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Insulin resistance and steatosis in humans

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Abstract

Insulin resistance is commonly found in a large number of adults—in particular, those with android obesity, the metabolic syndrome or type 2 diabetes. Strong adverse relationships between adipose tissue, liver and muscles in these patients result in lipotoxicity, with deposition of triglycerides (TG) within the liver and muscles together with insulin resistance. Such a situation is also seen in lipodystrophic patients with fat loss. Insulin signals in the liver through its tyrosine-kinase receptors to negatively control hepatic glucose production (HGP), replenish glycogen stores and synthesize fatty acids (FA), leading to TG exported as VLDL. In liver insulin resistance, HGP is increased mainly by activation of the gluconeogenic pathway, resulting in increased fasting glycemia. Lipogenesis is also increased possibly due to direct activation of the SREBP-1 transcription factor and together with increased FA availability results in an increased production of VLDL-TG. An imbalance between the pathways of TG synthesis and oxidation or export results in 'metabolic' steatosis. Increased cellular FA derivatives activate stress kinases, leading to phosphorylation of serine in insulin receptor substrate (IRS) proteins and, hence, insulin resistance. A number of studies in normal subjects and patients have revealed a strong association between insulin resistance and metabolic steatosis. Moreover, when insulin resistance is decreased by weight loss in obese subjects or by treatment with insulin sensitizers such as thiazolidinediones, the levels of liver fat and insulin resistance vary accordingly. An important question that remains unanswered concerns the relationship between steatosis and non-alcoholic steatohepatitis (NASH), and the potential roles of insulin resistance together with inflammation and oxidative stress in such a setting.

Résumé

Insulinorésistance et stéatose hépatique chez l'homme

La résistance à l'insuline est une situation fréquente en clinique, en particulier chez les sujets qui présentent une obésité androïde, un syndrome métabolique ou un diabète de type 2. Les interactions délétères entre tissu adipeux, foie et muscle chez ces patients induisent un état dit « lipotoxicité » avec dépôt intrahépatique et musculaire de triglycérides et résistance à l'insuline. Une situation semblable est observée chez les patients lipodystrophiques qui présentent un défaut de tissu adipeux. L'insuline agit en activant son récepteur membranaire à activité tyrosine-kinase qui réprime la production hépatique de glucose (PHG), remplit les stocks de glycogène et active la synthèse des acides gras et aboutit à la production de triglycérides exportés sur les VLDL. En cas de résistance à l'insuline, la PHG est élevée, du fait de l'activation de la voie de la gluconéogenèse aboutissant à une hyperglycémie à jeun. La lipogenèse reste également élevée, du fait sans doute d'une activation directe du facteur de transcription SREBP-1, et avec la disponibilité accrue en acides gras libres, induit une augmentation de la production de VLDL riches en TG. Un déséquilibre entre la voie de synthèse et les voies d'oxydation ou d'export des TG aboutit à une stéatose métabolique. Dans ce cas, les dérivés d'acides gras présents dans la cellule activent des kinases de stress qui vont phosphoryler les protéines substrats du récepteur, IRS, sur des résidus sérine, inhibant la transmission du signal insuline. Plusieurs études réalisées chez des sujets en bonne santé et des patients ont mis en évidence une association étroite entre le degré de résistance à l'insuline et de stéatose métabolique. De plus, lorsque l'insulinorésistance est améliorée par une perte de poids chez le patient obèse ou par des traitements insulinosensibilisateurs comme les thiazolidinediones, la quantité de lipides hépatiques et la résistance à l'insuline varient en parallèle. Une question importante, et qui reste non résolue, concerne la relation entre la stéatose simple et la stéatohépatite non alcoolique et le rôle potentiel qu'y jouent le degré de résistance à l'insuline ainsi que l'état inflammatoire et le stress oxydant.

Keywords: Insulin; Steatosis; Adipose tissue; Liver; Triglycerides; Lipogenesis; Hepatic glucose production; Free fatty acids; Lipotoxicity; Review.

Mots clés : Insuline ; Stéatose ; Tissu adipeux ; Foie ; Triglycérides ; Lipogenèse ; Production hépatique de glucose ; Acides gras libres ; Lipotoxicité ; Revue.

