

Etiology and Pathophysiology

Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle

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Received 22 July 2008; revised 21 October 2008; accepted 23 October 2008

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Summary

Lipotoxicity in skeletal muscle plays a critical role in the aetiology of insulin resistance and type 2 diabetes mellitus by interference of lipid metabolites with insulin signalling and action. The dynamics of lipid oxidation and fine tuning with fatty acid uptake and intramyocellular triacylglycerol turnover may be very important to limit the accumulation of lipid intermediates. The use of metabolic inflexibility, defined as the impaired capacity to increase fat oxidation upon increased fatty acid availability and to switch between fat and glucose as the primary fuel source after a meal, does more justice to the complexity of changes in fuel oxidation during the day. Fatty acid availability, uptake and oxidation all play a role in metabolic flexibility and insulin resistance. During high fatty acid availability, fatty acid transporters may limit cellular and mitochondrial fatty acid uptake and thus limit fat oxidation. After a meal, when the demand for fatty acids as fuel is low, an increased fractional extraction of lipids from plasma may promote intramyocellular lipid accumulation and insulin resistance. Furthermore, defects in fuel switching cluster together with impaired mitochondrial content and/or function. Lifestyle changes in dietary fat intake, physical activity and weight loss may improve metabolic flexibility in skeletal muscle, and thereby contribute to the prevention of type 2 diabetes.

Keywords: Fat oxidation, insulin resistance, lifestyle, metabolic flexibility.

