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TOPIC HIGHLIGHT

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Hepatic steatosis, low-grade chronic inflammation and hormone/growth factor/adipokine imbalance

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Abstract

Non-alcoholic fatty liver disease (NAFLD), a further expression of metabolic syndrome, strictly linked to obesity and diabetes mellitus, is characterized by insulin resistance (IR), elevated serum levels of free fatty acids and fatty infiltration of the liver, which is known as hepatic steatosis. Hepatocyte apoptosis is a key feature of this disease and correlates with its severity. Free-fatty-acid-induced toxicity represents one of mechanisms for the pathogenesis of NAFLD and hormones, growth factors and adipokines influence also play a key role. This review highlights the various pathways that contribute to the development of hepatic steatosis. Circulating concentrations of inflammatory cytokines are reckoned to be the most important factor in causing and maintaining IR. Low-grade chronic inflammation is fundamental in the progression of NAFLD toward higher risk cirrhotic states.

Key words: Hepatic steatosis; Low-grade chronic inflammation; Adipokines; Hormones; Growth factors

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INTRODUCTION

There are many genetic, evolutionary, environmental, behavioral and physiological factors that induce and exacerbate obesity. Teleologically, our early ancestors had a survival advantage if they were able to store energy to be used in famine or during very stressful situations. Those individuals with 'thrifty genes' survived and reproduced more metabolically efficient offspring. In modern society, thrifty genes are coupled with increased food availability and sedentary lifestyle. This often results in a net positive energy balance. The current view is that obesity leads to hyperinsulinemia/insulin resistance (IR) and IR exacerbates obesity, which frustrates most attempts at weight loss. Adipocytes, while utilizing glucose and fat excess during nutritional affluence and storing them as triglycerides, release energy as free fatty acids (FFAs) and glycerol by lipolysis. These FFAs do not produce significant metabolic disturbances as long as they are oxidized in target tissues by the leptins of adipocytes. Long-chain fatty acids (LCFAs) in non-adipocytes, which cannot undergo mitochondrial β-oxidation, cause signal transduction defects[1] or result in apoptosis *via* accumulation as cytosolic triglycerides. The consequences of increased lipid delivery to peripheral tissues are multiple and the integrated response

to central adiposity is complex and involves several organs and tissues.

Concerning the cardiovascular risk, some observations suggest that different lipid accumulation processes begin in the subendothelial or deep intimal regions, which contributes to complicated atheroma core formation in peripheral arteries. Furthermore, it has been demonstrated that the presence of deep intimal lipid accumulation is associated with reduced endothelium-dependent relaxation in large arteries. The functional and morphological abnormalities might contribute to human coronary atherogenesis that progresses slowly with age[2]. Besides, adipocytes can also prevent atherosclerotic vascular damage by its product adiponectin[3].

LIPOTOXICITY: THE CLASSICAL VIEW

Acute elevations in FFAs can provoke peripheral IR in humans[4]. In addition, acute lowering of FFAs with the antilipolytic drugs can enhance peripheral insulin-mediated glucose uptake[5]. A defect at the level of reduced glucose transport *per se* results from an effect of FFAs to inhibit proximal insulin signaling steps, including tyrosyl phosphorylation of insulin receptor substrates[6]. Obesity results in the accumulation of muscle (intramyocellular) triglycerides and activated lipids in the form of long-chain fatty acyl-CoA esters[7]. This accumulation is also implicated in the impairment of insulin signaling, possibly *via* activation of selected protein kinase C isoforms[8]. Similar effects occur in the liver in association with hepatic lipid accumulation, a ubiquitous finding in obese patients. Lipids can also accumulate in pancreatic islets, which impairs insulin secretion, such as in the development of diabetes in ZDF rats[7]. In addition, decreased catabolism contributes to tissue lipid accumulation, because hepatic and intramyocellular lipid content is associated with reduced mitochondrial oxidative and phosphorylation activity in muscle of elderly humans[9]. Obesity is clearly associated with increased levels of circulating FFAs. Patients with various grades of obesity and IR are generally resistant to the antilipolytic effects of insulin[10]. Furthermore, adipocyte constituents of visceral fat are more metabolically active and have a higher rate of lipolysis[11]. Increases in FFAs can provoke peripheral IR in animals and humans[4]. In addition to the role of FFAs in producing IR in muscle, the impaired ability of insulin to suppress FFA release boosts hepatic glucose production, because insulin-mediated anti-lipolysis contributes to insulin regulation of hepatic glucose output[12]. The glucose-fatty acid cycle that has the potential to increase fatty acid utilization to inhibit glucose oxidation in muscle was first proposed by Randle *et al*[13] in 1963.

