Métabolisme postprandial des acides gras dans le développement de la lipotoxicité et du diabète de type 2.

La résistance à l’insuline et la diminution de la sécrétion d’insuline stimulée par le glucose sont les deux principales anomalies physiopathologiques qui sous-tendent le diabète de type 2. Au cours des deux dernières décennies, un grand nombre de travaux de recherche ont souligné l’importance de l’exposition excessive des tissus aux acides gras dans le développement de la résistance à l’insuline de même que la diminution de sécrétion de l’insuline. Puisque l’importation nette d’acides gras se produit en période postprandiale, la surexposition tissulaire aux acides gras survient lors de cette période physiologique. Bien des avancées ont été effectuées dans notre compréhension des mécanismes cellulaires toxiques qui surviennent lors de la surexposition des tissus aux acides gras. Cependant, les mécanismes mis en jeu au niveau du corps entier chez l’humain pendant l’évolution vers le diabète de type 2 ne sont pas totalement élucidés. Des avancées récentes qui permettent de mieux comprendre...
les mécanismes qui régulent le transport des acides gras aux tissus ainsi que leur métabolisme sont revues dans cet article. L’implication de leurs anomalies dans l’installation de la lipotoxicité tissulaire et le développement du diabète de type 2 est discutée.

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Mots clés : Diabète type 2 ; Résistance à l’insuline ; Sécrétion d’insuline ; Métabolisme des lipides ; Acides gras libres ; Triglycérides ; Lipotoxicité ; Métabolisme postprandial ; Revue

1. Introduction

The evolution of type 2 diabetes (T2D) is characterised by a slow progression from normal fasting glucose and glucose tolerance to impaired fasting glucose and/or impaired glucose tolerance and, finally, to postprandial hyperglycaemia, with or without fasting hyperglycaemia [1]. Although the cause of T2D remains unclear, research efforts especially in the field of integrative physiology in humans have clearly identified its two main pathophysiological features: (1) IR in several tissues; and (2) impaired GSIS in the pancreatic β-cell. IR is classically defined as the relative incapacity of insulin to stimulate glucose removal from the circulation, a phenomenon mainly attributable to impaired insulin-stimulated glucose use in skeletal muscles [2]. Impaired GSIS is a sine qua non for the presence of T2D because a normal β-cell response to glucose is able to compensate for IR and maintain normal blood glucose levels. This is best illustrated by a reciprocal relationship between GSIS and IR, quantified by most investigators using a hyperbolic function described mathematically as the product of GSIS and IR (the so-called disposition index, or DI) [3]. A lower DI represents failure of GSIS to increase as expected as IR develops, and is characteristic of patients with impaired glucose tolerance and established T2D and non-diabetic subjects who will progress towards the development of impaired glucose tolerance in the future. There is ample evidence from preclinical and experimental studies in humans that both insulin sensitivity and GSIS are markedly affected by excess exposure of tissues to fatty acids, a process generally referred to as ‘lipotoxicity’.

2. The concept and mechanisms of lipotoxicity

The term ‘lipotoxicity’ refers to the processes leading to end-organ damage and/or dysfunction following excess exposure to fatty acids, and was first coined in the context of fat-induced IR and impaired GSIS leading to T2D [4]. Since then, however, the process has also been implicated in endothelial dysfunction and atherosclerosis, heart failure, kidney failure, steatohepatitis and liver failure, autoimmune inflammatory disorders, susceptibility to infections, apoptosis in various cell types, cancer and ageing. The overwhelming evidence for fat-induced IR and impaired GSIS from experimental work in preclinical models has accumulated over the past two decades [5]. Several different and potentially overlapping cellular mechanisms have been implicated in fatty acid-induced IR and/or impaired GSIS (Fig. 1). Only a brief overview of these mechanisms is included here.

It has been shown that IR induced by experimental elevation of NEFA in human muscle is due to impaired glucose transport at the level of the plasma membrane [6]. In turn, this is closely associated with ectopic fat deposition such as increased IMTG and fatty infiltration of the liver. Accumulation of reactive lipids such as DAG occurs in different tissues during NEFA-induced IR and may lead to activation of nPKC that, in turn, reduces insulin-mediated glut4 cell membrane translocation through serine/threonine phosphorylation of intracellular insulin signalling pathways. Accumulation of ceramides in muscle has also been seen in some human studies during experimental elevation of plasma NEFA. The synthesis of ceramides is largely dependent on long-chain saturated-fatty acid availability in the cell and can alter insulin signalling pathways in muscle [7]. Ceramides have been implicated in palmitate-induced β-cell mitochondrial defects and apoptosis [8,9], and in palmitate-induced reduction of insulin-gene expression [10]. Interestingly, both DAG (via activation of nPKC) and ceramides (via activation of JNK) can lead to impaired insulin-IRS-2-mediated β-cell-survival signalling through serine/threonine phosphorylation [11]. Thus, fatty acid-induced IR and impaired β-cell function may stem from similar abnormalities of insulin signalling pathways.