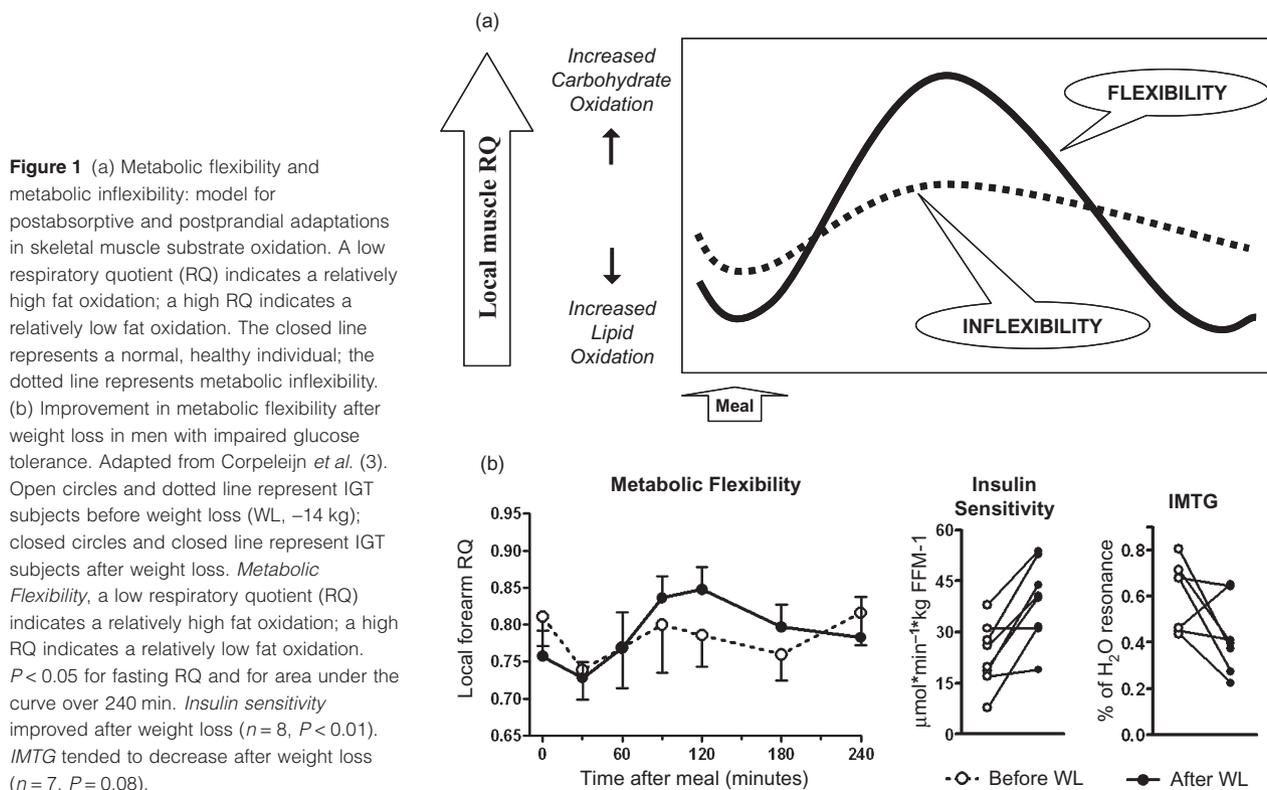
obesity reviews (2009) **10**, 178–193

Introduction

Insulin resistance and diabetes mellitus type 2 (T2D) are strongly associated with excess lipid accumulation in non-adipose tissues like skeletal muscle, most likely by interference of the accumulated lipid metabolites diacylglycerol (DAG), ceramides and long chain fatty acyl-CoA (LCFA-CoA) with insulin signalling (1,2). The dynamics of lipid oxidation and fine tuning with fatty acid uptake and intramyocellular triacylglycerol (IMTG) turnover may be very important to limit the accumulation of lipid intermediates. This may be particularly relevant in situations when energy demand does not challenge the fat oxidative capacity of skeletal muscle, for example during fasting or after a meal. Recently, it has become more and more clear that the

obese, insulin resistant and T2D phenotype is associated with an impaired fat oxidation during fasting, with an impaired switch from fat to glucose oxidation after a meal (3) or after insulin stimulation (4,5) (Fig. 1a), and an impaired rise in fat oxidation after beta-adrenergic stimulation (6,7) or during exercise (8,9). In this review, the impairments in the regulation of fuel oxidation are referred to as metabolic inflexibility, more precisely defined as the impaired capacity to increase fat oxidation upon increased fatty acid availability and to switch between fat and glucose as the primary fuel source. The use of metabolic inflexibility rather than impaired fat oxidation does more justice to the complexity of changes in fat oxidation during the day.

The interplay between glucose and fatty acids on substrate oxidation in skeletal muscle *in vivo* has been studied



for many years. First, the glucose-fatty acid cycle was proposed by Randle and colleagues, showing the ability of exogenous fatty acids to reduce glucose oxidation (10). An increase in free fatty acid (FFA) availability would lead to an increased FFA oxidation, inhibiting pyruvate dehydrogenase and phosphofructokinase. A subsequent accumulation of glucose-6-phosphate inhibits hexokinase activity, and the rise in intracellular glucose concentrations would then result in a negative feedback to glucose uptake. Second came the observation that hyperglycaemia can reduce fatty acid oxidation in skeletal muscle, designated the reverse Randle cycle (11,12). Later, however, studies based on molecular genomic technologies have shown that although the observation of Randle and colleagues was right, the mechanistic explanation for the suppressive effect of fatty acids on glucose metabolism is different. It turned out to be the direct effect of accumulated lipid and lipid intermediates (DAG, ceramides, LCFA-CoA) that interfere with insulin signalling (1,2). Low grade intralipid infusion preceding a hyperinsulinemic euglycemic clamp reduced insulin sensitivity, and also reduced metabolic flexibility, measured as the suppression of whole body fat oxidation and the stimulation of whole body glucose oxidation (13). Other studies with lipid infusions during a hyperinsulinemic euglycemic clamp show a delay in the effect of fatty acids on muscle insulin resistance. This delay may be explained by the time that is needed for lipids and lipid intermediates, such as LCFA-CoA, DAG and ceramides,

to accumulate in skeletal muscle (14) and interfere with insulin signalling (15). Interestingly, lipid infusion increased malonyl-CoA concentrations (16). Malonyl-CoA is proposed as the master switch in a fuel-sensing pathway (19,20), which is explained in Fig. 2. Malonyl-CoA was elevated in muscle biopsies from obese and obese T2D subjects, when compared with lean controls (17), although in another study malonyl-CoA was not significantly reduced in obese T2D subjects compared with overweight controls (18).

Underlying causes for (obesity-induced) metabolic inflexibility may be predominantly found in lifestyle factors and their interaction with genetic or intrinsic characteristics of skeletal muscle. Changes in diet, physical activity or adiposity certainly have specific effects on metabolism, and may offer opportunities to improve metabolic flexibility. Dysfunction of other organs may play a role via changes in the neuro-endocrine environment of skeletal muscle, of which the effect will depend on intrinsic muscle characteristics. This paper reviews the role of impaired metabolic flexibility of skeletal muscle in the development of insulin resistance and T2D with a specific focus on human studies. It summarizes the human studies that have specifically investigated *in vivo* or *ex vivo* skeletal muscle fatty acid uptake and oxidation or used stable isotope methods to study fatty acid metabolism, in human subjects with insulin resistance and/or impaired glucose metabolism. Further, we will explore the potential of lifestyle interventions with

