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Molecular Mechanisms of Adipogenesis and Adipocyte Biology - Possible role of MKPs and STAMPs

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INTRODUCTION

During the last 25 years, our scientific view of adipose tissue physiology has changed completely. From solely being described as a depot for uptake and release of triglycerides (TG) [1], it is now regarded a complex multifunctional compartment with many similarities to an organ [2]. Pioneering experiments in the mid-80s by the Spiegelman laboratory [3] and the discovery of the leptin obesity gene in the early 90s [4], started this paradigm shift in adipose tissue biology by showing that adipocytes, in response to various stimuli, secrete physiologically relevant signaling molecules into the circulation which control global energy homeostasis. Today, we know that a wide range of peptide and fatty acid hormones are secreted from adipose tissue regulating diverse physiological functions, such as immune responses, blood pressure control, bone mass, thyroid and reproductive functions, fat mass and nutrient homeostasis [5, 6]. This new knowledge of normal adipocyte biology not only increased our understanding of mammalian physiology, it has also awarded us with new possibilities to combat major health problems, as it has become evident that excess adipose tissue plays central roles in diseases, such as type 2 diabetes, atherosclerosis, and cancer [7-9].

The following overview discusses the developmental origin of adipose tissue and the differentiation program that turns precursor cells into functional adipocytes, the molecular details of how adipocytes release and store fat, the characteristics and functional importance of the many molecules that adipocytes secrete, and how all these properties are related to human health and disease. Finally, an introduction to the STAMP family connects recent knowledge about these proteins to the growing universe of adipose tissue biology.

ADIPOSE TISSUE DEVELOPMENT

General features

Adipose tissue is a loose connective tissue located either subcutaneously just under the skin, in neck regions, or at several intra-abdominal, or visceral, locations inside the thorax and abdomen in close proximity to major internal organs [10] (Figure 1A). In mice, the largest visceral adipose depot is, in contrast to the human body, connected to the gonads (Figure 1B), but for this exception the adipose tissue anatomy is similar [11]. The amount, position and type of adipose tissue can be precisely determined by computed tomography (CT), magnetic resonance imaging (MRI) or ultrasound (US) imaging techniques [12]. By using these techniques it is now evident that increased amount of abdominal adipose tissue, but not subcutaneous adipose tissue, is correlated with higher incidence of obesity-related type 2 diabetes and atherosclerosis [13]. The visceral depot shows higher gene expression for secretory and energy related proteins [14], higher lipolytic activity [15], and secrete more protein per adipocyte than subcutaneous adipose tissue [16]. In addition, the visceral depot is drained by the portal circulation system connecting it directly to the liver [12].

Adipose tissue can be divided into brown adipose tissue (BAT) and white adipose tissue (WAT), in addition to depot-specific differences.

Brown adipose tissue

Brown adipocytes are only found in mammals and have a multilocular distribution of TG droplets and a vast number of specialized mitochondria which contain the uniquely BAT-expressed protein, uncoupling protein-1 (UCP1), which generates heat at the expense of ATP [6, 17]. Until recently, the existence of BAT was thought to be restricted to small mammals and infants, but has now been shown to be present also in adult humans [18-20].

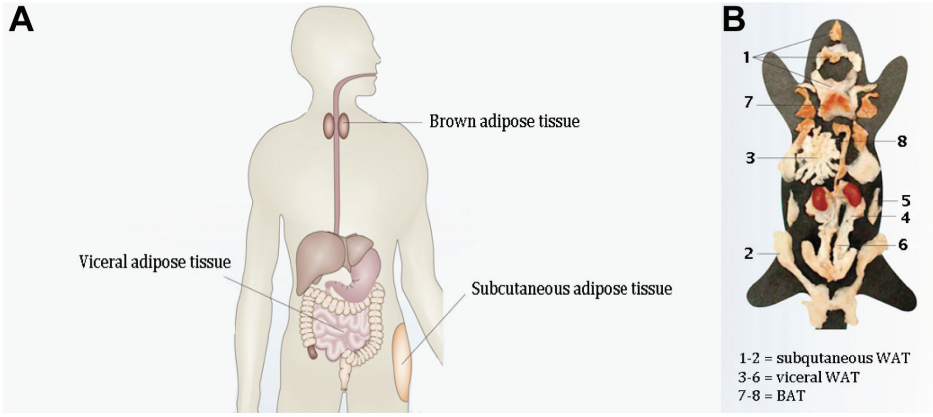

Figure 1. The main types of adipose tissue in humans and mice.
(A) In humans, visceral adipose tissue is localized in connection with the intestines, subcutaneous adipose tissue is under the skin, and brown adipose tissue is located in the neck.
(B) In mice, the largest visceral adipose depot is in connection to the gonads, also called epididymal adipose tissue (nr 6), but there is also large visceral depots in the omental region (nr 3) and in connection to the kidneys and other internal organs (nr 4 and 5). Subcutaneous WAT is mainly located at the anterior and dorsal ends. The figure was taken from [11] and used with permission from Nature Publishing Group.

White adipose tissue

The WAT consists mainly of mature white adipocytes, but stromal vascular cells can constitute up to 50% of the cellular content [21]. The adipocyte and stromal vascular fraction (SVF) can be separated by collagenase digestion followed by floatation of the adipocyte fraction by low speed centrifugation [22]. The stromal vascular fraction contains endothelial cells, pericytes, monocytes, macrophages, pluripotent stem cells, and other cell types, all with important functions to maintain homeostasis in the adipose tissue [23]. Pericytes and endothelial cells build up the vascular system that also retains adipocyte-committed precursor cells that differentiate into adipocytes upon stimulation [24]. The function of immune cells in adipose tissue is still not completely understood [25], but probably involves removal of necrotic cells [26].

Adipose tissues are highly dynamic, expanding and shrinking in response to various homeostatic, pharmacologic, and dietary stimuli [27]. A continuous positive energy balance will induce hypertrophic adipocytes within the WAT by increasing the size of the lipid droplet as more TG is taken up from the blood. But because adipocytes are postmitotic, a parallel hyperplastic response will take place to further increase the

adipose tissue TG storage capacity. Interestingly, the total number of adipocytes has been reported to be constant in adulthood, both in lean and obese subjects, but varies between different individuals [28]. Thus, the total adipose tissue number is set during childhood and adolescence. However, adipocytes do die and need to be replaced by new ones. Consequently, there is a continuous turnover of adipocytes throughout life [28]. What are the cellular characteristics of these progenitor adipose stem cells that develop into adipocytes and where do they come from?

Stem cells with potential to develop into adipocytes have been argued to occupy specific locations outside the adipose tissue, for example in the bone marrow, and then be recruited to WAT upon specific stimuli [29], but others argue that only adipose resident macrophages show bone marrow origin [30, 31]. Alternatively, progenitor cells can co-exist with adipocytes in the adipose tissue itself, as seen for neuronal and muscle stem cells [27]; there is currently strong experimental evidence that adipose stem cells exist in the WAT itself [27]. Consistently, the SVF contains adipose-derived stem cells (ADSCs) that can be differentiated to a variety of cell types, including bone, fat, cartilage, muscle, endothelial cells and neurons [32, 33]. Importantly, further subdivision of the SVF revealed a specific subpopulation of cells with the ability to develop into adipocytes containing a unilocular lipid droplet and to form physiologically active WAT in vivo [24, 34]. The Peroxisome Proliferator-Activated Receptor (PPAR) γ is necessary and sufficient for development of new adipocytes [35]. Interestingly, lineage analysis on PPARγ-expressing progenitor cells found that these cells reside near the vasculature in close proximity to pericytes [24]. The current view is therefore that adipocytes develop from progenitor cells of vasculature origin that are present in adipose tissue.

These recently identified precursor cells are not committed to become adipocytes as they can also be differentiated into other cell types of the mesenchymal lineage [34]. However, several molecules have previously been shown to induce commitment to the adipocyte linage by use of bone marrow derived mesenchymal stem cells (MSCs). For example, Bone Morphogenic Proteins (BMPs), through their intracellular mediators, the Smad proteins, trigger MCSs to enter the osteogenic and/or adipogenic lineage, while preventing commitment into the myogenic lineage [31]. Other

intracellular proteins, such as Schnurri-2 (Shn-2), modify the action of BMPs in the determination of the osteogenic and adipogenic lineages [36]. In addition, the action of BMPs can be modulated by members of the heparan sulphate proteoglycan family which are cell surface and extracellular matrix proteins [37]. Wnts comprise another family of highly conserved secreted proteins that act in a paracrine or autocrine manner by binding cell-surface receptors that increase commitment in the myogenic and osteogenic lineages and prevent adipogenesis [38]. Once MCSs are committed, they give rise to undifferentiated precursors (osteoblast, pre-adipocyte, and myoblast), which upon the expression of key transcription factors enter a differentiation program to acquire their specific functions [31]. The relationship between MSCs, ASCs and the adipocyte precursor cells, however, is still unclear.

Isolation of fibroblasts from Swiss mouse embryos gave rise to the 3T3 cell line that can be continuously propagated in culture and which starts to accumulate lipid droplets at confluency [39]. Later, subcloning experiments gave rise to the 3T3-F422A and 3T3-L1 sublines which accumulate high amounts of lipids upon adipogenic stimuli and develop into cells of adipocyte morphology although they contain multilocular lipid droplets and not the unilocular droplet seen in WAT *in vivo* [40]. Since their original establishment more than 30 years ago, they have been the most widely used model systems to study the differentiation of pre-adipocytes into mature adipocytes [31, 41].

Differentiation of a preadipocyte into a mature adipocyte is commonly divided into 3 stages: growth arrest, mitotic clonal expansion (MCE), and terminal differentiation [42]. Growth arrest of the pre-adipocytes (in G_0/G_1 phase) occurs by contact-inhibition. At this point, addition of prodifferentiative hormones will signal the arrested pre-adipocytes to re-enter the cell cycle and undergo several rounds of cell division, known as the MCE. There has been some controversy as to whether the MCE is required for differentiation [42]. Following the MCE, pre-adipocytes enter a unique growth arrested stage, G_D (D for differentiation), considered to be a poorly defined point of no return for commitment to terminal differentiation. During terminal differentiation, 3T3-L1 cells transform from their fibroblastic morphology into the appearance associated with mature adipocytes, with a round shape and lipid filled vacuoles, as well as with their biochemical characteristics [43]. Below is a review of

the most important known transcription factors that influence adipogenesis which are summarized in Figure 2.

The Peroxisome Proliferator-Activated Receptor (PPAR) gamma

The PPAR protein family consists of the α, δ and γ proteins. PPARs belong to the nuclear receptor (NR) superfamily and it is only PPARγ that is relevant for adipogenesis [44]. In order to bind DNA and regulate transcription PPARγ must heterodimerize with the retinoid X receptor (RXR) [45]. Multiple free fatty acids (FFAs) and their derivatives, as well as certain eicosanoids (e.g. the prostaglandin J_2), act as low affinity ligands for PPARγ; however, an endogenous PPARγ ligand of high affinity has not yet been identified. Nevertheless, several synthetic agonists are available, e.g. the thiazolidinediones (TZD), which are used in the clinic as insulin sensitizers [44]. PPARγ is responsible for activating many of the genes involved in fatty acid uptake and storage. The PPARγ gene gives rise to three different mRNA isoforms. PPARγ1 and PPARγ3 code for the same protein but from different transcripts that do not affect the open reading frame and are ubiquitously expressed, while PPARγ2 use a different promoter and alternative splicing and is unique to the WAT. The specific role of the different isoforms during adipogenesis is still unclear [42, 46]. The important role of PPARγ in adipocyte differentiation has been demonstrated through multiple experiments including *in vitro* overexpression and knockdown, as well as *in vivo* gene targeting in mice [46]. Knowledge drawn from these experiments suggests that PPARγ is necessary and sufficient for adipogenesis.

CCAAT/Enhancer Binding Protein (C/EBP)

C/EBP is a family of six highly conserved basic-leucine zipper transcription factors (α, β, γ, δ, ε and ζ). They act as homo- or heterodimers, and they have a ubiquitous tissue distribution. In adipocytes, three members of the C/EBP family regulate early phases of adipogenesis. C/EBPα acts as an activator for many adipocyte genes, such as GLUT4, leptin and aP2 [47]. Studies in fibroblasts lacking PPARγ found that C/EBPα alone is unable to induce differentiation, suggesting that C/EBPα and PPARγ participate in the same pathway [48]. C/EBPβ and C/EBPδ are expressed early after induction of adipogenesis [49]. Ectopic expression of C/EBPβ, but not C/EBPδ alone, has proven to be sufficient to induce adipocyte differentiation in vitro [49-51]. In

defining the order of actions towards terminal differentiation, recent studies have shown that C/EBPβ, but not C/EBPα, induces PPARγ expression. C/EBPβ is induced by the cAMP Response Element Binding Protein (CREB) in response to changes in cAMP levels [52]. DNA binding of C/EBPβ at the centromeres appears to be a prerequirement for initiation of MCE [53, 54].