Proinflammatory cytokines such as TNFα are also well-known to impair insulin action through serine/threonine phosphorylation of the insulin signalling pathways [12]. Major mediators of the cytokine-induced proinflammatory cellular response are activation of NFκB and AP1 through phosphorylation of IκB and JNK, respectively. Fatty acid-induced muscle IR is prevented by salicylates, inhibitors of IκB and JNK [13,14]. There is evidence that saturated fatty acids may activate TLRs, offering a potential mechanism between excess exposure to fatty acids, low-grade inflammation and IR [15]. TLRs are a group of CD14-associated cell surface receptors that are activated by components of microorganisms such as LPS, bacterial lipopolysaccharides, peptidoglycans and lipoteichoic acid. Upon activation, TLRs are known to phosphorylate JNK and IκB, and activate the AP1 and NFκB pathways that, in turn, activate the expression of various proinflammatory cytokines (such as IL-6 and TNFα), adhesion molecules, chemokines, acute-phase proteins and anti-microbial proteins. Recent experimental evidence suggests that TLR-2 and TLR-4 may be implicated in palmitate-induced insulin resistance in both muscles and blood vessels through JNK, PKC and IκB activation [16,17].

FABPs may provide another important cellular mechanism that links tissue exposure to fatty acids and the development of low-grade inflammation and IR [18]. This family of fatty acid
cellular chaperones is highly expressed in tissues that metabolise fatty acids such as liver and adipose tissue, but also in macrophages. Combined knockout of aP2 (FABP4) and MAL1 (FABP5) reduces fatty acid transport in macrophages and adipocytes and protects against lipid-induced hepatic steatosis, IR and inflammation in different tissues [19,20]. Recently, pharmacological inhibition of aP2 was shown to reduce IR and glucose intolerance as well as inflammation in adipose tissue and liver in genetic and diet-induced models of T2D [21]. Adipose tissue aP2 expression was reduced with weight loss in obese individuals [22], and a gene polymorphism at the aP2 locus was recently associated with reduced adipose tissue aP2 expression, lower plasma TG, and a lower risk of diabetes and coronary artery disease [23]. In contrast, adipose tissue mRNA expression of aP2 was reduced in non-obese, non-diabetic insulin-resistant relatives of subjects with T2D [24]. The impact of modulation of aP2 expression on whole-body and organ-specific fatty acid metabolism has yet to be studied in humans.

Abnormal mitochondrial function has been implicated in IR and impaired GSIS [25]. Increased exposure to long-chain fatty acids can lead to mitochondrial dysfunction and impaired oxidative capacity through a reduced proton gradient, matrix swelling and enhanced production of ROS. In addition, long-chain fatty acid anions trapped in the matrix can produce mitochondrial inner membrane leaks with reduced production of ATP through a detergent effect, opening of transition pores and/or alteration of cardiolipins. Cellular oxidative stress is also associated with fatty acid-induced reduction of PGC-1, a major regulator of mitochondrial biogenesis and oxidative capacity [26]. ROS produced during high mitochondrial fatty acid oxidation and/or endoplasmic reticulum (ER) stress can activate cellular inflammatory pathways (JNK–AP1, and IkKβ–NFkB) leading to cellular inflammation and apoptosis [27]. Production of ROS and oxidative stress was recently shown to trigger IR induced by glucocorticoids or TNFα [28]. Thus, ROS production may be a fundamental link between the various diabetogenic conditions, including excess long-chain fatty acid tissue exposure and the development of IR.