LOW-GRADE CHRONIC INFLAMMATION: THE MAIN ROLE OF INTERLEUKIN 6

Growing evidence links a low-grade, chronic inflammatory state to obesity and its coexisting conditions such as IR, type 2 diabetes, metabolic syndrome and non-alcoholic fatty liver disease (NAFLD)[14,15] that includes a large spectrum that ranges from fatty liver (FL), non-alcoholic steatohepatitis (NASH) and cryptogenetic cirrhosis. The spleen also plays a key role in this chronic process[16]. Anti-inflammatory drugs can reverse IR[17], which suggests that inflammation is directly involved in its pathogenesis. Inflammatory mediators that are biosynthesized in the liver and increased in NAFLD patients include C-reactive protein (CRP)[15], interleukin (IL)-6[16], fibrinogen and plasminogen activator inhibitor-1 (PAI-1)[18]. Fat in the liver represents a site beyond adipose tissue that independently contributes to synthesis of inflammatory mediators. In support of a sequence of cellular and molecular events that mediate hepatic IR in NAFLD, recent data lend credence to the fact that hepatic steatosis activates IκB kinase (IKK)-β and nuclear factor (NF)-κB[19]. Among the inducible transcription factors that control inflammatory gene expression, NF-κB plays a central and evolutionarily conserved role in coordinating the expression of various soluble pro-inflammatory mediators (cytokines and chemokines) and leukocyte adhesion molecules. In non-stimulated cells, NF-κB is sequestered in the cytosol by the inhibitor of NF-κB (IκB) that masks the nuclear localization signal present along the NF-κB protein sequence. Treatment of cells with pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-1, or with bacterial products such as lipopolysaccharide, leads to the activation of a specific-IKK complex that phosphorylates IκB and thereby tags it for ubiquitination and degradation by the proteasome[20]. The degradation of IκB thus allows NF-κB to translocate into the nucleus where it can act as a transcription factor that upregulates IL-6 production and secretion. IL-6 works locally through paracrine and/or endocrine mechanisms to activate IL-6 signaling in the liver. IL-6 is known to induce IR in hepatocytes[21]. Hepatic production of IL-6 also provides a further pathogenic link to extrahepatic organs such as muscle. NF-κB target genes are not upregulated in transgenic mouse muscle, but IL-6 target genes are, including suppressor of cytokine signaling (SOCS) and signal transducer and activator of transcription (STAT) proteins. These genes are reversed during IL-6 neutralization, which is consistent with the pathogenic involvement of IL-6. Activation of NF-κB leads to a severe syndrome of muscle wasting, without IR[22].

VISCERAL ADIPOSITY AND ADIPOCYTE DIFFERENTIATION

Although many studies have reported strict correlations between insulin sensitivity and visceral fat deposition[23], associations between the amount of subcutaneous fat on the trunk and IR have also been reported in obese non-diabetic men[24] and those with type 2 diabetes[25]. Thus, subcutaneous fat, not draining into the portal vein, determines IR by a mechanism that is not linked to the liver. At

the same time, IR in obese women is strictly related to increased overall fat mass, or to an elevation in truncal subcutaneous fat mass as measured by skinfold thickness[26] or magnetic resonance imaging[27]. Moreover, IR is predicted independently by an enlargement of truncal subcutaneous fat mass and an increased amount of visceral fat[26]. Total and subcutaneous fat mass is important to the IR syndrome; in fact, adipocytes, while responding sensitively to systemic influences, affect important target tissues with their secretions. They specifically detect the changes in energy equilibrium of the organism and adequately respond by drawing excess glucose and lipids from the bloodstream[28], storing them as triglycerides[29], releasing depot fat to non-adipocytes as FFAs and glycerol[30], and increasing fatty acid oxidation in adipose and non-adipose tissues to maintain fat homeostasis and cellular integrity[31]. The main transcription factor designating the characteristics of adipocytes is most likely the adipocyte determination and differentiation factor 1 (ADD1)/sterol regulatory element binding protein-1c (SREBP-1c)[32]. Its regulatory functions consist in sensing the glucose and fat excess and drawing them into the adipocytes to preserve energy and maintain constant blood levels. Otherwise, fat accumulation in non-adipocytes could be deleterious to their functions[33]. An inverse correlation has been found between cytosolic cholesterol concentration and ADD1/SREBP-1c, meanwhile, plasma insulin and glucose levels have a positive impact on ADD1/SREBP-1c[34].