Krüppel Like Factors (KLFs)

Another group of transcription factors that regulate adipogenesis is the KLFs. Several members of this large C2H2-zinc finger family play a role during adipocyte differentiation. KLF2 binds the promoter of PPARγ2 and represses its activation, thereby inhibiting adipogenesis [46]. After induction of differentiation, KLF5 expression, induced by C/EBPβ and C/EBPδ, dispatch KLF2 which promotes PPARγ2 expression. Later in development KLF5 is downregulated and expression of the proadipogenic KLF15 increases. KLF15 also promotes PPARγ2 expression in addition to promoting expression of genes associated with mature adipocytes (e.g. GLUT4) [55]. Other KLFs that have been shown to act during adipogenesis are KLF3, KLF4, and KLF6 [38]. KLF4 expression has been shown to be induced in response to cAMP and in cooperation with KROX20, which is a proadipogenic factor [56] that promotes C/EBPβ expression [57].

The Mitogen Activated Protein Kinases (MAPKs)

The MAPKs are ubiquitously expressed, highly conserved serine/threonine kinases and involved in pathways controlling embryogenesis, cell differentiation, cell proliferation and cell death [58]. In mammals there are three main MAPK families, the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 [58].

The ERK pathway was linked to adipogenesis in 1991 when it was shown that ectopic expression of a constitutively active Ras mutant led to growth arrest and terminal differentiation of 3T3-L1 cells in the absence of insulin and IGF-1 [59]. This was later confirmed by the discovery that inhibition of ERK expression suppresses adipogenesis [60] by inhibition of C/EBPβ [61]. However, in response to various growth factors and Preadipocyte Factor 1 (PREF-1), ERK has also been shown to

inhibit PPARγ activity by direct phosphorylation and thereby suppressing adipogenesis [62, 63]. In vivo studies with ERK1-/- knockout mice have indicated that ERK1, but not ERK2, is the isoform active in adipogenesis [121]. ERK1-/- mice are protected from insulin resistance and high fat diet-induced obesity supporting the *in vitro* findings [122].

Studies of p38 inhibitors effect in 3T3-L1 cells have linked p38 activation to phosphorylation of C/EBPβ leading to its activation, as seen with ERK, and subsequent promotion of adipogenesis [64]. Further evidence for p38 acting in a proadipogenic manner was shown by overexpression of active MKK6 which is upstream of p38 [65]. This dominant active mutant was sufficient to induce activation without any hormonal stimulation. However, prolonged overexpression induced massive cell death. Two additional reports further support p38 as an enhancer of adipocyte development [66, 67]. In contrast to these results, p38 has been found to activate CHOP leading to inhibition of C/EBPβ [64]. In addition, a study of p38 action in adipocyte development using multiple cell lines found that its knockdown and inhibition promoted adipogenesis [68]. These conflicting results could be due to the different cell lines that are used in these studies [68].

MAPK Phosphatases (MKPs)

The dual specificity phosphatases (DUSPs), also known as MAPK Phosphatases (MKPs), belong to the superfamily of protein tyrosine phosphatases (PTP) that can dephosphorylate both phospho-tyrosine and phospho-threonine residues. It is common to separate the MKPs in a group of typical MKPs and atypical MKPs, with 11 and 19 human family members, respectively [69]. All typical MKPs regulate MAPK activity through dephosphorylation of the TXY-motif. Several atypical MKPs have also been shown to have MAPK as their substrate, discussed below [70].

MKPs have a catalytic dual specific phosphatase (DSP) domain at the C-terminus with the conserved motif HCXXXXXR (histidine, cysteine, X as any amino acid and arginine). The DSP motif has no strict preference for any of the MAPKs; therefore, typical MKPs have a MAPK binding (MKB) motif at the N-terminal end.

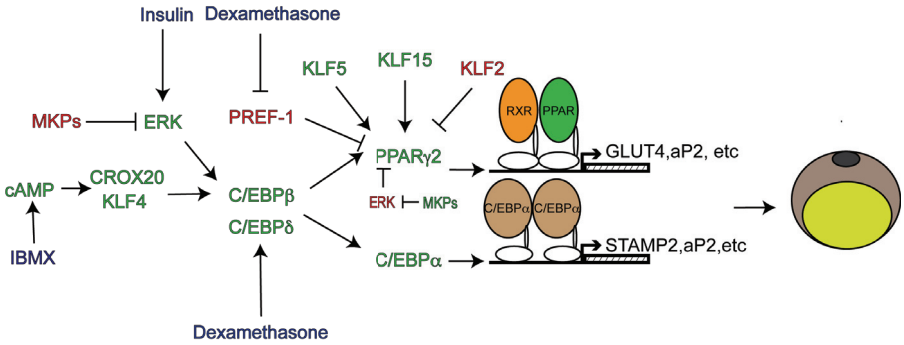

Figure 2. Overview of transcription factors that are involved in adipogenesis in the 3T3-L1 cell line and some of their regulators. Inducers of adipogenesis (blue) activate intracellular signaling cascades that induce transcription factor (TF) mobilization and transcriptional activation at promoters of pro-adipogenic factors and metabolic genes with functions in adipocytes. Molecules with a positive effect on adipogenesis are shown in green and those with a negative effect in red. See text for details.

To date, only two MKPs, MKP-1 and MKP-4, have been studied and found to affect adipogenesis and adipocyte function [71-74]. The effect of MKP-1 on adipogenesis was tested in both 3T3-L1 and 3T3-F442A cells where MKP-1 was found to regulate the essential down-regulation of ERK activity during adipogenesis [161]. MKP1 knockout mice display resistance to diet-induced obesity. This resistance was proposed to be due to lack of MKP-1 nuclear action, and not its cytosolic activity, as MAPK action in the cytosol was similar in wild type and knockout mice [75]. MKP-4 was present in murine adipocytes and was upregulated in ob/ob mice. In addition, ectopically expressed MKP4 inhibited adipogenesis and glucose uptake in 3T3-L1 cells. MKP-1 and MKP-4 were found to be induced by dexamethasone in 3T3-L1 cells, and a concominant block of p38 was followed by a reduction in insulin-induced glucose uptake [74]. In summary, these studies suggested that MKP-1 and MKP-4 are involved in adipogenesis by modulating MAPK activity.

ADIPOCYTE FUNCTION

Adipocytes control whole-body metabolism by regulating 95% of total body TG concentration in the body [76]. During fasting, there is a dynamic balance between the release of fatty acids from WAT and their uptake and oxidation by, most dramatically, the liver and skeletal muscle [77]. In states of high caloric intake WAT will increase uptake of fatty acids to avoid hyperlipidema and toxic lipid accumulation in

peripheral tissues, which can have devastating effects [6]. How is this dynamic uptake and release of fatty acids regulated in the WAT?

Adipocyte metabolism

Insulin signaling

Increased plasma glucose levels induce insulin secretion from pancreatic β cells into the bloodstream where it increases fatty acid and glucose uptake in the liver, muscle and adipocytes and decreases gluconeogenesis in the liver [78, 79]. In mature adipocytes insulin binds to the insulin receptor (IR), which then phosphorylates IR substrate 1 (IRS-1) (Figure 3). This leads to the recruitment and activation of the phosphatidylinositol-3 kinase (PI3K) and Akt [80]. Akt signaling induces translocation of GLUT4 from intracellular compartments to the plasma membrane where it facilitates transport of glucose into the cell [81]. Similarly, Akt also induces the translocation of fatty acid transport proteins (FATPs) 1 and 4 to the cell surface for import of fatty acids [76]. The increased concentrations of glucose and fatty acids that accumulate due to insulin stimulation rapidly induce synthesis of TG for long-term fat storage. In addition, insulin-induced serine-273 phosphorylation of phosphodiesterase 3B (PDE3B) inhibits lipolysis [82, 83].

Adipocyte precursor cells, positioned in the WAT, are also affected by insulin signaling. Here, proteins involved in cell proliferation and differentiation, especially the ERK and mTOR, are activated and initiate cell division and differentiation of precursor cells into adipocytes thereby increasing the storage capacity for fatty acids [80].

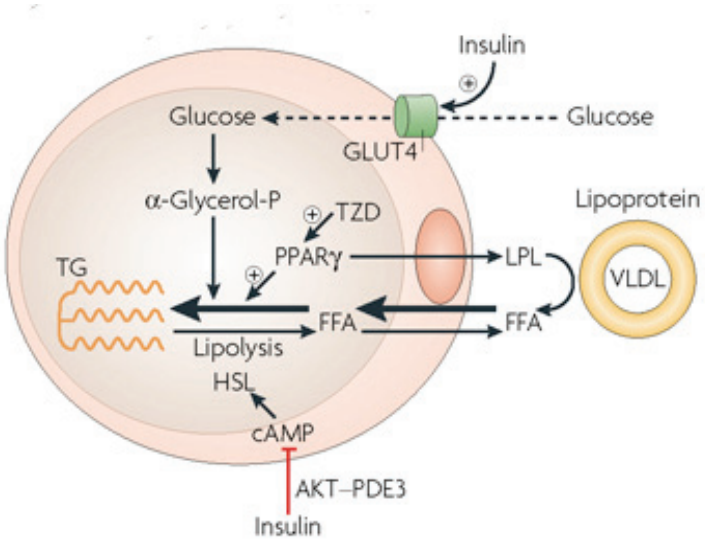

Figure 3. Insulin increases lipogenesis and inhibits lipolysis in adipocytes. Insulin promotes free fatty acid (FFA) esterification into triglycerides through stimulation of glucose transporter type 4 (GLUT4)-mediated glucose uptake. Glucose can be converted to 3-glycerol phosphate, the main source of the glycerol backbone of TG. Peroxisome proliferator-activated receptor-γ (PPARγ) activates lipoprotein lipase (LPL) expression and the TG biosynthetic pathway. Secreted LPL hydrolyzes TG from circulating very low-density lipoprotein (VLDL), releasing FFAs to be re-esterified. Several thiazolidinediones (TZDs) can activate PPAR. Insulin signalling also downregulates TG lipolysis through hormone-sensitive lipase (HSL). Insulin stimulation of the phosphatidylinositol 3-kinase (PI3K)–AKT pathway leads to activation of the enzyme phosphodiesterase-3 (PDE3). This enzyme catalyzes the breakdown of cyclic AMP (cAMP) which in turn reduces activation of HSL. Figure taken from [77] with permission from Nature Publishing Group.

Lipogenesis

FFAs have multiple essential physiological functions in all living organisms and influence the behavior of all cell types. They are the most energy-dense source of ATP, they can function as signaling molecules acting both as paracrine factors or as hormones that bind specifically to receptor proteins, and they are essential building blocks for all lipid synthesis and membrane formation [84]. FFAs can either be synthesized *de novo* from glucose or be recycled from the cytosol and the extracellular fluid. Excess FFAs are however toxic and the concentration of FFAs in blood and in the cell need to be kept low. This is achieved by conversion of FFAs into TG that can be stored as non-toxic lipoprotein complexes in the blood stream or inside cells in specialized lipid droplets [10, 85]. These organelles form as out-buds from the ER, but whether lipid droplets and ER stay in contact or are physically separated is not yet clear [85]. Lipid droplets consist of a core of TG and cholesterol esters surrounded by a phospholipid monolayer containing PAT

(Perilipin/APRP/TIP47) domain proteins which stabilize the TG storage, in addition to trafficking proteins like Rab GTPase and SNARE proteins involved in intracellular positioning of the lipid droplet [85]. Although virtually every cell type store triglycerides in lipid droplets, adipocytes are by far the most efficient cell type for the uptake and release of FFA. In fact, up to 85% of an adipocyte consists of TG, which is located in a single unilocular lipid droplet. Lipoprotein lipase (LPL) is secreted from adipocytes and acts on the surface of endothelial cells in the WAT where it hydrolyzes the lipoprotein bound TG to FFAs which are then transported into adipocytes by passive diffusion [86] or by fatty acid transporter proteins (FATPs) [87]. Inside the adipocyte, the fatty acids become chaperoned by adipocyte fatty acid binding protein 4 (FABP4 or aP2) for re-esterification and conjugation to coenzyme A (coA) catalyzed by acyl CoA synthetases (ACS) [10]. *De novo* synthesis of TG mainly takes place in the liver and to a lesser extent in WAT, but dietary uptake of TG contributes, under normal conditions, as the major source of TG accumulation . Both *de novo* synthesized FFA and recycled FFA are joined with 3-glycerol phosphate (G3P) by G3P acyltransferases (GPAT), 1-acylglycerol-3-phosphate acyltransferases (AGPAT), and diacylglycerol acyltransferases (DGATs) to produce TG. Adipocytes have very low glycerokinase activity and cannot recycle glycerol for the TG synthesis process and therefore use glucose as the main source of G3P.