ER stress is a recently recognised mechanism leading to cell death that is brought about by conditions associated with accumulation of misfolded proteins in the ER, including excess protein synthesis, hypoxia, oxidative stress, Ca++ loading, viral infection and excess energy-substrate utilisation [29,30]. When this occurs, a complex adaptive response, the UPR, is initiated, leading to a reduction of protein synthesis, an increase in proteasomal protein degradation and activation of chaperones that promote protein folding to alleviate ER stress. However, UPR has been associated with increased ER-associated NADPH activity and production of ROS, JNK activation and activation of cellular inflammatory pathways. High-fat-fed and ob/ob mice display ER stress in adipose tissues and liver that has been implicated in the development of IR in those tissues [31,32]. ER stress can be induced by palmitate exposure through increased production of ROS and also by increased palmitate incorporation into ER that may alter its functional integrity [33–35].

Finally, GPR40/FFAR1 and GRP120, cell membrane medium- to long-chain fatty acid receptors expressed in β-cells and gastrointestinal tract, respectively, were shown to mediate, at least in part, acute NEFA-induced stimulation of GSIS [36]. GRP40 knockout was shown to confer protection, while GRP40 overexpression can potentiate impaired β-cell function and hepatic steatosis induced by a high-fat intake in mice [37]. However, GRP40 was not implicated in palmitate-induced apoptosis, but was shown to mediate oleate-induced protection of mouse β-cells in a recent study [38]. The role of this family of fatty acid receptors in the development of lipotoxicity is unclear at present.

In addition to these mechanisms, it is possible that excess fatty acid exposure may alter lipid membrane composition,
which might result in altered cellular signalling, exocytosis and/or fatty acid transport via modification of the dynamics of plasma membrane microdomains (lipid rafts and caveoleae) [39,40]. Furthermore, gene expression may be modulated at the transcriptional level by fatty acids (especially polyunsaturated fatty acids) through interaction with PPARs, LXRs, HNF4 and/or SREBP [41]. Significant cross-talk between several of these mechanisms is expected, and may influence the final outcome of excess exposure to fatty acids in a given tissue. For example, activation of PPARs by polyunsaturated fatty acids during positive energy balance may mitgiate lipotoxicity by inducing mitochondrial biogenesis, increasing fatty acid oxidation, reducing tissue accumulation of DAG and ceramides, down-regulating AP1 and NFκB activation to reduce low-grade inflammation, increasing adipose tissue expansion and fatty acid storage (see discussion below), and promote adaptation of β-cells to the development of IR [12,41–43]. This already highly complex scenario is further complicated by the fact that these mechanisms can differ from one tissue or cell type to another due to their intrinsic properties (such as receptor expression, substrate utilisation and available fatty acid metabolic pathways) and/or their likelihood of interaction with other tissues (according to proximity to adipose tissues and the possibility of close contact with macrophages and other proinflammatory cells of the immune system) and access to fatty acid pools (expression of fatty acid transporters and/or cytosolic chaperones, expression of intracellular and/or extracellular lipolytic enzymes to hydrolyse esterified fatty acids). Thus, lipotoxicity should be viewed as a highly complex interplay between numerous cellular mechanisms — some potentially toxic and others adaptive — that is further modulated by multiorgan interactions in response to mismatched fatty acid intake and production versus utilisation. The precise hierarchy of events leading to fatty acid-induced IR and impaired GSIS in vivo is currently poorly understood.

3. Evidence for lipotoxicity leading to IR and impaired GSIS in humans

Several research groups have provided extensive experimental evidence for the development of peripheral tissue IR (including muscle and liver) using Intralipid and heparin intravenous infusion to raise plasma NEFA levels in humans [5,44]. In this experimental paradigm, Intralipid (a lipid emulsion rich in linoleate) provides TG in excess to the circulation, and heparin activates lipoprotein lipase to increase intravascular lipolysis of circulating TG, thus increasing tissue exposure to fatty acids by releasing NEFA into the systemic circulation. This experimental protocol leads to the development of IR within six hours in humans, a phenomenon associated with reduced insulin-mediated glucose transport to muscles. Increased in vivo exposure of muscles to plasma NEFA leads to IMPG accumulation prior to or concomitantly with the development of reduced insulin-stimulated glucose disposal. In particular, it was shown that similar elevation of IMTG and reduction of insulin-stimulated glucose disposal also occurs after two days following a very high-fat, high-calorie diet [45]. Elevation of plasma NEFA levels leads to reduced mitochondrial gene expression, including PGC-1 and UCP-3, and to a reduced insulin-mediated increase in ATP flux in muscles [46–48], suggesting that increased fatty acid exposure causes mitochondrial dysfunction in vivo in humans. Induction of tissue oxidative stress by long-chain fatty acids may also lead to tissue IR [13,14]. Furthermore, systemic lipid exposure by intravenous infusion of lipid emulsion can lead to activation of inflammatory pathways in circulating leucocytes [49], and to reduced activation and proliferation of circulating T cells [40]. Thus, elevation of plasma NEFA levels may lead to inflammation and immune abnormalities associated with IR and T2D.