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1. Introduction

Liver steatosis indicates the presence of triglycerides (TG) as lipid droplets within hepatocytes. In the case of metabolic disorders, metabolic steatosis has a macrovesicular pattern. The presence of steatosis, while normal in the liver of migrating birds, such as ducks and geese, that require energy stores to overcome prolonged fasting periods when flying south in autumn, is not physiologically normal in humans. Steatosis is generally associated with increased adipose-tissue stores—in particular, in the abdominal visceral and subcutaneous depots, such as found in android obesity, the metabolic syndrome and type 2 diabetes—and is also characterized by insulin resistance. This means that the association between insulin resistance and steatosis is clear. Indeed, the condition is very common and probably, when mild, requires lifestyle changes, but not aggressive pharmacological interventions. The severity of steatosis is driven by its possible evolution towards steatohepatitis (NASH) and the long-term consequences, and the roles played by insulin resistance and inflammation need to be determined, as discussed in the report by K. Clément and colleagues (also in this issue).

Insulin resistance is a common feature present in a number of physiological and pathological conditions in humans. It plays a leading role in diseases related to adipose-tissue dysfunction such as abdominal obesity and the metabolic syndrome, which are characterized by increased amounts of abdominal fat that lead to insulin resistance, and have repercussions on metabolic parameters such as altered glycemia, dyslipidemia with decreased circulating HDL and increased LDL, and raised blood pressure. Insulin resistance is also central to diseases that associate adipose-tissue dysfunction and endocrine pancreas deficiency such as type 2 diabetes. It is now thought that non-alcoholic fatty liver disease (NAFLD) is a component of the metabolic syndrome and type 2 diabetes that may progress to NASH in the long-term, along with complications of fibrosis and cirrhosis. Severe insulin resistance is observed in patients with less common diseases such as lipodystrophy, with decreases in some fat depots such as subcutaneous adipose tissue and, sometimes, with increases in other depots such as visceral fat. This abnormal fat repartitioning results in severe metabolic alterations with dyslipidemia and insulin-resistant diabetes together with NAFLD and a frequent evolution towards NASH. The origin of human lipodystrophy may be genetic—in particular, disorders leading to complete lipodystrophy such as Berardinelli– Seip congenital lipodystrophy (BSCL), or partial forms such as familial partial lipodystrophy (FPLD), that are linked to mutations of the gene encoding lamin A/C or PPARγ. Lipodystrophy may also be acquired such as observed in HIV-infected patients receiving antiretroviral drugs or in patients treated with corticoids [1]. Insulin resistance is also present in diseases that primarily affect the liver such as chronic hepatitis C.

In the liver, insulin is involved in a number of actions responsible for glucose control and lipid metabolism. In case of insulin resistance, insulin levels are raised to overcome this resistance. The resulting effects depend on the metabolic pathway: a deficient insulin response for glucose metabolism leads to increased glucose production in the fasting state, while elevated insulin leads to activation of the lipid biosynthetic pathway, resulting in increased VLDL production and dyslipidemia. Given the central role played by the liver in lipid metabolism, any imbalance between the entry and export of lipid derivatives results in steatosis.

Steatosis and insulin resistance have a number of reciprocal relationships and can enhance each other. Increased oxidative stress and stress of the endoplasmic reticulum are probably some of the altered mechanisms in this setting. Insulin resistance at the adipose-tissue level plays an important role in hepatic insulin resistance: increased free fatty acid (FFA) production favors lipid deposition in the liver. The inflammatory signals released in adipose-tissue diseases also play a leading role, with increased proinflammatory and decreased adiponectin signalling in the liver.

Inflammatory signals within the liver have also to be considered: activation of the immune system and Kupffer cells results in the local release of proinflammatory cytokines.

In addition, a role for mitochondria has recently emerged, and the close link between mitochondrial dysfunction and insulin resistance has been clearly outlined. The most likely mechanism to explain such a connection is increased oxidative stress. Furthermore, increased stress of the endoplasmic reticulum due to lipid overload is involved in hepatic dysfunction, thus linking steatosis and insulin resistance.

