Obesity
An overview of the complex biological effects of expanding adipose tissue

T.W. van Haeften*

Abstract

Obesity has a more complex biology than previously thought. Feeding behaviour is regulated via a integrative circuit of afferent input from the stomach, the pancreas and the intestines via hormonal influences and the vagal nerve, which affect hypothalamic nuclei. This will lead to satiety and inhibition of hunger combined with other effects such as energy expenditure and growth promoting effects. Moreover, upon sufficient caloric intake, triglycerides are taken up in adipocytes, which in their turn will produce more leptin which inhibits hypothalamic nuclei and which will add to these hypothalamic effects. It has been suggested that more macrophages enter the adipose tissue which would alter adipocyte secretion. Adipocytes are now known to produce an array of proteins with hormonal effects calledadipo(cyto)kines. In obesity, many of these adipokines are produced in substantially higher amounts with potentially deleterious effcets notably on the risk of atherosclerosis, type 2 diabetes and cancer.

* Dept Internal Medicine F 02.126. UMC Utrecht
PO Box 8085 NL 3508 GA Utrecht The Netherlands

INTRODUCTION

Many countries now see a rapidly expanding "pandemic" of obesity.

While this started mainly in the Western world, since some 20 years virtually all countries now see their populations literally growing fatter.

The causes for this are more complex than was initially thought.

A major concern has become the development of "visceral obesity" that is the phenomenon that adipose tissue has enlarged around the "viscera" i.e. within the abdomen.

Visceral obesity may lead to larger risks for a number of diseases, notably cardiovascular (atherosclerotic) disease, diabetes mellitus, and various forms of cancer (1).

Although the biology behind this is still a matter of intense research, the complexity of this becomes gradually apparent. The unique position of visceral fat as to the portal vein circulation has led to intense research into the relationship of visceral fat and liver function and more generally to the "endocrinological" role of visceral fat tissue.

Fat tissue is now known not only to act as storage pool for calories in the form of triglyceride, but also to secrete a number of proteins which act as cytokines at distal sites, known as adipocytokines or adipokines. Although obesity is a recent phenomenon, adipocytokines date back over many millions of years and are present in all mammals and even other species.

BIOLOGY OF ADIPOCYTES

Adipose tissue exists mainly of adipocytes, which are formed from precursor cells, so called pre-adipocytes . Adipocytes can rapidly take up fatty acids (FA) and can store fat is triglycerides.

FA circulate in the plasma mainly in the form of triglycerides,bound to Very Low Density Lipoproteins (VLDL) and chylomicrons. Upon activation by insulin, Lipoprotein Lipase (LPL) located in the vascular tissue will exert a lipolytic effect on VLDL-bound triglyceride, forming glycerol and three molecules of FA. FA'sare then taken up into the adipocyte by Fatty Acid Binding Proteins (FABP) which are located at the cell surface.

Within the adipocyte, FA's and glycerol can form triglyceride,again under stimulation of insulin.Upon uptake of fat, adipocytes become larger , but obesity will not induce further development of preadipocytes to adipocytes.

Inversely, adipocyte triglyceride can rapidly undergo lipolysis in which the FA's and glycerol are formed; FA's can then leave the adipocyte. Lipolysis occurs by enzymatic effects of adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and monoglyceride lipase (2). This process is under stimulatory influence of (nor-)epinephrine by virtue of the activation of hormone sensitive lipase (HSL) (3); insulin is notable in that it has a strong inhibitory effect on HSL and thus on lipolysis. The latter inhibitory mechanism underlines actually the weight losing effect of a calorie-restricted diet:caloric restriction will lead to less insulin production ; thereby less inhibition of lipolysis ensues.

Interestingly, a slight increase in size will induce adipocytes to produce larger quantities of most of its proteins. In apparent contrast to the situation of obesity, leptin's major physiological effects are notable when adipocytes are small.

Adipocytes are now known to produce an array of adipocytokines with effects not only on appetite and energy expenditure (leptin) but also on the vasculature (adiponectin, visfatin, resistin, omentin, chemerin, apelin), the immune system (interleukin-6) or have effects on inflammation (Tumor Necrosis Factor-alpha (TNF-alpha), adipsin) (4). Many adipokines may have a role in cell division (leptin, interleukins).