Lipolysis

TG are released from lipid droplets by the action of TG hydrolases and their associated proteins in a process called lipolysis [84]. Prolonged fasting empties the TG content in most non-adipose tissues and induce lipolysis of TG in WAT. The end products of lipolysis are FA and glycerol, which are delivered to tissues with high energy demand, such as muscle and liver. The two main enzymatic reactions in TG release are adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). HSL was for a long period thought to be the only TG hydrolase that could cleave TG. Activation of perilipin by phosphorylation of at least 5 different serine residues, achieved by protein kinase A (PKA), 5′-AMP activated protein kinase (AMPK) or ERK translocates HSL to the lipid droplet. In 2004 ATGL was discovered to be an additional TG hydrolase with specific activity for the first step in TG hydrolysis [88].

Activation of ATGL also seems to involve PKA and perilipin but the molecular details are still unclear [84].

Endocrine action of the adipocyte

The basics for the fat storage and metabolism in the adipocyte have been known since the 1970s. However, the adipocyte gained much more attention after the surprising discoveries in the 1990s made it clear that WAT also acts as an endocrine organ playing a major part in the systemic regulation of energy balance by the release of adipocyte specific hormones called adipokines.

After the identification of leptin as an adipocyte secreted protein, several additional hormones and secreted products of WAT origin have been identified called adipokines. These molecules regulate whole body metabolic homeostasis by signaling to the brain, pancreas, liver, muscle, reproductive tract, immune system, and the vasculature [23]. The main known adipokines are reviewed briefly below.

Leptin

On a normal diet, the ob/ob and db/db strains of mice weigh three times more than wild type mice and contain five times more fat mass [89]. It was not until 1994 that the cause of the ob/ob phenotype was found to be a mutation in the gene encoding leptin [4], which is highly expressed in WAT and from where it is secreted into circulation. When WAT accumulates during periods of a positive energy balance, leptin concentration in plasma also increases. Similarly, in periods of energy insufficiency, leptin levels increase [90]. In addition to fat mass, insulin signaling and PPARγ agonists induce secretion of leptin. The db/db locus was found to contain the receptor for leptin shortly after the discovery of leptin itself [91]. The leptin receptor is highly expressed in hypothalamic neurons involved in regulation of food intake and is probably the most physiologically relevant target for leptin since brain specific deletion of leptin receptor is sufficient to cause severe obesity [92] (Figure 4). From the circulation, leptin is transported across the blood brain barrier to reach the brain where binding to the leptin receptor activates intracellular JAK/STAT3 (Janus kinase/Signal Transducer and Activator of Transcription) signaling which stimulates energy expenditure and inhibits food intake and weight gain [93].

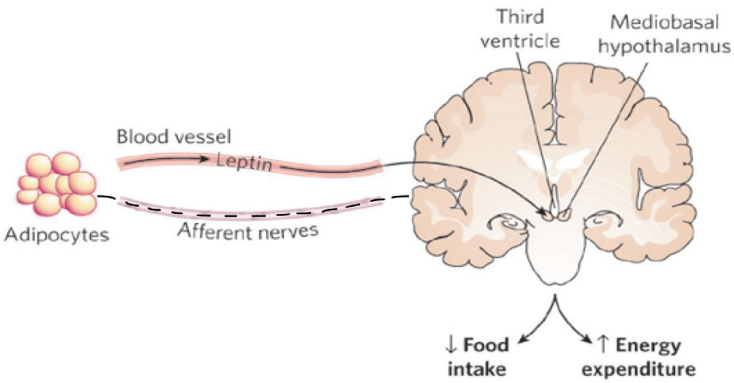

Figure 4. Leptin action. Leptin is released from adipocytes and binds to leptin receptors in the mediobasal hypothalamus, which then stimulate a systemic decrease in food intake and increase in energy expenditure. Afferent nerves can signal to adipocytes so that communication is bidirectional. The figure was taken from [6] with permission from Nature Publishing Group.

Except for hypothalamic expression, leptin receptor is present in a variety of tissues such as cancer cells, hepatocytes, heart muscle, pancreatic β cells, several types of immune cells and adipocytes; however, the functional significance of leptin action in these cells is still being explored [93]. Interestingly, in lean animals leptin administration induces complete loss of WAT by STAT-3 induced fatty acid oxidation in adipocytes [94, 95]. However, mice on a high fat diet very early develop leptin resistance and continue to store triglycerides although circulating leptin levels increase [96]. A recent report, however, raise doubts about the hypothesis that leptin directly binds leptin receptor in WAT to induce lipolysis by showing that instead it is the sympathetic innervation signals from the hypothalamus to the WAT that may be responsible for this effect [97].

Adiponectin

Adiponectin is an adipocyte specific protein that belongs to the collagen superfamily and is the adipokine secreted in highest amounts [98]. In contrast to leptin, adiponectin levels are reduced with obesity and elevated during starvation. Biologically, adiponectin is rarely found as a monomer; instead it forms homotrimers, which then dimerize to yield adiponectin hexamers and even higher molecular weight complexes [93]. Adiponectin impacts body metabolism by increasing insulin sensitivity in metabolic tissues, such as muscle, WAT and liver. In muscle it binds to

Adiponectin Receptor 1 (AdipoR1) and promotes glucose uptake and FFA oxidation; in liver it binds AdipoR2, promotes FFA oxidation and decreases gluconeogenesis [99]. Downstream signaling of AdipoR1 is not well characterized, but is known to involve activation of p38 and PPARα [100]. Aside from metabolic effects, adiponectin executes anti-inflammatory effects through inhibition of NF-κB signaling and reduced secretion of several inflammatory cytokines released from monocytes, macrophages and dendritic cells [98].

Retinol binding protein 4 (RBP4)

RBP4 is a plasma transport protein for retinol and is up-regulated in WAT in mice deficient in GLUT4 [101]. RBP4 is elevated in obese and obese-diabetic human and mice, and overexpression of RBP4 in mice leads to increased insulin resistance [102]. RBP4 secretion by WAT is suggested to be a response to low glucose blood levels detected by GLUT4. RBP4 suppresses insulin signals in muscle inhibiting the activity of PI-3K and IRS-1 phosphorylation, while increasing the glucose production in the liver leading to higher plasma glucose concentration [101] .

Visfatin

Visfatin, originally identified as pre-B-cell colony-enhancing factor (PBEF) over a decade ago, is expressed in bone marrow, liver and muscle, and has been re-identified in WAT as a factor that is up-regulated during development of obesity. The visceral tissue specificity of visfatin is still controversial, and its supposed role in binding and activation of the insulin receptor need further proof, but its connection to adiposity is still strong [103, 104].

Plasminogen Activator Inhibitor-1 (PAI-1)

PAI-1 regulates the coagulation cascade as an inhibitor of fibrinolysis and inactivation of urokinase- and tissue-type plasminogen activator. PAI-1 also has proposed roles in atherogenesis and angiogenesis. PAI-1 is expressed in many cell types within the WAT and its levels correlate with visceral adiposity [105, 106].

Monocyte chemoattractant protein 1 (MCP-1)

MCP-1 is involved in recruiting monocytes and T lymphocytes to sites of injury and infection. This chemokine is expressed in multiple cell types including endothelial, skeletal and smooth muscle cells, as well as adipocytes. The target of MCP-1 is chemokine CC motif receptor 2 (CCR2). Expression of MCP-1 rises with obesity in most fat tissue types, especially in visceral fat. Insulin, TNFα, GH and IL-6 all induce MCP-1 expression in 3T3-L1 cells [107, 108].

ADIPOSE TISSUE AND OBESITY

It is estimated that there are currently more than 400 million obese adults worldwide and that this number can rise to 1.12 billion by 2030 if the current trend continues [109]. In the Unites States the prevalence of obesity (assessed by Body Mass index (BMI) ≥30, which is the weight in kilograms divided by the square of the height in meters) for people older than 20 years was 33.9% in 2008 [110], more than twice as many reported in 1962 [111]. In Europe there are large regional differences with a BMI ≥30 prevalence ranging from 4% to 28.3% in men and 6.2%-36.5% in women with highest rates in Central, Eastern and Southern Europe [112]. In parallel 16.9% of children in the United States were at or above the 95th percentile in the BMI-for-age growth charts [113]. Although these latest data suggest that the upward trend in obesity is slowing, the numbers are still alarmingly high [114].

As noted above, abdominal adipose tissue expansion is closely associated with chronic diseases while excess subcutaneous adipose tissue accumulation show no such association [13]. The BMI measurement does not accurately take this important difference into account and measuring the waist-to-hip ratio is a better indicator for the amount of adipose tissue in relation to its health effects. Current guidelines recommend measuring the waist circumference in persons with a BMI between 25.0 and 34.9 and proposing cutoff points for waist circumference of 102 cm in men and 88 cm in women [115].

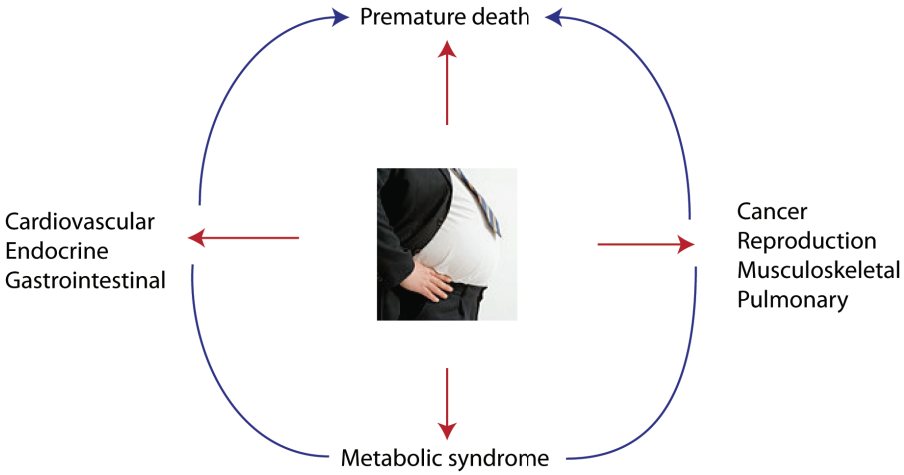

Figure 5. Obesity has been associated with a wide range of diseases. On the right side are organ systems/disease states with an unclear relationship to obesity. Positioned on the left side are diseases which have been more robustly linked to obesity [116, 117].

Obesity is a disorder of energy balance. Excess energy intake and less energy expenditure eventually lead to an accumulation of adipose tissue and deregulation of metabolic activity. Environmental factors probably play the most significant role in the development of obesity, but polygenic inheritance also contributes to this [118]. A wide range of genome–wide association studies have been targeted to identify the genetic contributors for development of human obesity, but a clear picture have not yet emerged from these studies [118]. Whatever the causes for obesity, the consequences are development of metabolic syndrome, insulin resistance, and other defects in metabolism that can lead to cardiovascular disease, type 2 diabetes, certain forms of cancer, reduced fertility, asthma, and muscle degeneration, all of which significantly can reduce life expectancy [7, 13] (Figure 5).

There are several different definitions of metabolic syndrome and also some discrepancies regarding medical diagnosis, but the definition by the International Diabetes Federation is currently the most prevailing [119]. It defines a person to have the metabolic syndrome if central obesity as well as two of the following conditions are present:

- Raised plasma triglycerides
- Reduced HDL cholesterol

- Raised blood pressure
- Raised fasting plasma glucose

In addition, a proinflammatory state and prothrombotic state are considered to be signs of metabolic syndrome. Patients with metabolic syndrome have a 2-fold increased risk of developing cardiovascular disease and a 5-fold increased risk of developing type 2 diabetes [120]. However, a large portion of patients diagnosed with cardiovascular disease and type 2 diabetes is non-obese and many severely obese subjects do not develop any chronic diseases. This tells us that it is not the accumulation of adipose tissue itself that causes metabolic syndrome, but that functional disruption of normal metabolic tissue causes the onset of disease [121]. Current non-obese risk factors for metabolic syndrome, type 2 diabetes and cardiovascular disease, are stress, a sedentary lifestyle, aging, diabetes mellitus, coronary heart disease, and lipodystrophy. Consequently, there is some confusion as to how the term metabolic syndrome should be used in a clinical setting and if it is a good term at all [120].

Insulin resistance and type 2 diabetes

The hallmarks of insulin insensitivity are decreased glucose uptake into skeletal muscle, impaired insulin-mediated inhibition of glucose production in the liver, and a reduced ability of insulin to inhibit lipolysis in adipose tissue [7]. These impairments precede the development of systemic hyperglycemia [122]. In general, increased adipose tissue mass, either by hypertrophy or hyperplasia, will increase the storage capacity for FAs and this alone is not associated with onset of metabolic disease [6]. But as chronic nutritional overload continues, adipocytes can no longer take up all the circulating FAs and liver and muscle FA disposal will increase [7]. FAs entering muscle and liver can either be broken down by β-oxidation in the mitochondria or they can be stored as TG in the lipid droplet. In the liver, the TG pool can either be stored intracellularly in hepatocytes or in VLDL TG pools, which is released into to the circulation. The consequence of increased FA uptake in liver and muscle is accumulation of various metabolic by-products, which over time induce insulin resistance [7].