A number of pathways have been explored in animal models, and a number of studies have outlined the potential mechanisms resulting in liver insulin resistance in murine models of obesity (see the report by Postic and Girard in this issue). However, while steatosis is easily produced in animal models, NASH is not, thereby limiting analysis of the pathophysiological mechanisms responsible for the transition between the two stages. Nevertheless, transversal clinical studies can reveal the presence of an association between several dysfunctions, although a causal link is more difficult to demonstrate. More important, longitudinal studies in patients who are losing weight or being treated with insulin sensitizers have revealed a correlation between various stages of steatosis and insulin resistance, thereby clarifying the mechanisms acting at that level. However, the factors involved in the evolution of steatosis to NASH have yet to be confirmed in patients.

2. Insulin signalling in the liver

Hepatocytes are one of three types of insulin target cells (along with myocytes and adipocytes) that carry a large number of insulin receptors on their cell surfaces. Insulin signalling can only take place through insulin receptors [2,3].

2.1. Insulin signalling pathways

One molecule of insulin is able to bind and activate one insulin receptor, a transmembranous protein comprising four subunits—two α and two β. The β subunits possess tyrosine-kinase activity in their intracytoplasmic domain, which is activated after linkage with insulin and phosphorylated on specific tyrosine residues of the receptor itself. This phosphotyrosine signal is recognized by protein substrates of the receptor that become activated and transmit the insulin signal in the cell. The main family of substrates is the 'insulin receptor substrate' (IRS) family, which has four members (IRS1-4). In addition, phosphotyrosines of the insulin receptor β subunit can also be recognized by substrates from the SHC and CAP/cbl families. Two main pathways diverge from the receptor. One leads to activation of phosphatidylinositol 3-kinase (PI3-kinase), then PKB/Akt or atypical PKC for insulin metabolic activities such as increased glycogen synthesis, lipogenesis and inhibition of gluconeogenesis. The other—the 'canonical MAP kinase pathway'—leads to activation of cell proliferation and differentiation.

The main IRS isoforms involved in hepatocytes are IRS1 and IRS2. Recent studies have clarified their respective roles in these cells. IRS1 is always present, and has a major role after feeding in controlling glycogen synthesis and lipogenesis where there is excess glucose in the circulation that needs to be stored in hepatocytes as glycogen or exported from hepatocytes as TG on VLDL. IRS2 is involved in control during the fasting state, when its level is markedly upregulated to allow insulin to limit hepatic glucose production (HGP) by controlling the expression of two gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase [4-6].

2.2. Insulin controls hepatic glucose and lipid metabolism

Insulin controls glucose metabolism in the liver. After meals, glucose and other sugars from nutriments, released as monosaccharides in the intestine, enter hepatocytes from the portal blood through glucose transporter GLUT2, always present in the hepatocyte plasma membrane; this allows the entry of glucose when circulating levels are increased and its export during fasting periods, when glucose levels are lowered. Within hepatocytes, glucose is directed towards glycolysis and ATP production, as required for energy by the cell and to replenish glycogen stores. When glycogen stores and energy requirements are fulfilled, glucose is diverted towards lipid synthesis, first by glycolysis, then by the initiation of the mitochondrial tricarboxylic cycle and, finally, by lipogenesis in the cytosol to produce fatty acids (FA). Insulin is a strong activator of these pathways and especially of the lipogenesis pathway through activation of the expression and proteolytic maturation of the transcription factor SREBP-1, acting in conjunction with the glucose-responsive transcription factor CHREB, thereby leading to the increased expression of the enzyme glucokinase in glucose metabolism, acetyl-CoA carboxylase and FA synthase in the lipogenesis pathway. FAs are esterified as TG associated with apolipoprotein B (apoB) and exported as VLDL lipoproteins. This means that lipid metabolism is strongly related to glucose metabolism in hepatocyte and biosynthetic pathways leading to glycogen and TG synthesis activated by insulin. Circulating TG-rich lipoproteins, VLDL from the liver, and chylomicrons produced by the intestines and bearing lipids directly from the diet are all deprived of their TG by the activity of adipocyte lipoprotein lipase, activated by insulin. FAs released from TG enter adipocytes and are stored in lipid droplets as TG.