The roles of adiponectin and the effects of leptin for food intake will first be described, followed by the influences of other organs on food intake.

Adiponectin

Adiponectin is produced by adipose tissue and skeletal muscle. While larger adipocytes will produce larger quantities of most adipocytokines, the production of adiponectin is *lower* in larger adipocytes. Adiponectin has anti-inflammatory actions in macrophages via inhibition of nuclear factor kappa-light chain enhancer of B cells (NF-kB) (5). A similar inhibition may also have an anti-atherogenic effect in endothelial cells. By virtue of its effect on endothelial nitric oxide synthase (eNOS), which regulates the production of nitric oxide (NO), adiponectin may diminish proliferation of vascular smooth muscle cells. Some studies point also to a role for adiponectin in myocardial remodeling protecting the myocardium from injury (6).

Adiponectin may increase insulin sensitivity, fatty acid oxidation and inhibit hepatic gluconeogenesis. These protective effects (anti-atherogenic, anti-diabetic, anti-inflammatory) make adiponectin a" good"

adipokine. It is of particular note that since adipocytes become larger in obesity, they produce less adiponectin possibly adding to the augmented risk of obese subjects for atherosclerosis and diabetes.

Leptin and food intake

Leptin is exclusively produced from adipocytes.Its production is regulated over the course of weeks. Plentitude of food intake will lead to increased production of leptin by the (enlarged) adipocytes.

Leptin acts on leptin receptors present in various tissues (7). Upon binding with leptin, leptin receptors present in the hypothalamus will lead to activation of the melanocortin 4 receptor (MC4) which will decrease hunger (with reduction of food intake) and at the same time will lead to expenditure of energy. Leptin will further diminish neuropeptide Y (NPY) in arcuate nucleus cells, which will then suppress hunger.

In addition, leptin receptors in the hypothalamus are nicely situated to also influence pituitary function (7,8). Leptin can stimulate gonadotrophin production which activates gonadal function. Upon continued food intake , increasing levels of leptin will then enable the animals gonadal function and ultimately its breeding. Similarly, leptin appears to influence growth hormone and possibly also Thyrotropin Releasing Hormone (TRH) and thereby Thyroid Stimulating Hormone (TSH) levels and thyroidal function. This constellation would lead to the proposition that feeding itself will activate in the animal a complex hormonal interplay ultimately leading to growth and procreation.

HORMONAL RELATIONS BETWEEN OTHER ORGANS AND FOOD INTAKE

Stomach

*Ghrelin*Interestingly, the effects of leptin are largely opposed by gastric production of ghrelin, a protein which is produced under circumstances of hunger. Ghrelin can stimulate the release of Growth Hormone (GH), hence its name. Upon binding to specific receptors in the hypothalamus, Ghrelin stimulates Neuropeptide Y (NPY) release and induces food intake (9). It was originally thought that the stomach would secrete ghrelin simply because the stomach was empty and therefore the person was hungry. The thus secreted ghrelin would then stimulate the person via the hypothalamus to go and eat. However, it now appears that in humans this relationship is more complex and that the simple knowledge of a person that "it is time to eat" may be sufficient for cerebral stimulation of the stomach to secrete more ghrelin, which indeed in turn will activate the hypothalamus to make the person feel hungry. Therefore, the clinical importance of ghrelin is still under debate.

Pancreas

Insulin secretion Insulin is secreted in low amounts during the fasting state by B cells in the pancreas islets. Upon eating insulin is secreted in larger amounts, both due to the effects of swallowing and the stimulation of food on the intestines and by the mere effect of the rise in plasma glucose. Insulin will then lead to decreases in hepatic glucose production and uptake of glucose and formation of glycogen in muscle and adipose tissue.