Insulin crucially controls almost all aspects of adipocyte pathobiology. Almost all anabolic effects of insulin in the adipocytes are regulated by the transcription factor ADD1/SREBP-1c that also controls other mature adipocyte markers *via* transactivation of peroxisome proliferator activated receptor (PPAR)-γ and leptin[35]. PPAR-γ has major influences on various aspects of adipogenesis, such as adipocyte differentiation from preadipocytes and differentiation of fibroblasts into mature adipocytes[36]. ADD1/SREBP-1c and PPAR-γ also regulate the genetic expression of the enzymes for *de novo* lipogenesis and glucose transporter GLUT4[37].

Adipocytes do not have unlimited capacity for expansion by storing fat as triglycerides. When adipocytes reach a critical fat cell size, adipogenesis is triggered that increases the number of fat cells[38]. ADD1/SREBP-1c, and in particular PPAR-γ, efficiently cause the differentiation of pre-adipocytes and even fibroblasts or myoblasts into mature adipocytes[32]. Old adipocytes are protected by diverting the fuel excess to more competent (in terms of lipogenesis) younger adipocytes. When adipogenesis cannot occur, fat cells produce factors that strongly inhibit the anabolic actions of insulin. Two of these are TNF-α[39] and resistin[40]. These adipocyte products might result in the development of metabolic syndrome by creating insensitivity to insulin action, mainly in the fat tissue, and partly in the liver and muscle[33]. Resistin, a novel signaling molecule isolated in mice has been suggested as the putative hormone that links obesity with type 2 diabetes. Research confirms the expression of resistin in human adipose tissue and increased expression in abdominal fat[41]. Glucose firstly determines the fate of nutritional energy, whether it is oxidized or stored as triglycerides[34]. It then inhibits oxidation of LCFAs, which causes accumulation of LCFAs and their metabolites in the cytosol, which results in impaired signal transduction[42], and finally stimulates apoptosis, when unoxidized LCFA metabolites continue to accumulate in the long term (so-called glucolipotoxicity)[43].

ADD1/SREBP-1c, which is mostly dependent on insulin as outlined above, has central importance in fat tissue for the regulation of energy metabolism[30]. The amount and type of fat and associated cholesterol content of food generally inhibit ADD1/SREBP-1c expression *via* an effect on cytosolic cholesterol level[44]. Although activation of this transcription factor changes mainly according to the same intracellular cholesterol concentrations[45], the glucose component of the nutritional excess and insulin are the predominant stimulators for the genetic expression of ADD1/SREBP-1c in fat tissue[34]. It has been proposed that the amount and quality of nutritional carbohydrate, which determines the glycemic index and the responding insulin level, control the activation of ADD1/SREBP-1c. Extracellular glucose level is not only the operating lipogenic machinery of fat cells, but also controls the adipocyte secretions that are effective in non-adipocytes and their energy regulation, which determines fuel partitioning, and oxidation or storage in cells, including fat tissue. Ultimately, chronic over-nutrition, particularly rich in carbohydrates, causes impairment in transcriptional regulatory effects of ADD1/SREBP-1c, which leads to the development of a variety of metabolic disorders. In this way, IR has the potential to provide information on healthy and non-healthy obesity[46].

GROWTH FACTORS AND ADIPOKINES

Differentiation of precursor cells into mature fat cells is stimulated by multiple hormones, including glucocorticoids, growth hormone (GH), insulin-like growth factor (IGF)- I , and insulin.

As reported in a recent published review[47], a relevant role for GH in metabolism has been known since the late 1940s, when its effects on lipid and protein metabolism were demonstrated in experimental animal models[48]. After the binding of GH to specific monomeric or dimeric receptors, members of the cytokine receptor superfamily, the GH intracellular signal that is involved in lipid metabolism results in activation by trans-phosphorylation of adjacent Janus-kinase 2, with subsequent recruitment of the STAT pathway[49]. The GH signal cascade induces ultimately the transcription of specific genes, such as those for IGF- I , IGF-binding proteins (IGFBPs), acid-labile subunit (ALS), or SOCS proteins[47].

IGF- I , the main anabolic mediator of GH effects, is primarily GH-dependent and influences GH secretion through a negative feedback system[50]. IGF- I exhibits a 45% amino acid homology with insulin, acts as an insulin

sensitizer, and is a member of the IGF family, along with IGF-Ⅱ. Consequently, IGF-Ⅰ and insulin, apart from high affinity for their specific receptors, share lower affinity for their cognate insulin and IGF-Ⅰ receptors, respectively. The IGFBPs, present in serum, other biological fluids, and tissue extracts, bind IGF-Ⅰ and IGF-Ⅱ with affinities comparable to those of IGF-Ⅰ receptors. IGF-Ⅰ, in particular, circulates in plasma as a ternary complex along with IGFBP-3 or -5 and ALS, which prolongs the half-life of IGF-Ⅰ and modulates its bioavailability to peripheral tissues[51]. IGF-Ⅰ intracellular signal triggers metabolic mechanisms different from GH, by inducing tyrosine phosphorylation of the insulin receptors substrate proteins, with subsequent activation of phosphatidylinositol-triphosphate kinase and mitogen-activated protein kinase pathways[50,52]. However, a close interplay between the signaling pathways activated by GH, IGF-Ⅰ, and insulin has been demonstrated *in vitro*[47].