Insulin is a pleiotropic hormone that also activates protein synthesis and cell proliferation, and inhibits cell apoptosis. In the postabsorptive and fasting states, insulin levels are low and liver energy metabolism is regulated by glucagon in priority. In adipose tissue, the lipolysis of TG, which is strongly inhibited by insulin, is activated by epinephrine and allows the release of FFA. These substrates are used by the liver and muscles to produce energy as ATP, after being oxidized, which occurs mainly inside the mitochondria through the β-oxidation pathway, the tricarboxylic cycle and the respiratory chain. In the postabsorptive state, the liver produces glucose to feed the brain and glucose-dependent tissues. Glycogen breakage is activated to replenish circulating glucose, then HGP uses the glyconeogenic pathway. The key enzyme, PEPCK, is regulated at the transcriptional level and activated by transcription factor FOXO1, present in the nucleus. Glucagon activates FOXO1 and the gluconeogenic pathway. Conversely, insulin signalling leads to FOXO1 phosphorylation by the kinase Akt/PKB, which induces its cytosolic retention and degradation by the proteasome, thereby impeding PEPCK activation and HGP. Insulin inhibits FA oxidation at the level of its entry into mitochondria by its action of increasing malonyl-CoA, an inhibitor of enzyme CPT1 (carnitine palmitoyltransferase type 1) in charge of FA mitochondrial import. Glucagon decreases malonyl-CoA levels and, thus, allows the entry of FFA from adipose tissue into mitochondria to be oxidized.

3. Insulin resistance in the liver

3.1. Consequences for glucose metabolism

Insulin resistance in hepatocytes results in less storage of glycogen in the postprandial state and more HGP in the fasting state. Indeed, glucose production by glycogen degradation is normally inhibited by insulin but, when glycogen stores are reduced, the increased expression of PEPCK allows an increase of HGP by the gluconeogenic pathway [7]. Interestingly, a recent study measured the expression of FOXO1 in NASH. The authors found evidence that, in humans, fatty liver and NASH are associated with a progressive increase in the expression of PEPCK and glucose-6-phosphatase. The expression of PEPCK correlates with insulin resistance as measured by the HOMA index. Interestingly, FOXO1 expression and activity are also increased in patients with NASH, and mRNA levels correlate with hepatic insulin resistance. In the presence of oxidative stress, FOXO1 is hypophosphorylated by the insulin-signalling pathway and is localized in the nucleus. Moreover, the expression of FOXO1 is correlated with the severity of steatosis and necroinflammation [8].

3.2. Lipid metabolism in hyperinsulinaemia

A number of studies have reported that, with insulin resistance as seen in visceral obesity, the metabolic syndrome and type 2 diabetes, lipogenesis—an insulin-responsive pathway—is enhanced and not decreased as expected [9]. Although difficult to understand, one explanation could be that the different insulin-signalling pathways in the liver are differentially sensitive to insulin: for example, the pathway leading to SREBP-1 activation remains sensitive while that suppressing HGP is non-responsive. Another element to consider is the activation of lipogenesis by CHREB and SREBP-1 transcription factors, which are responsive to high glucose levels, as seen in diabetes, as well as to insulin. In addition, recent studies suggest that SREPB-1 could be activated independently of insulin due to the presence of increased stress of the endoplasmic reticulum, as observed in steatotic liver in response to fat overload and, in particular, with saturated FAs, leading to increased lipogenesis [10,11]. Finally, TNF-α, a proinflammatory cytokine that is increased with hepatic inflammation, can also activate SREBP-1, resulting in increased lipid synthesis within the liver [12].

Increased deposition of TG occurs in several tissues outside of adipose tissue in insulin resistance and contributes to lipotoxicity [7].

Given the increased production of FFA by adipose tissue together with decreased adiponectin secretion, FA derivatives such as acyl-CoA and diacylglycerol accumulate in the cytosol in muscle, liver, pancreas and cardiac cells. Indeed, most FFA are driven towards mitochondria where they are oxidized to provide energy but, with high levels, mitochondrial dysfunction can occur in some subjects (depending on, for example, age, a family history of diabetes or a high-fat diet); if adiponectin, which controls mitochondrial entry and oxidation, is deficient, then FA derivatives can accumulate in the cytosol, although most will enter the mitochondria. Increased oxidation could result in increased generation of reactive oxygen species (ROS), leading to an oxidative stress response and activation of Jun kinase. Also, in the cytosol, they may activate atypical PKC, which can phosphorylate IRS1 on serine residues, leading to insulin resistance. This pathway was first shown by Petersen and Shulman in human muscles [13], but it is also present in human liver and other tissues. The fact that TG is stored in these tissues probably represents a protective mechanism that buffers toxic FA derivatives by storing less toxic TG within lipid droplets.