Interestingly, insulin is now known to have more effects: it has cell nucleus effects leading to changes in protein production and even mitosis in various tissues. Moreover, insulin

has multiple effects on the hypothalamus leading to enhancing satiety after a meal , and inhibition of hunger (10). Indeed , intranasal insulin administration reduces body weight in men (but not in women) (11). Thus, insulin appears to have leptin-like actions on hypothalamic centers although its time course is possibly more rapid. Indirectly, it may enhance energy expenditure possibly via enhanced sympathetic tone.

The gut

The presence of food in the intestines will lead to production of gastric inhibitory polypeptide also known as glucose-independent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP1) which will activate the B-cells via specific cell receptors to produce and secrete insulin especially at times that plasma glucose levels are (slightly) elevated (12).

*GLP1 and GIP*GLP1 has multiple effects presumably on cardiac tissue and especially on food intake. In rodents, GLP1 diminishes food intake by activation of specific receptors on hypothalamic centers by effects reminiscent of leptin. However, in humans GLP1 is metabolized to inactive metabolites within minutes by dipeptidyl peptidase 4 (DPP4). In humans the effects of GLP1 on food intake have been suggested to be indirect via stimulation of the autonomic nervous system especially the afferent branches of the vagal nerve (13). However, subcutaneous administration of analogues of GLP-1, that are not readily metabolized by DPP4, are effective in reducing hunger and consequently in reducing food intake presumably by direct hypothalamic effects.

In rodents GIP has also an effects on the hypothalamus leading to satiety and less hunger.

In the lower intestines Peptide YY (PYY) is also released in response to food and , just as GLP1, notably in response to protein (14).

Taken together the leptin pathways imply a useful feedback of adipocytes on appetite and food intake. Various hormonal and neuronal effects from the gut further influence food intake. They also enable the animal to expand energy, and appear to fulfill roles in enabling the animal to grow and to breed under circumstances with sufficient food.

ROLE OF INFLAMMATION IN EXPANDING ADIPOSE TISSUE

Normal fat tissue contains a low amount of inflammatory cells, notably macrophages of the M2 type and eosinophils. In obesity, these cells are relatively scarce, while other inflammatory cells predominate, notably M1-macrophages, neutrophils , lymphocytes, mast cells and others (15). This apparently leads to augmented secretion of cytokines, among which Tumor Necrosis Factor –alpha (TNF-α), Interleukin-6 (IL-6) and others, which have been reported to impair insulin signaling.

Visceral fat and subcutaneous tissue express a large number of genes in an identical manner. However, subtle differences exist (1), with preponderance of leptin production in subcutaneous fat tissue, while no differences exist in TNF-alpha; complement factors, angiotensinogen and others are produced predominantly in visceral tissue. Many of the deleterious effects of obesity seem to relate mainly to the development of visceral adiposity.

BIOLOGICAL IMPACT OF OBESITY

Obesity has a slowly evolving effect on alarge number of systems in mammals. It has gradually become clear that obesity increases

the risk of atherosclerosis, type 2 diabetes mellitus (Type 2 DM) and of certain types of cancer.These three disorders will be described only briefly, since an elaborate review of these complex disorders is outside the scope of this overview.

Atherosclerosis

Epidemiological studies have concluded to a augmented risk for atherosclerotic disease for obese people (1). However, the largest risk seems to relate to visceral adiposity, that is the existence of adiposity around the "viscera" within the abdomen, as opposed to subcutaneous fat. Atherosclerosis is a complex disease with disturbances in the arteries among which in the intima media . A large number of mechanisms is involved, including macrophage activation in the arterial wall, enhanced inflammatory changes, pro-thrombotic changes etc.

Adipokines have multiple effects which can augment the atherosclerotic process. For example, *leptin* has an NO-dependent vasodilatory effect but it can also upregulate endothelin-1 , a vasoconstrictor (16). It has also been proposed to lead to cellular changes in vascular cells. *Resistin* has been proposed to activate endothelin (15), and to have multiple effects leading to endothelial dysfunction and possibly proliferative effects on the vasculature (6). *Adiponectin* has been proposed to exert anti-atherosclerotic effects via its inhibitory effects on TNF-alpha in the vascular wall. Unfortunately, in obesity, less adiponectin is produced in adipocytes. Visfatin is also produced less in obesity.