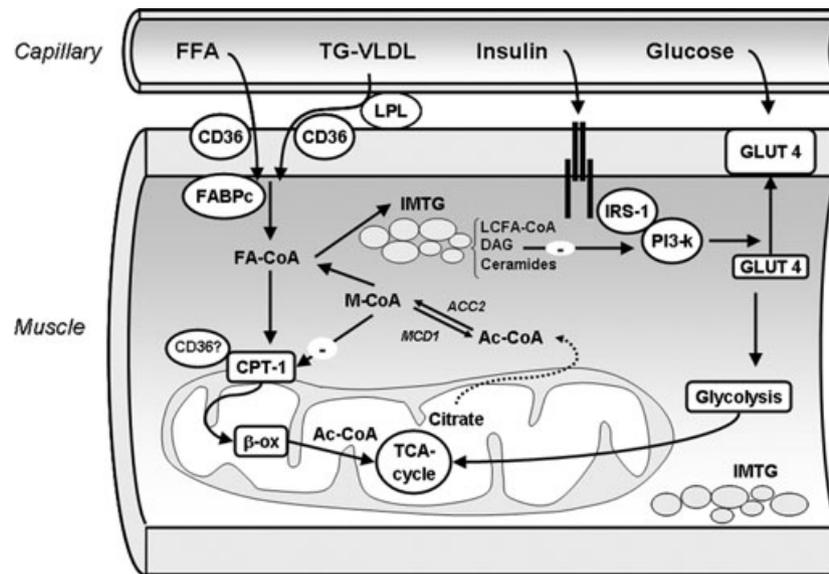


Figure 2 Cell metabolism in the myocyte: key actors in the pathogenesis of fat-induced muscular insulin resistance and metabolic flexibility. Key actors are presented here: fatty acid transporters, the malonyl-CoA fuel-sensing pathway, mitochondrial function, IMTG acculation and interferences of lipid intermediates with insulin signalling. The fractional extraction of free fatty acids (FFA) and FFA derived from very low density lipoproteins triacylglycerols (TG-VLDL) hydrolysis by lipoprotein lipase (LPL) is regulated by fatty acid transporters (CD36). The intra-muscular fuel-sensing pathway may be represented by the *malonyl-CoA* (M-CoA) metabolic pathway. Intra-muscular malonyl-CoA is a potent allosteric inhibitor of carnitine-palmitoyl transferase-1 (CPT-1). Malonyl-CoA concentration is synthesized by the acetyl-CoA carboxylase-2 (ACC2) and broken down by malonyl-CoA decarboxylase (MCD). When nutrients are plentiful, glucose and fatty acid fluxes increase intra-mitochondrial citrate – through the tricarboxylic acid (TCA) cycle – and of cytosolic citrate, which is able to stimulate ACC2 and which in turn increases the production of malonyl-CoA. Malonyl-CoA, inhibiting CPT-1, will then block mitochondrial FA-CoA uptake and therefore limit β -oxidation. A mismatch between oxidation and uptake results in increased levels of long chain fatty acyl-CoA (FA-CoA), diacylglycerol (DAG), triacylglycerol (IMTG) and ceramide, which interact with insulin signalling and reduce insulin-stimulated glucose uptake. When nutrients are not in excess, e.g. owing to increased demand during exercise, the energy demand will cause a drop in citrate levels, a subsequent drop in malonyl-CoA levels, disinhibition of CPT-1 activity, increased mitochondrial fatty acyl-CoA uptake and an increase in fatty acid oxidative disposal. FABPc, cytosolic fatty acid binding protein; FFA, free fatty acid.

changes in diet, physical activity and weight loss to improve metabolic flexibility and lipid handling in skeletal muscle.

Fatty acid handling in insulin resistance and diabetes mellitus type 2

Fatty acid metabolism in skeletal muscle is complex and depends on many factors at whole body and at organ level. The most important factors will be discussed here in relation to metabolic flexibility. This includes fatty acid availability – mainly determined by lipolysis in adipose tissue and capillaries, fatty acid uptake – which is dependent on fatty acid availability but is additionally regulated by specific fatty acid transport proteins (FATP), fatty acid storage – which plays a major role in the hypothesis of lipid-induced insulin resistance, and fatty acid oxidation, which is impaired in many ways in the obese, insulin resistant phenotype.