Thus, insulin resistance can result from a number of adverse situations, such as hyperinsulinemia, and lead to the production of molecules able to decrease insulin action such as proinflammatory cytokines like TNF-α or IL-6, or increased FFA.

3.3. Molecular mechanisms of insulin resistance

The molecular mechanisms underlying insulin resistance have been partially described. The number of insulin receptors present on the surface of hepatocytes is regulated by the kinetics of insulin receptor biosynthesis and degradation that are normally equivalent, leading to a consistent number of receptors on the cell surface. The degradation pathway involves receptor internalization into clathrin-coated vesicles, then endocytosis and either recycling to plasma membrane or degradation. This pathway allows insulin linked to the receptor to be degraded in late endosomes or lysosomes. When insulin levels are increased, as found in insulin resistance, the insulin– insulin receptor complexes are internalized at an increased rate, thus allowing insulin degradation. But even if most receptors are recycled back to the membrane, some will be degraded together with insulin, leading to decreased receptor numbers, the so-called ‘downregulation phenomenon’, resulting in fewer surface receptors as long as insulin levels are high and, therefore, in insulin resistance. Deactivation of insulin receptors and IRS proteins requires the action of tyrosine phosphatases, and several phosphatases that decrease the insulin signal have been proposed, including PTP1B and LAR. Otherwise, increased insulin levels characteristic of insulin resistance will further decrease insulin signalling by acting on IRS protein serine phosphorylation through the activation of stress kinases such as Jun kinase or IKKβ kinase in the NFκB pathway. A number of studies have shown that serine phosphorylation of critical residues

on IRS1—in particular, serine 307 or 312—blocks transmission of the insulin signal [3,14,15]. Thus, instead of being a natural enhancer of insulin action through tyrosine phosphorylation, IRS when phosphorylated on serine becomes an important step in impeding the action of insulin and leading, in turn, to further desensitization to insulin.

The proinflammatory cytokines are also able to induce insulin resistance [14,16,17]. TNF-α, produced either locally by activated Kupffer cells or at a distance in adipose tissue, is able, through binding to surface receptors, to activate stress kinases such as Jun kinase and IKKβ, leading again to serine phosphorylation of IRS proteins and the blocking of insulin signal transduction. IL-6 can induce insulin resistance by acting on the suppressor of cytokine signalling (SOCS) protein family and, in particular, by inducing SOCS3, a protein that inhibits insulin signalling through insulin receptors and IRS proteins driven towards degradation by the proteasome pathway.

The ability of FFA to induce insulin resistance has been explained by the ability of their intracellular derivatives, acyl-CoA and diacylglycerol, to activate isoforms of atypical PKC that are able to phosphorylate IRS proteins on serine residues, thereby leading to insulin resistance. In addition, recent studies have revealed that FFA can also activate toll-like receptor 4 (TLR4), present on Kupffer cells [18]. These receptors, activated also by bacterial lipopolysaccharides, are connected to the proinflammatory pathways in macrophages and, through activation of the NFκB pathway, lead to increased production of the proinflammatory cytokines TNF-α, IL-6 and IL-1β.

3.4. The role of adipokines

Adiponectin and leptin produced by adipose tissue may also play a role in the liver. Adiponectin is the most abundant adipokine, and its production by adipose tissue is strongly related to insulin sensitivity. It acts on signalling pathways within a number of cell types by binding to two surface receptors—AdipoR1 and AdipoR2—which are involved in the activation of PPARα in hepatocytes, leading to the expression of genes involved in FA oxidation and activation of AMP kinase, which also increases FA oxidation, in mitochondria. In addition, adiponectin inhibits the expression of the gluconeogenic enzymes PEPCK and glucose-6-phosphatase, thus decreasing HGP. In general, adiponectin favors insulin action, is protective against FA-derivative accumulation in the cytosol and prevents stress-kinase activation. In NAFLD patients, circulating adiponectin levels were reported to be negatively related to hepatic insulin resistance and the amount of fat [19]. Accordingly, in human studies of lipoatrophic and obese individuals, adiponectin concentration was inversely related to liver fat content and insulin resistance [20-22].

The role of leptin in the liver is complex. This adipokine is secreted by adipose tissue, and its levels are elevated in obesity. Leptin also activates AMP kinase, allowing increased FA oxidation. However, obese subjects are resistant to leptin.

