregu.00348.2005

Spector, R., & Johanson, C. (2006). Micronutrient and urate transport in choroid plexus and kidney: implications for drug therapy. *Pharmaceutical research*, *23*(11), 2515–2524. https://doi.org/10.1007/s11095-006-9091-5

Speliotes, E. K., Willer, C. J., Berndt, S. I., Monda, K. L., Thorleifsson, G., Jackson, A. U., Lango Allen, H., Lindgren, C. M., Luan, J., Mägi, R., Randall, J. C., Vedantam, S., Winkler, T. W., Qi, L., Workalemahu, T., Heid, I. M., Steinthorsdottir, V., Stringham, H. M., Weedon, M. N., Wheeler, E., … Loos, R. J. (2010). Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nature genetics*, *42*(11), 937–948. https://doi.org/10.1038/ng.686

St-Pierre, J., & Tremblay, M. L. (2012). Modulation of leptin resistance by protein tyrosine phosphatases. *Cell metabolism*, *15*(3), 292–297. https://doi.org/10.1016/j.cmet.2012.02.004

Stanley, B. G., & Leibowitz, S. F. (1985). Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(11), 3940–3943. https://doi.org/10.1073/pnas.82.11.3940

Stark, R., Ashley, S. E., & Andrews, Z. B. (2013). AMPK and the neuroendocrine regulation of appetite and energy expenditure. *Molecular and cellular endocrinology*, *366*(2), 215–223. https://doi.org/10.1016/j.mce.2012.06.012

Ste Marie, L., Miura, G. I., Marsh, D. J., Yagaloff, K., & Palmiter, R. D. (2000). A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(22), 12339–12344. https://doi.org/10.1073/pnas.220409497

Steculorum, S. M., Ruud, J., Karakasilioti, I., Backes, H., Engström Ruud, L., Timper, K., Hess, M. E., Tsaousidou, E., Mauer, J., Vogt, M. C., Paeger, L., Bremser, S., Klein, A. C., Morgan, D. A., Frommolt, P., Brinkkötter, P. T., Hammerschmidt, P., Benzing, T., Rahmouni, K., Wunderlich, F. T., … Brüning, J. C. (2016). AgRP Neurons Control Systemic Insulin Sensitivity via Myostatin Expression in Brown Adipose Tissue. *Cell*, *165*(1), 125–138. https://doi.org/10.1016/j.cell.2016.02.044

Steegmaier, M., Lee, K. C., Prekeris, R., & Scheller, R. H. (2000). SNARE protein trafficking in polarized MDCK cells. *Traffic (Copenhagen, Denmark)*, *1*(7), 553–560. https://doi.org/10.1034/j.1600-0854.2000.010705.x

Steinberg, G. R., & Carling, D. (2019). AMP-activated protein kinase: the current landscape for drug development. *Nature reviews. Drug discovery*, *18*(7), 527–551. https://doi.org/10.1038/s41573-019-0019-2

Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., Patel, H. R., Ahima, R. S., & Lazar, M. A. (2001). The hormone resistin links obesity to diabetes. *Nature*, *409*(6818), 307–312. https://doi.org/10.1038/35053000

Steppan, C. M., Wang, J., Whiteman, E. L., Birnbaum, M. J., & Lazar, M. A. (2005). Activation of SOCS-3 by resistin. *Molecular and cellular biology*, *25*(4), 1569–1575. https://doi.org/10.1128/MCB.25.4.1569-1575.2005

Stevanovic, D., Janjetovic, K., Misirkic, M., Vucicevic, L., Sumarac-Dumanovic, M., Micic, D., Starcevic, V., & Trajkovic, V. (2012). Intracerebroventricular administration of metformin inhibits ghrelin-induced Hypothalamic AMP-kinase signalling and food intake. *Neuroendocrinology*, *96*(1), 24–31. https://doi.org/10.1159/000333963

Strobel, A., Issad, T., Camoin, L., Ozata, M., & Strosberg, A. D. (1998). A leptin missense mutation associated with hypogonadism and morbid obesity. *Nature genetics*, *18*(3), 213–215. https://doi.org/10.1038/ng0398-213

Sudo, M., Minokoshi, Y., & Shimazu, T. (1991). Ventromedial hypothalamic stimulation enhances peripheral glucose uptake in anesthetized rats. *The American journal of physiology*, *261*(3 Pt 1), E298–E303. https://doi.org/10.1152/ajpendo.1991.261.3.E298

Sukumaran, S., Xue, B., Jusko, W. J., Dubois, D. C., & Almon, R. R. (2010). Circadian variations in gene expression in rat abdominal adipose tissue and relationship to physiology. *Physiological genomics*, *42A*(2), 141–152. https://doi.org/10.1152/physiolgenomics.00106.2010

Sun, J., Gao, Y., Yao, T., Huang, Y., He, Z., Kong, X., Yu, K. J., Wang, R. T., Guo, H., Yan, J., Chang, Y., Chen, H., Scherer, P. E., Liu, T., & Williams, K. W. (2016). Adiponectin potentiates the acute effects of leptin in arcuate Pomc neurons. *Molecular metabolism*, *5*(10), 882–891. https://doi.org/10.1016/j.molmet.2016.08.007