Experimental elevation of plasma NEFA for 8 to 48 h was shown to impair GSIS in several [50–59], but not all, human studies [60–65]. Prolonged reduction of plasma NEFA levels with acipimox (an inhibitor of intracellular lipolysis) results in improvement of insulin sensitivity and GSIS in individuals at high risk of developing type 2 diabetes [51,66]. While it may impair GSIS, prolonged overexposure to plasma NEFA using Intralipid and heparin did not impair arginine-stimulated insulin secretion in vivo [67].

4. Factors associated with enhanced susceptibility to lipotoxicity in vivo in humans

All individuals may not display the same susceptibility to NEFA-induced diabetogenic effects. For example, we have shown that obese, insulin-resistant individuals display an absolute NEFA-mediated reduction in GSIS whereas individuals with established T2D do not [53], and that healthy insulin-sensitive individuals display only a reduced DI without an absolute reduction in GSIS [52]. We found that non-diabetic Oji-Cree Native Canadians, who have a very high risk of developing T2D, were not more susceptible to NEFA-mediated IR and impaired DI [54]. Susceptibility to a lipid-induced reduction in DI was also found to be similar in women with previous gestational diabetes versus controls [59]. Using an intravenous infusion of lipid emulsion without heparin, Kashyap et al. [68] found a NEFA-mediated reduction in GSIS only in non-diabetic subjects with a strong family history of T2D, and not in healthy subjects without such a family history. Similar findings were observed by Storgaard et al. [56]. A NEFA-mediated reduction in insulin-stimulated glucose disposal and reduced muscle insulin signalling was found only in insulin-sensitive, but not in insulin-resistant, individuals [68,69]. An association was reported between plasma NEFA levels during infusion of lipid emulsion and impaired insulin-stimulated glucose disposal and reduced muscle insulin signalling, although such changes already have an impact on low increases in plasma NEFA within the physiological range [70,71]. Both plasma NEFA and TG levels tended to be higher during infusion of lipid emulsion in subjects with a strong family history of T2D in the study by Kashyap et al. [68], although the relationship between lipid levels and GSIS change was not reported by these authors. Experimental work also supports a role for hyperglycaemia in the potentiation of the toxic effects of fatty acids [72]. However, the role of hyperglycaemia with regards to lipotoxicity in vivo in
studies in animal models have nevertheless shown the potential deleterious for glucose homoeostasis, it should be noted that dies suggest that saturated fat intake could be potentially more unsaturated versus saturated fat diet occurred only in subjects the latter study, the metabolic benefits associated with mono-
totistic effects in vitro than other long-chain fatty acids, perhaps because of an enhanced production of ceramides (see discussion among diets with Intralipid and heparin infusion could be attributable to saturated, polyunsaturated or even monounsaturated fats. Howe-
ver, NEFA are heterogeneous in terms of their biological and potential cellular toxic effects. Risk of IGT and T2D is higher in individuals who have a lower proportion of polyunsaturated- to-saturated fatty acid serum content [74], and lower dietary polyunsaturated-to-saturated fatty acid ratios [75]. Total and saturated dietary fat intake was recently found to predict the incidence of T2D, independent of weight gain and other lifestyle factors [76]. Palmitate has been associated with more proapoptotic effects in vitro than other long-chain fatty acids, perhaps because of an enhanced production of ceramides (see discussion above). Saturated fatty acids may also display more proinflammatory effects because of their capacity to activate TLRs (as discussed above), whereas linoleate and oleate may reduce activation of NFκB by lipopolysaccharides [77,78]. Frequent oral saturated, monounsaturated or polyunsaturated fat intake over 30h all lead to reduced insulin clearance [79]. In the latter study, the development of IR with reduced DI was observed only during feeding with meals containing saturated fat. Lower GSIS was observed with polyunsaturated fat feeding, but this was in the face of lower IR (DI was unaffected), suggesting a compensatory reduction in insulin secretion. In a 5-week, crosso-
ver, randomised diet intervention, Summers et al. found significant reductions in abdominal subcutaneous fat and in IR on substituting polyunsaturated fat for saturated fat in the diet, although total energy intake also tended to be reduced with the polyunsaturated versus saturated fat diet [80]. In another ran-
domised, controlled dietary intervention, Vessby et al. found an approximately 10% reduction in insulin sensitivity and 9% increase in GSIS with a 3-month diet rich in saturated fat, whereas insulin sensitivity and GSIS did not change significantly after a diet rich in monounsaturated fat [81]. Interestingly, in the latter study, the metabolic benefits associated with mono-
unsaturated versus saturated fat diet occurred only in subjects with a total fat intake below the median. While these human stud-
ies suggest that saturated fat intake could be potentially more deleterious for glucose homoeostasis, it should be noted that studies in animal models have nevertheless shown the potential of excess exposure to oleate and linoleate to induce β-cell lipo-
toxicity [82]. Thus, it is possible that all prevalent long-chain fatty acids have diabetogenic potential when sufficient overex-
posure occurs. Fatty acid composition together with the duration and magnitude of tissue exposure are also likely to be important factors in the development of lipotoxicity. However, the in vivo dose- and time-dependent lipotoxic responses of various fatty acids have thus far not been studied in humans.