Adiponectin and leptin are produced in stellate cells, the former when cells are not activated and exert antifibrogenic properties, the latter in myofibroblasts which participate in liver fibrosis [12].

4. Cellular stress

In addition to these molecules acting at the extracellular level and inducing a decreased response to insulin, other intracellular mechanisms are also probably involved. Mitochondrial dysfunction has been shown to induce insulin resistance. Mitochondria play a central role in glucose and FA oxidation to synthesize energy. During the process of respiratory chain function, some ROS are generated, which is useful for the cell. However, in situations of defective mitochondrial function in terms of required FA oxidation, the level of ROS is increased within mitochondria and leaks out of the mitochondria into the cytosol, where it activates stress kinases and leads to insulin resistance. Mitochondrial dysfunction is present in tissues during the aging process, which leads to age-related insulin resistance. It has also been observed in relatives of type 2 diabetic patients even before the occurrence of diabetes, suggesting a role for genetic factors in mitochondrial defects [13]. It may even be induced by a number of drugs acting on the liver. In addition, it may result from the increased FA pool leading to steatosis in humans. An important role has been established for the transcription factor PGC1α, activated by PPAR receptors and involved in mitochondrial biogenesis in the face of increased oxidative requirements [23].

The importance of oxidative stress and mitochondrial dysfunction in NASH has been outlined by Sanyal *et al.* [24], who studied liver samples from patients with steatosis compared with NASH. Both NASH and steatosis were associated with insulin resistance in muscle and adipocytes, as indicated by decreased glucose control during a euglycemic– hyperinsulinemic clamp and abnormal glycerol production by adipose tissue. However, it is worth noting that only NASH samples showed mitochondrial alterations with loss of mitochondrial cristae and inclusions. This was associated with markers of increased oxidative stress in the liver. Therefore, mitochondrial dysfunction was present in NASH samples. Increased FA influx to the liver was associated with increased β-oxidation, which suggests that it could be responsible for increased ROS production.

Another stress pathway that has been more recently identified in the liver is endoplasmic reticulum stress. Increased fat and FAs in the liver could activate this pathway, which is ini-

tiated at the level of the endoplasmic reticulum. The synthesis of proteins is normally controlled by chaperone proteins such as BIP. In cases of increased saturated lipid contents in the endoplasmic reticulum, its integrity and morphology are compromised. BIP dissociates from neosynthesized proteins and stops protein synthesis, then initiates a stress pathway that could activate stress kinases and, thus, lead to insulin resistance. ER stress can also activate SREBP-1, leading to increased lipogenesis and steatosis [11].

5. What is the origin of the agents acting on hepatocytes to control insulin sensitivity?

Both extrahepatically and intrahepatically produced agents are involved in hepatic insulin resistance, and adipose tissue is among the main contributors. In the presence of increased visceral as well as upper-body subcutaneous fat, it has been observed that adipose tissue presents a low-grade state of inflammation that results in an increased production of FFA and proinflammatory cytokines, and a decreased production of adiponectin. These alterations result in increased insulin resistance in the liver (see the report by K. Clément and colleagues in this issue).

Diet is also important. The level of saturated FAs from diet can increase insulin resistance and liver fat content. Studies in humans have revealed that about 15% of dietary FAs are incorporated in hepatic fat and secreted as VLDL-TG after a meal [25]. This suggests that the liver takes up a significant fraction of dietary lipids in the postprandial period. A study by Westerbacka *et al.* evaluated liver fat by proton spectroscopy and markers of insulin sensitivity in 10 non-diabetic obese women at baseline and after 2 weeks of isocaloric periods containing either 16% or 56% of total energy intake as fat. Interestingly, liver fat content decreased in the low-fat period together with insulin levels, and increased in the high-fat period in parallel with insulinemia. The other fat depots and metabolic parameters were not altered. This means that dietary fat influences liver fat content and possibly also hepatic insulin sensitivity [26].

The production of proinflammatory cytokines by activated Kuppfer cells are also involved.