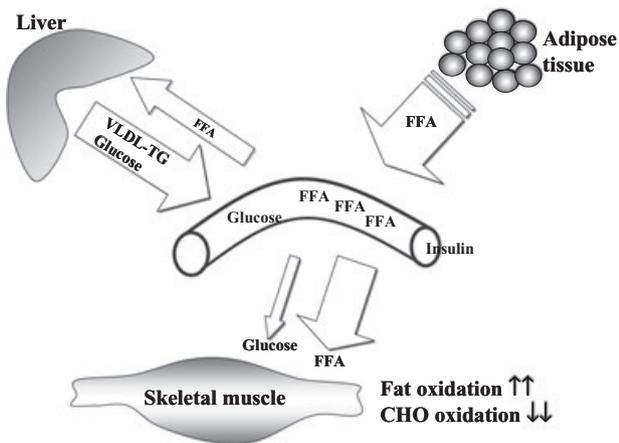
Fatty acid availability

An increased flux of fatty acids (lipid overflow) from adipose to non-adipose tissue may contribute to an increased IMTG storage in tissues like skeletal muscle and liver. Fatty acids

are present in the circulation as non-esterified fatty acids released by adipose tissue, bound to plasma proteins, as VLDL-triglycerides produced by the liver or as triglycerides in chylomicrons that are produced in the intestine after a meal. Fatty acids may also be available from intracellular lipid stores. Whereas the amount of fat that can be stored in adipose tissue is almost unlimited, glucose can only be stored as glycogen in relatively small amounts in liver and muscle. The consequence of this is that during fasting conditions, glucose will be saved for organs that are glucose-dependent, whereas other organs like skeletal muscle will rely on fatty acid oxidation. In the fasting state, lipolysis of stored triglycerides in adipocytes delivers FFAs to the plasma, from where they are taken up by the consuming tissues, mainly skeletal muscle, heart and liver (Fig. 3a). In addition, lipolysis of circulating VLDL-triglycerides by lipoprotein lipase in the capillary endothelium may contribute significantly to the circulating FFA pool (Fig. 3a) (21,22). In the postprandial phase, it becomes more complicated because FFA can also be derived from lipolysis of chylomicron-triglycerides (Fig. 3b). Lipolysis in adipose tissue is rapidly suppressed by insulin, which reduces the FFA release from adipose tissue and thus circulating FFA concentrations. After a meal, the

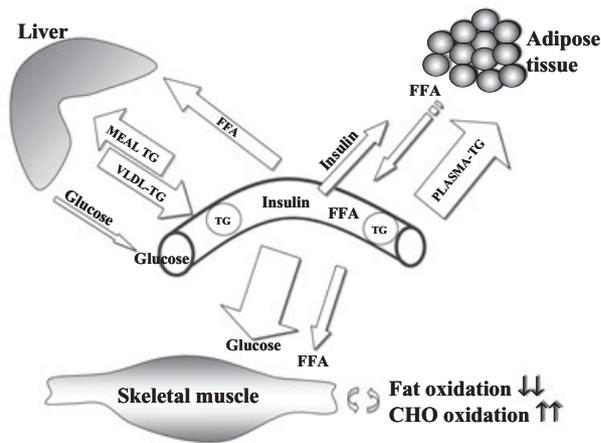
(a) Healthy; Fasting

Fatty acid supply from adipose tissue is high, skeletal muscle oxidizes predominantly fatty acids.



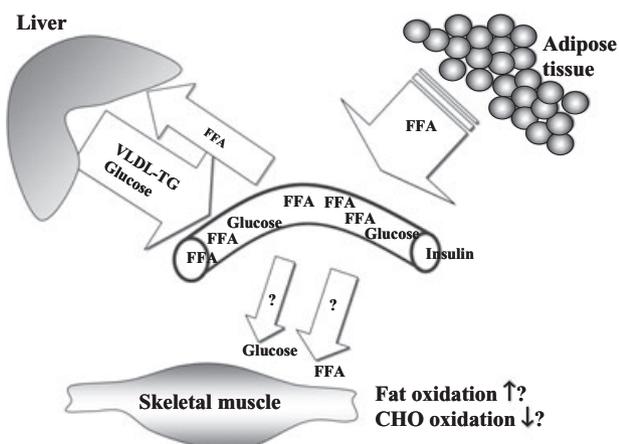
(b) Healthy; Postprandial

Fatty acid supply from adipose tissue is suppressed by insulin, skeletal muscle switches from fat oxidation to carbohydrate oxidation.



(c) Insulin Resistant; Fasting

Fatty acid supply from the expanded adipose tissue is increased, skeletal muscle fat oxidation is blunted.



(d) Insulin Resistant; Postprandial

Fatty acid supply from the expanded adipose tissue is less suppressed, the ability of skeletal muscle to switch from fat oxidation to carbohydrate oxidation is blunted.