Under chronic over-nutrition, infiltration of activated macrophages into adipose tissue is observed [123, 124]. Exactly why this happens is not completely understood, but both adipocytes and resting macrophages secrete cytokines and chemokines such as CCL2, Interleukin-6 (IL-6), IL-1β, and TNF-α under stress conditions and this could attract more immune cells. In addition, increased adipose cell death caused by various forms of stress has been suggested to serve as a signal for infiltration of immune cells into adipose tissue [7]. Interestingly, increased numbers of infiltrating immune cells could also explain why cytokines such as TNF-α and IL-6 are overexpressed in adipose tissue of obese mice and humans [116]. Mechanistic insight into the inflammatory cytokine action in adipocytes was first shown for TNF-α, which induces activation of the JNK kinase followed by phosphorylation of specific serine residues on IRS-1 to inhibit downstream insulin signaling [125]. In later studies, a wide range of molecules, such as fatty acids and amino acids, have been shown to inhibit IRS-1 signaling by a similar mechanism or by activation of another pro-inflammatory signaling cascade, that involving NF-κB [126, 127].

Despite this knowledge, exactly what causes the inflammatory state in adipose tissue is unclear. One initiating mechanism could be ER stress. The ER is a vast network of membranes in which all the secretory and membrane proteins are assembled into their secondary and tertiary structures [116]. Proper folding, maturation, storage and transport of these proteins take place in this organelle. Unfolded or misfolded proteins are detected, removed from the ER and degraded by the 26S proteasome system in a process called the unfolded protein response (UPR).

The UPR is mediated by three different stress-sensing pathways that are initiated by three transmembrane proteins which are located in the ER: pancreatic ER kinase (PERK), inositol-requiring kinase 1 (IRE1), and activating transcription factor 6 (ATF6) [128]. Activation of PERK leads to the phosphorylation of eukaryotic translation initiation factor 2α (eIF2 α) and inhibition of translation [129]. In addition to its kinase activity, which leads to autophosphorylation, IRE1 also possesses endoribonuclease activity that splices X-box binding protein 1 (XBP1) mRNA; this results in the production of the active transcription factor XBP1s [130, 131]. The ATF6-mediated branch of the UPR cooperates with IRE1 by upregulating the

expression of XBP1 mRNA [132]. The expression and activation of XBP1s, as well as the production of active ATF6 and its translocation to the nucleus, where it acts as a transcription factor, leads to a complex transcriptional programme that has a central role in the UPR. This programme includes the upregulation of ER-resident chaperone proteins, which promote protein folding, and the production of components of the protein-degradation apparatus that assist in the re-establishment of ER homeostasis [129].

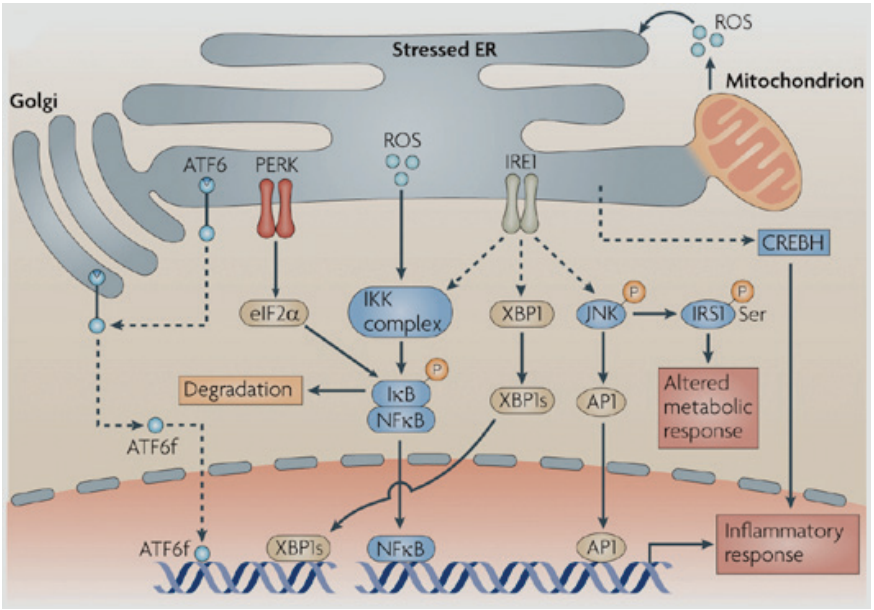

Figure 6. ER stress perturbs insulin signaling. The UPR is mediated by three different pathways that are initiated by three transmembrane proteins that are located in the ER — activating transcription factor 6 (ATF6), pancreatic ER kinase (PERK) and inositol-requiring kinase 1 (IRE1). ER stress is linked to inflammation through the activation of the JUN N-terminal kinase (JNK) and the IκB kinase (IKK)–nuclear factor-κB (NFκB) pathways, and through cyclic-AMP-responsive-element-binding protein H (CREBH) activation by the UPR. These pathways result in the induction of an inflammatory response. Activation of JNK can also serine phosphorylate insulin receptor substrate 1 (IRS1), resulting in altered metabolic responses. Key organelles for cellular metabolism, such as the ER, Golgi, mitochondria and peroxisomes (not shown) are connected through an endomembrane network, which provides functional continuity between organelles that can therefore share functional information in the form of lipids and proteins at specific contact sites. This functional and molecular integration between the organelles can mediate the spread of stress from one organelle to the other, resulting in exacerbation of inflammation and cytotoxicity during chronic metabolic stress conditions such as obesity and dyslipidaemia. AP1, activator protein 1; eIF2α, eukaryotic translation initiation factor 2α; IKKB, inhibitor of NFκB; XBP1s, spliced X-box binding protein 1. The figure was reprinted from [25] with permission from Nature Publishing Group.

In addition to these protective responses and stimulation of ER synthesis, these UPR pathways can also induce important inflammatory signals. If ER homeostasis can not be restored, the ER activates apoptotic pathways to initiate cell death [129]. ER stress

activates both JNK and NF-κB signaling in adipose tissue and liver, which are directly linked to development of insulin resistance. Indeed, mice on a high fat diet show increased levels of ER stress and JNK-mediated insulin resistance [133]. Furthermore, deficiency of one Xbp1allele is sufficient to induce insulin resistance [133].

Atherosclerosis

Increased amount of visceral adipose tissue is associated with increased cardiovascular morbidity and mortality including stroke, congestive heart failure, myocardial infarction and cardiovascular death, and this is independent of the association between obesity and other cardiovascular risk factors [8]. Increased levels of very low-density lipoprotein (VLDL) cholesterol, triacylglyerols, and total cholesterol together with decreased levels of high-density lipoprotein (HDL) cholesterol are characteristics of obesity [134].

The link between insulin resistance and atherosclerosis is more controversial [8]. Although increased adiposity is directly linked to heart failure, it probably involves malfunction of other aspects of adipose function, in addition to insulin resistance. Several of the possible mechanisms linking obesity to cardiovascular disease, such as increased levels of FFA, lipotoxicity and disturbances in adipokine secretion, are believed to be related to insulin resistance [8]. Increased levels of FFA might also affect endothelial nitric oxide production, thereby impairing endothelium-dependent vasodilation. They may also increase myocardial oxygen requirements — and therefore lead to ischaemia — decrease myocardial contractility and induce cardiac arrhythmias [135]. To what extent visceral fat exerts a direct effect on risk, of mortality in particular, or indirect effects, through insulin resistance or the effects of adipokines, remains an open question [8]. Ectopic fat storage in the heart, blood vessels and kidneys can impair their function, contributing to the increased cardiovascular risk in obesity [136].

Cancer

The association between increased adiposity and cancer may not be as strong and is less well characterized than for type 2 diabetes and cardiovascular disease [137], but nevertheless a wide range of cancer types has a connection to obesity. This includes some forms of colon cancer, postmenopausal breast cancer, endometrial cancer, kidney cancer, adenocarcinoma of the oesophagus, adenocarcinoma of the gastric cardia, gallbladder cancer, liver cancer, pancreatic cancer, haematopoietic cancers , and advanced prostate cancer [9, 138]. An estimated 15-20% of all cancer incidents have been linked to obesity [139, 140]. Below is a more detailed description of evidence that link obesity to individual types of cancer.

Breast Cancer and Obesity

Obesity has been consistently shown to increase rates of breast cancer in postmenopausal women by 30 to 50% but in premenopausal women there is an inverse relationship [139, 141]. Some studies have found central adiposity to be an independent predictor of postmenopausal breast cancer risk beyond the risk attributed to overweight alone, but a recent systematic review has indicated that this is not the case [138]. Both BMI and weight gain are more strongly related to risk of breast cancer among postmenopausal women who have never used hormone replacement therapy, compared with women who have used hormones [138]. This finding lends support to the hypothesis that adiposity increases breast cancer risk through its estrogenic effects. Studies of breast cancer mortality and survival among breast cancer cases illustrate that adiposity is associated with both increased likelihood of recurrence and reduced likelihood of survival among those with the disease, regardless of menopausal status and after adjustment for stage and treatment. There are substantial data to suggest that adiposity is associated with a more aggressive tumor; obese women are more likely than lean women to have increased tumor size, lymph node involvement, and later stage disease at diagnosis [138].

Colorectal Cancer and Obesity

Obesity increases the risk of colorectal cancer in men by approx 50-100% and in women by 20-50% in both case–control and cohort studies [142]. A gender difference, in which obese men are more likely to develop colorectal cancer than

obese women, has consistently been observed across studies and populations. The reasons for this gender difference is not clear, but one hypothesis is that central adiposity, which occurs more frequently in men, is a stronger predictor of colon cancer risk than peripheral adiposity or general overweight. Support for the role of central obesity in colorectal cancer comes from studies reporting that waist circumference is related strongly to risk of colorectal cancer and large adenomas in men [142]. One mechanistic hypothesis is that high body mass, and central obesity in particular, increase colon cancer risk through their effect on insulin production [143]. Insulin and Insulin-like Growth Factors (IGFs) have been shown to promote the growth of colonic mucosal cells and colonic carcinoma cells in in vitro studies [138]. Elevated levels of serum leptin have recently been found to be associated with increased risk of colon cancer, independent of circulating insulin levels [144]. Low levels of plasma adiponectin have also been found to be associated with increased risk of colorectal cancer and colorectal adenoma.

Liver Cancer and Obesity

Studies that have examined obesity and liver cancer or hepatocellular carcinoma (HCC) have found excess relative risk in both men and women in the range of 50-400%, but the magnitude of the observed relative risk from existing studies is not consistent [138]. Obesity, and especially visceral adiposity, is strongly associated with nonalcoholic fatty liver disease (NAFLD), a chronic liver disease that occurs in nondrinkers but that is histologically similar to alcohol-induced liver disease [145]. NAFLD is characterized by a spectrum of liver tissue changes ranging from accumulation of fat in the liver to fatty liver disease, nonalcoholic steatohepatitis (NASH), and HCC. Visceral adiposity thus likely contributes to the risk of HCC by promoting NAFLD and NASH [146].

Pancreatic Cancer and Obesity

Several recent studies suggest that high body mass is associated with increased risk for pancreatic cancer in men and women, with relative risk estimates for obesity generally in the range of 50-100% [139]. However, other studies found smaller positive associations or, in some cases, no association [138]. Smoking is an important potential confounder of the relationship between adiposity and pancreatic cancer, and

the smoking habits of the various study populations and differential adequacy of control for smoking may partly explain differences across studies [138].

Prostate Cancer and Obesity

There are conflicting results regarding an association between body mass and prostate cancer incidence [10, 142]. However, there is accumulating evidence that obesity is associated with an increase in risk of advanced prostate cancer or death from prostate cancer [147]. Recent studies consistently indicated that obese men with prostate cancer are more likely to have aggressive disease that recurs after radical prostatectomy than non-obese men. As with breast cancer, "nonbiological" issues of screening, detection, and treatment are important to the evaluation of the impact of adiposity on prostate cancer prognosis. It can be harder to perform a digital rectal examination in obese men because of their general adiposity in combination with larger prostate size [148]. Additionally, despite larger prostate sizes, obese men may have lower serum levels of prostate-specific antigen (PSA) [149], potentially biasing them toward later stage at diagnosis even in the presence of PSA screening. Surgery is more difficult to perform in obese men, with a greater risk of positive surgical margins [150].