Another possible mechanism for modulation of susceptibility to accumulation of IMTG and reactive lipids is the capacity of muscles to oxidise fatty acids. In vivo, muscles from insulin-resistant subjects display a reduced capacity to oxidise plasma NEFA during fasting, β-adrenergic stimulation and exercise [6,25, 26]. Muscle mitochondria in T2D were smaller and displayed reduced FA oxidation at the CPT-1 and post-CPT-1 levels. Reduced mitochondrial biogenesis has been invoked as a possible cause for impaired muscle plasma NEFA oxidation based on the demonstration that muscles from prediabetic and diabetic individuals have reduced mRNA levels of PGC1-α and -β, important coactivators implicated in adaptive fatty acid oxidation. It is possible, however, that impaired muscle fatty acid oxidative capacity could be a secondary phenomenon, not a primary pathogenic feature of IR. Muscle PGC1-α gene expression can be increased, and expression of acetyl-CoA carboxylase and β-hydroxyacyl-CoA reduced, by physical training [83–85] whereas PGC1-α expression can be reduced by fatty acid exposure [86]. Furthermore, IR is associated with leptin resistance and reduced adiponectin levels, two factors that may reduce fatty acid oxidation [87–89]. Thus, impaired muscle fatty acid oxidation in IR could also be due to leptin resistance and/or low adiponectin levels. Whether impaired muscle oxidative capacity is a consequence of reduced fitness, increased fatty acid delivery to muscles and/or impaired adipose tissue function during the development of IR, or whether it is a primary defect, remains to be determined in humans.

5. Mechanisms of fatty acid delivery to non-adipose tissues

Non-adipose tissue delivery of fatty acids occurs via two distinct sources: (1) the plasma NEFA pool; and (2) circulating TG (and presumably: to a much smaller degree, other circulating fatty acid esters). Adipose tissue plays an important role in the regulation of other tissue exposure to fatty acids by storing dietary fatty acids transported by chylomicron-TG in the post-
prandial state and by releasing NEFA into the circulation through intracellular lipolysis of stored TG [5]. Insulin is the major regul-
ator of the balance between net adipose fatty acid storage in the postprandial state and net release during the postabsorptive state. In the fasting state, as plasma insulin levels are low, intracellular lipolysis in adipose tissue is high, and plasma NEFA levels and appearance are at maximum. In the fasting state, however, there is no net entry of fatty acids via chylomicron-TG and, by defini-
tion, the energy balance is negative. The combination of high plasma NEFA with low insulin-stimulated glucose transport in muscles thus leads to maximum fatty acid oxidative metabolism during fasting. Increased insulin levels strongly suppress intra-
cellular lipolysis in adipose tissue, limiting the appearance of NEFA derived from adipose tissue after meals. Simultaneously, insulin stimulates adipose tissue LPL activity and intracellular fatty acid esterification, resulting in net and preferential storage of dietary fatty acids from chylomicron-TG in adipose tissue [90]. The latter study also provided evidence for storage of fatty acids from plasma NEFA in adipose tissue during the postprandial state, indicating stimulation of post-LPL fatty acid storage capacity. We have recently shown that, in healthy subjects, the most important post-LPL site of insulin action to limit plasma NEFA appearance during enhanced intravascular lipolysis is inhibition of intracellular lipolysis, not stimulation of fatty acid esterification [73].