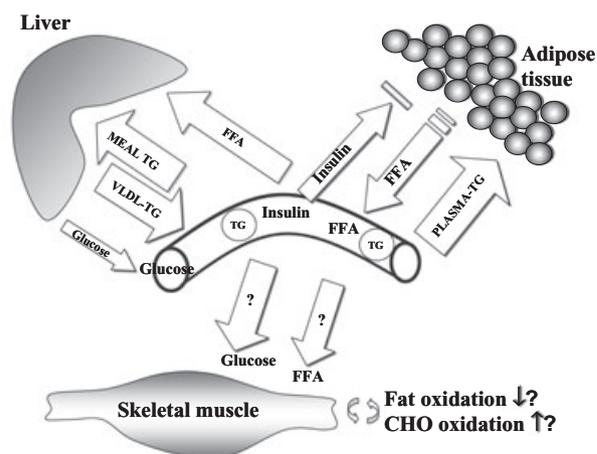


Figure 3 Schematic overview of metabolic fluxes of fatty acids and glucose between organs in healthy subjects during fasting (a) and in the postprandial phase (b) and in the same conditions characterized by insulin resistance (c and d). CHO, carbohydrate; FFA, free fatty acids; VLDL-TG, very low density lipoproteins; meal-TG, chylomicrons.

efficiency of trapping dietary fatty acids from meal-derived triglycerides in adipose tissue is variable with time. In the early postprandial period (0–2 h) most FFAs are trapped (100–80%) but in the late postprandial period (4–6 h), more FFAs escape entrapment (50–80%) (23,24). Thus, a considerable part of the circulatory postprandial FFAs (30–40%) are spillover from triglyceride hydrolysis in the vasculature of adipose tissue (23) and, as recently demonstrated, from spillover of VLDL-triglyceride hydrolysis in skeletal muscle

(24). Tissue perfusion is an important modulator for the extraction of fatty acids from plasma by adipose tissue (25). This is demonstrated by an increased extraction of circulating triglycerides by adipose tissue when adipose tissue blood flow is increased pharmacologically (26). Additionally, an insulin-mediated increase in skeletal muscle perfusion may increase the delivery of insulin and other hormones to muscle tissue and may account for increased glucose uptake (27), although controversial data have been reported (28).

Recent evidence shows that capillary recruitment in response to insulin may be impaired in skeletal muscle of insulin resistant human subjects (29,30).

It is generally suggested that the origins of insulin resistance lie in increased supply of FFA from increased adipose tissue mass (31,32) (Fig. 3c), and postprandially, in an impaired capacity of adipose tissue to trap dietary fatty acids or to suppress endogenous lipolysis (reduced lipid buffering capacity of adipose tissue, Fig. 3d). This lipid overflow to other organs than adipose tissue may lead to ectopic fat storage (32). The traditional concept of lipid overflow caused by elevated plasma FFA is challenged by evidence that in insulin resistant overweight and obese subjects, plasma FFA are not necessarily elevated (33,34). It is currently under investigation to what extent lipid overflow is inflicted by increased FFA or rather by elevated plasma TG, and/or whether 'lipid overflow' should be considered relatively to fat oxidative capacity rather than in an absolute sense. Also the question in which time frame lipid overflow can play a critical role in the development of insulin resistance may be important when FFA levels are studied once insulin resistance has already developed. At the same time, lipid infusion studies provide strong evidence that lipid overflow increases intramyocellular lipid content and can cause insulin resistance (13,14). Important is that these lipid infusions were given during a hyperinsulinemic euglycemic clamp, when fatty acid availability and uptake largely exceed lipid oxidation.

Fatty acid uptake

Fatty acids are taken up by passive and facilitated diffusion, which is dependent on the concentration gradient between the interstitial and intracellular fluid. For this, plasma concentration and intracellular metabolism (oxidation and esterification) are key factors. The diffusion is facilitated by specific transport proteins that alter fractional fatty acid uptake, i.e. the percentage of fatty acids available from plasma that are extracted by the organ (35). Intracellular processes such as activation to acyl-CoA, e.g. coupled to transport as shown for FATP1, and esterification may enhance uptake. Evidence from cultured adipocytes suggests that active ATP-dependent transport is involved (36), although it is uncertain whether this is similar for muscle and adipose tissues, given their different metabolic roles (for review on mechanisms, see (37–39)).