Biological mechanisms for a link between obesity and cancer

As described above, obesity-induced insulin resistance in liver and muscle increase insulin secretion from the pancreas and thereby raising plasma insulin levels. This also increases insulin-like growth factor (IGF) secretion from the liver [151] (see Figure 7 for an overview of IGF and insulin action). IGFs are cell proliferation inducers that regulate energy-dependent growth processes at the whole organism and at the cellular level [152]. IGF-I stimulates cell proliferation and inhibits apoptosis and has been shown to have strong mitogenic affects in a wide variety of cancer cell lines. The synthesis of IGF-I and its main binding protein, IGFBP-3, are regulated primarily by growth hormone (GH) [152]. In the circulation, more than 90% of IGF is bound to IGF binding protein 3 (IGFBP-3). Chronic hyperinsulinemia results in elevated blood glucose levels, decreased levels of IGFBP and higher levels of free plasma IGF. Obesity does not increase absolute plasma IGF-I levels, and the mild decrease in IGF-I levels observed in obese and hyperinsulinemic individuals can be explained by the negative feedback of free IGF on GH secretion, which is also lower

in obese individuals [138]. Insulin and free IGF interact with and regulate the synthesis and bioavailability of sex steroids that affect the development and progression of certain cancers [153]. Chronic hyperinsulinemia inhibits hepatic synthesis of sex hormone-binding globulin (SHBG), which increases the concentration of androgens and estrogens freely available in the circulation. This free fraction determines the actual biological activity of androgens and estrogens, hormones essential for the growth, differentiation and function of many tissues in both men and women, and implicated in breast and prostate cancer, respectively. There is a strong inverse association between the amount and distribution of body fat and circulating levels of SHBG [138].

At the whole organism level, circulating IGF1 and IGF2 are produced mainly in the liver (the former under dominant growth hormone control), whereas the pancreatic β cells produce insulin. In general, the only source of insulin in neoplastic tissue is that delivered by the circulation, whereas IGF1 and IGF2, as well as being delivered from the circulation, are also frequently produced in autocrine and paracrine manners [152].

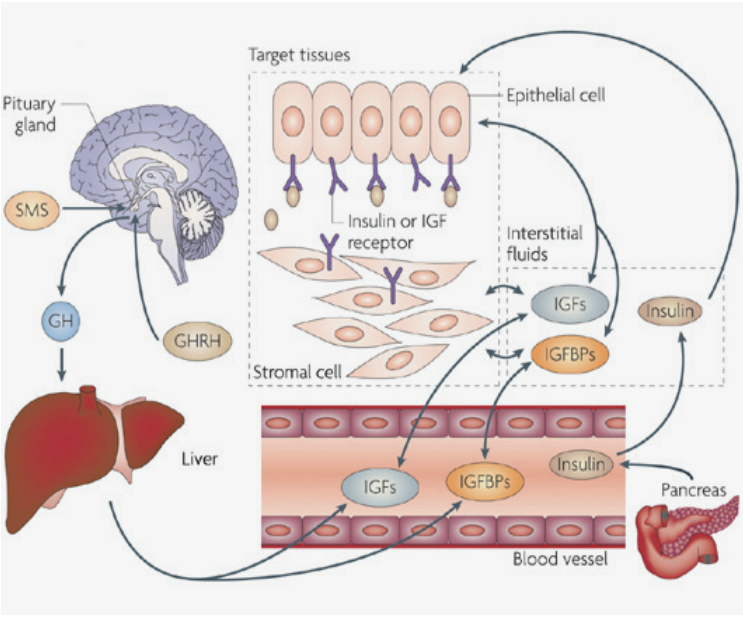

Figure 7. Systemic Insulin-growth factor (IGF) regulation. At the whole organism level, circulating IGF1 and IGF2 are produced mainly in the liver, whereas insulin is produced by the pancreatic β-cells.

In general, the only source of insulin in neoplastic tissue is that delivered by the circulation, whereas IGF1 and IGF2, as well as being delivered from the circulation, are also frequently produced in an autocrine and paracrine manner. GH, growth hormone; GHRH, GH-releasing hormone; SMS, somatostatin. The figure is reprinted from [152] with permission from Nature Publishing Group.

Adipokines and Cancer

Adiponectin levels are decreased in obese and type II diabetic subjects and low levels of circulating adiponectin has been associated with increased angiogenesis, increased hyperinsulinemia and also increased proliferation of neoplastic tissue [141]. *In vitro* experiments has confirmed this by showing that adiponectin receptors are expressed in breast cancer cell lines and that recombinant adiponectin treatment suppresses cell proliferation and induces apoptosis through increased AMPK signaling and inhibition of MAPK signaling [154, 155]. Additionally, adiponectin has anti inflammatory properties that also could explain its anticancer effect [156]. Leptin treatment seems to have the opposite effect as it increases the growth of both normal and malignant cells by stimulating the MAPK and JAK2-STAT3 pathway, at least in breast, colon and prostate cancer cell lines in vitro [157-159].

SIX TRANSMEMBRANE PROTEINS OF PROSTATE (STAMPs)

General features of the STAMPs

Androgens and the androgen receptor (AR) are cornerstones in prostate carcinogenesis [160]. However, the molecular details as to how AR function directly connects to prostate biology are still incomplete. Especially, the physiological and biochemical functions of the many target genes that are transcriptionally controlled by AR remain elusive. While screening for new AR regulated genes, STAMP1 was discovered [161]. Sequence databases indicated that at least two other proteins share sequence similarity to STAMP1. cDNA cloning of these two sequences and their *in silico* analysis confirmed them to belong to the same family and thus they were named STAMP2 and STAMP3 (reference [162], and Saatcioglu laboratory, unpublished data). STAMP proteins are known by several other names assigned by different groups which cloned them in different species: STAMP1 (STEAP2) [161, 163], STAMP2 (STEAP4, TIARP) [162, 164], and STAMP3 (STEAP3, TSAP6, pHYDE) [165, 166].

All three STAMPs share the putative six transmembrane (6TM) region flanked by a large amino-terminal and a short carboxyl-terminal domain, a structure reminiscent of channel and transporter proteins [162]. The STAMP family members have significant similarity at the *C*-terminal 6TM domain to the TM domains of the yeast FRE metalloreductases [167, 168] which have heme-binding capabilities and are involved in electron transfer chains [169]. In addition, they share high sequence similarity at the *N*-terminus to the archaeal and bacterial F420:$NADP^+$ Oxidoreductase (FNO) binding proteins [167, 168]. *STEAP* (Six-Transmembrane Epithelial Antigen of the Prostate) does not belong to the *STAMP* family, but is a *STAMP*-related gene. *STEAP* contains the conserved 6TM domain as in the STAMP family members, but does not have the FNO domain at its *N*-terminal portion. The STAMP family members have been suggested to function as ferrireductases and cupric reductases [167, 168]. They reduce iron from the ferric (Fe^{3+}) to ferrous (Fe^{2+}) state, copper from the cupric (Cu^{2+}) to cuprous (Cu^{+}) state, and stimulate cellular uptake of both iron and copper in 293T cells, suggesting that they may play potential roles in metal metabolism [167, 168]. Whether STAMPs have these activities *in vivo*, and whether this is linked to their biological function is currently under investigation. Below is a general background on each STAMP member and their known roles in non-metabolic tissue, followed by possible roles of STAMPs in adipose tissue.

STAMP1

STAMP1 is located on chromosome 7 (Chr7q21). This region contains a cluster of genes predicted to encode 6TM proteins. In addition to *STAMP1*, *STAMP2* and *STEAP* both lie in this locus. *STAMP1* is transcribed in the same direction as *STEAP,* but in the opposite direction to *STAMP2* [162]. The use of GFP-tagged STAMP1 in quantitative time-lapse and immunoflourescence confocal microscopy imaging studies indicated that STAMP1 is primarily localized to the Golgi, trans-Golgi network and the plasma membrane. It also co-localizes to the early endosomes, but not late endosomes or lysosomes, and shuttles between the Golgi and the plasma membrane, suggesting that it may be involved in secretory/endocytic pathways [161, 163].

STAMP1 is not detected in AR-negative prostate cancer cell lines PC-3 and DU145, but is highly expressed in the androgen-sensitive prostate cancer cell line LNCaP; however, its expression is not significantly androgen regulated. In human prostate cancer xenograft models, *STAMP1* showed significant expression in androgen-independen CWR22R tumors and lower levels of expression in androgen-dependent CWR22 tumors [161]. In situ hybridization of human prostate cancer specimens showed that *STAMP1* is expressed only in the epithelial cells of the prostate and its expression is significantly increased in prostate tumors compared with normal glands, suggesting that STAMP1 may play a role in prostate cancer development and may serve as a potential diagnostic marker [161, 163].

STAMP2

Similar to STAMP1, STAMP2 is primarily localized to the Golgi, *trans*-Golgi network, the plasma membrane, vesicular-tubular structures in the cytosol and early endosomes, but not late endosomes or lysosomes [162]. It also shuttles between the plasma membrane and the Golgi, suggesting that it may be involved in the secretory/endocytic pathways. Differently from STAMP1, STAMP2 also displays an ER-specific localization. *STAMP2* has a tissue-restricted expression with high levels in placenta, WAT, lung, heart, liver and prostate. *STAMP2* expression is highly androgen regulated in the androgen-sensitive, AR-positive prostate cancer cell line LNCaP, but in androgen receptor-negative prostate cancer cell lines (PC-3, DU145, CA-HPV10, PZ-HPV7 and YPEN-1) its expression was not detected. Moreover, in human prostate specimens STAMP2 is over-expressed in cancer cells compared with normal prostate epithelial cells suggesting that it may be useful as a diagnostic marker and may have a role in prostate cancer progression [162].

STAMP3

STAMP3, also known as TSAP6 (tumor suppressor activated protein 6), Steap3 (in mouse) and pHyde (in rat) is a p53-inducible human protein which regulates apoptosis and the cell cycle via direct interactions with Nix (a BCL-2 related protein) and Myt1 kinase (a negative regulator of the G2/M transition) [165]. STAMP3 is also reported to interact with and facilitate secretion of the translationally controlled tumor

protein (TCTP, also called histamine-releasing factor, HRF) by a nonclassical pathway, independent of the ER and Golgi apparatus [170]. The rat homologue of STAMP3, pHyde, has been shown to cause apoptosis in prostate cancer cells upon ectopic expression through a caspase-dependent pathway [166]. Adenoviral delivery of pHyde into human prostate cancer cells caused growth suppression and induced apoptosis synergistically with the chemotherapeutic agent cisplatin [171]. These data suggest that STAMP3 functions in the opposite direction to STAMP1 and STAMP2 in prostate carcinogenesis. Steap3 was shown to be the major ferrireductase having an important role in iron uptake in erythrocytes [168]. Consistently, mice lacking Steap3 exhibit hypochromic microcytic anemia [168].

STEAP1

Although formally not part of the STAMP family, some brief information on STEAP1 is also included here. STEAP1 was originally identified as a prostate-specific cell-surface antigen and found to be located at the cell-cell junction of the secretory epithelium of the prostate and overexpressed in prostate cancer [172]. STEAP1 is also overexpressed in multiple cancer cell lines, including bladder, colon, ovarian, and Ewing sarcoma, compared with normal cells [172]; it could therefore be a potential diagnostic/prognostic marker or a therapeutic target in cancer [173, 174]. Consistent with this hypothesis, STEAP1 mRNA is detectable in serum of patients with different solid tumours whereas it is not found in donors without known disease [175].

STAMPs' role in adipose tissue and metabolism

At the time STAMP2 was discovered as an androgen-regulated gene in prostate cancer cells, evidence was provided for a role of the murine STAMP2 ortholog TIARP in adipocytes [164]. Mouse STAMP2 shares the same general predicted protein domains and tissue expression profile as the human gene, but with some significant differences. Expression in lung tissue, for example, is high in human samples [162], but in contrast could not be detected in the mouse lung. STAMP2 is not expressed in 3T3-L1 pre-adipocytes, but is induced to high levels three days after initiation of adipogenesis. Interestingly, TNF-α increased STAMP2 expression

several fold in differentiated adipocytes. This is somewhat surprising as TNF-α presumably inhibits most adipocyte-specific gene expression and STAMP2 is induced during adipogenesis [176]. Immunofluorescence staining showed localization of STAMP2 to the plasma membrane and perinuclear regions and addition of TNF-α two days after differentiation increased plasma membrane specific staining [164]. Subsequently, several other hormones and cytokines have been reported to induce STAMP2 in differentiating 3T3-L1 cells, including growth hormone [177], interleukin-6 [178] and interleukin-1β [179]. Although these results suggest a role for STAMP2 in inflammatory signaling in metabolic tissue, they rely on *in vitro* data that do not provide any mechanistic model for the link between STAMP2 and the inflammatory cytokines.