Human adipocytes express GH receptors. Adult patients with GH deficiency (GHD) characteristically develop an increase in abdominal obesity, total cholesterol, triglycerides and fibrinogen levels, and a decrease in high-density lipoprotein (HDL)-cholesterol levels, which indicates the metabolic syndrome[53]. GHD is correlated with the severity of alterations in lipid metabolism[54] and GH treatment in GHD patients is associated with improved lipid profiles and cardiovascular risk[55]. GH exerts insulin-like and insulin-antagonistic metabolic effects[56], which include increased gluconeogenesis, enhanced lipolysis, and inhibition of insulin action. In particular, GH displays its lipolytic effect mainly in the visceral adipose tissue, by increasing adipose tissue hormone-sensitive lipase activity *via* enhanced stimulation of the β-adrenergic receptors[49]. This effect results in increased FFA flux from the adipose to peripheral tissues[48]. No definite effects have been reported on lipoprotein lipase (LPL), thus suggesting that GH might not affect triglyceride uptake in adipose tissue. GH might also directly induce adipogenesis *via* activation of STAT-5/PPAR-γ pathway[47], although its role has been demonstrated only during the early phase of the process[47]. Finally, GH inhibits serum leptin and increases circulating resistin levels, while the effect on adiponectin remains controversial[47]. In this context, it is still not yet clear whether GH metabolic actions are exerted directly, or indirectly *via* IGF-1, or are part of GH antagonism of insulin signaling.

The effects of IGF-Ⅰ on lipolysis, gluconeogenesis, and SOCS protein are the opposite of those of GH. In particular, metabolic IGF-Ⅰ effects are similarly to those of insulin, and mainly consist of increased tissue glucose uptake, inhibition of gluconeogenesis, and enhanced adipogenesis[56]. IGF-Ⅰ has been suggested to be a major regulator of cell proliferation, differentiation and metabolism, thus regulating, among other biological processes, adipose tissue growth and differentiation of pre-adipocytes into adipocytes. The role of IGF-Ⅰ in the accumulation of adipose tissue has been investigated using transgenic mice that overexpress the *IGFBP-1* gene. In response to a sucrose-enriched diet, the transgenic mice gain significantly less body weight, and adipocyte size and epididymal fat mass are significantly reduced compared with wild-type mice[57]. Moreover, fewer colonies are generated from adipose tissue of transgenic mice, and the mitogenic response of these cells to IGF-Ⅰ is significantly lessened compared with those from wild-type mice[57]. Finally, the induction of glycerol-3-phosphate dehydrogenase, a measure of adipocyte differentiation, is reduced in pre-adipocytes from transgenic mice by IGF-Ⅰ, but not insulin. In line with the lipogenic properties of IGF-Ⅰ, long-term IGF-Ⅰ treatment of patients with GH insensitivity syndrome results in increased adipose tissue[58]. Although it has been shown that *in vitro* GH treatment of 3T3-L1 pre-adipocyte cultures is associated with a concomitant increase in IGF-Ⅰ expression[57], the effects of GH on lipolysis are not mediated by IGF-Ⅰ, because there are no functional IGF-Ⅰ receptors in adipocytes[59]. Nevertheless, a direct and independent effect of GH-induced IGFBP-3 on adipocytes has also been reported[60]. These data indicate that IGF-Ⅰ has a crucial role in the proliferation of adipocyte precursors, the differentiation of pre-adipocytes, and the development of obesity in response to caloric excess[61]. However, in healthy individuals, IGF-Ⅰ levels are inversely related to the percentage of body fat[62], and epidemiological studies have demonstrated the relationship, in subjects without pituitary or cardiovascular diseases, between low IGF-Ⅰ and hypertension and type 2 diabetes[63], and cardiovascular risk[64].