Sun, Y., Wang, P., Zheng, H., & Smith, R. G. (2004). Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(13), 4679–4684. https://doi.org/10.1073/pnas.0305930101

Swanson L. W. (2000). Cerebral hemisphere regulation of motivated behavior. *Brain research*, *886*(1-2), 113–164. https://doi.org/10.1016/s0006-8993(00)02905-x

Szabo, O., & Szabo, A. J. (1975). Studies on the nature and mode of action of the insulin-sensitive glucoregulator receptor in the central nervous system. *Diabetes*, *24*(4), 328–336. https://doi.org/10.2337/diab.24.4.328

Tainter, M. L., Cutting, W. C., & Stockton, A. B. (1934). Use of Dinitrophenol in Nutritional Disorders : A Critical Survey of Clinical Results. *American journal of public health and the nation's health*, *24*(10), 1045–1053. https://doi.org/10.2105/ajph.24.10.1045

Talbott JH. (1970) A Biographical History of Medicine: Excerpts and Essays on the Men and Their Work., *New York: Grune & Stratton.*

Tamura, H., Kamegai, J., Shimizu, T., Ishii, S., Sugihara, H., & Oikawa, S. (2002). Ghrelin stimulates GH but not food intake in arcuate nucleus ablated rats. *Endocrinology*, *143*(9), 3268–3275. https://doi.org/10.1210/en.2002-220268

Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G. J., Campfield, L. A., Clark, F. T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K. J., Smutko, J. S., Mays, G. G., Wool, E. A., Monroe, C. A., & Tepper, R. I. (1995). Identification and expression cloning of a leptin receptor, OB-R. *Cell*, *83*(7), 1263–1271. https://doi.org/10.1016/0092-8674(95)90151-5

Terry, P., Gilbert, D. B., & Cooper, S. J. (1995). Dopamine receptor subtype agonists and feeding behavior. *Obesity research*, *3 Suppl 4*, 515S–523S. https://doi.org/10.1002/j.1550-8528.1995.tb00221.x

Theander-Carrillo, C., Wiedmer, P., Cettour-Rose, P., Nogueiras, R., Perez-Tilve, D., Pfluger, P., Castaneda, T. R., Muzzin, P., Schürmann, A., Szanto, I., Tschöp, M. H., & Rohner-Jeanrenaud, F. (2006). Ghrelin action in the brain controls adipocyte metabolism. *The Journal of clinical investigation*, *116*(7), 1983–1993. https://doi.org/10.1172/JCI25811

Thomzig, A., Laube, G., Prüss, H., & Veh, R. W. (2005). Pore-forming subunits of K-ATP channels, Kir6.1 and Kir6.2, display prominent differences in regional and cellular distribution in the rat brain. *The Journal of comparative neurology*, *484*(3), 313–330. https://doi.org/10.1002/cne.20469

Thon, M., Hosoi, T., & Ozawa, K. (2016). Possible Integrative Actions of Leptin and Insulin Signaling in the Hypothalamus Targeting Energy Homeostasis. Frontiers in endocrinology, 7, 138. https://doi.org/10.3389/fendo.2016.00138

Thorens B. (2014). Neural regulation of pancreatic islet cell mass and function. *Diabetes, obesity & metabolism*, *16 Suppl 1*, 87–95. https://doi.org/10.1111/dom.12346

Tokubuchi, I., Tajiri, Y., Iwata, S., Hara, K., Wada, N., Hashinaga, T., Nakayama, H., Mifune, H., & Yamada, K. (2017). Beneficial effects of metformin on energy metabolism and visceral fat volume through a possible mechanism of fatty acid oxidation in human subjects and rats. *PloS one*, *12*(2), e0171293. https://doi.org/10.1371/journal.pone.0171293

Tolle, V., & Low, M. J. (2008). In vivo evidence for inverse agonism of Agouti-related peptide in the central nervous system of proopiomelanocortin-deficient mice. *Diabetes*, *57*(1), 86–94. https://doi.org/10.2337/db07-0733

Torres, S. J., & Nowson, C. A. (2007). Relationship between stress, eating behavior, and obesity. *Nutrition (Burbank, Los Angeles County, Calif.)*, *23*(11-12), 887–894. https://doi.org/10.1016/j.nut.2007.08.008

Tovar, S., Nogueiras, R., Tung, L. Y., Castañeda, T. R., Vázquez, M. J., Morris, A., Williams, L. M., Dickson, S. L., & Diéguez, C. (2005). Central administration of resistin promotes short-term satiety in rats. *European journal of endocrinology*, *153*(3), R1–R5. https://doi.org/10.1530/eje.1.01999

Travagli, R. A., Hermann, G. E., Browning, K. N., & Rogers, R. C. (2006). Brainstem circuits regulating gastric function. *Annual review of physiology*, *68*, 279–305. https://doi.org/10.1146/annurev.physiol.68.040504.094635