In obesity, adipocytes grow larger and produce larger amounts of most of the adipokines. It has been proposed that this is related to hypoxia within the adipose cell (16).

Dyslipidemia Obesity is associated with disturbances in plasma lipids, with a notable increase in Low Density Lipoprotein cholesterol (LDL-cholesterol) and plasma triglycerides, and a decrease in High Density Lipoprotein cholesterol (HDL-cholesterol) especially in insulin resistant subjects. Presumably, the underlying insulin resistance leads to slightly elevated lipolysis with production of fatty acids in adipose tissue. Upon uptake in the liver, fatty acids will be bound to triglyceride. In the liver this is process is related to the production of cholesterol and its binding lipoprotein (Apo-B 100); this leads to Very-Low-Density-Lipoprotein (VLDL) formation. VLDL is transported into the blood, and it can release its cholesterol and its triglycerides in the vessel wall, potentially leading to atherosclerosis.

Hypertension .*Angiotensinogen* and *leptin* have been proposed to lead to hypertension. Indeed, although obesity itself clearly relates to hypertension, leptin-deficient humans (who are very obese) are not hypertensive. Leptin can augment sympathetic tone and can upregulate Na/K ATP-ase in the kidney. Large adipocytes produce more angiotensinogen, which would activate aldosterone and thereby lead to hypertension.

Insulin resistance

Insulin has a number of secondary effects: upon activation of the insulin receptor, autophosphorylation of the receptor activates Insulin Receptor Substrate-1 (IRS-1), which in its turn will phosphorylate subsequent molecules, Phospho- Inositol-3 phosphate Kinase (PI3-Kinase) leading to activation of Akt, ultimately leading to transcription of other factors at the DNA level, which will lead to production of various protein involved in diverse cellular processes as glycogen formation, (inhibition of) gluconeogenesis,

and to stimulation of glucose uptake. Insulin will also activate IRS-2. Via separate pathway of activating Mitogen Activating Protein Kinase (MAPK), activation of the IRS-1 will also lead to processes involved in expression of other genes notably involved in cellular growth and mitosis.

The activity of IRS1 is of major importance for overall insulin signaling. IRS-1 activity can be decreased by serine phosphorylation by Protein Kinase C (PKC), C-JunKinase (JNK), mammalian target of rapamycin (m-Tor), Suppressor of cytokine signaling-3 (Socs3)and others (17). This will decrease phosphorylation of PI-3K and activateproteasome dependent degradation.

Although the precise mechanisms of the occurrence of insulin resistance is still a matter of intense research, Free Fatty Acids (FFA), interleukin -6 and TNF-alpha have been proposed as causing insulin resistance by inducing transcription of factors leading to serine phosphorylation of IRS-1(and possibly IRS-2) at sites which will decrease the activation of down-stream molecules. The (separate) pathway leading to activation of mitogen activated protein kinase (MAPK) and cellular growth is presumably less or not involved in the process of insulin resistance. This has led to the hypothesis that under states of insulin resistance the MAPK dependent mechanisms have deleterious effects, for example in the myocardium (18). Similarly, it has been proposed that vasoconstrictor effects of insulin is regulated by MAPK dependent signaling while nitric oxide (NO) dependent vasodilator effects are regulated via PI-3K. In states of insulin resistance the MAPK effects would predominate possibly leading to endothelial dysfunction (19).

Type 2 Diabetes

Obesity is strongly associated with Type 2 diabetes.Type 2 diabetes ensues as a consequence of the combination of insulin resistance and pancreas beta cell failure (20). The above mentioned mechanisms for the development of insulin resistance are certainly of importance for the development of diabetes. During the development of type 2 diabetes beta cell mass is probably diminished in part by apoptosis (21).

It is now suspected that a number of adipokines have deleterious effects in beta-cells.