Membrane-bound fatty acid transporters in muscle are CD36 (fatty acid translocase, FAT/CD36, glycoprotein IV), membrane-bound fatty acid binding protein (FABPm) and FATP. The most important cytosolic proteins are cytosolic FABPc and acyl-CoA binding protein (35). CD36 is considered the most important membrane-bound protein for long chain fatty acid (LCFA) uptake (Fig. 2). In CD36 knockout mice, the relative contribution of CD36 to fatty

acid uptake was larger when the fatty acid : albumin ratio is low (40), thus at low FFA availability like after a meal. CD36 may be involved in the clearance of triglyceride-derived fatty acids from plasma in particular in the late phase after a meal (24). CD36 may also facilitate fatty acid uptake, and thus lipid oxidation, during exercise (41). FABPc is considered the most important cytosolic protein for guiding LCFAs inside the cell (Fig. 2). Studies with FABPc knockout mice indicated the involvement of FABPc in shuttling LCFAs from the sarcolemma to intracellular sites of oxidation or esterification, but rather in a permissive than in a regulatory way (42,43). Even a reduction of FABPc protein of 50% was sufficient to maintain LCFA trafficking. Recently, it was suggested that the fatty acid transporter CD36 may act as a LCFA acceptor in the carnitine palmitoyl transferase-1 (CPT-1) system for mitochondrial uptake of LCFAs (44) (Fig. 2). CD36 is present on human skeletal muscle mitochondrial membranes. It may play a role in the rate of mitochondrial palmitate oxidation during exercise (41) and mitochondrial CD36 content was strongly correlated with mitochondrial palmitate oxidation, although not specifically reduced in obese women (45). Rodent studies have shown that the fatty acid transporter CD36 is sensitive to insulin. Both insulin and muscle contraction can induce the translocation of CD36 from an intracellular compartment to the plasma membrane (35). In rat cardiomyocytes, insulin can increase mRNA expression as well as CD36 protein content already after 1 h (46). Recently it was shown that also in humans, CD36 protein content is up-regulated *in vivo* by insulin and that insulin resistance was associated with an altered (increased) insulin-mediated up-regulation of CD36 (47).

Fatty acid uptake links to metabolic flexibility in several ways. A limited fatty acid uptake in myocytes or mitochondria can lead to an impaired ability to stimulate fatty acid oxidation, e.g. during fasting or during exercise. Most fatty acids (95%) are oxidized in mitochondria. Fatty acid transporters can enhance fatty acid uptake into the cell even when the demand for fatty acids is low, and cause a relative overflow of fatty acids. This may promote lipid accumulation, insulin resistance and impaired metabolic flexibility (13,48). Moreover, a promoter polymorphism in the CD36 gene was associated with a decreased insulin sensitivity and an increased prevalence of T2D (49), suggesting a genetic basis for disturbances in fatty acid transport. Thus, data are indicating that the increased fat storage in the insulin resistant muscle is determined not only by the lipid overflow in the circulation, but also by genetic variation in the regulation of fatty acid transport at the level of skeletal muscle. This may predetermine a higher or lower fractional extraction.

To measure fatty acid uptake is complex, and sophisticated research methods, like the use of stable isotope tracers and arterio-venous differences over muscle or

adipose tissue, are necessary to gain insight in the utilization of fatty acids by different tissues. In Table 1, we summarized studies that specifically investigated *in vivo* or *ex vivo* skeletal muscle uptake and oxidation, or used stable isotope methods to study fatty acid metabolism, in human subjects with insulin resistance and/or impaired glucose metabolism. Initially, it was believed that in obese subjects, lipid overflow would increase FFA uptake and cause IMTG accumulation and insulin resistance. The first studies using stable isotope tracer methods to investigate FFA metabolism at organ level reported that, unexpectedly, fatty acid uptake by skeletal muscle was reduced in insulin resistant or diabetic subjects. Blaak and co-workers were the first to report an impaired ability to take up fatty acids in skeletal muscle of obese (insulin resistant) subjects during beta-adrenergic stimulation (6). Colberg and co-workers (50) showed that in visceral obesity, fasting fatty acid uptake into skeletal muscle was reduced. In diabetic subjects, the suppression of fatty acid uptake was blunted after meal intake, indicating an impaired regulation of fatty acid uptake (51). This was not accompanied by an increased fat oxidation. A reduced capacity to mobilize fatty acids (whole body rate of appearance, expressed per unit fat free mass) and a reduced oxidation of plasma-derived fatty acids during fasting and during exercise were found in obese diabetic patients when compared with obese controls (8). These impairments proved to be present already in men with impaired glucose tolerance (IGT), a prediabetic state (9,52), indicating a role in the early stages of development of T2D. In diabetic subjects, muscle FABPc content was lower, and during beta-adrenergic stimulation, the uptake as well as the oxidation of fatty acids were reduced when compared with lean controls (7). After a period of energy restriction, the FABPc content of skeletal muscle increased in obese premenopausal women. The increase correlated very well with a decrease in basal respiratory quotient (RQ), representative of a higher fat oxidation and improved metabolic flexibility (53). In diabetic patients, thiazolidinedione treatment restored insulin sensitivity in parallel with protein-mediated palmitate uptake in skeletal muscle fibres *ex vivo*. This was strongly associated with an up-regulation in CD36 mRNA expression and protein content (54). As CD36 is sensitive to insulin, Bonen and co-workers suggested that due to hyperinsulinemia, obesity and T2D are associated with an increased baseline translocation of the fatty acid transporter CD36 to the plasma membrane (55). Fatty acid uptake capacity was investigated with the giant vesicle model in abdominal muscle strips. Compared with overweight and lean controls, fatty acid uptake capacity was increased fourfold in the fasted obese and diabetic condition (55).