Tschöp, M. H., Speakman, J. R., Arch, J. R., Auwerx, J., Brüning, J. C., Chan, L., Eckel, R. H., Farese, R. V., Jr, Galgani, J. E., Hambly, C., Herman, M. A., Horvath, T. L., Kahn, B. B., Kozma, S. C., Maratos-Flier, E., Müller, T. D., Münzberg, H., Pfluger, P. T., Plum, L., Reitman, M. L., … Ravussin, E. (2011). A guide to analysis of mouse energy metabolism. *Nature methods*, *9*(1), 57–63. https://doi.org/10.1038/nmeth.1806

Tschöp, M., Smiley, D. L., & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature*, *407*(6806), 908–913. https://doi.org/10.1038/35038090

Tschöp, M., Smiley, D. L., & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature*, *407*(6806), 908–913. https://doi.org/10.1038/35038090

Tschöp, M., Wawarta, R., Riepl, R. L., Friedrich, S., Bidlingmaier, M., Landgraf, R., & Folwaczny, C. (2001). Post-prandial decrease of circulating human ghrelin levels. *Journal of endocrinological investigation*, *24*(6), RC19–RC21. https://doi.org/10.1007/BF03351037

Tsuneki, H., Tokai, E., Nakamura, Y., Takahashi, K., Fujita, M., Asaoka, T., Kon, K., Anzawa, Y., Wada, T., Takasaki, I., Kimura, K., Inoue, H., Yanagisawa, M., Sakurai, T., & Sasaoka, T. (2015). Hypothalamic orexin

prevents hepatic insulin resistance via daily bidirectional regulation of autonomic nervous system in mice. *Diabetes*, *64*(2), 459–470. https://doi.org/10.2337/db14-0695

Uddén, J., Björntorp, P., Arner, P., Barkeling, B., Meurling, L., & Rössner, S. (2003). Effects of glucocorticoids on leptin levels and eating behaviour in women. *Journal of internal medicine*, *253*(2), 225–231. https://doi.org/10.1046/j.1365-2796.2003.01099.x

Ueno, M., Akiguchi, I., Hosokawa, M., Kotani, H., Kanenishi, K., & Sakamoto, H. (2000). Blood-brain barrier permeability in the periventricular areas of the normal mouse brain. *Acta neuropathologica*, *99*(4), 385–392. https://doi.org/10.1007/s004010051140

Ulken, V., Püschel, G. P., & Jungermann, K. (1991). Increase in glucose and lactate output and perfusion resistance by stimulation of hepatic nerves in isolated perfused rat liver: role of alpha 1-, alpha 2-, beta 1- and beta 2-receptors. *Biological chemistry Hoppe-Seyler*, *372*(6), 401–409. https://doi.org/10.1515/bchm3.1991.372.1.401

Ulrich H, Trapp CE, Vidgoff B. (1936) The Treatment of Narcolepsy with Benzedrine Sulphate. Annals of Internal Medicine.;9(9):1213-1221. DOI: .10.7326/0003-4819-9-9-1213

Üner, A. G., Keçik, O., Quaresma, P. G. F., De Araujo, T. M., Lee, H., Li, W., Kim, H. J., Chung, M., Bjørbæk, C., & Kim, Y. B. (2019). Role of POMC and AgRP neuronal activities on glycaemia in mice. *Scientific reports*, *9*(1), 13068. https://doi.org/10.1038/s41598-019-49295-7

Uranga, R. M., Millán, C., Barahona, M. J., Recabal, A., Salgado, M., Martinez, F., Ordenes, P., Elizondo-Vega, R., Sepúlveda, F., Uribe, E., & García-Robles, M. L. Á. (2017). Adenovirus-mediated suppression of hypothalamic glucokinase affects feeding behavior. *Scientific reports*, *7*(1), 3697. https://doi.org/10.1038/s41598-017-03928-x

Vaisse, C., Halaas, J. L., Horvath, C. M., Darnell, J. E., Jr, Stoffel, M., & Friedman, J. M. (1996). Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. *Nature genetics*, *14*(1), 95–97. https://doi.org/10.1038/ng0996-95

van Baak M. A. (2001). The peripheral sympathetic nervous system in human obesity. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, *2*(1), 3–14. https://doi.org/10.1046/j.1467-789x.2001.00010.x

van Can, J., Sloth, B., Jensen, C. B., Flint, A., Blaak, E. E., & Saris, W. H. (2014). Effects of the once-daily GLP-1 analog liraglutide on gastric emptying, glycemic parameters, appetite and energy metabolism in obese, non-diabetic adults. *International journal of obesity (2005)*, *38*(6), 784–793. https://doi.org/10.1038/ijo.2013.162

van den Hoek, A. M., van Heijningen, C., Schröder-van der Elst, J. P., Ouwens, D. M., Havekes, L. M., Romijn, J. A., Kalsbeek, A., & Pijl, H. (2008). Intracerebroventricular administration of neuropeptide Y induces hepatic insulin resistance via sympathetic innervation. *Diabetes*, *57*(9), 2304–2310. https://doi.org/10.2337/db07-1658

van den Pol A. N. (2003). Weighing the role of hypothalamic feeding neurotransmitters. *Neuron*, *40*(6), 1059–1061. https://doi.org/10.1016/s0896-6273(03)00809-