Although intracellular lipolysis in adipose tissue is the major source of plasma NEFA, especially during the fasting state, evidence has accumulated to suggest that intravascular TG lipolysis also contributes significantly to plasma NEFA in the fasting and postprandial states [5,90–92]. The source of this spillover of NEFA from intravascular TG lipolysis is most likely the adipose tissues because the net NEFA balance across adipose tissue circulation is positive, whereas the net NEFA balance across the forearm (muscle) circulation in humans [90,91,93] and across the liver circulation in dogs [94] is negative. Furthermore, a recent study using arteriovenous gradient measurements of dietary and plasma fatty acid tracers has suggested that lipolysis of chylomicron-TG, not VLDL-TG, is the dominant source of NEFA spillover [90]. Using heparin + Intralipid for maximum stimulation of this LPL-mediated spillover of plasma NEFA shows that insulin reduces this spillover independent of its effect on LPL activity [73]. In the latter study, we have shown that most of the insulin-mediated reduction of NEFA spillover at the post-LPL level could be accounted for by insulin-mediated suppression of intracellular lipolysis in healthy humans.

To add to the complexity of determining postprandial exposure of tissues to fatty acids, plasma NEFA clearance may also be stimulated by insulin at the whole-body level [73,95,96]. Insulin acutely increases NEFA transport within cardiac and skeletal muscle cells through stimulation of expression and translocation of the fatty acid transporter CD36 to the plasma membrane, independent of intracellular metabolism of fatty acids [97]. We recently demonstrated that insulin increases NEFA clearance at the whole-body level indirectly by reducing plasma NEFA, but no direct effect of insulin was detectable independent of changes in NEFA appearance [98]. In the latter study, clearance of plasma NEFA was inversely proportional to the rate of its appearance, and plasma NEFA levels rose as an exponential growth function of those rates. This observation suggests that plasma NEFA level does not accurately reflect NEFA flux: total tissue exposure to NEFA over time may be overestimated when levels are higher such as during fasting, and underestimated when levels are lower, such as postprandial, if assessed by relative plasma NEFA concentration alone.

In addition to the plasma NEFA pool, circulating TG may also contribute directly to non-adipose tissue exposure to fatty acids. Direct extraction of TG has been recently demonstrated in the microcirculation of non-adipose tissues such as skeletal muscle, heart and liver [90,94,99]. However, little information is available on the relative contribution of plasma NEFA versus plasma TG to the total fatty acid exposure of different organs in humans. In a recent study conducted in the fasting state, when plasma TG is mainly transported into VLDL, plasma NEFA contributed to more than 80% of fatty acids used by the heart in vivo in humans during the fasting state whereas circulating TG contributed less than 20% [99]. Fatty acid delivery to tissues from circulating TG should be viewed as a dynamic process, as suggested by changes in tissue TG extraction rate over time in the postprandial state [90]. The degree of tissue fatty acid delivery directly from circulating TG is likely to depend on plasma TG concentration, whether it is carried in chylomicrons or VLDL, and on the degree of LPL activity in the microcirculation of the tissue. In both adipose tissue and muscle, there is a clear preference of LPL for chylomicron-TG over VLDL-TG in the postprandial state in vivo in humans [90]. Because insulin is well-known to control tissue LPL translocation and activity in the adipose tissue microcirculation, this hormone is thought to play a critical role in TG extraction and storage in this tissue [5].

Some dietary fatty acids are recovered into VLDL-TG within a few hours after a meal [90,92,100,101] and contribute to a substantial proportion of VLDL-TG after several hours [102]. This rapid postprandial redistribution of dietary fatty acids to the liver was directly observed using magnetic resonance spectroscopic studies, showing a rapid increase in liver incorporation of dietary fatty acids peaking at 6 h and returning to baseline level 8 h after meal consumption [103]. Experimental studies using intravenous administration of 11C-palmitate and positron emission tomography in pigs demonstrated that insulin accelerates clearance of plasma NEFA and reduces incorporation of labeled palmitate into plasma TG during fasting [104]. Our studies in rats also showed that insulin reduces plasma NEFA mitochondrial and TG uptake in the liver [105]. These results are in keeping with the well-demonstrated insulin-mediated suppression of VLDL secretion in humans [106]. De novo lipogenesis — synthesis of long-chain fatty acids from acetyl-CoA — rapidly increases after meals and contributes significantly to circulating TG in the postprandial state [107]. In the latter study, postprandial insulin levels correlated positively with the relative contribution of de novo lipogenesis to fatty acids contained into circulating TG. Thus, insulin controls dietary fatty acid tissue partition at all levels — from LPL-mediated lipolysis of chylomicron-TG and plasma NEFA uptake in tissues to secretion of dietary fatty acids and fatty acids synthesized de novo into VLDL.