Hepatic GH signaling is also essential to regulate intrahepatic lipid metabolism. In contrast with its effects on adipose tissue, GH induces triglyceride uptake in the liver by increasing LPL and hepatic lipase expression in a STAT-5 independent manner; however, the net effects of GH in intrahepatic lipid metabolism might be affected by GH antagonism of insulin signaling in the liver, or by GH-mediated secretion of IGF-Ⅰ[47]. Intrahepatic lipid accumulation and other histological liver markers characterize patients with NAFLD. NAFLD represents a spectrum of disease that ranges from simple steatosis to NASH, NAFLD-associated cirrhosis and end-stage liver disease. Ninety percent of circulating IGF-Ⅰ originates in the liver, and hepatocytes are the largest source of IGFBP-1 and IGFBP-3. Thus, both NASH and liver cirrhosis result in a progressive decline of hepatic IGF-Ⅰ output[65]. In this context, a possible link between hepatic steatosis, GH/IGF-Ⅰ axis, and inflammatory cytokines, probably *via* SOCS signaling, might be suggested as one of the mechanisms involved in the development and/or progression of metabolic syndrome and its cardiovascular and hepatic consequences[66-68].

Transforming growth factor (TGF)-β1, in addition to playing a certain role as a pro-fibrogenetic cytokine mainly in NAFLD[69], is an anti-proliferative and pro-apoptotic factor for mammary epithelial cells, in which it acts in an auto/paracrine manner and is thus considered an important local regulator of mammary tissue involution. A recent study has supported additional evidence that stimu-

lation of IGF- I is associated with complete abrogation of TGF-β1-induced activation of pro-apoptotic Bad and Bax and in the consequent protection against apoptosis. In conclusion, apoptotic effects of TGF-β1 are mediated by IGFBPs and occurs through IGF- I sequestration, which results in inhibition of the protein kinase B/Akt-dependent survival pathway[70].

Leptin, which has autocrine, paracrine and endocrine effects, is one of the most important substances secreted by fat cells[71]. Leptin controls peripheral fatty acid oxidation *via* PPAR-α stimulation, and plays a key metabolic regulatory role in fat tissue more than in muscle, liver and pancreatic β cells. Leptin levels correlate directly with the severity of hepatic steatosis but not with inflammation or fibrosis in NAFLD patients[72].

Adipocytes that increase lipogenic activity by sensing the fuel excess also secrete leptin to prevent cytosolic fat accumulation that would compromise functions of non-adipocytes[31]. Leptin limits the lipid accumulation by its autocrine effect in adipocytes, thus maintaining cellular fat balance[30]. Its fatty acid oxidative effects are also enhanced by upregulation of mRNA of uncoupling protein-2 (UCP-2) in adipocytes and in some non-adipocytes[73], such as muscle and β cells[74]. UCP leads to energy loss by converting the energy from the Kreb's cycle to thermogenic heat dissipation in the mitochondrial electron transport chain[75]. Recent studies have indicated that leptin is an independent risk factor for coronary artery disease[73]. Although leptin is highly correlated to the overall adipose tissue, leptin is associated with IR independently of fat mass, which suggests that hyperleptinemia is an independent component of the metabolic syndrome[76,77].

Although the exact interactions between insulin and leptin are still confusing, a putative leptin resistance, like IR, has been postulated in obesity[78], and a few data also suggest peripheral leptin resistance[79]. A soluble form of the soluble leptin receptor (sOb-R) has been demonstrated. sOb-R represents the main leptin-binding compound in plasma, which results in a fraction of bound and free leptin in plasma[80]. The exact function of the sOb-R is not clear. In obesity, levels of the sOb-R are decreased compared with lean controls, which resulted in an increased fraction of free leptin[81]. A reduction in body weight through diet or bariatric surgery significantly increases the concentration of circulating sOb-R, and therefore, increases the fraction of bound leptin[82].

Thus, sOb-R might act as a modulating factor of leptin action and plays an important role in leptin resistance. The high concentrations of free leptin are indicative of leptin resistance[83]. Recent findings suggest that the insulin-degrading capacity of morbidly obese patients is linked to venous leptin levels. Insulin controls leptin synthesis but leptin can, in turn, influence insulin cleavage and thus insulinemia. If insulin cleavage is to be interpreted as a way to decrease hyperinsulinemia, leptin could be a signal that limits the extent of insulin physiological actions. The fact that leptin is produced in the same insulin-degrading tissue (i.e. visceral adipose fat pad) supports this association[84]. IR and clustering of components of the metabolic syndrome decrease the concentration of the sOb-R and increase leptin levels in obese or overweight middle-aged men, which results in a decreased fraction of bound leptin, which further emphasizes the close relationship between the insulin and leptin axes. It is likely that low levels of sOb-R and a high concentration of free leptin are independent components of the metabolic syndrome.