To further characterize STAMP2 function in metabolic tissues, experiments were performed in cultured adipocytes *in vitro* and in mouse models of obesity. It has been postulated that regulatory molecules would respond to nutritional status and inflammatory signals. Investigation of STAMP2 expression in 3T3-L1 adipocytes by various nutritional stimuli showed that STAMP2 expression was markedly induced by high serum and fatty acids whereas there was minimal regulation in response to glucose or insulin [180]. Additional experiments established that fluctuations in nutritional status also results in regulation of STAMP2 expression in vivo. In lean mice, STAMP2 expression was elevated in the fed, as compared to fasted, state, particularly in visceral adipose depots, a site often considered as the most relevant depot for metabolic pathologies [13, 180].

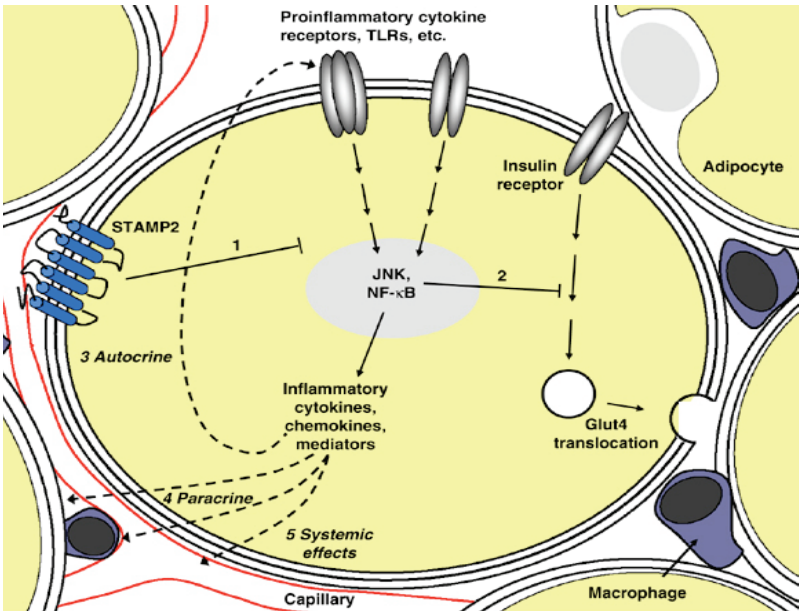

Figure 8. Potential functions of STAMP2 in adipose tissue. Based on the initial characterization of STAMP2 knockout mice [180], several functions of STAMP2 have been suggested: 1. Inhbition of pro-inflammatory signalling, e.g. JNK and NF-κB. 2. Increased insulin sensitivity and glucose uptake. 3. Decreased secretion of pro-inflammatory mediators (autocrine effect). 4. Decreased macrophage infiltration (paracrine effect). 5. Increased metabolic function of liver and muscle (systemic effect). TLR, Toll-like receptors. Figure was taken from [181] and used with permission from Nature Publishing Group.

To explore the role of STAMP2 function *in vivo*, a STAMP2 knockout mouse model was established. Characterization of these mice showed elevated levels of inflammatory cytokines and reduced levels of metabolic markers selectively in the visceral WAT at 3 months of age on a regular chow diet [180] (Figure 8). In addition, glucose uptake in muscle, WAT and liver was impaired in STAMP2 knockout animals *in vivo,* as well as in an adipocyte cell line upon siRNA-mediated knockdown of STAMP2. Tissue sections of the visceral WAT and liver from the same mice demonstrated high macrophage infiltration into WAT and lipid accumulation in the liver even though the body weight did not differ significantly between the knockout and the control groups [180]. Macrophage infiltration and fatty liver are commonly seen in obese subjects on a high fat diet and are both involved in the development of local metabolic inflammation and insulin resistance, but are not common in mice on a chow diet [116, 182]. These results therefore indicate that STAMP2 plays an important role in limiting local stress that is induced by feeding; long term accumulation of such stress in the absence of STAMP2 results in the metabolic

syndrome. Oxidative stress could be one type of stress that STAMP2 normally counteracts as elevated levels of TBARS and NADPH oxidase was reported in STAMP2 knockout animals as a sign of accumulated oxidative stress [180].

Expression of STAMP2 is high in human WAT ([183], T Lindstad, unpublished data). However, it remains to be determined whether the nutritional regulation of STAMP2 is disrupted in obese humans in the same manner as in mouse models of obesity, as there are conflicting reports on this [183, 184]. Addressing this question is crucial, but is also challenging, as the nutritional regulation of STAMP2 expression occurs in the visceral WAT which is generally not readily available in experimental protocols [25].

REFERENCES

1. Krause, B.R. and A.D. Hartman, Adipose tissue and cholesterol metabolism. J Lipid Res, 1984. 25(2): p. 97-110.
2. Cinti, S., Anatomy of the adipose organ. Eat Weight Disord, 2000. 5(3): p. 132-42.
3. Cook, K.S., et al., Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. Science, 1987. 237(4813): p. 402-5.
4. Zhang, Y., et al., Positional cloning of the mouse obese gene and its human homologue. Nature, 1994. 372(6505): p. 425-32.
5. Trayhurn, P., Endocrine and signalling role of adipose tissue: new perspectives on fat. Acta Physiol Scand, 2005. 184(4): p. 285-93.
6. Rosen, E.D. and B.M. Spiegelman, Adipocytes as regulators of energy balance and glucose homeostasis. Nature, 2006. 444(7121): p. 847-53.
7. Schenk, S., M. Saberi, and J.M. Olefsky, Insulin sensitivity: modulation by nutrients and inflammation. J Clin Invest, 2008. 118(9): p. 2992-3002.
8. Van Gaal, L.F., I.L. Mertens, and C.E. De Block, Mechanisms linking obesity with cardiovascular disease. Nature, 2006. 444(7121): p. 875-80.
9. Calle, E.E. and R. Kaaks, Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer, 2004. 4(8): p. 579-91.
10. Fantuzzi, G. and T. Mazzone, Adipose tissue and adipokines in health and disease. 2007, Totowa, N.J.: Humana Press. xxi, 397 s.
11. Tran, T.T. and C.R. Kahn, Transplantation of adipose tissue and stem cells: role in metabolism and disease. Nat Rev Endocrinol, 2010.
12. Wajchenberg, B.L., Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev, 2000. 21(6): p. 697-738.
13. Despres, J.P. and I. Lemieux, Abdominal obesity and metabolic syndrome. Nature, 2006. 444(7121): p. 881-7.
14. Maeda, K., et al., Analysis of an expression profile of genes in the human adipose tissue. Gene, 1997. 190(2): p. 227-35.
15. Bjorntorp, P., Metabolic implications of body fat distribution. Diabetes Care, 1991. 14(12): p. 1132-43.
16. Fain, J.N., et al., Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology, 2004. 145(5): p. 2273-82.
17. Cannon, B. and J. Nedergaard, Brown adipose tissue: function and physiological significance. Physiol Rev, 2004. 84(1): p. 277-359.
18. Virtanen, K.A., et al., Functional brown adipose tissue in healthy adults. N Engl J Med, 2009. 360(15): p. 1518-25.
19. Cypess, A.M., et al., Identification and importance of brown adipose tissue in adult humans. N Engl J Med, 2009. 360(15): p. 1509-17.
20. van Marken Lichtenbelt, W.D., et al., Cold-activated brown adipose tissue in healthy men. N Engl J Med, 2009. 360(15): p. 1500-8.
21. Trayhurn, P., Adipocyte biology. Obes Rev, 2007. 8 Suppl 1: p. 41-4.
22. Rodbell, M., Metabolism of Isolated Fat Cells. I. Effects of Hormones on Glucose Metabolism and Lipolysis. J Biol Chem, 1964. 239: p. 375-80.

23. Trujillo, M.E. and P.E. Scherer, Adipose tissue-derived factors: impact on health and disease. Endocr Rev, 2006. 27(7): p. 762-78.
24. Tang, W., et al., White fat progenitor cells reside in the adipose vasculature. Science, 2008. 322(5901): p. 583-6.
25. Hotamisligil, G.S. and E. Erbay, Nutrient sensing and inflammation in metabolic diseases. Nat Rev Immunol, 2008. 8(12): p. 923-34.
26. Cinti, S., et al., Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res, 2005. 46(11): p. 2347-55.
27. Zeve, D., W. Tang, and J. Graff, Fighting fat with fat: the expanding field of adipose stem cells. Cell Stem Cell, 2009. 5(5): p. 472-81.
28. Spalding, K.L., et al., Dynamics of fat cell turnover in humans. Nature, 2008. 453(7196): p. 783-7.
29. Crossno, J.T., Jr., et al., Rosiglitazone promotes development of a novel adipocyte population from bone marrow-derived circulating progenitor cells. J Clin Invest, 2006. 116(12): p. 3220-8.
30. Koh, Y.J., et al., Bone marrow-derived circulating progenitor cells fail to transdifferentiate into adipocytes in adult adipose tissues in mice. J Clin Invest, 2007. 117(12): p. 3684-95.
31. Gesta, S., Y.H. Tseng, and C.R. Kahn, Developmental origin of fat: tracking obesity to its source. Cell, 2007. 131(2): p. 242-56.
32. Boquest, A.C., et al., Isolation and transcription profiling of purified uncultured human stromal stem cells: alteration of gene expression after in vitro cell culture. Mol Biol Cell, 2005. 16(3): p. 1131-41.
33. Zuk, P.A., et al., Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell, 2002. 13(12): p. 4279-95.
34. Rodeheffer, M.S., K. Birsoy, and J.M. Friedman, Identification of white adipocyte progenitor cells in vivo. Cell, 2008. 135(2): p. 240-9.
35. Tontonoz, P. and B.M. Spiegelman, Fat and beyond: the diverse biology of PPARgamma. Annu Rev Biochem, 2008. 77: p. 289-312.
36. Jin, W., et al., Schnurri-2 controls BMP-dependent adipogenesis via interaction with Smad proteins. Dev Cell, 2006. 10(4): p. 461-71.
37. Gesta, S., et al., Evidence for a role of developmental genes in the origin of obesity and body fat distribution. Proc Natl Acad Sci U S A, 2006. 103(17): p. 6676-81.
38. Lefterova, M.I. and M.A. Lazar, New developments in adipogenesis. Trends Endocrinol Metab, 2009. 20(3): p. 107-14.
39. Todaro, G.J. and H. Green, Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. J Cell Biol, 1963. 17: p. 299-313.
40. Green, H. and M. Meuth, An established pre-adipose cell line and its differentiation in culture. Cell, 1974. 3(2): p. 127-33.
41. Farmer, S.R., Transcriptional control of adipocyte formation. Cell Metabolism, 2006. 4(4): p. 263-73.
42. Otto, T.C. and M.D. Lane, Adipose development: from stem cell to adipocyte. Crit Rev Biochem Mol Biol, 2005. 40(4): p. 229-42.
43. Rosen, E.D. and B.M. Spiegelman, Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol, 2000. 16: p. 145-71.

44. Rosen, E.D. and B.M. Spiegelman, PPARgamma : a nuclear regulator of metabolism, differentiation, and cell growth. J Biol Chem, 2001. 276(41): p. 37731-4.
45. Aranda, A. and A. Pascual, Nuclear hormone receptors and gene expression. Physiol Rev, 2001. 81(3): p. 1269-304.
46. Rosen, E.D. and O.A. MacDougald, Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol, 2006. 7(12): p. 885-96.
47. Darlington, G.J., S.E. Ross, and O.A. MacDougald, The role of C/EBP genes in adipocyte differentiation. J Biol Chem, 1998. 273(46): p. 30057-60.
48. Rosen, E.D., et al., C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. Genes Dev, 2002. 16(1): p. 22-6.
49. Tang, Q.Q. and M.D. Lane, Activation and centromeric localization of CCAAT/enhancer-binding proteins during the mitotic clonal expansion of adipocyte differentiation. Genes Dev, 1999. 13(17): p. 2231-41.
50. Wu, Z., N.L. Bucher, and S.R. Farmer, Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. Mol Cell Biol, 1996. 16(8): p. 4128-36.
51. Wu, Z., et al., Conditional ectopic expression of C/EBP beta in NIH-3T3 cells induces PPAR gamma and stimulates adipogenesis. Genes Dev, 1995. 9(19): p. 2350-63.
52. Zhang, J.W., et al., Role of CREB in transcriptional regulation of CCAAT/enhancer-binding protein beta gene during adipogenesis. J Biol Chem, 2004. 279(6): p. 4471-8.
53. Zhang, J.W., et al., Dominant-negative C/EBP disrupts mitotic clonal expansion and differentiation of 3T3-L1 preadipocytes. Proc Natl Acad Sci U S A, 2004. 101(1): p. 43-7.
54. Tang, Q.Q., T.C. Otto, and M.D. Lane, CCAAT/enhancer-binding protein beta is required for mitotic clonal expansion during adipogenesis. Proc Natl Acad Sci U S A, 2003. 100(3): p. 850-5.
55. Oishi, Y., et al., Kruppel-like transcription factor KLF5 is a key regulator of adipocyte differentiation. Cell Metabolism, 2005. 1(1): p. 27-39.
56. Chen, Z., et al., Krox20 stimulates adipogenesis via C/EBPbeta-dependent and -independent mechanisms. Cell Metabolism, 2005. 1(2): p. 93-106.
57. Birsoy, K., Z. Chen, and J. Friedman, Transcriptional regulation of adipogenesis by KLF4. Cell Metabolism, 2008. 7(4): p. 339-47.
58. Pearson, G., et al., Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev, 2001. 22(2): p. 153-83.
59. Benito, M., et al., Differentiation of 3T3-L1 fibroblasts to adipocytes induced by transfection of ras oncogenes. Science, 1991. 253(5019): p. 565-8.
60. Sale, E.M., P.G. Atkinson, and G.J. Sale, Requirement of MAP kinase for differentiation of fibroblasts to adipocytes, for insulin activation of p90 S6 kinase and for insulin or serum stimulation of DNA synthesis. EMBO J, 1995. 14(4): p. 674-84.
61. Tang, Q.Q., et al., Sequential phosphorylation of CCAAT enhancer-binding protein beta by MAPK and glycogen synthase kinase 3beta is required for adipogenesis. Proc Natl Acad Sci U S A, 2005. 102(28): p. 9766-71.
62. Hu, E., et al., Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma. Science, 1996. 274(5295): p. 2100-3.

63. Kim, K.A., et al., Pref-1 (preadipocyte factor 1) activates the MEK/extracellular signal-regulated kinase pathway to inhibit adipocyte differentiation. Mol Cell Biol, 2007. 27(6): p. 2294-308.
64. Engelman, J.A., M.P. Lisanti, and P.E. Scherer, Specific inhibitors of p38 mitogen-activated protein kinase block 3T3-L1 adipogenesis. J Biol Chem, 1998. 273(48): p. 32111-20.
65. Engelman, J.A., et al., Constitutively active mitogen-activated protein kinase kinase 6 (MKK6) or salicylate induces spontaneous 3T3-L1 adipogenesis. J Biol Chem, 1999. 274(50): p. 35630-8.
66. Patel, N.G., et al., Differential regulation of lipogenesis and leptin production by independent signaling pathways and rosiglitazone during human adipocyte differentiation. Diabetes, 2003. 52(1): p. 43-50.
67. Aouadi, M., et al., p38MAP Kinase activity is required for human primary adipocyte differentiation. FEBS Lett, 2007. 581(29): p. 5591-6.
68. Aouadi, M., et al., Inhibition of p38MAPK increases adipogenesis from embryonic to adult stages. Diabetes, 2006. 55(2): p. 281-9.
69. Alonso, A., et al., Protein tyrosine phosphatases in the human genome. Cell, 2004. 117(6): p. 699-711.
70. Jeffrey, K.L., et al., Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. Nat Rev Drug Discov, 2007. 6(5): p. 391-403.
71. Ito, A., et al., Role of MAPK phosphatase-1 in the induction of monocyte chemoattractant protein-1 during the course of adipocyte hypertrophy. J Biol Chem, 2007. 282(35): p. 25445-52.
72. Emanuelli, B., et al., Overexpression of the dual-specificity phosphatase MKP-4/DUSP-9 protects against stress-induced insulin resistance. Proc Natl Acad Sci U S A, 2008. 105(9): p. 3545-50.
73. Sakaue, H., et al., Role of MAPK phosphatase-1 (MKP-1) in adipocyte differentiation. J Biol Chem, 2004. 279(38): p. 39951-7.
74. Bazuine, M., et al., Mitogen-activated protein kinase (MAPK) phosphatase-1 and -4 attenuate p38 MAPK during dexamethasone-induced insulin resistance in 3T3-L1 adipocytes. Mol Endocrinol, 2004. 18(7): p. 1697-707.
75. Wu, J.J., et al., Mice lacking MAP kinase phosphatase-1 have enhanced MAP kinase activity and resistance to diet-induced obesity. Cell Metabolism, 2006. 4(1): p. 61-73.
76. Stahl, A., et al., Insulin causes fatty acid transport protein translocation and enhanced fatty acid uptake in adipocytes. Dev Cell, 2002. 2(4): p. 477-88.
77. Guilherme, A., et al., Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol, 2008. 9(5): p. 367-77.
78. Badman, M.K. and J.S. Flier, The gut and energy balance: visceral allies in the obesity wars. Science, 2005. 307(5717): p. 1909-14.
79. Cheatham, B. and C.R. Kahn, Insulin action and the insulin signaling network. Endocr Rev, 1995. 16(2): p. 117-42.
80. Laviola, L., et al., Insulin signalling in human adipose tissue. Arch Physiol Biochem, 2006. 112(2): p. 82-8.
81. Watson, R.T., M. Kanzaki, and J.E. Pessin, Regulated membrane trafficking of the insulin-responsive glucose transporter 4 in adipocytes. Endocr Rev, 2004. 25(2): p. 177-204.

82. Degerman, E., et al., Evidence that insulin and isoprenaline activate the cGMP-inhibited low-Km cAMP phosphodiesterase in rat fat cells by phosphorylation. Proc Natl Acad Sci U S A, 1990. 87(2): p. 533-7.
83. Kitamura, T., et al., Insulin-induced phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B by the serine-threonine kinase Akt. Mol Cell Biol, 1999. 19(9): p. 6286-96.
84. Zimmermann, R., et al., Fate of fat: the role of adipose triglyceride lipase in lipolysis. Biochim Biophys Acta, 2009. 1791(6): p. 494-500.
85. Martin, S. and R.G. Parton, Lipid droplets: a unified view of a dynamic organelle. Nat Rev Mol Cell Biol, 2006. 7(5): p. 373-8.
86. Hamilton, J.A. and F. Kamp, How are free fatty acids transported in membranes? Is it by proteins or by free diffusion through the lipids? Diabetes, 1999. 48(12): p. 2255-69.
87. Abumrad, N., C. Coburn, and A. Ibrahimi, Membrane proteins implicated in long-chain fatty acid uptake by mammalian cells: CD36, FATP and FABPm. Biochim Biophys Acta, 1999. 1441(1): p. 4-13.
88. Zimmermann, R., et al., Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science, 2004. 306(5700): p. 1383-6.
89. Coleman, D.L., Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia, 1978. 14(3): p. 141-8.
90. Friedman, J.M. and J.L. Halaas, Leptin and the regulation of body weight in mammals. Nature, 1998. 395(6704): p. 763-70.
91. Tartaglia, L.A., et al., Identification and expression cloning of a leptin receptor, OB-R. Cell, 1995. 83(7): p. 1263-71.
92. Cohen, P., et al., Selective deletion of leptin receptor in neurons leads to obesity. J Clin Invest, 2001. 108(8): p. 1113-21.
93. Badman, M.K. and J.S. Flier, The adipocyte as an active participant in energy balance and metabolism. Gastroenterology, 2007. 132(6): p. 2103-15.
94. Orci, L., et al., Rapid transformation of white adipocytes into fat-oxidizing machines. Proc Natl Acad Sci U S A, 2004. 101(7): p. 2058-63.
95. Wang, M.Y., Y. Lee, and R.H. Unger, Novel form of lipolysis induced by leptin. J Biol Chem, 1999. 274(25): p. 17541-4.
96. Wang, M.Y., et al., Fat storage in adipocytes requires inactivation of leptin's paracrine activity: implications for treatment of human obesity. Proc Natl Acad Sci U S A, 2005. 102(50): p. 18011-6.
97. Buettner, C., et al., Leptin controls adipose tissue lipogenesis via central, STAT3-independent mechanisms. Nat Med, 2008. 14(6): p. 667-75.
98. Scherer, P.E., Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes, 2006. 55(6): p. 1537-45.
99. Reshef, L., et al., Glyceroneogenesis and the triglyceride/fatty acid cycle. J Biol Chem, 2003. 278(33): p. 30413-6.
100. Kadowaki, T. and T. Yamauchi, Adiponectin and adiponectin receptors. Endocr Rev, 2005. 26(3): p. 439-51.
101. Graham, T.E. and B.B. Kahn, Tissue-specific alterations of glucose transport and molecular mechanisms of intertissue communication in obesity and type 2 diabetes. Horm Metab Res, 2007. 39(10): p. 717-21.
102. Yang, Q., et al., Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature, 2005. 436(7049): p. 356-62.
103. Fukuhara, A., et al., Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science, 2005. 307(5708): p. 426-30.

104. Berndt, J., et al., Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. Diabetes, 2005. 54(10): p. 2911-6.
105. Kershaw, E.E. and J.S. Flier, Adipose tissue as an endocrine organ. J Clin Endocrinol Metab, 2004. 89(6): p. 2548-56.
106. Tilg, H. and A.R. Moschen, Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol, 2006. 6(10): p. 772-83.
107. Fasshauer, M., et al., Monocyte chemoattractant protein 1 expression is stimulated by growth hormone and interleukin-6 in 3T3-L1 adipocytes. Biochem Biophys Res Commun, 2004. 317(2): p. 598-604.
108. Sell, H., U. Kaiser, and J. Eckel, Expression of chemokine receptors in insulin-resistant human skeletal muscle cells. Horm Metab Res, 2007. 39(4): p. 244-9.
109. Kelly, T., et al., Global burden of obesity in 2005 and projections to 2030. Int J Obes (Lond), 2008. 32(9): p. 1431-7.
110. Flegal, K.M., et al., Prevalence and trends in obesity among US adults, 1999-2008. JAMA, 2010. 303(3): p. 235-41.
111. Flegal, K.M., et al., Overweight and obesity in the United States: prevalence and trends, 1960-1994. Int J Obes Relat Metab Disord, 1998. 22(1): p. 39-47.
112. Berghofer, A., et al., Obesity prevalence from a European perspective: a systematic review. BMC Public Health, 2008. 8: p. 200.
113. Ogden, C.L., et al., Prevalence of high body mass index in US children and adolescents, 2007-2008. JAMA, 2010. 303(3): p. 242-9.
114. Gaziano, J.M., Fifth phase of the epidemiologic transition: the age of obesity and inactivity. JAMA, 2010. 303(3): p. 275-6.
115. Executive summary of the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. Arch Intern Med, 1998. 158(17): p. 1855-67.
116. Hotamisligil, G.S., Inflammation and metabolic disorders. Nature, 2006. 444(7121): p. 860-7.
117. Brown, W.V., et al., Obesity: why be concerned? Am J Med, 2009. 122(4 Suppl 1): p. S4-11.
118. Walley, A.J., J.E. Asher, and P. Froguel, The genetic contribution to non-syndromic human obesity. Nat Rev Genet, 2009. 10(7): p. 431-42.
119. Alberti, K.G., P. Zimmet, and J. Shaw, The metabolic syndrome--a new worldwide definition. Lancet, 2005. 366(9491): p. 1059-62.
120. Grundy, S.M., Does a diagnosis of metabolic syndrome have value in clinical practice? Am J Clin Nutr, 2006. 83(6): p. 1248-51.
121. Kahn, S.E., R.L. Hull, and K.M. Utzschneider, Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature, 2006. 444(7121): p. 840-6.
122. Stumvoll, M., B.J. Goldstein, and T.W. van Haeften, Type 2 diabetes: pathogenesis and treatment. Lancet, 2008. 371(9631): p. 2153-6.
123. Weisberg, S.P., et al., Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest, 2003. 112(12): p. 1796-808.
124. Xu, H., et al., Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest, 2003. 112(12): p. 1821-30.
125. Hirosumi, J., et al., A central role for JNK in obesity and insulin resistance. Nature, 2002. 420(6913): p. 333-6.
126. Cai, D., et al., Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med, 2005. 11(2): p. 183-90.

127. Arkan, M.C., et al., IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med, 2005. 11(2): p. 191-8.
128. Schroder, M. and R.J. Kaufman, The mammalian unfolded protein response. Annu Rev Biochem, 2005. 74: p. 739-89.
129. Ron, D. and P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol, 2007. 8(7): p. 519-29.
130. Cox, J.S., C.E. Shamu, and P. Walter, Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. Cell, 1993. 73(6): p. 1197-206.
131. Urano, F., et al., Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science, 2000. 287(5453): p. 664-6.
132. Yoshida, H., et al., XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell, 2001. 107(7): p. 881-91.
133. Ozcan, U., et al., Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science, 2004. 306(5695): p. 457-61.
134. Howard, B.V., G. Ruotolo, and D.C. Robbins, Obesity and dyslipidemia. Endocrinol Metab Clin North Am, 2003. 32(4): p. 855-67.
135. Smith, S.R. and P.W. Wilson, Free fatty acids and atherosclerosis--guilty or innocent? J Clin Endocrinol Metab, 2006. 91(7): p. 2506-8.
136. Montani, J.P., et al., Ectopic fat storage in heart, blood vessels and kidneys in the pathogenesis of cardiovascular diseases. Int J Obes Relat Metab Disord, 2004. 28 Suppl 4: p. S58-65.
137. Calle, E.E., Obesity and cancer. BMJ, 2007. 335(7630): p. 1107-8.
138. Calle, E.E., Adiposity and Cancer, in Nutrition and Health: Adipose Tissue and Adipokines in Health and Disease, G.F.a.T. Mazzone, Editor. 2007, Humana Press. p. 307-325.
139. Calle, E.E., et al., Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med, 2003. 348(17): p. 1625-38.
140. Reeves, G.K., et al., Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study. BMJ, 2007. 335(7630): p. 1134.
141. Rose, D.P., S.M. Haffner, and J. Baillargeon, Adiposity, the metabolic syndrome, and breast cancer in African-American and white American women. Endocr Rev, 2007. 28(7): p. 763-77.
142. Giovannucci, E. and D. Michaud, The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. Gastroenterology, 2007. 132(6): p. 2208-25.
143. Sandhu, M.S., D.B. Dunger, and E.L. Giovannucci, Insulin, insulin-like growth factor-I (IGF-I), IGF binding proteins, their biologic interactions, and colorectal cancer. J Natl Cancer Inst, 2002. 94(13): p. 972-80.
144. Stattin, P., et al., Obesity and colon cancer: does leptin provide a link? Int J Cancer, 2004. 109(1): p. 149-52.
145. Fabbrini, E., S. Sullivan, and S. Klein, Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology, 2010. 51(2): p. 679-89.
146. Caldwell, S.H., et al., Obesity and hepatocellular carcinoma. Gastroenterology, 2004. 127(5 Suppl 1): p. S97-103.

147. Neugut, A.I., A.C. Chen, and D.P. Petrylak, The "skinny" on obesity and prostate cancer prognosis. J Clin Oncol, 2004. 22(3): p. 395-8.
148. Freedland, S.J., Obesity and prostate cancer: a growing problem. Clin Cancer Res, 2005. 11(19 Pt 1): p. 6763-6.
149. Banez, L.L., et al., Obesity-related plasma hemodilution and PSA concentration among men with prostate cancer. JAMA, 2007. 298(19): p. 2275-80.
150. Freedland, S.J., et al., Stronger association between obesity and biochemical progression after radical prostatectomy among men treated in the last 10 years. Clin Cancer Res, 2005. 11(8): p. 2883-8.
151. Giovannucci, E., Nutrition, insulin, insulin-like growth factors and cancer. Horm Metab Res, 2003. 35(11-12): p. 694-704.
152. Pollak, M., Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer, 2008. 8(12): p. 915-28.
153. Kaaks, R., Nutrition, hormones, and breast cancer: is insulin the missing link? Cancer Causes Control, 1996. 7(6): p. 605-25.
154. Kang, J.H., et al., Adiponectin induces growth arrest and apoptosis of MDA-MB-231 breast cancer cell. Arch Pharm Res, 2005. 28(11): p. 1263-9.
155. Dieudonne, M.N., et al., Adiponectin mediates antiproliferative and apoptotic responses in human MCF7 breast cancer cells. Biochem Biophys Res Commun, 2006. 345(1): p. 271-9.
156. Brakenhielm, E., et al., Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc Natl Acad Sci U S A, 2004. 101(8): p. 2476-81.
157. Yin, N., et al., Molecular mechanisms involved in the growth stimulation of breast cancer cells by leptin. Cancer Res, 2004. 64(16): p. 5870-5.
158. Fenton, J.I., et al., Leptin, insulin-like growth factor-1, and insulin-like growth factor-2 are mitogens in ApcMin/+ but not Apc+/+ colonic epithelial cell lines. Cancer Epidemiol Biomarkers Prev, 2005. 14(7): p. 1646-52.
159. Onuma, M., et al., Prostate cancer cell-adipocyte interaction: leptin mediates androgen-independent prostate cancer cell proliferation through c-Jun NH2-terminal kinase. J Biol Chem, 2003. 278(43): p. 42660-7.
160. Heinlein, C.A. and C. Chang, Androgen receptor in prostate cancer. Endocr Rev, 2004. 25(2): p. 276-308.
161. Korkmaz, K.S., et al., Molecular cloning and characterization of STAMP1, a highly prostate-specific six transmembrane protein that is overexpressed in prostate cancer. J Biol Chem, 2002. 277(39): p. 36689-96.
162. Korkmaz, C.G., et al., Molecular cloning and characterization of STAMP2, an androgen-regulated six transmembrane protein that is overexpressed in prostate cancer. Oncogene, 2005. 24(31): p. 4934-45.
163. Porkka, K.P., M.A. Helenius, and T. Visakorpi, Cloning and characterization of a novel six-transmembrane protein STEAP2, expressed in normal and malignant prostate. Lab Invest, 2002. 82(11): p. 1573-82.
164. Moldes, M., et al., Tumor necrosis factor-alpha-induced adipose-related protein (TIARP), a cell-surface protein that is highly induced by tumor necrosis factor-alpha and adipose conversion. J Biol Chem, 2001. 276(36): p. 33938-46.
165. Passer, B.J., et al., The p53-inducible TSAP6 gene product regulates apoptosis and the cell cycle and interacts with Nix and the Myt1 kinase. Proc Natl Acad Sci U S A, 2003. 100(5): p. 2284-9.

166. Steiner, M.S., et al., Growth inhibition of prostate cancer by an adenovirus expressing a novel tumor suppressor gene, pHyde. Cancer Res, 2000. 60(16): p. 4419-25.
167. Ohgami, R.S., et al., The Steap proteins are metalloreductases. Blood, 2006. 108(4): p. 1388-94.
168. Ohgami, R.S., et al., Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nat Genet, 2005. 37(11): p. 1264-9.
169. Sanchez-Pulido, L., et al., ACRATA: a novel electron transfer domain associated to apoptosis and cancer. BMC Cancer, 2004. 4: p. 98.
170. Amzallag, N., et al., TSAP6 facilitates the secretion of translationally controlled tumor protein/histamine-releasing factor via a nonclassical pathway. J Biol Chem, 2004. 279(44): p. 46104-12.
171. Zhang, X., et al., Apoptosis induction in prostate cancer cells by a novel gene product, pHyde, involves caspase-3. Oncogene, 2001. 20(42): p. 5982-90.
172. Hubert, R.S., et al., STEAP: a prostate-specific cell-surface antigen highly expressed in human prostate tumors. Proc Natl Acad Sci U S A, 1999. 96(25): p. 14523-8.
173. Alves, P.M., et al., STEAP, a prostate tumor antigen, is a target of human CD8+ T cells. Cancer Immunol Immunother, 2006. 55(12): p. 1515-23.
174. Garcia-Hernandez Mde, L., et al., In vivo effects of vaccination with six-transmembrane epithelial antigen of the prostate: a candidate antigen for treating prostate cancer. Cancer Res, 2007. 67(3): p. 1344-51.
175. Valenti, M.T., et al., STEAP mRNA detection in serum of patients with solid tumours. Cancer Lett, 2009. 273(1): p. 122-6.
176. Xu, H. and G.S. Hotamisligil, Signaling pathways utilized by tumor necrosis factor receptor 1 in adipocytes to suppress differentiation. FEBS Lett, 2001. 506(2): p. 97-102.
177. Fasshauer, M., et al., GH is a positive regulator of tumor necrosis factor alpha-induced adipose related protein in 3T3-L1 adipocytes. J Endocrinol, 2003. 178(3): p. 523-31.
178. Fasshauer, M., et al., Interleukin-6 is a positive regulator of tumor necrosis factor alpha-induced adipose-related protein in 3T3-L1 adipocytes. FEBS Lett, 2004. 560(1-3): p. 153-7.
179. Kralisch, S., et al., Interleukin-1beta is a positive regulator of TIARP/STAMP2 gene and protein expression in adipocytes in vitro. FEBS Lett, 2009. 583(7): p. 1196-200.
180. Wellen, K.E., et al., Coordinated regulation of nutrient and inflammatory responses by STAMP2 is essential for metabolic homeostasis. Cell, 2007. 129(3): p. 537-48.
181. Abedini, A. and S.E. Shoelson, Inflammation and obesity: STAMPing out insulin resistance? Immunol Cell Biol, 2007. 85(6): p. 399-400.
182. Wellen, K.E. and G.S. Hotamisligil, Inflammation, stress, and diabetes. J Clin Invest, 2005. 115(5): p. 1111-9.
183. Arner, P., et al., Expression of six transmembrane protein of prostate 2 in human adipose tissue associates with adiposity and insulin resistance. J Clin Endocrinol Metab, 2008. 93(6): p. 2249-54.
184. Zhang, C.M., et al., Downregulation of STEAP4, a highly-expressed TNF-alpha-inducible gene in adipose tissue, is associated with obesity in humans. Acta Pharmacol Sin, 2008. 29(5): p. 587-92.

185. Arnoldussen, Y.J., et al., The mitogen-activated protein kinase phosphatase vaccinia H1-related protein inhibits apoptosis in prostate cancer cells and is overexpressed in prostate cancer. Cancer Res, 2008. 68(22): p. 9255-64.
186. Haslam, D., Obesity: a medical history. Obes Rev, 2007. 8 Suppl 1: p. 31-6.
187. Olshansky, S.J., et al., A potential decline in life expectancy in the United States in the 21st century. N Engl J Med, 2005. 352(11): p. 1138-45.
188. Flegal, K.M., et al., Excess deaths associated with underweight, overweight, and obesity. JAMA, 2005. 293(15): p. 1861-7.
189. Wolf, A.M. and K.A. Woodworth, Obesity prevention: recommended strategies and challenges. Am J Med, 2009. 122(4 Suppl 1): p. S19-23.
190. Aronne, L.J., et al., When prevention fails: obesity treatment strategies. Am J Med, 2009. 122(4 Suppl 1): p. S24-32.
191. Flier, J.S., Obesity wars: molecular progress confronts an expanding epidemic. Cell, 2004. 116(2): p. 337-50.
192. Hirsch, J. and B. Batchelor, Adipose tissue cellularity in human obesity. Clin Endocrinol Metab, 1976. 5(2): p. 299-311.
193. Xu, H., et al., Dual specificity mitogen-activated protein (MAP) kinase phosphatase-4 plays a potential role in insulin resistance. J Biol Chem, 2003. 278(32): p. 30187-92.
194. Wu, J.J., et al., Mice lacking MAP kinase phosphatase-1 have enhanced MAP kinase activity and resistance to diet-induced obesity. Cell Metab, 2006. 4(1): p. 61-73.
195. Owens, D.M. and S.M. Keyse, Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. Oncogene, 2007. 26(22): p. 3203-13.
196. Kondoh, K. and E. Nishida, Regulation of MAP kinases by MAP kinase phosphatases. Biochim Biophys Acta, 2007. 1773(8): p. 1227-37.
197. Liu, Y., E.G. Shepherd, and L.D. Nelin, MAPK phosphatases--regulating the immune response. Nat Rev Immunol, 2007. 7(3): p. 202-12.
198. Christie, G.R., et al., The dual-specificity protein phosphatase DUSP9/MKP-4 is essential for placental function but is not required for normal embryonic development. Mol Cell Biol, 2005. 25(18): p. 8323-33.
199. Alonso, A., et al., Tyrosine phosphorylation of VHR phosphatase by ZAP-70. Nat Immunol, 2003. 4(1): p. 44-8.
200. Kang, T.H. and K.T. Kim, Negative regulation of ERK activity by VRK3-mediated activation of VHR phosphatase. Nat Cell Biol, 2006. 8(8): p. 863-9.
201. Sabio, G., et al., A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. Science, 2008. 322(5907): p. 1539-43.
202. Solinas, G., et al., JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. Cell Metabolism, 2007. 6(5): p. 386-97.
203. Lefterova, M.I., et al., PPARgamma and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. Genes Dev, 2008. 22(21): p. 2941-52.
204. Sendamarai, A.K., et al., Structure of the membrane proximal oxidoreductase domain of human Steap3, the dominant ferrireductase of the erythroid transferrin cycle. Proc Natl Acad Sci U S A, 2008. 105(21): p. 7410-5.
205. Joe, A.W., et al., Depot-specific differences in adipogenic progenitor abundance and proliferative response to high-fat diet. Stem Cells, 2009. 27(10): p. 2563-70.

206. Cawthorn, W.P. and J.K. Sethi, TNF-alpha and adipocyte biology. FEBS Lett, 2008. 582(1): p. 117-31.
207. Gregor, M.F., et al., Endoplasmic reticulum stress is reduced in tissues of obese subjects after weight loss. Diabetes, 2009. 58(3): p. 693-700.
208. Vembar, S.S. and J.L. Brodsky, One step at a time: endoplasmic reticulum-associated degradation. Nat Rev Mol Cell Biol, 2008. 9(12): p. 944-57.
209. Mantovani, A., et al., Cancer-related inflammation. Nature, 2008. 454(7203): p. 436-44.

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