6. Pathophysiology of steatosis in humans

The occurrence of fat as TG in liver cells results from an imbalance between TG synthesis and degradation. The synthesis pathway results from the *de novo* synthesis of FAs, then TG from glucose, and is activated by hyperinsulinemia, as seen in patients with the metabolic syndrome, type 2 diabetes or lipodystrophy syndromes. Increased TG also results from increased FFA in the liver due to increased lipolysis by insulin-resistant adipose tissue in cases of increased visceral and subcutaneous abdominal fat depots. The sources of FAs stored in the liver and secreted via lipoproteins in patients with NAFLD have been identified: 60% comes from FFA; 26% from *de novo* lipogenesis; and 15% from diet [25]. It has been shown that the contribution of splanchnic lipolysis to hepatic FFA delivery ranged from < 10% in lean subjects to almost 50% in obese subjects, and increased as a function of visceral fat amounts in both women and men. Leg and splanchnic tissues contributed to a greater release of systemic FFA in obese *vs* lean men and women. Nevertheless, visceral fat does not account for the majority of portal FFA, and subcutaneous fat from the upper body plays a leading role [27]. In addition, as indicated above, about 15% of dietary lipids are recovered in VLDL. Indeed, increased TG synthesis also results from the increase in TG-rich lipoproteins due to an increased production of chylomicrons with a fat-rich diet and an increased production of VLDL by the liver. Insulin resistance in adipose tissue leads to less exportation of lipoprotein lipase of the vessel surface and less lipoprotein hydrolysis, thereby increasing levels of remnant TG-rich lipoproteins going back to the liver. In addition to increased FA flux, another factor that controls the fat pool is the efficiency of FA oxidation in the mitochondria. In studies of patients with steatosis or NASH, FA oxidation was not impaired [24].

Finally, TG equilibrium requires the efficient export of TG on VLDL, which is controlled by levels of apoB, the protein required to recover the VLDL particle. An increased degradation of apoB has been reported in response to insulin relative to the level required to sort VLDL, therefore leading to increased TG storage within hepatocytes i.e. in steatosis [9]. This impairment of apoB is relative as, in cases of massive FA availability due to increases in the different biosynthetic pathways, a relative deficiency of apoB leads to steatosis together with an increased release of VLDL and increased circulating TG levels.

For these reasons, steatosis appears to be a consequence of insulin resistance both at the level of adipose tissue and in the liver.

7. Relationship between steatosis and insulin resistance in humans

The link between steatosis and insulin resistance has been reported in several studies in patients with the metabolic syndrome or diabetes as a reciprocal, positive relationship. Interestingly, such a link has also been observed in young, lean, healthy subjects of different ethnic origins: Asian-Indian men present an increased prevalence of insulin resistance

and NAFLD compared with other ethnic groups, and this is associated with an increased circulating level of IL-6 [28]. Also, insulin-resistant fatty liver overproduces a number of molecules that are deleterious at the vascular level and generate a state of low-grade inflammation that is prothrombotic, increasing cardiovascular risk and including high glucose levels with the production of advanced glycation end-products (AGE) involved in diabetic complications [12], VLDL-TG, plasminogen activator inhibitor-1 (PAI-1), coagulation factors, C-reactive protein (CRP) and fibrinogen [17,29].

Several studies have revealed an association between levels of liver fat and insulin resistance in patients with obesity, the metabolic syndrome or type 2 diabetes [29,30]. The presence of NAFLD predicts type 2 diabetes: in the NHANES-III survey, adults with NAFLD were twice as likely to have type 2 diabetes than those without NAFLD, after adjustment for age, gender, race and BMI [29].

Yki-Jarvinen evaluated whether the amount of liver fat was related to components of the metabolic syndrome and to insulin sensitivity in 271 non-diabetic subjects. Liver fat was fourfold higher in those with *vs* without the metabolic syndrome, and this association was independent of age, gender and BMI. Liver fat was associated with levels of transaminases ASAT and ALAT as surrogate markers of NAFLD. The amount of liver fat has been related to the amount of subcutaneous fat in both men and women [31], although the relationship was more striking with visceral fat. In fact, the best correlate of liver fat was fasting plasma insulin and C-peptide in both men and women, indicating that it is not the visible fat, but rather the fat hidden in the liver that is the most accurate indicator of insulin resistance [29].