6. Abnormal regulation of plasma fatty acid transport in IR and T2D

Given the importance of insulin in the control of fatty acid access to tissues both in the fasting and postprandial state, as discussed above, it is no surprise that IR with or without impaired GSIS is associated with disordered fatty acid metabolism [5]. Increases in plasma TG and NEFA levels during fasting are well-documented in IR and T2D. Elevation of plasma TG
during fasting in these pathological conditions is attributable mainly to increased VLDL secretion, and may require the presence of both hepatic and peripheral IR. In particular, hepatic insulin signaling through IRS-2 appears to be important for the regulation of liver and systemic fatty acid homoeostasis [108]. Most in vivo studies have shown that plasma NEFA appearance in obese individuals during fasting is similar or even lower per quantity of fat mass than in lean subjects, but is higher when expressed per fat-free mass [5,96], suggesting that increased fatness per se is a cause of elevated fasting plasma NEFA in IR and T2D. Nevertheless, fasting plasma NEFA flux was elevated, and its suppression by insulin impaired, in prediabetic and diabetic individuals compared with healthy subjects of similar age and BMI in most studies. We have shown that impaired insulin-mediated suppression of plasma NEFA during fasting is associated with peripheral, but not hepatic, insulin resistance, independent of age and BMI [109]. Differences in visceral fat content could partly account for the increase in fasting plasma NEFA in IR. Visceral fat may contribute up to 50% of all hepatic NEFA delivery in obese subjects, but accounts for only around 6% of systemic NEFA during fasting versus 15% in lean subjects, with or without T2D [110]. Patients with uncontrolled T2D also have reduced whole-body and renal plasma NEFA clearance that may, at least in part, be related to hyperglycaemia [111,112]. Thus, total adipose tissue mass, visceral fat and peripheral tissue IR, by increasing plasma NEFA appearance, and hyperglycaemia, by reducing plasma NEFA clearance, may contribute to elevated plasma NEFA levels during fasting in T2D. The relative contribution of fatty acid spillover from circulating TG lipolysis versus intracellular adipose-tissue lipolysis to plasma NEFA during fasting in IR and T2D is currently unknown.

The adipose tissue of lean subjects can switch from a net export to a net import of NEFA from fasting to postprandial state in the presence of a net export of NEFA postprandially in insulin-resistant subjects [113]. Plasma NEFA appearance rate is elevated postprandially in obese insulin-resistant individuals [114,115] and in obese patients with T2D compared with younger, lean, healthy individuals [96,116]. Most of this excess appears to originate from non-splanchnic upper-body fat [115]. Subjects with T2D display higher and faster hepatic and muscle accumulation of dietary fatty acids in the postprandial state compared with non-diabetic controls matched for age and body composition [103]. In the latter study, it was estimated that the liver and skeletal muscles of patients with T2D comprised 13 and 35% of dietary fatty acids, respectively, at peak uptake, in contrast to 9 and 4%, respectively, in controls. In addition, there were only slight, non-significant differences in plasma TG, lipoproteins and plasma NEFA levels between the two groups in the study. Keeping in mind that relative changes in plasma NEFA levels during intravascular TG lipolysis underestimates NEFA flux [98], and because fatty acid oxidation was not measured in the study by Ravikumar et al. [103], the precise mechanism leading to excess postprandial dietary fatty acid uptake by the liver and skeletal muscles in T2D is currently unclear.