Although these findings seem conflicting with the previously reported reduced FFA uptake, observations of *in vivo* FFA uptake are very diverse and depend on the type of

subject and condition, and the comparison that is made (Table 1). FFA uptake was inversely related to visceral fat (50). Comparing obese or obese IGT/T2D subjects to lean, fatty acid uptake during fasting was reduced and impaired in response to stimuli in most (6,7,51,52,56) but not all (4) studies. Comparing IGT/T2D subjects to BMI-matched controls, some studies show a reduced whole body FFA disposal (expressed per unit fat free mass) (8,9) and a lower fasting FFA uptake over leg muscle (57). Others show no differences between the groups over the forearm muscle (3,57), whereas some shown an increased palmitate uptake (18) or an increased whole body FFA disposal at rest and during exercise (58). Therefore, the role of fatty acid uptake in metabolic flexibility, IMTG accumulation and insulin resistance is not clear yet. Much may depend on the interaction between FFA uptake and oxidation under different conditions, e.g. fasting, postprandial, during exercise or stress (beta-adrenergic stimulation).

In summary, cellular and mitochondrial fatty acid uptake are facilitated by fatty acid transporters, e.g. CD36. *In vivo* fatty acid uptake may be reduced in insulin resistant subjects, but *in vitro* capacity for fatty acid uptake was increased. The fatty acid transporter CD36 may play a role in metabolic inflexibility by limiting cellular and mitochondrial fatty acid uptake and thus fatty acid oxidation, and possibly by increasing the fractional extraction of lipids from plasma when the demand is low, promoting IMTG accumulation and insulin resistance.

Fatty acid oxidation

Skeletal muscle is the most important organ concerning the uptake and oxidation of fatty acids. At rest, about 60–80% of muscle energy production is provided by fat oxidation. Furthermore, in absolute terms, skeletal muscle is a major consumer of O₂ owing to its large total mass, although the oxygen consumption of skeletal muscle per unit of mass is rather low in rest (4 mL O₂/kg wet weight). During exercise, the oxygen consumption can increase enormously, up to ~350 mL O₂/kg wet weight. After a meal, glucose will be present in abundance. High glucose concentrations have potential toxic effects, and skeletal muscle will prevent toxic glucose concentrations by increasing glucose uptake and oxidation at the cost of fatty acid uptake and oxidation (Fig. 3b). In insulin-stimulated conditions ~80% of the glucose utilization is accounted for by skeletal muscle (59). Also high FFA levels are toxic. However, after a meal, the body has – in contrast to glucose – the option to shuttle large amounts of excess FFA to the adipose tissue.

Metabolic flexibility is influenced by both uptake and oxidation of glucose and plasma-derived fatty acids. Table 1 presents studies which report on substrate oxidation and investigate muscle fatty acid metabolism with arterio-venous differences and/or whole body fatty acid

Table 1 Design and outcomes of studies that specifically investigated *in vivo* or *ex vivo* skeletal muscle fatty acid uptake and oxidation, or used stable isotope methods to study fatty acid metabolism, in human subjects with insulin resistance and/or impaired glucose metabolism