The possible involvement of dietary fat in liver fat content and insulin resistance has already been mentioned. Bugianesi *et al.* [19] investigated insulin sensitivity in non-obese, non-diabetic patients with NAFLD, but without dyslipidemia, increased visceral fat or hypertension, who were matched with controls by age, BMI and body composition. Insulin resistance was present in patients with NAFLD in a pattern consistent with accelerated lipolysis, the result of resistance in adipose tissue leading to an increased FFA supply and oxidative use of lipid at the whole-body level. In the liver, which normally extracts FFA with high efficiency, higher rates of lipid oxidation and impaired suppression of hepatic lipid oxidation by insulin indicated the presence of insulin resistance. This study suggested that, in such patients, most of the hepatic insulin resistance results from the increased fatty substrate delivery by adipose tissue.

As previously described, insulin resistance was present in patients with NAFLD in the study of Sanyal *et al.* [24], who compared them with patients without steatosis, but with increased oxidative stress and FA oxidation. Indeed, only patients with NASH presented altered mitochondrial function.

8. Steatosis and insulin resistance after weight loss or treatment with insulin sensitizers

Weight loss is effective in reducing liver fat. Eight obese patients with type 2 diabetes were studied before and after weight stabilization using a moderately hypocaloric very low-fat diet. Weight losses of about 8 kg resulted in normalization of fasting plasma glucose and HGP together with an 81% reduction in intrahepatic lipid. However, there were no significant changes in either insulin-stimulated peripheral glucose uptake or intramyocellular lipid levels. Therefore, moderate weight loss was able to reverse liver fat and liver insulin resistance independently of any change in peripheral glucose metabolism [32]. Similar data have been reported in other studies [22,30], indicating that steatosis and insulin resistance evolve in parallel.

Moreover, longitudinal studies of patients with NASH have evaluated the ability of PPARγ agonists, such as pioglitazone or rosiglitazone, and metformin to improve steatosis and insulin resistance. Metformin is used in the treatment of type 2 diabetes, and its positive action on liver fat and insulin sensitivity has been revealed in several studies, but was not confirmed in a double-blind, randomized study of type 2 diabetics, where metformin treatment improved basal hepatic insulin sensitivity, but did not change the amount of liver fat [22].

Thiazolidinediones (TZD) lower levels of FFA by reducing adipose-tissue lipolysis while dramatically increasing circulating levels of adiponectin, which is correlated with changes in liver fat independent of BMI and increased insulin sensitivity [20,29,30].

A recent randomized, double-blind study compared the effects of rosiglitazone and placebo on steatosis and insulin sensitivity. In this so-called FLIRT trial, 63 NASH patients received either rosiglitazone 8 mg/d or placebo for 1 year. Rosiglitazone improved steatosis and transaminase levels despite weight gains, an effect related to improvement in insulin sensitivity. However, other parameters of liver injury were not modified [33].

In rare situations of lipodystrophy with massive steatosis and severe insulin resistance, treatment with recombinant human leptin in such patients devoid of leptin resulted in a spectacular decrease in liver fat quantities and steatosis together with major improvements in insulin resistance [34].

These studies reveal that, in most cases, the quantity of liver fat is closely related to the degree of insulin resistance. Also, that they vary in parallel suggests a strong link between these two parameters.

9. Conclusion

A number of arguments suggest that insulin resistance leads to steatosis and that steatosis, or rather the presence of

FA derivatives used for TG synthesis, enhances insulin resistance. In humans, steatosis and insulin resistance are clearly associated, and lead to an increased prevalence of diabetes and cardiovascular risk. A number of mechanisms might explain this association, some of which are extrahepatic, such as adipose tissue or diet, while some are intrahepatic.

An important question is to ask why, in some patients, does steatosis evolve towards NASH while insulin resistance is present in both entities. The degree of insulin resistance is greater in patients with NASH than with steatosis [35,36], and its involvement in the transition has been reported in an animal model [37]. It is evident that the presence of abnormal mitochondria and increased oxidative stress in NASH compared with steatosis [24] favors an important role for mitochondrial dysfunction. However, adipokines and cytokines may also play major roles. Adiponectin is thought to be more decreased in NASH than in steatosis, and an adiponectin gene polymorphism can predict the severity of disease in NASH [35,36,38]. In addition, TNF-α is overexpressed at the mRNA level both in adipose tissue and the liver, suggesting an important role for TNF-α in the pathogenesis of NASH [39]. The roles of inflammation (see the report by K. Clément and colleagues in this issue) and immunity also need to be considered in such a setting [40].

Conflicts of interest: The author has none to declare.

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