Leptin itself can augment insulin secretion. There is conflicting evidence regarding its potential to affect B cell apoptosis. On one side leptin has been shown to decrease uncoupling protein 2 (UCP2) in Bcells which would decrease apoptosis, but othershave not found an effect on apoptosis (22). However, leptin has multiple effects on gene transcription in various cell types both via Janus Kinase / Signal Transducer and Activator Transcription (JAK/STAT) and MAPK/extracellular signal-regulated kinase (ERK) pathways which may have more subtle effects in apoptosis and/or in B cell function.

Fatty acids, among which notably palmitate, have been shown to be deleterious for B cells and to be able to cause apoptosis. *Adiponectin* has been proposed to diminish the deleterious effects of palmitate . In its turn, palmitate appears to lower adiponectin receptor 2 mRNA. Adiponectin may also have effects on gene transcription via ERK pathways (22). Others have proposed that adiponectin (and leptin) increase proliferation of B cells.

TNF-alpha is able to induce apoptosis in B cells (22) either directly or possibly also via augmenting amylin expression; amylin has

been proposed to lead to accumulation of amyloid in islets.

Although a possible role of *visfatin* is far from clear, it now appears that visfatin may stimulate B cell proliferation; it may have additional effects possibly via gene regulation of PDX1 and the insulin gene (22). Visfatin is notable since its production is decreased in obesity and therefore its potential beneficial effects on B cells might be of less importance in obesity.

Cancer

Obesity is associated with a markedly increased risk for a number of cancer types ,such as colon, esophageal, breast, uterine, prostate and other cancers. In view of the marked hyperinsulinemia associated with insulin resistance, insulin is suspected to play a major role. Indeed, *insulin* has proliferative and anti-apoptotic effects in various tissues. Whether this effect is via the actual insulin receptor or via the Insulin-like Growth Factor –I (IGF-1) receptor is so far less clear; insulin can actually be bound to both receptor types (23). The growth promoting properties of IGF-1 are complex, since IGF-1 is bound to a number of binding proteins, the levels of which are partly dependent on plasma insulin.

A number of studies have looked into the potential role of *leptin* for breast cancer. Leptinappears to augment estrogen production and expression , and it has a proliferative effect on breast tumour cells (23,24). By its MAPK and STAT signaling effects leptin is relevant for cell proliferation and differentiation (23).

Adiponectin has been proposed to protect against carcinogenesis by its insulin sensitizing effects (counteracting hyperinsulinemia), and by inactivating MAPK.

Plasminogen activator inhibitor-1 (PAI-1)

which is also augmented in obesity has also been hypothesized to relate to cancer by promoting tumour growth and/or by effects on the vasculature such as adhesion and angiogenesis (22). Although more adipokines have been named as potentially involved in tumorigenesis, more research will be needed before this would become clearer (22).

CONCLUSION

Recent years have seen the exciting discovery of the fundamental role of fat tissue hormonal regulation of food intake, satiety, and energy expenditure, as exemplified by the role of leptin. This has subsequently led to the discovery of several of hormones from other organs , especially the gut , with important effects in the regulation of food intake. Expanding adipose tissue, as is seen in obesity, has now been proposed to be under the influence of inflammatory cells such as macrophages. This leads presumably to production of deleterious substances such as TNF-alpha, interleukins and others. Several adipokines may be involved in the development of insulin resistance. Moreover, in obesity, adipocytes produce high amounts of deleterious adipokines that may have deleterious effects on the risk of atherosclerosis, type 2 diabetes and cancer. Obese subjects are generally hyperinsulinemic in response to the prevailing insulin resistance. It has been suggested that insulin may have growth proliferative effects involved in tumour growth. Unfortunately, in obesity lower amounts of adiponectin are produced; adiponectin may have protective effects both against the development of atherosclerosis, and cancer and possibly type 2 diabetes.

Legends

Figure 1. A schematic representation of the effects of various hormones influencing

feeding behaviour.

Figure 2. Hypothalamic effects of leptin on feeding behaviour are associated with other effects notably on energy expenditure, and complex interactions with growth, and procreation.

Figure 3 .Insulin resistance: Effects of adipokines on insulin signaling

Figure 4. Schematic representation of potential effects of adipokines on atherosclerosis, cancer and type 2 diabetes mellitus

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