Van Heek, M., Compton, D. S., France, C. F., Tedesco, R. P., Fawzi, A. B., Graziano, M. P., Sybertz, E. J., Strader, C. D., & Davis, H. R., Jr (1997). Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *The Journal of clinical investigation*, *99*(3), 385–390. https://doi.org/10.1172/JCI119171

van Houten, M., Posner, B. I., Kopriwa, B. M., & Brawer, J. R. (1979). Insulin-binding sites in the rat brain: in vivo localization to the circumventricular organs by quantitative radioautography. *Endocrinology*, *105*(3), 666–673. https://doi.org/10.1210/endo-105-3-666

van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., & Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *The New England journal of medicine*, *360*(15), 1500–1508. https://doi.org/10.1056/NEJMoa0808718

Varela, L., & Horvath, T. L. (2012). Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis. *EMBO reports*, *13*(12), 1079–1086. https://doi.org/10.1038/embor.2012.174

Vázquez, M. J., González, C. R., Varela, L., Lage, R., Tovar, S., Sangiao-Alvarellos, S., Williams, L. M., Vidal-Puig, A., Nogueiras, R., López, M., & Diéguez, C. (2008). Central resistin regulates hypothalamic and peripheral lipid metabolism in a nutritional-dependent fashion. *Endocrinology*, *149*(9), 4534–4543. https://doi.org/10.1210/en.2007-1708

Velásquez, D. A., Martínez, G., Romero, A., Vázquez, M. J., Boit, K. D., Dopeso-Reyes, I. G., López, M., Vidal, A., Nogueiras, R., & Diéguez, C. (2011). The central Sirtuin 1/p53 pathway is essential for the orexigenic action of ghrelin. *Diabetes*, *60*(4), 1177–1185. https://doi.org/10.2337/db10-0802

Vembar, S. S., & Brodsky, J. L. (2008). One step at a time: endoplasmic reticulum-associated degradation. *Nature reviews. Molecular cell biology*, *9*(12), 944–957. https://doi.org/10.1038/nrm2546

Verty, A. N., Boon, W. M., Mallet, P. E., McGregor, I. S., & Oldfield, B. J. (2009). Involvement of hypothalamic peptides in the anorectic action of the CB receptor antagonist rimonabant (SR 141716). *The European journal of neuroscience*, *29*(11), 2207–2216. https://doi.org/10.1111/j.1460-9568.2009.06750.x

Viollet, B., Andreelli, F., Jørgensen, S. B., Perrin, C., Geloen, A., Flamez, D., Mu, J., Lenzner, C., Baud, O., Bennoun, M., Gomas, E., Nicolas, G., Wojtaszewski, J. F., Kahn, A., Carling, D., Schuit, F. C., Birnbaum, M. J., Richter, E. A., Burcelin, R., & Vaulont, S. (2003). The AMP-activated protein kinase alpha2 catalytic subunit controls whole-body insulin sensitivity. *The Journal of clinical investigation*, *111*(1), 91–98. https://doi.org/10.1172/JCI16567

Volkow, N. D., Wang, G. J., & Baler, R. D. (2011). Reward, dopamine and the control of food intake: implications for obesity. *Trends in cognitive sciences*, *15*(1), 37–46. https://doi.org/10.1016/j.tics.2010.11.001

Vong, L., Ye, C., Yang, Z., Choi, B., Chua, S., Jr, & Lowell, B. B. (2011). Leptin action on GABAergic neurons prevents obesity and reduces inhibitory tone to POMC neurons. *Neuron*, *71*(1), 142–154. https://doi.org/10.1016/j.neuron.2011.05.028

Wadden, T. A., Bailey, T. S., Billings, L. K., Davies, M., Frias, J. P., Koroleva, A., Lingvay, I., O'Neil, P. M., Rubino, D. M., Skovgaard, D., Wallenstein, S. O. R., Garvey, W. T., & STEP 3 Investigators (2021). Effect of Subcutaneous Semaglutide vs Placebo as an Adjunct to Intensive Behavioral Therapy on Body Weight in Adults With Overweight or Obesity: The STEP 3 Randomized Clinical Trial. *JAMA*, *325*(14), 1403–1413. https://doi.org/10.1001/jama.2021.1831

Wadden, T. A., Hollander, P., Klein, S., Niswender, K., Woo, V., Hale, P. M., Aronne, L., & NN8022-1923 Investigators (2013). Weight maintenance and additional weight loss with liraglutide after low-calorie-diet-induced weight loss: the SCALE Maintenance randomized study. *International journal of obesity (2005)*, *37*(11), 1443–1451. https://doi.org/10.1038/ijo.2013.120

Wakonig, P., Költringer, P., Lind, P., Eber, O., & Wawschinek, O. (1988). Methode zur Bestimmung der Proteinbindung der Schilddrüsenhormone und zum Nachweis von Antikörpern gegen T4 und T3 [Method for the determination of protein binding of thyroid gland hormones and the detection of antibodies to T4 and T3]. *Acta medica Austriaca*, *15*(1), 15–19.