Adiponectin is a novel adipose-specific molecule that possesses possible anti-atherogenic and anti-inflammatory properties. The plasma levels of adiponectin are lower in obese subjects and in patients with type 2 diabetes, which contributes to the development of atherosclerotic complications[85]. It has been demonstrated that secretion of adiponectin from adipocytes is stimulated by insulin[86]. Furthermore, ADD1/SREBP-1c has been recognized as being responsible for the control of adiponectin at the transcriptional level[87].

In NAFLD patients, adiponectin and adiponectin receptor Ⅱ (AdipoRⅡ) staining is less evident in biopsies from those suffering from NASH than FL. Hepatic expression of adiponectin and AdipoRⅡ is reduced in NAFLD[88]. Patients with NASH have significantly lower levels of serum adiponectin than do controls. Although no significant correlation exists between serum adiponectin and anthropometric data, it is independently associated with age, HDL, and triglycerides. Type of meal has no effect on serum adiponectin either in patients with NASH or in controls. There is no expression of adiponectin mRNA in liver samples. However, AdipoRⅡ mRNA expression is higher in NASH than in FL and normal liver tissue[89]. Obesity, particularly visceral adiposity, might also contribute to IR by altering the levels of key adipocyte-derived circulating proteins, referred to as adipokines, including adiponectin and resistin. Adiponectin is produced by adipocytes, and its levels are generally lower in patients with IR and the metabolic syndrome[90]. A role in the regulation of insulin sensitivity and glucose homeostasis was demonstrated in studies that have shown that recombinant adiponectin lowers glucose in diabetic rodents and enhances insulin action in hepatocytes[91]. Exogenous adiponectin, or transgenic adiponectin overexpression, also can reduce lipid accumulation in muscle and liver and enhance insulin sensitivity in mice. Importantly, adiponectin-deficient mice display delayed FFA clearance and are sensitive to diet-induced IR[72]. The mechanisms that mediate the beneficial effects of this protein might involve the activation of AMP-activated protein kinase in muscle or liver; adiponectin signaling *via* this pathway might involve two recently identified, distinct receptors[91].

Administration of exogenous resistin to rodents increases plasma glucose and hepatic glucose production, whereas resistin-null mice have reduced fasting glucose levels[92]. Resistin is reckoned as an adipocyte-specific secretory factor that can cause IR and decrease adipocyte differentiation. Conversely, based on various studies, IGFs can improve IR and stimulate adipocyte adipogenesis. Whether IGFs exert their effects by controlling resistin

production or modulating resistin action is not known. These data demonstrate that IGF- I downregulates resistin gene expression *via* IGF-1R-dependent and MEK1-, p38 MAPK-, and phosphoinositide 3-kinase-independent pathways, and probably modifies the distribution of resistin protein between the intracellular and extracellular compartments *via* a p38 MAPK-dependent pathway. Decreases in resistin production and secretion induced by IGF- I might be related to the mechanism by which IGF- I modulates body weight and diabetes in animals[93].

Adipose tissue was once thought of as a reservoir for surplus energy, but more recently, it has been recognized as an active endocrine organ that contributes to metabolic homeostasis by secreting several adipokines such as leptin, adiponectin, TNF-α, IL-6, PAI-1 and resistin. Initially, resistin was reported as an adipose-tissue-specific protein[40] but ensuing studies *in vitro* and *in vivo* have shown conflicting data regarding the expression of resistin in relation to IR or obesity[94]. Moreover, a longitudinal analysis has shown that serum resistin is higher in obese than in lean subjects, and that changes in serum resistin are positively correlated with changes in body mass index (BMI), fat mass, plasma glucose and insulin levels after a weight reduction program entailing dieting and exercise[95]. In normal control rats, *in vivo* insulin infusion and *ex vivo* administration of TNF-α to cultured fat pads increases resistin gene expression significantly. These results imply that hyperinsulinemia and increased TNF-α levels might upregulate the adipose resistin gene in bile-duct-ligation-induced liver cirrhosis[96].

CARDIOVASCULAR RISK

Obesity is considered to be a major contributor to overall and cardiovascular morbidity and mortality[97]. Epidemiological studies have demonstrated that the incidence and prevalence of obesity are increasing. Metabolic syndrome, which comprises IR, visceral obesity, hypertension, dyslipidemia, and microalbuminuria, is considered a major risk factor for atherosclerosis in obesity[98,99]. Fat accumulation in the visceral depot and liver are strongly correlated, and both are highly correlated with the development and severity of IR[100].

