Insulin sensitivity : modulation by neuropeptides and hormones

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Chapter 1

General introduction

Obesity and diabetes.

Most adult animals and humans tend to keep their body weight within a relative narrow range, despite large variations in daily food intake and physical activity. This indicates that body weight is tightly regulated. However, the growing percentage of people that are overweight or obese shows that this regulatory mechanism is not flawless. There is considerable evidence that during evolution, this regulation system has evolved as a system intended for conservation of energy, seeking food in times of famine and storing energy in times of plenty. This increased the survival chance during long periods of energy deprivation. There has been little evolutionary pressure to increase energy expenditure or reduce food intake once energy stores are replete. Therefore, this regulatory system is biased strongly towards weight gain and storage of fat, with few mechanisms that encourage weight loss [1].

Nowadays, in our Western society food is in abundance and energy-rich with high levels of sugar and saturated fats. At the same time, large shifts towards less physically demanding work have been observed [2]. These environmental changes are reflected in the percentages of overweight/obese people. The prevalence of overweight and obesity is commonly assessed by using body mass index (BMI), defined as the weight in kilograms divided by the square of the height in meters (kg/m^2). A BMI over 25 kg/m^2 is defined as overweight and a BMI over 30 kg/m^2 as obese. Globally, obesity has reached epidemic proportions with more than 1 billion overweight adults, at least 300 million of them obese (World Health Organization, 2003). In The Netherlands, 47% of the adults are overweight with 11% being obese (CBS, 2004).

Overweight and obesity are caused by a disturbed balance between energy/food intake and energy expenditure. Overweight and obesity pose a major risk for chronic diseases, particularly type 2 diabetes mellitus, cardiovascular disease, hypertension, stroke and certain forms of cancer [3]. The likelihood of developing type 2 diabetes mellitus and hypertension rises steeply with increasing body fatness. Approximately 85% of patients with diabetes mellitus have type 2 and of these patients, 90% are obese or overweight (WHO, 2003). Type 2 diabetes mellitus is no immediate life threatening disease, but the increased glucose levels ultimately lead to complications, such as cardiovascular disease, retinopathy,

nephropathy and cognitive dysfunction. These complications will reduce the overall quality of life, and also form an increased risk of premature death.

Regulation of food intake.

Hypothalamic regulation of food intake.

Energy/food intake is regulated by a highly complex system, that integrates several signals concerning the metabolic status and energy expenditure, but also the availability of food, memory of food and the social situation. This regulatory mechanism involves several brain regions ranging from cortex to brainstem, but most interest has focused on the hypothalamus, which is considered as the main regulatory feeding center of the brain.

The hypothalamus consists of several nuclei, that are involved in the regulation of food intake. One of them is the arcuate nucleus, which lies around the base of the third ventricle, immediately above the median eminence. Due to this position, the neurons of the arcuate nucleus have easy access to peripheral satiety factors. First of all, peripheral signals can gain access to the arcuate nucleus from the cerebrospinal fluid (csf) in the third ventricle (either by diffusion or via receptors)[4;5]. Secondly, peripheral signals can easily reach the arcuate axon terminals, because the endothelial barrier within the median eminence lacks tight junctions [6]. Therefore, the blood-brain-barrier is not present in this region and arcuate axon terminals are in direct contact with signals from the bloodstream. The neurons of the arcuate nucleus are called first order neurons because of this direct contact with peripheral satiety factors. The arcuate nucleus contains two distinct groups of neurons with opposing effects on food intake (Fig. 1). One group consists of neurons that co-express neuropeptide Y (NPY) and agouti-related peptide (AgRP), neuropeptides, that activate appetite [7;8]. The other group consists of neurons that co-express pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), both neuropeptides that inhibit appetite [9].

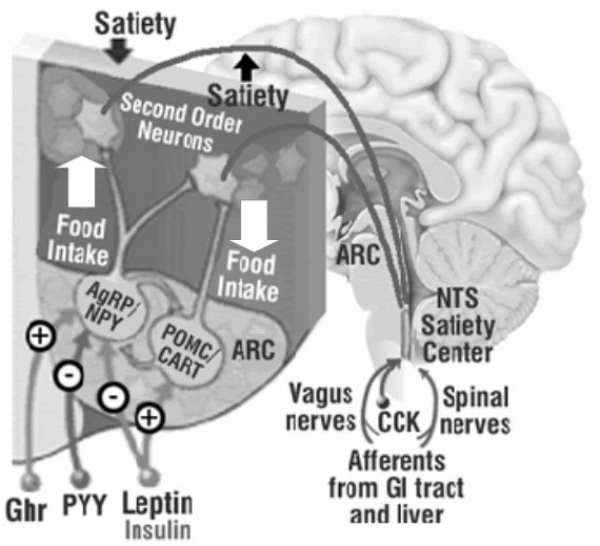

Figure 1. Central command centers. The arcuate nucleus of the brain contains two sets of neurons with opposing effects. Activation of the NPY/AgRP neurons increases appetite, whereas activation of the POMC/CART neurons has the opposite effect.
Adapted from Marx J, 2003. *Science* 299, 846-849. Republished with permission.