Walker, D. L., Miles, L. A., & Davis, M. (2009). Selective participation of the bed nucleus of the stria terminalis and CRF in sustained anxiety-like versus phasic fear-like responses. *Progress in neuro-psychopharmacology & biological psychiatry*, *33*(8), 1291–1308. https://doi.org/10.1016/j.pnpbp.2009.06.022

Wang, D. S., Jonker, J. W., Kato, Y., Kusuhara, H., Schinkel, A. H., & Sugiyama, Y. (2002). Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *The Journal of pharmacology and experimental therapeutics*, *302*(2), 510–515. https://doi.org/10.1124/jpet.102.034140

Wang, D., He, X., Zhao, Z., Feng, Q., Lin, R., Sun, Y., Ding, T., Xu, F., Luo, M., & Zhan, C. (2015). Whole-brain mapping of the direct inputs and axonal projections of POMC and AgRP neurons. *Frontiers in neuroanatomy*, *9*, 40. https://doi.org/10.3389/fnana.2015.00040

Wang, H., Ni, Y., Yang, S., Li, H., Li, X., & Feng, B. (2013). The effects of gliclazide, metformin, and acarbose on body composition in patients with newly diagnosed type 2 diabetes mellitus. *Current therapeutic research, clinical and experimental*, *75*, 88–92. https://doi.org/10.1016/j.curtheres.2013.10.002

Wang, J., Ren, J., Wu, B., Feng, S., Cai, G., Tuluc, F., Peränen, J., & Guo, W. (2015). Activation of Rab8 guanine nucleotide exchange factor Rabin8 by ERK1/2 in response to EGF signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(1), 148–153. https://doi.org/10.1073/pnas.1412089112

Wang, M. Y., Zhou, Y. T., Newgard, C. B., & Unger, R. H. (1996). A novel leptin receptor isoform in rat. *FEBS letters*, *392*(2), 87–90. https://doi.org/10.1016/0014-5793(96)00790-9

Wang, T. J., Larson, M. G., Vasan, R. S., Cheng, S., Rhee, E. P., McCabe, E., Lewis, G. D., Fox, C. S., Jacques, P. F., Fernandez, C., O'Donnell, C. J., Carr, S. A., Mootha, V. K., Florez, J. C., Souza, A., Melander, O., Clish, C. B., & Gerszten, R. E. (2011). Metabolite profiles and the risk of developing diabetes. *Nature medicine*, *17*(4), 448–453. https://doi.org/10.1038/nm.2307

Wauman, J., & Tavernier, J. (2011). Leptin receptor signaling: pathways to leptin resistance. *Frontiers in bioscience (Landmark edition)*, *16*(7), 2771–2793. https://doi.org/10.2741/3885

Weber, G. W., Lukeneder, A., Harzhauser, M., Mitteroecker, P., Wurm, L., Hollaus, L. M., Kainz, S., Haack, F., Antl-Weiser, W., & Kern, A. (2022). The microstructure and the origin of the Venus from Willendorf. *Scientific reports*, *12*(1), 2926. https://doi.org/10.1038/s41598-022-06799-z

Weghuber, D., Barrett, T., Barrientos-Pérez, M., Gies, I., Hesse, D., Jeppesen, O. K., Kelly, A. S., Mastrandrea, L. D., Sørrig, R., Arslanian, S., & STEP TEENS Investigators (2022). Once-Weekly Semaglutide in Adolescents with Obesity. *The New England journal of medicine*, *387*(24), 2245–2257. https://doi.org/10.1056/NEJMoa2208601

Weintraub, M., Hasday, J. D., Mushlin, A. I., & Lockwood, D. H. (1984). A double-blind clinical trial in weight control. Use of fenfluramine and phentermine alone and in combination. *Archives of internal medicine*, *144*(6), 1143–1148.

Weir, G. C., & Bonner-Weir, S. (2004). Five stages of evolving beta-cell dysfunction during progression to diabetes. *Diabetes*, *53 Suppl 3*, S16–S21. https://doi.org/10.2337/diabetes.53.suppl_3.s16

Wen, L., & Duffy, A. (2017). Factors Influencing the Gut Microbiota, Inflammation, and Type 2 Diabetes. *The Journal of nutrition*, *147*(7), 1468S–1475S. https://doi.org/10.3945/jn.116.240754

Widdowson, P. S., Upton, R., Buckingham, R., Arch, J., & Williams, G. (1997). Inhibition of food response to intracerebroventricular injection of leptin is attenuated in rats with diet-induced obesity. *Diabetes*, *46*(11), 1782–1785. https://doi.org/10.2337/diab.46.11.1782

Wiernsperger, N. F., & Bailey, C. J. (1999). The antihyperglycaemic effect of metformin: therapeutic and cellular mechanisms. *Drugs*, *58 Suppl 1*, 31–82. https://doi.org/10.2165/00003495-199958001-00009

Wilcock, C., & Bailey, C. J. (1994). Accumulation of metformin by tissues of the normal and diabetic mouse. *Xenobiotica; the fate of foreign compounds in biological systems*, *24*(1), 49–57. https://doi.org/10.3109/00498259409043220

Wilding, J. P. H., Batterham, R. L., Calanna, S., Davies, M., Van Gaal, L. F., Lingvay, I., McGowan, B. M., Rosenstock, J., Tran, M. T. D., Wadden, T. A., Wharton, S., Yokote, K., Zeuthen, N., Kushner, R. F., & STEP 1 Study Group (2021). Once-Weekly Semaglutide in Adults with Overweight or Obesity. *The New England journal of medicine*, *384*(11), 989–1002. https://doi.org/10.1056/NEJMoa2032183

Wiley, J. W., Lu, Y. X., & Chung, O. Y. (1991). Mechanism of action of peptide YY to inhibit gastric motility. *Gastroenterology*, *100*(4), 865–872. https://doi.org/10.1016/0016-5085(91)90257-l

Williams, K. W., Margatho, L. O., Lee, C. E., Choi, M., Lee, S., Scott, M. M., Elias, C. F., & Elmquist, J. K. (2010). Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *30*(7), 2472–2479. https://doi.org/10.1523/JNEUROSCI.3118-09.2010

Williams, K. W., Zsombok, A., & Smith, B. N. (2007). Rapid inhibition of neurons in the dorsal motor nucleus of the vagus by leptin. *Endocrinology*, *148*(4), 1868–1881. https://doi.org/10.1210/en.2006-1098

Wilsey, J., & Scarpace, P. J. (2004). Caloric restriction reverses the deficits in leptin receptor protein and leptin signaling capacity associated with diet-induced obesity: role of leptin in the regulation of hypothalamic long-form leptin receptor expression. *The Journal of endocrinology*, *181*(2), 297–306. https://doi.org/10.1677/joe.0.1810297

Wilsey, J., Zolotukhin, S., Prima, V., & Scarpace, P. J. (2003). Central leptin gene therapy fails to overcome leptin resistance associated with diet-induced obesity. *American journal of physiology. Regulatory, integrative and comparative physiology*, *285*(5), R1011–R1020. https://doi.org/10.1152/ajpregu.00193.2003

Wing, R. R., Lang, W., Wadden, T. A., Safford, M., Knowler, W. C., Bertoni, A. G., Hill, J. O., Brancati, F. L., Peters, A., Wagenknecht, L., & Look AHEAD Research Group (2011). Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. *Diabetes care*, *34*(7), 1481–1486. https://doi.org/10.2337/dc10-2415

Wirth, M. M., Olszewski, P. K., Yu, C., Levine, A. S., & Giraudo, S. Q. (2001). Paraventricular hypothalamic alpha-melanocyte-stimulating hormone and MTII reduce feeding without causing aversive effects. *Peptides*, *22*(1), 129–134. https://doi.org/10.1016/s0196-9781(00)00367-3

Witters L. A. (2001). The blooming of the French lilac. *The Journal of clinical investigation*, *108*(8), 1105–1107. https://doi.org/10.1172/JCI14178

Wittkowski, W., Scheuer, A. (1974). Functional changes of the neuronal and glial elements at the surface of the external layer of the median eminence. *Z. Anat. Entwickl. Gesch.* 143, 255–262. https://doi.org/10.1007/BF00519869

Wittmann, G., Hrabovszky, E., & Lechan, R. M. (2013). Distinct glutamatergic and GABAergic subsets of hypothalamic pro-opiomelanocortin neurons revealed by in situ hybridization in male rats and mice. *The Journal of comparative neurology*, *521*(14), 3287–3302. https://doi.org/10.1002/cne.23350

Wolburg, H., Noell, S., Mack, A., Wolburg-Buchholz, K., & Fallier-Becker, P. (2009). Brain endothelial cells and the glio-vascular complex. *Cell and tissue research*, *335*(1), 75–96. https://doi.org/10.1007/s00441-008-0658-9

Woods, S. C., Stein, L. J., McKay, L. D., & Porte, D., Jr (1984). Suppression of food intake by intravenous nutrients and insulin in the baboon. *The American journal of physiology*, *247*(2 Pt 2), R393–R401. https://doi.org/10.1152/ajpregu.1984.247.2.R393

Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., Dhillo, W. S., Ghatei, M. A., & Bloom, S. R. (2001). Ghrelin enhances appetite and increases food intake in humans. *The Journal of clinical endocrinology and metabolism*, *86*(12), 5992. https://doi.org/10.1210/jcem.86.12.8111