Given that CRP level is a strong predictor of cardiovascular events in men[101], the mechanisms that underlie elevated CRP levels among unhealthy subjects are important. CRP is the main acute phase protein and is a marker of systemic inflammation. Adipose tissue secretes pro-inflammatory cytokines, such as IL-6 and TNF-α. The synthesis of CRP, mostly under the control of IL-6[102] and TNF-α, can stimulate the production of CRP[103]. About 30% of total circulating levels of IL-6 originate from adipose tissue in healthy Caucasian subjects[104]. Adipose tissue is an important factor in the increased CRP levels, *via* IL-6.

VISCERAL ADIPOSITY

Given that omental adipose tissue is a pure depot of visceral adipose tissue, it is of interest to investigate the regulation of lipid metabolism in human omental tissue *in vivo*. It has been proposed that IR of the liver derives from a relative increase in the delivery of FFA from the omental fat depot to the liver (*via* the portal vein). Increased delivery results from: (1) more stored lipid in the omental depot; (2) severe IR of the central fat depot; and (3) possible regulation of visceral lipolysis by the central nervous system. The significance of portal FFA delivery results from the importance of FFAs in the control of liver glucose production. Insulin regulates liver glucose output primarily *via* control of adipocyte lipolysis. Thus, because FFAs regulate the liver, it is expected that visceral adiposity will enhance delivery of FFAs to the liver and make the liver relatively insulin resistant. It is of interest how the intact organism compensates for IR secondary to visceral fat deposition. Although part of the compensation is enhanced B-cell sensitivity to glucose, an equally important component is reduced liver insulin clearance, which allows for a greater fraction of β-cell insulin secretion to bypass liver degradation, to enter the systemic circulation, and to result in hyperinsulinemic compensation. The signals that result in β-cell upregulation and reduced liver insulin clearance with visceral adiposity are unknown, but it appears that the glucagon-like peptide hormone plays an important role[105].

In patients who have undergone abdominal surgery, two specific adipokine concentrations have been measured in venous blood from the omentum to obtain information on some processes of synthesis in the presence of abdominal obesity. Although vascular endothelial growth factor (VEGF) and IL-6 concentrations are increased in the systemic circulation, the contribution of visceral adipose tissue to circulating levels of VEGF and IL-6 is modest[106]. In contrast, in a recent study on rat tissues, the omentum has been found to have the greatest VEGF concentrations of those examined and the highest VEGF secretion rate. Fractionation studies of the omentum furthermore have demonstrated that omental adipocytes, rather than the stromal-vascular cells, are the primary source of VEGF. An endothelial cell mitogenic assay has showed that a major portion of the mitogenic activity of heparin-binding proteins and conditioned media derived from omentum is abolished by VEGF antibody. Additional studies with the transcription inhibitor actinomycin D have demonstrated that the VEGF gene is continuously transcribed in the rat omental adipocytes. Incubation of the omental adipocytes under hypoxic conditions has induced approximately a 1.7-fold increase in VEGF protein expression, which is abolished by actinomycin D[107]. However, what is the importance of VEGF? Liver regeneration is dependent upon coordinated proliferation of hepatocytes and endothelial cells. VEGF promotes angiogenesis. Hepatic steatosis increases liver resection morbidity and delays regeneration. As a counter-reacting mechanism, serum VEGF concentration increases in more severe forms of NAFLD. Some researchers have hypothesized that VEGF overexpression stimulates hepatic regeneration[108].

Another debate has arisen over the so-called "portal hypothesis" that implicates increased lipolytic activity in visceral fat, and therefore, increased delivery of FFA to the liver, which ultimately leads to hepatic IR. The mechanism by which increased central adiposity causes hepatic IR has been clarified by research at the transcriptional level, by studying the expression of several genes that are involved in glucose and lipid metabolism in the fat-fed canine model. Northern blot analysis has revealed an increase in the ratio of visceral to subcutaneous mRNA expression of LPL and PPAR-γ. In addition, the ratio for SREBP-1 tends to be higher in fat-fed dogs, which suggests enhanced lipid accumulation in the visceral fat depot. The visceral to subcutaneous ratio of HSL increases significantly, which implies a higher rate of lipolysis in visceral adipose tissue despite hyperinsulinemia in obese dogs. Liver SREBP-1 expression is increased significantly, with a tendency for increased fatty-acid-binding protein expression. In addition, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase increases significantly, consistent with enhanced gluconeogenesis[109].