During fasting or a fall in the body's energy stores, the mRNA expression of the two orexigenic peptides, NPY and AgRP, is increased. NPY and AgRP will produce a shift towards a positive energy balance by increasing food intake and decreasing energy expenditure [10;11]. From the two orexigenic neuropeptides, NPY is the most potent one. Currently, six different NPY receptors have been identified, that mediate the effects of NPY [12;13]. Most of the NPY neurons (~90%) also contain AgRP [8]. AgRP acts as a high affinity antagonist of the melanocortin 3 and 4 receptors (MC3R and MC4R), 2 receptors downstream of the POMC pathway [14;15]. Furthermore, NPY/AgRP neurons can inhibit their neighbouring POMC/CART neurons by means of the neurotransmitter GABA [16].

During the fed condition or a state of positive energy balance, the mRNA expression of the two anorexigenic neuropeptides, POMC and CART is increased. These neuropeptides will produce a shift towards a negative energy balance by decreasing food intake and increasing energy expenditure [10;11]. POMC is a precursor molecule that is cleaved into several peptides that are called melanocortins (MC). Of these melanocortins, α-melanocyte-stimulating hormone (α-MSH) is considered to be the most important one for regulation of food intake. The effects of melanocortins are mediated by melanocortin receptors of which currently five are cloned. Two of them, MC3R and MC4R, are mainly expressed within the brain where they interfere with food intake. Both receptors have a high affinity for α-MSH, but also for AgRP. CART is co-localized with POMC in the arcuate nucleus. However, the mechanisms that

mediate the effects of CART are still poorly understood and until now there has not been a receptor identified.

The neurons from the arcuate nucleus project to second order neurons in the paraventricular nucleus, ventromedial nucleus, dorsomedial hypothalamic nucleus and the lateral hypothalamic area [10;11]. The second order neurons in these areas are also divided into neurons that contain orexigenic or anorexigenic neuropeptides. Second order orexigenic neuropeptides are melanin-concentrating hormone (MCH) and orexins (or hypocretins), second order anorexigenic neuropeptides are corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH). The second order neurons project to different autonomic centers in the brainstem. In these areas satiety signals are processed and the hypothalamic signals are integrated with afferent information related to satiety [17].

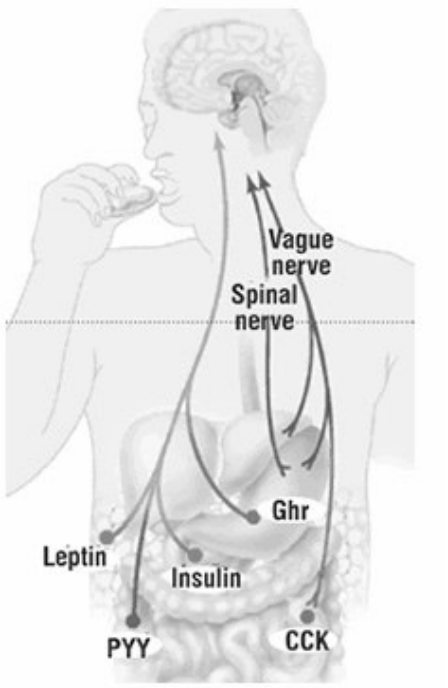

Figure 2. Appetite conrollers.
The body produces several hormones that act through the brain to regulate short- and long-term appetite.
From Marx J, 2003. *Science* 299, 846-849. Republished with permission.

The hypothalamic pathways, that regulate food intake are essential for the long-term regulation of food intake and energy homeostasis. Apparently, in the obese situation these pathways are not functioning properly. Indeed, it has been shown that the balance between orexigenic and anorexigenic neuropeptides is profoundly altered in several animal models of obesity [18].

Peripheral signals that regulate food intake.

Numerous peripheral signals act on the central regulatory centers, and, thereby, contribute to the regulation of food intake and energy expenditure (Fig. 2). These peripheral signals can be divided in long-term and short-term signals [19]. Long-term signals provide information about body fat stores and the amount of energy

consumed over a more prolonged period of time. Short-term signals do not reflect body adiposity, but provide information about hunger and satiety.

Leptin and insulin are examples of long-term signals. Leptin is secreted from adipocytes in proportion to the amount of adipose tissue [20]. Although insulin is secreted from pancreatic ß–cells, the circulating concentrations of insulin are also proportional to adipose tissue [21]. However, the overall insulin concentration should be taken into account, because insulin concentration can rise rapidly in a short period of time in response to a meal, and then return to basal levels [22]. Nevertheless, insulin transport into the brain is not rapid, but occurs over a period of hours, consistent with a role for insulin as a long-term regulator of energy balance [23]. Leptin and insulin both bind to receptors located in the arcuate nucleus and thereby affect the NPY- and POMC-pathway leading to an inhibitory effect on appetite [5;24].

Ghrelin, cholecystokinin (CCK) and peptide YY (PYY) are examples of short-term signals. Ghrelin is a circulating hormone that is synthesized in the stomach and that increases food intake [25]. Ghrelin levels increase during fasting, rising sharply before and falling within one hour of a meal, suggesting that ghrelin plays a role in hunger and meal initiation [26]. CCK is a hormone that is produced in the upper part of the small intestine in response to the presence of ingested food. It is released postprandialy and inhibits food intake [27]. CCK induces satiety and decreases meal size by stimulating the vagal nerve projecting to the nucleus of the solitary tract (NTS) in the brainstem [28]. PYY is a hormone that is produces in the distal part of the gastrointestinal tract and is released into the circulation in response to a meal [29]. PYY can be cleaved into PYY_{3-36}, the isoform of PYY that inhibits food intake. PYY_{3-36} inhibits food intake by acting directly on the arcuate nucleus via the Y2R, a presynaptic inhibitory receptor on NPY neurons [30].

These long-term and short-term signals are regulated by interacting mechanisms. They cooperate, in order to integrate energy expenditure and energy intake, to ensure that energy homeostasis is maintained. In the obese situation, the mechanism fails to preserve energy homeostasis and several of these peripheral signals have been shown to be dysregulated as well in obesity [31-33].

Insulin resistance.

The metabolic syndrome comprises a cluster of anomalies that increase the risk of cardiovascular disease and type 2 diabetes mellitus: hyperglycemia, abdominal obesity, hypertriglyceridemia, hypertension and low levels of high-density lipoprotein (HDL) cholesterol [34-36]. Insulin resistance may underlie the majority of these pathologies [37] and therapies that effectively reinforce insulin action may therefore ameliorate the risk profile of metabolic syndrome patients [38;39]. Insulin resistance is defined as the requirement of an abnormally large amount of insulin (endogenous or exogenous) for a biological response [40]. Insulin resistance describes a condition that is characterized by decreased tissue sensitivity to the action of insulin and therefore affects multiple organs.

Insulin resistance in the liver leads to the failure of insulin to suppress the hepatic glucose production sufficiently. Insulin affects glucose production directly via signaling through the hepatic insulin receptor to inhibit glycogenolysis and gluconeogenesis. However, it has also been suggested that insulin suppresses glucose production indirectly through extrahepatic actions of insulin on muscle and adipose tissue to inhibit release of gluconeogenic substrates (lactate, alanine and glycerol) and gluconeogenic energy substrates (FFAs) [41-43]. In addition, insulin suppresses the hepatic production of very-low-density lipoprotein (VLDL) particles. These inhibitory effects are also directly on the liver through the effects of insulin on synthesis and secretion of VLDL [44] and indirectly because insulin affects the FFA release from adipose tissue [45].

Insulin resistance in muscle and adipose tissue leads to a diminished ability of insulin to stimulate glucose uptake in these tissues. In muscle, insulin stimulates the uptake and oxidation of glucose and the formation of glycogen. Skeletal muscle can use both glucose and FFA as energy source and the shift between these two depends primarily on the availability of FFAs and exercise level. In adipose tissue, glucose is needed for the formation of glycerol-3-phosphate, which is necessary for the formation of triglycerides (TG). Insulin stimulates the glucose uptake and therefore promotes adipocyte TG synthesis. Insulin also inhibits the rate of TG lipolysis through inhibition of the lipolytic enzyme hormone sensitive lipase.

Outline of this thesis.

The studies described in this thesis all involve the hypothesis that the hypothalamus is not only involved in the regulation of food intake, but also regulates insulin sensitivity (independent of its effects on food intake). In obesity, dysregulation of several hypothalamic neuropeptides and peripheral hormones that regulate food intake, has been observed and leads to an increased food intake. Perhaps the same dysregulation of these neuropeptides and hormones can cause insulin resistance as well. All studies described here where performed in mice.

The effects of both the NPY and POMC pathway on insulin sensitivity were studied. In **chapter 2** we describe the effects of a continuous intracerebroventricular (icv) infusion of NPY on insulin sensitivity. In **chapter 3** the effects of icv injections of MTII, an agonist of the POMC pathway, is described. In **chapter 4** the acute effects of the peripheral hormone PYY_{3-36} on insulin sensitivity are described. In **chapter 5** the long-term effects of PYY_{3-36} are investigated to examine whether PYY_{3-36} could be of use in the clinical management of obesity and insulin resistance. Finally, in **chapter 6**, the role of the peripheral hormone leptin and the role of its central signalling on insulin sensitivity is examined in *ob/ob* mice and evaluated against the contribution of the obese phenotype itself on insulin sensitivity.

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