7. Adipose tissue fatty acid spillover in the pathogenesis of type 2 diabetes

The above-mentioned evidence suggests a critical role for disordered fatty acid storage in adipose tissues in the pathogenesis of T2D. Tissues such as skeletal muscle, heart, β cells and liver that use fatty acids as a major energy source are likely to accumulate fatty acids and reactive lipids only during positive energy balance — during the postprandial state [103]. To prove a causal link between adipose tissue fatty acid storage and the development of T2D, however, it is first required to demonstrate

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**Fig. 2.** Hypothetical model for induction of adipose tissue inflammation, insulin resistance, reduced storage of fatty acids and impaired adiponectin secretion by unabated and excessive NEFA flux generated by chylomicron-triglyceride lipolysis as a consequence of chronic excess energy and fat intake.
that a postprandial defect in adipose tissue fatty acid storage is present very early in the natural history of the disease. Postprandial plasma NEFA and TG levels are increased concomitantly with elevated postprandial insulin levels in healthy offspring of T2D parents [117], subjects whose lifetime risk of developing T2D is high. A recent study in our laboratory suggests that these high-risk subjects display increased plasma NEFA levels and appearance during enhanced intravascular lipolysis with heparin + Intralipid at high postprandial plasma insulin levels [120]. Using nicotinic acid, we demonstrated that impaired insulin-mediated suppression of intracellular adipose tissue lipolysis is not responsible for the higher plasma NEFA appearance in the offspring of parents with T2D under these experimental conditions. Thus, increased spillover of plasma NEFA from circulating TG lipolysis may occur very early in the natural history of T2D.

Reduced expression of adipogenic transcription factors such as C/EBPα, β, and δ, PPARγ2 and SREBP-1 was demonstrated in non-obese, non-diabetic insulin-resistant men with at least two relatives with T2D [24]. Experimental work supports the concept of ‘adipose tissue expandability’ through activation of genes such as PPARγ2 in response to a positive energy balance as an important mechanism of protection against lipotoxicity and the development of IR and T2D [43]. Expression of several components of the Wnt signalling pathway, an important regulator of mesenchymal cell differentiation, is reduced in proportion to increased adipocyte size (a marker of adipocyte IR and dysfunction) in first-degree relatives of subjects with T2D [24]. Although the role of the Wnt pathway in the development of adipose tissue IR and dysfunction in humans has not been elucidated, transgenic expression of Wnt10b in adipocytes and myeloid cells has been associated with reduced adipose tissue mass and inflammation, and improved insulin sensitivity in ob/ob and agouti mice [118].

Macrophage recruitment from the circulation and infiltration into adipose tissues is associated with obesity and may lead to impaired adipose tissue insulin signalling and impaired fatty acid metabolism through secretion of proinflammatory cytokines such as TNFα, IL-6 and MCP-1 [12]. NEFA may activate JNK and IκBα via TLRs, leading to activation of NFκB in both adipocytes and macrophages, and to IR in adipocytes [15]. Loss of function of TLR-4 in mice is associated with resistance to diet- and fatty acid-induced IR and adipose tissue inflammation [119]. The existence of mechanisms such as NEFA activation of TLRs render possible that inflammation and metabolic dysfunction in adipose tissues may be induced rapidly by the almost unabated influx of fatty acids from lipolysis of chylomicron-TG during excess dietary caloric and fat intake (Fig. 2). Alternatively, metabolic dysfunction of adipose tissue and postprandial spillover of fatty acids towards non-adipose tissues could perhaps occur only over the long term in association with the development of obesity and adipocyte hypertrophy. The exact sequence of events that initiates adipose tissue inflammation and metabolic dysfunction in vivo in humans remains to be determined. Whether postprandial spillover of dietary fatty acids is rapidly inductive by a sustained excess of fatty acids and caloric intake in humans is an important question that needs to be investigated.

8. Conclusion: translating the concept of lipotoxicity into clinical practice to prevent T2D

The ever-expanding links between increased tissue fatty acid exposure and the development of IR and impaired GSIS, and the considerable advances so far in our knowledge of cellular mechanisms of lipotoxicity have yet to be translated into practical prevention of T2D. No prospective study has yet determined the impact of postprandial tissue exposure to fatty acids on the early development of IR, β-cell dysfunction or deterioration of glucose tolerance in humans. In view of the pathophysiological importance of excess postprandial exposure to fatty acids in preclinical models and small-scale human metabolic studies, this needs to be ascertained in large-scale prospective cohort studies. Many questions remain concerning the regulation of postprandial partitioning of fatty acids and the mechanisms leading to increased fatty acid exposure of non-adipose tissues. The relative role played by fatty acid delivery to non-adipose tissues via plasma NEFA versus TG and to increased tissue uptake versus reduced oxidation in the development of T2D await more mechanistic studies in humans. A better understanding of these processes offers great promise for the future prevention and treatment of T2D.

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