MITHOCONDRIAL INVOLVEMENT

ATP is crucial for maintaining cellular integrity, therefore, abnormal production might predispose to hepatocellular injury, and mitochondrial dysfunction could be the key mechanism. Estimates of energy metabolism are mainly based on basal metabolic rate (BMR). BMR involves measurement of subjects at rest, under thermo-neutral temperatures (i.e. no thermogenic stress), in a post-absorptive (not digesting food) and inactive state. The underlying machinery that fuels BMR is identical to that which fuels all the other sources of energy utilization, namely, oxidative phosphorylation. ATP is generated in mitochondria, and is subsequently hydrolyzed to ADP and phosphate to release energy for useful work. This process of electron transport during oxidative phosphorylation is the primary source of oxygen radical species. Total energy expenditure (TEE) is commonly predicted on the basis of patient weight, activity level, and degree of metabolic stress (metabolic demands). BMR accounts for about 70% of TEE; the remainder is provided by energy dissipated by metabolism of food (10% of TEE), and energy expended during physical activity (20% of TEE). Conditions that increase metabolic stress, such as infection, critical illness, or trauma, having inflammation in common, can increase BMR. BMR in obese patients is generally augmented, in contrast to common belief, and it is a strong body response to overfeeding, probably cytokine-mediated. BMR is generally measured by indirect calorimetry using a canopy system and single-frequency bio-impedance analysis. Increased energy expenditure, observed in morbidly obese patients with NAFLD as a consequence of a systemic, low-grade, inflammatory process, might explain progression from obesity to metabolic syndrome, independent of the presence of NAFLD. In this context, increased BMR might be indicative of metabolic syndrome, strictly linked to IL-6 levels[110]. Indeed, energy expenditure in obese patients is increased not only because the increased fat-free mass results in a rise in BMR, but also because of the higher energy cost of weight-bearing activities. NAFLD, which is characterized by mitochondrial dysfunction, can predispose to drug-induced hepatotoxicity that probably shares the same pathophysiological mechanism[111].

An up-to-date study has documented that hepatic mitochondrial dysfunction precedes the development of NAFLD and IR in Otsuka Long-Evans Tokushima fatty rats. This evidence suggests that progressive mitochondrial dysfunction contributes to the natural history of obesity-associated NAFLD[112].

INTRINSIC FACTORS LEADING TO HEPATIC STEATOSIS

Although we have previously focused on adipocyte biology and development of obesity, with an emphasis on IR, steatosis of the liver could be independently influenced by some aforementioned transcription factors. It is now clear that several members of the nuclear receptor superfamily are co-expressed by macrophages, lymphocytes and other cell types that are involved in the regulation of inflammatory and immune responses. Beyond PPAR-γ and SREBP-1c, nuclear liver X receptors[113] are members of this family that are known to be activated by lipid-derived endogenous (such as fatty acids, eicosanoids and cholesterol) and pharmacological ligands. Such transcription factors, as well as PPAR-γ co-activator 1α (PGC-1α)[114], farnesoid X receptor[115] and AMP-activated protein kinase[116], a key regulator of fatty acid oxidation in the liver, represent fundamental issues in the development of NAFLD and hepatic IR. In addition to peripheral IR and pancreatic β-cell dysfunction, it should be emphasized that type 2 diabetes mellitus is also characterized by aberrant hepatic gluconeogenesis. cAMP response element-binding protein (CREB), a key regulator of hepatic gluconeogenesis, mediates its actions through transcriptional induction of the nuclear hormone receptor PGC-1α. Recently, CREB-induced activation of the NR4A orphan nuclear receptor family, including the three highly homologous isotypes, NR4A1, NR4A2, and NR4A3, has been identified as a novel PGC-1α-independent mechanism for regulating hepatic gluconeogenesis.

CONCLUSION

It remains to be established whether IR is also a phenotypic expression and to what extend it has a genetic determinant. Although it is generally thought that organ fat deposition begins when visceral and subcutaneous abdominal adipose tissue stores are full, a recent study has not been able to confirm this. Given that IR is not related to fat deposition, it has been hypothesized that the chain of events does not presuppose that obesity is the cause of IR. This is supported by the clear association between inflammatory status (CRP level and spleen volume) and the hepatic score

at ultrasound[15]. Could the high fat liver content be the breaking point between benign and progressive obesity? This is the first intriguing question that could be answered by successive follow-up of the obese population. A possible confirmation of these findings is found in a study that suggests that the contribution of visceral fat to inflammation might not be completely accounted for by clinical measures of obesity (BMI and waist circumference)[117]. A second point to stress is whether weight control can slow down the progression of IR and the worsening of fat deposition in organs in obese patients and overweight subjects as soon as possible, such as in adolescence. Could a possible anti-inflammatory approach be used to cure metabolic syndrome and NAFLD? In fact, inflammatory mechanisms are fundamental to the progression of NAFLD towards higher-risk cirrhotic states. Finally, does hepatic IR follow peripheral IR[118]? In other words, are hepatocytes the last adipocytes? It is likely that they are[119].

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