Würtz, P., Kangas, A. J., Soininen, P., Lehtimäki, T., Kähönen, M., Viikari, J. S., Raitakari, O. T., Järvelin, M. R., Davey Smith, G., & Ala-Korpela, M. (2013). Lipoprotein subclass profiling reveals pleiotropy in the genetic variants of lipid risk factors for coronary heart disease: a note on Mendelian randomization studies. *Journal of the American College of Cardiology*, *62*(20), 1906–1908. https://doi.org/10.1016/j.jacc.2013.07.085

Wynne, K., Park, A. J., Small, C. J., Patterson, M., Ellis, S. M., Murphy, K. G., Wren, A. M., Frost, G. S., Meeran, K., Ghatei, M. A., & Bloom, S. R. (2005). Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. *Diabetes*, *54*(8), 2390–2395. https://doi.org/10.2337/diabetes.54.8.2390

Wyse, B. M., & Dulin, W. E. (1970). The influence of age and dietary conditions on diabetes in the db mouse. *Diabetologia*, *6*(3), 268–273. https://doi.org/10.1007/BF01212237

Xu, B., Goulding, E. H., Zang, K., Cepoi, D., Cone, R. D., Jones, K. R., Tecott, L. H., & Reichardt, L. F. (2003). Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nature neuroscience*, *6*(7), 736–742. https://doi.org/10.1038/nn1073

Xu, J., Bernstein, A. M., Wong, A., Lu, X. H., Khoja, S., Yang, X. W., Davies, D. L., Micevych, P., Sofroniew, M. V., & Khakh, B. S. (2016). P2X4 Receptor Reporter Mice: Sparse Brain Expression and Feeding-Related Presynaptic Facilitation in the Arcuate Nucleus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *36*(34), 8902–8920. https://doi.org/10.1523/JNEUROSCI.1496-16.2016

Yamashita, M., Takayanagi, Y., Yoshida, M., Nishimori, K., Kusama, M., & Onaka, T. (2013). Involvement of prolactin-releasing peptide in the activation of oxytocin neurones in response to food intake. *Journal of neuroendocrinology*, *25*(5), 455–465. https://doi.org/10.1111/jne.12019

Yamauchi, T., Iwai, M., Kobayashi, N., & Shimazu, T. (1998). Noradrenaline and ATP decrease the secretion of triglyceride and apoprotein B from perfused rat liver. *Pflugers Archiv : European journal of physiology*, *435*(3), 368–374. https://doi.org/10.1007/s004240050525

Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., Yamashita, S., Noda, M., Kita, S., Ueki, K., Eto, K., Akanuma, Y., Froguel, P., Foufelle, F., Ferre, P., Carling, D., Kimura, S., Nagai, R., Kahn, B. B., & Kadowaki, T. (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature medicine*, *8*(11), 1288–1295. https://doi.org/10.1038/nm788

Yang, L., Qi, Y., & Yang, Y. (2015). Astrocytes control food intake by inhibiting AGRP neuron activity via adenosine A1 receptors. *Cell reports*, *11*(5), 798–807. https://doi.org/10.1016/j.celrep.2015.04.002

Yoo, S., Cha, D., Kim, D. W., Hoang, T. V., & Blackshaw, S. (2019). Tanycyte-Independent Control of Hypothalamic Leptin Signaling. *Frontiers in neuroscience*, *13*, 240. https://doi.org/10.3389/fnins.2019.00240

Yoo, S., Cha, D., Kim, S., Jiang, L., Cooke, P., Adebesin, M., Wolfe, A., Riddle, R., Aja, S., & Blackshaw, S. (2020). Tanycyte ablation in the arcuate nucleus and median eminence increases obesity susceptibility by increasing body fat content in male mice. *Glia*, *68*(10), 1987–2000. https://doi.org/10.1002/glia.23817

Yu, Q., Gamayun, I., Wartenberg, P., Zhang, Q., Qiao, S., Kusumakshi, S., Candlish, S., Götz, V., Wen, S., Das, D., Wyatt, A., Wahl, V., Ectors, F., Kattler, K., Yildiz, D., Prevot, V., Schwaninger, M., Ternier, G., Giacobini, P., Ciofi, P., … Boehm, U. (2023). Bitter taste cells in the ventricular walls of the murine brain regulate glucose homeostasis. *Nature communications*, *14*(1), 1588. https://doi.org/10.1038/s41467-023-37099-3

Zerial, M., & McBride, H. (2001). Rab proteins as membrane organizers. *Nature reviews. Molecular cell biology*, *2*(2), 107–117. https://doi.org/10.1038/35052055

Zhan, C., Zhou, J., Feng, Q., Zhang, J. E., Lin, S., Bao, J., Wu, P., & Luo, M. (2013). Acute and long-term suppression of feeding behavior by POMC neurons in the brainstem and hypothalamus, respectively. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *33*(8), 3624–3632. https://doi.org/10.1523/JNEUROSCI.2742-12.2013

Zhang, E. E., Chapeau, E., Hagihara, K., & Feng, G. S. (2004). Neuronal Shp2 tyrosine phosphatase controls energy balance and metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(45), 16064–16069. https://doi.org/10.1073/pnas.0405041101

Zhang, F., Basinski, M. B., Beals, J. M., Briggs, S. L., Churgay, L. M., Clawson, D. K., DiMarchi, R. D., Furman, T. C., Hale, J. E., Hsiung, H. M., Schoner, B. E., Smith, D. P., Zhang, X. Y., Wery, J. P., & Schevitz, R. W. (1997). Crystal structure of the obese protein leptin-E100. *Nature*, *387*(6629), 206–209. https://doi.org/10.1038/387206a0

Zhang, G., Bai, H., Zhang, H., Dean, C., Wu, Q., Li, J., Guariglia, S., Meng, Q., & Cai, D. (2011). Neuropeptide exocytosis involving synaptotagmin-4 and oxytocin in hypothalamic programming of body weight and energy balance. *Neuron*, *69*(3), 523–535. https://doi.org/10.1016/j.neuron.2010.12.036

Zhang, J., Wang, L., Yang, Y., Wang, S., Huang, C., Yang, L., Li, B., Wang, L., Wang, H., & Hao, S. (2023). Dissection of the bed nucleus of the stria terminalis neuronal subtypes in feeding regulation. *Physiology & behavior*, *271*, 114333. https://doi.org/10.1016/j.physbeh.2023.114333

Zhang, J., Wu, W., Li, D., Guo, Y., & Ding, H. (2010). Overactivation of NF-κB impairs insulin sensitivity and mediates palmitate-induced insulin resistance in C2C12 skeletal muscle cells. Endocrine, 37(1), 157–166. https://doi.org/10.1007/s12020-009-9283-y

Zhang, X., & van den Pol, A. N. (2016). Hypothalamic arcuate nucleus tyrosine hydroxylase neurons play orexigenic role in energy homeostasis. *Nature neuroscience*, *19*(10), 1341–1347. https://doi.org/10.1038/nn.4372

Zhang, X., Dong, F., Ren, J., Driscoll, M. J., & Culver, B. (2005). High dietary fat induces NADPH oxidase-associated oxidative stress and inflammation in rat cerebral cortex. *Experimental neurology*, *191*(2), 318–325. https://doi.org/10.1016/j.expneurol.2004.10.011

Zhang, X., Zhang, G., Zhang, H., Karin, M., Bai, H., & Cai, D. (2008). Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. *Cell*, *135*(1), 61–73. https://doi.org/10.1016/j.cell.2008.07.043

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, *372*(6505), 425–432. https://doi.org/10.1038/372425a0

Zinman, B., Bhosekar, V., Busch, R., Holst, I., Ludvik, B., Thielke, D., Thrasher, J., Woo, V., & Philis-Tsimikas, A. (2019). Semaglutide once weekly as add-on to SGLT-2 inhibitor therapy in type 2 diabetes (SUSTAIN 9): a randomised, placebo-controlled trial. *The lancet. Diabetes & endocrinology*, *7*(5), 356–367. https://doi.org/10.1016/S2213-8587(19)30066-X

Zubiaga, L., Briand, O., Auger, F., Touche, V., Hubert, T., Thevenet, J., Marciniak, C., Quenon, A., Bonner, C., Peschard, S., Raverdy, V., Daoudi, M., Kerr-Conte, J., Pasquetti, G., Koepsell, H., Zdzieblo, D., Mühlemann, M., Thorens, B., Delzenne, N. D., Bindels, L. B., … Pattou, F. (2023). Oral metformin transiently lowers post-prandial glucose response by reducing the apical expression of sodium-glucose co-transporter 1 in enterocytes. *iScience*, *26*(4), 106057. https://doi.org/10.1016/j.isci.2023.106057

Zubiaga, L., Briand, O., Auger, F., Touche, V., Hubert, T., Thevenet, J., Marciniak, C., Quenon, A., Bonner, C., Peschard, S., Raverdy, V., Daoudi, M., Kerr-Conte, J., Pasquetti, G., Koepsell, H., Zdzieblo, D., Mühlemann, M., Thorens, B., Delzenne, N. D., Bindels, L. B., … Pattou, F. (2023). Oral metformin transiently lowers post-prandial glucose response by reducing the apical expression of sodium-glucose co-transporter 1 in enterocytes. *iScience*, *26*(4), 106057. https://doi.org/10.1016/j.isci.2023.106057

# Acknowledgements

Thank you, Vincent, for the huge opportunity that you offer me every day.
Thank you, Monica, for teaching me how to never give up.
Thank you, dear member of the jury, for the time that you decided to dedicate in the examination of my work.
Thank you, all the current and ex members of the lab, scientific and administrative, that I crossed my path with. I love you all. A big up to Flo and Dani. Special mention to Ci Virgi, I'll thank you in private.
Thank you, all the non-scientific people of my life, that gave me their support when I most needed it.

Finally, I would like to thank the various organizations who have funded this work This work was supported by the European Research Council (ERC) Synergy Grant-2019-WATCH No 810331, EGID (no. ANR-10- LABEX-0046).