Subjects; model; conditions	Outcome on FA uptake	Outcome on lipid oxidation
Range		
(50) Lean to obese women; A-V over leg; StI; leg O ₂ and CO ₂ ; Fasting (overnight); moderate insulin stimulation	FFA uptake inversely related to visceral fat ($r = -0.60$, $P = 0.01$)	Postabsorptive leg fat oxidation rates positively related to insulin-mediated glucose storage in muscle ($r = 0.61$, $P < 0.05$), no direct correlation with visceral fat
Obese compared with lean		
(6) Obese vs. lean; A-V over forearm; StI; whole body O ₂ and CO ₂ ; Fasting (overnight); β -adrenergic stimulation	Fasting: n.a. Beta-adrenergic stimulation: lack of increase	Fasting: = Beta-adrenergic stimulation, whole body RQ: lack of decrease
(4) Obese vs. lean; A-V over leg; StI; leg O ₂ and CO ₂ ; Fasting (overnight); insulin stimulation; WL in obese	Fasting: = Insulin stimulation: equally \downarrow Obese before vs. after WL, fasting: \downarrow Obese before vs. after WL, clamp: \downarrow	Fasting: leg RQ \uparrow , fat oxidation \downarrow , glucose oxidation \uparrow Insulin stimulation: obese no change Obese before vs. after WL, fasting: fat oxidation =, leg RQ = Obese before vs. after WL, clamp: fat oxidation \downarrow , glucose oxidation =, leg RQ \uparrow
(88) Obese before and after exercise training whole body; StI; whole body O ₂ and CO ₂ Fasting (overnight); exercise (50% VO ₂ max, 1 h); after no training; training at 40% VO ₂ max, 12 weeks, 3 times per week; or training at 70% VO ₂ max, 12 weeks, 3 times per week	Whole body FFA disposal not presented	Fasting in all groups before and after training: = Exercise: total and non-plasma derived FFA oxidation \uparrow after training at 40% VO ₂ max
DM2/IGT compared with lean		
(51) Obese T2D (fGlu 10.2, HbA1c 8.9%) vs. lean A-V over leg; StI; leg O ₂ and CO ₂ Fasting (overnight); postprandial (mixed meal with 60 E% fat)	Fasting: \downarrow Postprandial: impaired suppression	Fasting: RQ \uparrow Postprandial: RQ \uparrow
(52) Obese IGT (fGlu 6.1) vs. lean Femoral regions Positron Emission Tomography, no femoral O ₂ Fasting (12–15 h)	Fasting: \downarrow	n.a.
(7) Obese T2D (fGlu 7.7, HbA1c 6.4%) vs. lean A-V over forearm; StI; whole body O ₂ and CO ₂ Fasting (overnight); β -adrenergic stimulation	Fasting: \downarrow Beta-adrenergic stimulation: impaired stimulation	Fasting: n.a. Beta-adrenergic stimulation: \downarrow of plasma-derived free fatty acids
(56) T2D*, obese, overweight and lean Giant vesicles from abdominal muscle; oxidation in muscle strips from lean and obese; fasting (16–18 h)	Fasting: \uparrow in T2D and obese	Fasting (lean and obese): =
DM2/IGT compared with BMI-matched controls		
(8) Obese T2D (fGlu 7.7) vs. obese control Whole body; StI; whole body O ₂ and CO ₂ Fasting (overnight); exercise (50% VO ₂ max, 1 h)	Fasting: whole body FFA disposal \downarrow Exercise: whole body FFA disposal \downarrow	Fasting: n.a. Exercise: total fat oxidation =; oxidation of plasma-derived FFA \downarrow
(9) Obese IGT (fGlu 6.2) vs. obese NGT Whole body; StI; whole body O ₂ and CO ₂ Fasting (overnight); exercise (50% VO ₂ max, 1 h)	Fasting: whole body FFA disposal \downarrow Exercise: whole body FFA disposal \downarrow	Fasting: oxidation of plasma-derived FFA \downarrow Exercise: oxidation of TG-derived FFA \uparrow
(61) Obese T2D before (fGlu 7.5) and after (fGlu 6.5) WL Whole body; StI; whole body O ₂ and CO ₂ Fasting (overnight); exercise (50% VO ₂ max, 1 h); WL (-15 kg)	Fasting: whole body FFA disposal \downarrow Exercise: whole body FFA disposal =	Fasting: oxidation of plasma-derived FFA \downarrow (trend $P = 0.10$) Exercise: oxidation of plasma-derived FFA =
(57) Obese T2D (fGlu 8.6) vs. overweight controls A-V over forearm and leg; StI, no O ₂ and CO ₂ data Fasting (overnight); insulin stimulation	Forearm: = Leg: \downarrow	n.a.
(18) Obese DM2 (fGlu 7.6, HbA1c 8.7%) vs. overweight controls A-V over leg; StI; leg and whole body O ₂ and CO ₂ Fasting (overnight); high glucose/moderate insulin/high FFA conditions	Fasting: palmitate uptake \uparrow High glucose clamp: less increase	Fasting: fat oxidation \uparrow , whole body RQ \downarrow High glucose clamp: fat oxidation \uparrow , whole body RQ \downarrow

Table 1 Continued

Subjects; model; conditions	Outcome on FA uptake	Outcome on lipid oxidation
(58) Overweight long-standing T2D (fGlu 9.4, HbA1c 7.3%) vs. overweight controls Whole body; StI; whole body O ₂ and CO ₂ Fasting (overnight); exercise (50% Wmax, 1 h); recovery after exercise (2 h)	Fasting: whole body FFA disposal ↑ Exercise: whole body FFA disposal ↑ Recovery: =	Fasting: plasma-derived FFA ