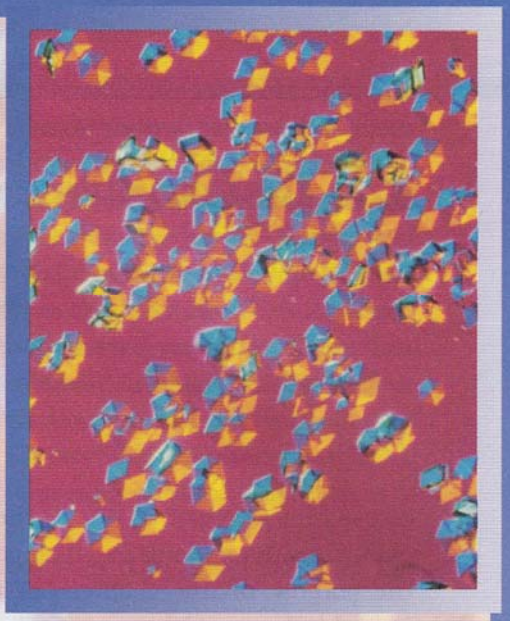

3. Obesity-related Diseases – Insulin Resistance – Metabolic Syndrome

József Mandl – Péter Csermely – Ágota Vér

3.1. Development of Obesity-Related Diseases

Obesity has emerged as one of the most important public health problems in developed countries. (For the purpose of this chapter obesity denotes diet-induced obesity. Other types or aspects of obesity are dealt with in other chapters of the book.) More than 60% of adults in the United States are classified as being overweight. According to WHO (World Health Organisation) estimations, there are 300 million clinically obese adults (Body Mass Index over 30 kg/m^2) worldwide. Since 1980 the worldwide prevalence of obesity has doubled. Even more alarming is the marked increase in obesity among children. The current obesity "epidemic" has reached India, China, and South Asia with the spread of urbanisation, and Western diet and lifestyle in the absence of appropriate physical exercise. This global epidemic has led to an increase in the incidence of associated obesity-related disorders, in particular insulin resistance/glucose intolerance, type 2 diabetes mellitus (T2DM), dyslipidemia and cardiovascular disease/hypertension.

Metabolic syndrome denotes a collection of risk factors which are in a complex inter-relationship with each other: dysglycemia, raised blood pressure, elevated triglycerol levels, low HDL cholesterol levels and obesity (particularly central obesity). Insulin resistance underlying T2DM is a possible link in this complex interrelationship. However,

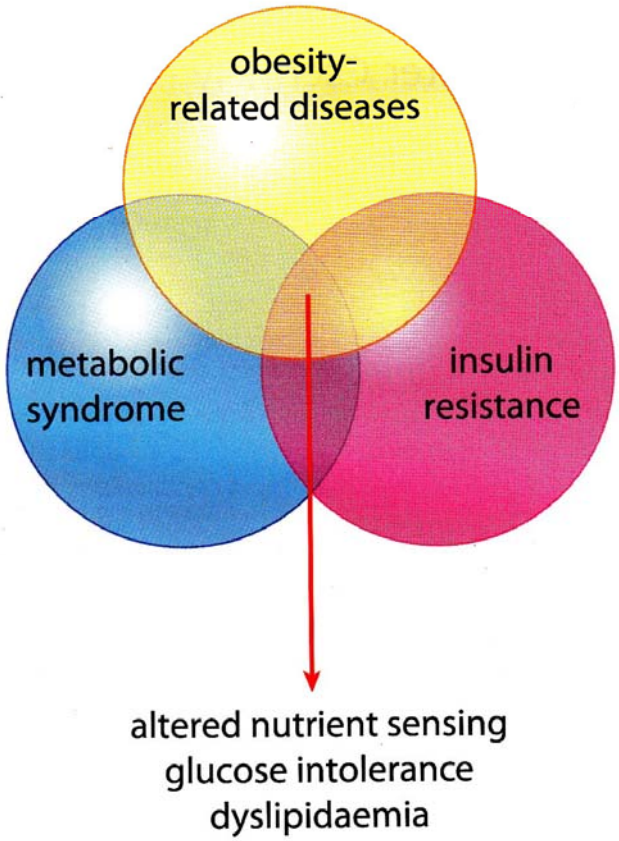

Figure 1. Obesity-related diseases, metabolic syndrome, diabetes mellitus overlap each other

diabetes mellitus (DM) itself is also a complex metabolic disorder; lean people may also become diabetics. Not all manifestations of diabetes are triggered by obesity or insulin resistance (*Fig. 1*). Several data support the existence of a polygenic background of DM.

However, dramatically altered living conditions, typified by chronic nutritional excess associated with a sedentary lifestyle, are also responsible for obesity, in addition to genetic determination.

Struggle for food is crucial aspect of life for all living organisms. Essentially, metabolism deals with the following questions: how to gain food and how to utilize it, how to minimize the loss of energy in the course of energy transformation and how to optimalize the effectivity of energy storage. Civilisation brought a major change in this ancient strategy: one of the essential problems (at least in so-called welfare societies) is not how to gain food, but how to avoid it.

3.2. Regulation of Metabolism is Dependent on Nutritional Status

There is a balance in energy utilization between energy consumption and energy production, which means cells/organisms must be capable of metabolic adaptation to different environmental conditions: starvation or energy supply. Energy status, nutritional supply, hormonal status, infections, etc. are all involved among the various stimuli regulating metabolism. There are various molecular mechanisms for sensing nutritional status and, particularly, various nutrients.

Adenosine 5'-monophosphate (AMP)-activat protein kinase (AMPK) is a master regulator a cellular energy sensor. Its role seems to be fun mental in the maintenance of glucose homeosta AMPK is expressed in those tissues where regu tion is essential for metabolic the homeosta liver, skeletal muscle and adipose tissue. Simila to other key enzymes of the intermediary metal lism, AMPK activity is regulated both by alloste means and by post-translational modifications.

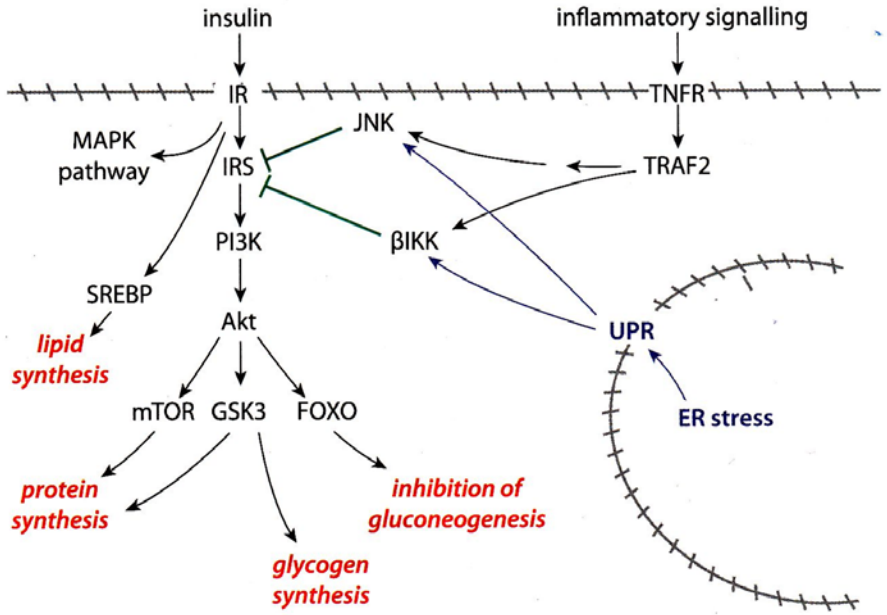

Figure 2. Cross-talk between insulin and proinflammatory tumour necrosis factor signalling.
Regulation of several metabolic processes are affected by insulin-dependent cellular signalling forming complex anabolic system mediated by different protein/ lipid kinases and transcription factors. Insulin si nalling interferes with proinflammatory signalling pathways. Proinflammatory pathways activate JNK a IKKβ, which phosphorylate IRS at serine residues, inhibiting its tyrosine phosphorylation by activated IR. this way, proinflammatory pathways block insulin signalling. JNK and IKKβ are also activated by ER stres induced UPR. Thus, both extracellular and endogenous (blue arrows) signalling interfere with each other. addition, SREBPs are also activated by ER stress.

Abbreviations: IR: insulin receptor, IRS: insulin receptor substrate, PI3K: phosphatidyl inositol 3 kina Akt: protein kinase B, mTOR: mammalian target of rapamycin, GSK3: glycogen synthase kinase 3, FoxO: for head transcription factor, JNK: c-Jun N-terminal kinase, IKKβ: inhibitor of nuclear factor κB (NF κB) kinase- MAPK: mitogen-activated protein kinase, TNFR: TNF receptor, TRAF 2: TNF receptor-associated factor UPR: unfolded protein response, SREBP: sterol response element-binding protein, ER: endoplasmic reticulu

one hand, it is a target of allosteric activation by AMP, thus sensing various conditions that lead to alterations in the intracellular AMP/ATP ratio (e.g. hypoxia, glucose deprivation). On the other hand, it senses energy status at a cellular level through modulation of its activities via post-translational modifications and by other means (increase in intracellular calcium/calmodulin-dependent protein kinase kinase 2 (CAMKK), TGF-β-activated kinase 1, etc.). Stimuli for activation of AMPK include hormones, cytokines and adipokines released in obesity-related diseases. In general, activation of stimulation of AMPK suppresses anabolism, energy storage and growth.

Besides the phosphorylation state of adenine nucleotides, the other fundamental ratio characteristic of actual intracellular energy homeostasis is that between NAD^+ and NADH. The dependence of sirtuins on NAD^+ directly links their enzymatic activity to the energy status of the cell *via* the cellular NAD^+/NADH ratio, given that sirtuins are NAD^+ dependent histone- deacetylases. There are several reports on a correlation between the activities of sirtuins and AMPK, longevity and caloric restriction, integrating sensing of metabolic status with adaptive transcriptional outputs. Moreover, sirtuins are involved in development of insulin resistance.

The regulation of metabolic homeostasis also occurs via appropriate extracellular (hormonal, cytokine, etc.) stimuli mediated by different plasma membrane receptors. These may be categorized in different ways. From a metabolic point of view, there are (i) anabolic signals which stimulate energy/nutrient uptake, storage of energy and cellular growth; and (ii) signals which mobilize the stored excess energy mainly for purposes of defense (e.g. inflammation). Insulin is an essential growth factor as its receptor is expressed in every tissue (see chapter 2.) to promote anabolism and growth. In contrast, inflammatory signal transducing pathways, such as the TNFα (tumour necrosis factor α) receptor, are activated when the mobilization of energy stores is needed (e.g. sepsis or infections). Equilibrium and cross-talk exists among these signalling routes. Interrelationship between insulin signalling and inflammatory pathways plays a major role in obesity-related diseases *(Fig. 2)*.

Furthermore, several (ligand-dependent intracellular receptor) transcription factors have been identified in the regulation of metabolism with special respect to carbohydrate and lipid metabolism. They include (partly ligand inducible) nuclear receptors: peroxisome proliferator-activated receptor (PPAR) family α, β, γ1, γ2; retinoid X receptor (RXR) α, activated by various lipids; liver X receptor (LXR) α, activated by glucose; hepatic nuclear receptor (HNF-4) α, γ, activated by various fatty acids; and basic helix-loop-helix leucine zipper transcription factors: sterol regulatory element-binding proteins (SREBP), activated by cholesterol and lipids; carbohydrate regulatory element-binding protein (ChREBP), activated by glucose; and Max-like factor X (MLX). Thus, a shift in metabolism towards fatty acid synthesis and storage or, conversely, contrary towards fatty acid oxidation is regulated at various metabolic and signalling levels. Moreover, these mechanisms are also involved in the process of nutrient sensing. Altered nutrient sensing regarding metabolically important lipids, carbohydrates and amino acids are essential components in the pathomechanism of obesity-related diseases.

3.3. Disorders of Insulin Signalling – Diabetes Mellitus

Insulin is the main anabolic hormone; tits role is fundamental for the maintenance of metabolic homeostasis. Obesity-related diseases and metabolic syndrome (and obviously insulin resistance) are connected to insulin signalling (see I.2.). Binding of insulin to its receptor (IR) activates insulin signalling through tyrosine phosphorylation of insulin receptor substrates (IRS). Tyrosine phosphorylation of IR induces a series of enzymatic activations and molecular interactions leading to different events in insulin signalling (Fig. 2). IR activation leads to the phosphorylation of several tyrosine residues on IRS proteins, some of which are recognised by the Src homology 2 (SH2) domain of the p85 regulatory subunit of PI3K (see also chapter 2.). The catalytic subunit of PI3K, p110, then phosphorylates phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], leading to the formation of Ptd(3,4,5)P_3. AKT (otherwise known as protein kinase B, PKB) is activated by Ptd(3,4,5)P_3, which is recruited to the plasma membrane. Activation of AKT also requires 3-phosphoinositide-dependent protein kinase 1 (PDK1), leading to the phosphorylation of AKT. Activated AKT leads to the phosphorylation and inactivation of GSK3. A major substrate of GSK3 is glycogen synthase, which is involved in glycogen synthesis. Phosphorylation of glycogen synthase inhibits glycogen synthesis. Thus, inactivation of GSK3 by AKT promotes glucose storage as glycogen. Alternatively, activation of FoxO transcription factors causes inhibition of gluconeogenesis. AKT also increases protein synthesis through mTOR (see later) and also GSK3. IR activation results in increased lipid synthesis mediated by SREBP transcription factors (see later). The various components (e.g. PI3K, mTOR) of this complex regulatory system can also be connected to numerous other signalling routes (see chapter 2).

Alternatively, IRS proteins can be phosphorylated at serine residues; this process is catalyzed by various other protein kinases, such as c-Jun N-terminal kinases (JNK) and IκB kinase β (IK
Serine phosphorylation of IRS inhibits ins
signalling. JNK and IKKβ are activated by pr
flammatory signalling such as the TNFR, and
in the course of the unfolded protein resp
(see later).

Essentially two types of insulin related diso
exist: insulin deficiency, type 1 DM (T1DM);
insulin resistance, type 2 DM (T2DM). T1DM
not belong to the category of obesity-relate
seases, but for didactical reasons it is detailed in
chapter. DM is diagnosed by its classical sympt
polyuria and polydipsia. At present the estim
number of people suffering from DM is appr
mately 150 million worldwide.

Insulin Deficiency – Type 1 Diabetes Mellitus

T1DM is caused by insulin deficiency resul
from the selective and progressive death o
sulin producing pancreatic beta cells via an
toimmune mechanism. Autoimmune mar
can be detected in all but a minority of case
Caucasian populations, development of the
case is mainly connected to genetic factors lir
to certain HLA antigens such as HLA-DR3
HLA-DR4. In Caucasian populations are
nected to the development of the disease. T
are responsible for about 50–70% of cases. M
over, there are about 20 genes, or gene regi
which can also be coupled to T1DM. The in
ited inclination to form autoantigens against
cell constituents, due to viral infections, imm
processes and inflammation due to toxic ag
lead to beta cell injury. Various viruses, suc
mumps, Coxsackie 4B- and 5B, rubeola, ecl
and 30 and hepatitis B and C might show st
tural similarities to certain regions of proteins
cluding the glutamate decarboxylase (GAD
kDa fragment, islet proteins 37 kDa, 38 kDa

kDa and 69 kDa, GLUT2 and insulin], leading to formations of autoantibodies. If 80–90% of pancreatic beta cells are impaired, T1DM develops.

Insulin Resistance – Type 2 Diabetes Mellitus

In the case of insulin resistance the focus on the essential significance of insulin-producing pancreatic β cells has also shifted towards those main insulin-responding tissues and organs such as the liver, muscle and adipose tissue that are essential for metabolic homeostasis.

Insulin resistance syndrome features central (especially visceral) obesity, glucose intolerance, dyslipidemia and hypertension, which promote the development of T2DM, cardiovascular disease, cancer, polycystic ovarian disease, and non-alcoholic fatty liver disease. this is also known as metabolic syndrome. From various statistical data, which show the enhanced prevalence of insulin resistance, perhaps the most concerning is that up to 50% of severely obese children develop this condition.

Alterations in glucose homeostasis are the focus of the disease. Hyperinsulinaemia, which evolves with insulin resistance, also acts on other non-insulin-resistant tissues, leading to several additional consequences.

Previously, the liver was considered as the "regulatory" organ in intermediary metabolism as compared to quantitatively more important "peripheral" tissues, adipose tissue and muscle. The significance of muscle has since been shown by several observations. Development of insulin resistance in muscle is among the earliest defects in T2DM. Glucose uptake into skeletal muscle accounts for 70–90% of the oral glucose load, while that of adipose tissue accounts for only 5–15%. However, our knowledge and view on adipose tissue has changed dramatically over the last decades.

Adipose Tissue in Obesity-Related Diseases

The increased release of adipokines, free fatty acids and inflammatory mediators due to alterations in adipose tissue result in adipocyte dysfunction, adverse effects on liver, pancreatic beta cells and skeletal muscle; moreover, the effects extend to the heart and vascular beds.

Storage of excess energy due to the increase in caloric consumption accompanied with a decrease in energy expenditure causes adipocyte hypertrophy and hyperplasia.

Metabolic Significance of Adipose Tissue

Adipose tissue is a connective tissue which has been differentiated to store energy in the form of lipids and to mobilize it upon appropriate stimuli. Adipose tissue is a metabolic and endocrine organ crucial to for whole-body insulin sensitivity. Furthermore, the maintenance and distribution of adipose tissue is also essential in energy homeostasis. White adipose tissue is the predominant type of fat in adults. It serves as a storage depot for excess energy. (In newborns brown adipose tissue generates heat through mitochondrial uncoupling of terminal oxidation and oxidative phosphorylation. This chapter deals only with white adipose tissue; therefore, "the term adipose tissue" denotes only white adipose tissue.) Due to a chronic nutritional surplus there is an increase of adipocytes in both number (hyperplasia) and size (hypertrophy). An increase in the number of preadipocytes and in the differentiation of preadipocytes into mature adipocytes underlies hyperplasia, or adipogenesis. Preadipocyte differentiation is stimulated via various pathways, such as by insulin and glucocorticoids among others. In obesity there is a change in the function of adipocyte-resident macrophages, as well. The amount of macrophages is dependent on hyperplasia and hypertrophy of adipose tissue. In the course of obesity, inflammation develops in the adipose tissue, and the production of cytokines and chemokines is therefore enhanced. Inflammatory mediators are also involved in preadipocyte differentiation (see below).

Adipose tissue is also a key regulator of systemic carbohydrate metabolism and plays a dominant role in glucose sensing, too. The importance of insulin-sensitive plasma membrane translocation of GLUT4 (for details see Chapter 2) in adipocytes and in skeletal muscle is well established in the development of systemic glucose intolerance and hyperinsulinemia, as characteristics of insulin resistance.

Adipocytes and macrophages are closely related and share several functions. Among others they both secrete adipokines and also react to pathogen-associated compounds. Adipokines participate in the maintenance of the metabol-

ic homeostasis of the organism. Moreover, they mediate metabolic communication between adipose tissue and muscle, liver or brain. Essential (patho)physiological processes such as as appetite (control of feeding), thermogenesis, glucose and lipid metabolism, inflammatory responses, blood pressure and angiogenesis are regulated by adipokines. In obesity the disposal of dietary glucose and lipids and the expression of adipokines are dysregulated. There are major differences in adipokine production in adipose tissues of d rent localization.

The regulation of energy homeostasis of th ganism by adipokines occurs through autocrin paracrine mechanisms and also via hormon effects on the central nervous system. Under mal conditions adipokines are a self-defense sy against excess energy intake. Obesity induces s alterations in their secretion, which result in r changes in insulin sensitivity and inflammatio

3.4. Adipokines

The principle insulin-sensitising adipokines are leptin and adiponectin. There are also several additional adipokines, including resistin, visfatin, apelin, omentin, chemerin. Furthermore, components of different systems such as proinflammatory cytokines [TNFα, interleukin-6 (IL-6), monocyte chemoattractant protein 1 (MCP-1)], plasminogen activator inhibitor -1 (PAI-1), and the renin-angiotensin system also have adipokine activities.

Leptin

Leptin is an integral component in the response to starvation. It regulates food intake as well as energy expenditure. In spite of the huge number of observations concerning its functions and effects, the full extent of its physiological and pathological role is unknown. Leptin is a 16 kDa protein, synthesized mainly in adipose tissue, placenta and the gastric wall. It decreases food intake and increases energy utilization. Part of this regulation involves the decrease in appetite, counteraction of insulin effects and decrease in storage of triglycerols (TG). Acting through its receptors in hypothalamic nucleus arcuatus, it decreases the production of appetite stimulatory peptides (neuropeptide Y, AGRP: Agouti-like peptide) and increases the expression of pro-opiomelanocortin and cocaine-amphetamine stimulatory proteins in neurons which decreases appetite. In secondary regulatory centers of appetite (nucleus paraventricularis, lateralis hypothalamus), it causes an increase in the level of CRH (corticotropin-releasing-hormone), TRH (thyreotropin-releasing hormone) and oxytocin which lower appetite, while decreasing the production of MCH (melanin-concentrating hormone), orexin-1 and -2 which stimulate appetite. Moreover, it regulates the cholecystokinin sensitivity of the solitary nucleus, which is responsible for regulating the determination of tsaturation with regard to dietary changes.

Leptin stimulates energy utilisation. It causes hyperpolarisation of pancreatic beta cells and inhibits basal and glucose-stimulated insulin secretion.

In the liver leptin stimulates gluconeogenesis by an increase in the activity of glucose-6-phosphatase and phosphoenolpyruvate-carboxykinase (PEPCK), and it increases the degradation of glycogen. Furthermore, it causes an increase in beta oxidation of fatty acids through a PPARα activation-mediated stimulation of carnitinpalmitoyl transferase and acyl-CoA oxidase. In accordance with these regulations, leptin blocks lipogenesis, represses stearoyl-CoA desaturase and decreases the expression of the sterol response element-binding proteins (SREBP). In muscle it stimulates AMP-activated protein kinase (AMPK), and by enhancing fatty acid oxidation it increases energy expenditure.

In adipose tissue leptin increases lipolysis, and enhances the activity of hormone-sensitive lipase via β3 receptors through the stimulation of catecholamines, thereby causing symphatic activity in the CNS.

In starvation and in cases of low calory intake there is a decrease in leptin serum concentration. However, there is no increase in leptin concentration in cases of acute overfeeding. Leptin concentration in the circulation is considered as an adiposity signal as it is dependent on the amount of the adipose tissue in the organism. Thus, in obesity the concentration of leptin in the blood is increased, but the appetite of obese people is not decreased. In obesity, similarly to insulin resistance, the increased leptin level results in leptin resistance. This is explained by leptin desensitisation. In spite of increased leptin levels leptin concentration in the brain of over weight people is low, as leptin cannot penetrate through the blood-brain barrier. Hence, there is a lack of leptin-caused regulation. Among others, consequences the down-regulation of appetite-stimulating endocannabioid production in the hypothalamus is missing.

Leptin receptors belong to the superfamily of cytokine receptors having a single transmembrane

region. The Ob-Rb isoform is expressed in the hypothalamus. This receptor in the hypothalamus is connected to JAK/STAT and MAPK signal transduction routes (see I. 2.). However, leptin also induces negative feed-back regulatory pathways For instance, SOCS3 (suppressor of cytokine signalling protein) promotes the proteasomal degradation of the Ob-Rb receptor and inhibits phosphorylation of STAT3 (and IRS). The other negative regulatory route is the stimulatory effect of leptin on the expression of phosphotyrosine-phosphatase-1-B (PTP1B), the substrate of which is JAK2. JAK2 is inactivated by dephosphorylation. Thus, SOCS3 and PTP1B are involved in development of leptin resistance.

Furthermore, Ob-Rb connected SHP2 also has impact on the PI3K pathway and MAPK signalling. Thus, leptin effects are also connected to insulin signalling also via SHP2.

Adiponectin

Adiponectin is a protein synthetized mainly in adipocytes which acts as an endogenous insulin-sensitizing hormone. Its receptors, AdipoR1 and AdipoR2, possess 7 tansmembrane domains, but are not G protein coupled. AdipoR1 and AdipoR2 are expressed prevalently in skeletal muscle and liver, respectively. AMPK is activated by adiponectin, a protein hormone which stimulates several processes in muscle and liver. In muscle, adiponectin enhances fatty acid oxidation, stimulates glucose uptake and lactate production, and causes acetyl-CoA carboxylase to become phosphorylated. In skeletal muscle, tyrosine-phosphorylation of IR is enhanced. In the liver, free fatty acid (FFA) uptake and VLDL production are reduced; fatty acid oxidation, aerobic glucose utilization and glycogen synthesis are increased; while gluconeogenesis is decreased. Thus, adiponectin acts as an insulin sensitizer in the liver and muscle, decreasing blood glucose levels. In vascular endothelium, monocyte adhesion is prevented, foam cell transformation is suppressed, and proliferation and migration of blood vessel smooth muscle cells is inhibited by adiponectin. Therefore, contrary to the other pro-inflammatory adipokines, adiponectin has atheroprotective effects.

Despite the fact that adiponectin is synthesized in adipose tissue, its serum concentration is lower in obese people than in lean people. Expression and secretion of adiponectin is inhibited by inflammatory cytokines such as TNFα. TNFα level is elevated in obesity (see later); thus, it represents a feedback mechanism in obese people.

The level of adiponectin is decreased in es tial hypertension and T2DM. This phenomen explained by the decrease in PI3K activity req for the secretion of adiponectin. Pima Indians lower adiponectin levels compared to other gr which correlates with the increased prevalen T2DM observed in this population. On the c hand, there is a negative correlation betweer culating adiponectin and triglycerol levels, wh positive correlation has been demonstrated bet adiponectin and HDL-cholesterol. These obs tions are associated with hypoadiponectinemia increased atherosclerosis in dyslipidemia.

Resistin, Visfatin, Apelin, Omer

Several recently discovered adipokines are duced in adipose tissue. They are mainly syn sized in adipose tissues and their supposed role influence insulin signalling. Most of them pc various cardiovascular effects, as well. Their r of action and exact molecular mechanism is st be elucidated. Inflammation is a key factor in sity. The number of macrophages is increased inflammatory-signalling pathways are activ resulting in an overproduction of proinflamm factors and cytokines, such as TNFα, IL-6, i and MCP-1. MCP-1 leads to further macrop infiltration into adipose tissue. Several inflar tory cytokines are also adipokines.

TNFα and IL-6

TNFα is a multifunctional cytokine. It is volved in the regulation of the immune resp (see chapter I. 3.3), differentiation, prolifera apoptosis and metabolism. TNFα is a proinflar tory cytokine that activates various signalling p ways (leading to the activation of NFκB, M cascades, JNK, etc.). In obesity its expressi increased; circulating TNFα level correlates BMI. TNFα is produced mainly by adipose t macrophages, but also by adipocytes. The d nature of adipose tissue and its close associ with muscle, pancreas and vasculature suggest local production also targets non-adipose tis Moreover, TNFα has autocrine and paracrin fects, and also acts on the liver.

TNFα acts through TNFR1 and TNFR2 (T receptors 1 and 2), cell surface receptors whos pression in adipose tissue also depends on nutr TNFα is active in impairing insulin signalli

adipocytes. It causes via the activation of various protein kinases (e.g. JNK and IKKβ), serine phosphorylation (S307) of IRS-1 (see chapter I. 2.), and induction of SOCS3, blocking tyrosine phosphorylation of IRS-1. Therefore, TNFα inhibits insulin signalling, the activation of PI3K *(Fig. 2)* and the translocation of GLUT4 to the plasma membrane in adipocytes.

TNFα inhibits the growth and differentiation of adipocytes, and also inhibits lipogenesis. It suppresses the expression of adipogenic transcription factors such as PPARγ and C/EBPα. TNFα stimulates lipolysis, and decreases the formation of lipoprotein-lipase (LPL), fatty acid transport protein and acetyl-CoA synthase. Secretion of leptin and IL-6 are stimulated, while that of adiponectin is inhibited. In the liver TNFα enhances the expression of enzymes involved in fatty acid synthesis and stimulates the release of VLDL. Finally, TNFα can alter the formation of several other cytokines.

IL-6 is a pro inflammatory cytokine which is also synthesized in adipose tissue. It inhibits insulin signalling by inducing SOCS3 in asimilar way to TNFα. Thus it participates in the maintenance of chronic inflammation, in impaired glucose tolerance, dyslipidemia, and finally in the development of insulin resistance. Decreased weight or decrease in calorie intake reduce the production of the cytokine.

Renin-Angiotensin System

The components of the renin-angiotensin system (RAS), including angiotensinogen, angiotensin-converting enzyme (ACE) 1A and 1B, both types of angiotensin II (AII) receptor, renin and renin-binding protein, are expressed in adipose tissue. ACE and AII is secreted by preadipocytes, and their secretion is induced during adipocyte differentiation. It is supposed that RAS is involved in adipocyte differentiation and proliferation, and also in the development of insulin resistance and in consequent hypertension. There is cross-talk between insulin and AII signalling. In T2DM it is supposed that AII-dependent signalling is involved in the regulation of insulin sensitivity.

3.5. Molecular Mechanisms of Insulin Resistance

Insulin resistance can develop via several pathways. Insulin signalling is amplified partly by different types of phosphorylation, catalyzed by various protein kinases. As mentioned before, components of insulin signalling can undergo post-translational modification that may increase or decrease their respective activities: serine phosphorylation of IR decreases its kinase activity, and serine phosphorylation of IRS proteins impairs their ability to be tyrosine phosphorylated. In addition, interaction of various inhibitory proteins with different members of the signalling pathway, e.g. SOCS proteins or PTP1b, act through different mechanisms against components of the kinase cascade.

Interrelationship with other signalling pathways also modifies insulin signalling. Obesity-induced inflammation promotes insulin resistance; its moderation improves it.

Organelle Dysfunctions in Insulin Resistance – Role of ER Stress

ER is the largest organelle in most eukaryotic cells and plays different roles in the integration, compartmentation and regulation of metabolism (see chapter I. 4.2.), linking nutrient sensing to cellular signalling especially with respect to protein and lipid metabolism. The primary aim of these responses is to adjust metabolism in response to environmental changes. Development of insulin resistance, metabolic syndrome and T2DM can be regarded as failures of adaptation attempts, resulting in a pathological transition of physiological regulatory processes. ER stress is provoked when a complex response is required for adaptation to various alterations. It can be induced by several stimuli and also by excess nutrients, glucose and fatty acids in obesity. States of energy imbalance and excessive demands on the ER result in ER dysfunction, which then leads to ER stress. The outcome might promote survival; conversely, failure can lead to ER stress-induced apoptosis.

Various steps, as well as the regulation of sev biosynthetic processes such as protein and lipid s thesis, biotransformation and glucose product are dependent on ER homeostasis.

Synthesis and folding of secretory and mer rane proteins occur in the luminal compartmen the ER. Various regulatory mechanisms are invol in the control of protein formation. To prevent aggregation of proteins and to enhance protein f ing, the lumen of the ER contains a high conc tration of ER chaperones and foldases. There balance between protein synthesis and the prot folding capacity in the ER. If this balance is alte correcting mechanisms are induced to maint protein homeostasis in the ER. UPR (unfolded p tein response) is a complex signalling response t aims to restore this balance (for details see chap I. 4.2.). ER membrane-bound receptors/protein s sors are activated and transformed to transcript factors that ignite several signalling routes. T UPR is comprised of three different pathways tiated by these ER transmembrane proteins aid cell survival under stress conditions. However, t physiological compensatory mechanism can a play a fundamental pathological role when comp sation is unsuccessful.

Prolonged and severe ER stress can stimul proapoptotic signals that interfere with nor cellular functions and might lead mitochond dysfunction. Prolonged ER stress is associated w the release of ER Ca^{2+} stores, which can pert mitochondria and stimulate oxidative stress. Mo over, Bcl-2 family members Bax and Bak may be leased from the ER during ER stress and may ca mitochondrial permeability transition (MPT) (chapter I. 4.3.). MPT might lead to apoptosis (chapter I. 7.).

ER stress markers have recently been discove in adipose tissue in obese patients. Hepatocytes, a pocytes and pancreatic beta cells, which are dee involved in the development of metabolic syndro and insulin resistance, are characterized by high p

tein secretory capacity. Therefore, ER stress plays a dominant role in the pathogenesis of insulin resistance in these cell types. In adipocytes it causes, for instance decreased adiponectin formation, while in pancreatic β cells insulin synthesis is deteriorated.

Adaptation to Nutritional Overload – Nutrient Sensing

In addition to protein synthesis, the ER is also the site of lipid synthesis and adaptation to altered conditions of lipid formation. Firstly, the ER is considered the site of triglycerol droplet formation in response to fatty acid accumulation within the cell. Droplets are spherical structures surrounded by a single phospholipid membrane layer and associated proteins. Droplet formation in different tissues is a subject of very intensive research.

In adipose tissue ER stress is linked to adipocyte differentiation. Adipocyte differentiation requires a complex coordinated interplay of several transcription factors. Optimal integration of metabolism, nutrition and adaptation to environmental conditions is achieved via nuclear receptors. The key regulator of adipocyte differentiation and function is PPARγ retinoid X receptor (RXR), a heterodimer, in cooperation with CCAAT/enhancer-binding proteins C/EBP-s) (see chapter I. 1.2.). A significant number of genes involved in lipid uptake, synthesis and storage, and in lipolysis are regulated directly by PPARγ. Moreover, endocrine functions of adipocytes are also targeted by PPARγ; for example it regulates adiponectin expression. PPARγ mutations in the development of insulin resistance are subjects of intensive research.

Secondly, ER stress can activate SREBPs, transcription factors involved in *de novo* lipid synthesis. The role of the ER in cholesterol sensing and regulation of lipid synthesis is mediated by the ER-resident SREBP (sterol response element-binding protein) family of transcription factors (SREBP–1a, –1c, –2). In response to low sterol levels (in the case of SREBP–1a, –2), or insulin signalling (in the case of SREBP–1c), ER membrane-bound proteins are released and translocated to the Golgi, where they are activated as transcription factors by proteolysis. The activated SREBPs then enter the nucleus and upregulate either cholesterol synthesis (SREBP–1a, –2), or fatty acid and triglycerol (SREBP–1c) synthesis, and additionally LDL receptor expression (SREBP–2). Thus, SREBP–1a and –2 are regulated by sterol supply, while the activation of SREBP–1c is determined by caloric intake via insulin and glucagon action. SREBP 1c is induced during differentiation in adipocytes, while its expression is downregulated in obesity, which might be due to insulin resistance.

The serine/threonine kinase mTOR pathway is involved in amino acid sensing. There is cross-talk between mTOR, insulin signalling and TNF signalling, as well. Through this, nutrient sensing related to extracellular stimuli, hormonal, nutritional and environmental information is integrated. mTOR also changes in obesity. mTOR, stimulated via JNK activation, suppresses insulin receptor signalling, thus contributing to the development of insulin resistance. The mTOR pathway upregulates protein synthesis and regulates numerous processes in cells, including the cell cycle, cellular growth and autophagy. Protein deprivation induces autophagy, while amino acid abundance inhibits autophagy mediated by the ER resident Bcl-2 proteins, which are involved in influencing mitochondrial membrane permeabilization and apoptosis.

Saturated fatty acids may lead to toxic consequences in the liver, adipose tissue and pancreatic beta cells. This is the phenomenon of lipotoxicity. Insulin resistant adipocytes do not take up fatty acids, lipolysis develops and serum FFA and lipid levels are increased. Higher concentrations of circulating FFA and triglycerols are associated with lipid accumulation in various tissues such as liver, skeletal muscle, heart and pancreas. In insulin resistance the effect of insulin, as the main antilipolytic hormone, is lacking, which further increases the concentration of circulating lipids.

Incomplete fatty acid oxidation and mitochondrial overload are responsible for intramyocellular lipid accumulation in skeletal muscle, leading to insulin resistance.

Obesity is associated with oxidative stress in mitochondria. Excess free fatty acids increase mitochondrial ROS production in obesity. Hyperglycemia can also lead to increased ROS production in mitochondria, which has a pathogenic role through oxidative stress in the pancreas, liver and muscle. Hyperglycemia is sensed by beta cells through an accelerated oxidative and ATP-generating metabolism that is accompanied by enhanced ROS production. Hypoglycemia also induces ER stress and UPR in liver cells.

Inflammation in Insulin Resistance

Chronic inflammation, oxidative stress, hypoxia, and mechanical stress due to hypertrophy are the main characteristics of insulin-resistant adipose tis-

sue. Inflammatory pathways are upregulated, leading to increased expression of adipokine-cytokines. These processes lead to organelle dysfunction in adipocytes, particularly in the ER and mitochondria. Organelle dysfunction is also induced in hepatocytes and pancreatic beta cells.

Inflammatory signalling induces the activation of JNK and IKKβ. These signalling pathways interfere with insulin signalling *(Fig. 2)*. An interrelationship exists between energy mobilization and energy storing anabolic signalling at the level of insulin receptor-mediated processes. This is part of the integration of metabolic and immune homeostasis, as well as of nutrient- and pathogen-sensing pathways.

Insulin Signalling Interferes with Inflammatory Signals and the Unfolded Protein Response

ER stress and UPR interfere with insulin signalling to induce insulin resistance *(Fig. 2)*. In the course of the UPR, JNK is activated, and this results in the activation of AP-1. Furthermore Iκb is activated, leading to the activation of NFκB. Both JNK and Iκb lead to serine phosphorylation of insulin receptor substrate-1 (IRS-1). Ser phosphorylation of IRS-1 impairs its Tyr phosphorylation, therefore inhibiting insulin responsiveness. JNK is activated in inflammation by cytokines and by TNFα as well. Taken together, characteristic manisfestations of insulin resistance are produced by the activation of JNK. In obesity JNK is activated in adipose tissue, the liver, pancreatic islets and muscle and appears to promote insulin resistance in these tissues.

The enlargement of fat stores in adipose tissue is associated with elevated glucose and free plasma fatty acid levels. Hyperglycemia and hyper-free fatty acidemia induce ER stress, and ER stress can further increase insulin resistance. A similar cross-talk between inflammatory and insulin signalling also exists in the liver. Inflammatory cytokines cause similar effects through toll-like receptors (TLR). TLR2 and TLR4 receptors are expressed in the plasma membranes of adipocytes, and they are also activated by saturated fatty acids. In obesity their expression in adipose tissue is stimulated and pro-inflammatory responses are induced. Fatty acids, through TLR, can induce the production of cytokines in macrophages. This establishes a connection between nutrient sensing and the action of inflammatory mediators.

UPR-inducing insulin resistance contributes to further increases in plasma glucose and lipid levels, generating ER stress in other tissues and ultimately worsening insulin resistance. TNFα produced in enlarged adipose tissue inhibits lipoprotein lipase activity and increases lipolysis.

Pancreatic Beta Cells in Insulin Resistance

Glucose homeostasis is maintained by insulin synthesis and secretion in pancreatic beta cells and by peripheral glucose utilization regulated by insulin. Hyperglycemia is the consequence of inadequate insulin secretion and/or insulin action. Dysfunctional beta cells are essential for the pathogenesis of T2DM. Hyperglycaemia leads to beta cell exhaustion and depletion of available insulin stores. Alterations in nutrient/glucose supply are of special importance in the responsiveness of pancreatic beta cells. ER stress seems to be crucial not only in physiological regulation, but also in beta cell failure in T2DM. Induction of ER stress is part of the physiological beta cell response to acute nutrient (glucose and fatty acid) abundance. Fifty percent of total protein synthesis is proinsulin production, and UPR is needed for proper proinsulin folding in cases of hyperglycemia, insulin resistance or high fat diet. Sustained or excessive ER stress reduces insulin expression and increases apoptosis, thus decreasing beta cell mass, thereby playing a role in the development of T2DM.

Oxidative stress due to hyperglycemia-induced ROS formation causes beta cell impairment. Beta cells are particularly sensitive to oxidative insults due to low expression levels of antioxidant enzymes such as superoxide dismutases, and virtually no catalase or glutathione peroxidase (see chapter I. 3.2.) Glucose toxicity and oxidative stress in T2DM is associated with insulin resistance. Chronic exposure to high glucose has adverse effects on insulin synthesis by decreasing the availability of insulin gene transcription factors and thus to insulin promoter activity, leading to reduced insulin formation and secretion. Insulin resistance due to obesity places an additional demand on beta cells to produce more insulin. (It should be noted that there are lean people with T2DM who have never been obese and are not resistant to insulin. These facts support a genetic disease concept of T2DM.)

Development of uncompensated ER stress-induced apoptosis in pancreatic beta cells is also related

to the rate of increased islet amyloid polypeptide. In addition, the effects of cytokines, similarly to their effect in T1DM, exert negative effects on beta cells through inflammatory signalling routes and activation of JNK. These together diminish beta cell insulin sensitivity, ultimately leading to a loss of beta cell mass. Lipotoxicity can be also exerted in pancreatic beta cells, which contributes to the onset of apoptosis.

The Liver in Metabolic Syndrome – Pathogenesis of Fatty Liver

The central role of the liver in carbohydrate and lipid metabolism is well known. Liver affects body fat composition. Alternatively, dietary fat modifies hepatic carbohydrate and lipid metabolism. Hepatic insulin resistance is thought to be largely responsible for the development of fasting hyperglycemia. Non-alcoholic fatty liver disease (NAFLD), characterized by enhanced alanine aminotransferase- and gamma-glutamyltransferase levels can be considered as a manifestation of insulin resistance in the liver.

As has been previously shown, several transcription factors mediate the impact of environmental changes on liver metabolism. Moreover, the role of ER stress has also been proposed to underpin the altered transcriptional regulation of metabolism leading to fatty liver disease. The role of the liver in protein homeostasis is well established, it being the key protein secretory organ of, among others, lipoproteins and numerous serum proteins of the internal environment. Moreover, liver functions maintaining lipid homeostasis are also essential.

ER stress also has a guarding function in lipid homeostasis; failure of compensation induces accumulation of lipid, lipid droplet formation and hepatic steatosis.

Insulin is known to upregulate acetyl-CoA carboxylase and fatty acid synthase via the activation of SREBP–1c in the liver, which affects hepatic triglycerol production and secretion. On the other hand, SREBP–1c is also induced during ER stress and by several other conditions such as hyperhomocysteinemia, alcoholism, high carbohydrate/fat diets, etc. Similarly to the activation of SREBP, which is induced by ER stress and occurs by proteolysis transcription factor, cyclic-AMP-responsive-element-binding protein H (CREBH) is also activated in the liver. CREBH stimulates production of acute phase response proteins. (see chapter I. 3.3.1.)

3.6. Obesity and Atherosclerosis

Atherosclerosis is discussed in another chapter in this book (chapter II. 2.). Diet-induced obesity is frequently connected to diet-induced atherosclerosis. TNFα is likely involved in adiposity-related vascular dysfunction. The metabolic connection between obesity and atherosclerosis has been targeted by several studies. These connections emphasized by the well-known increased card vascular risk in obese people. Patients with me bolic syndrome are at twice the risk of develop cardiovascular diseases as individuals without syndrome.

MEDICAL PATHOBIOCHEMISTRY

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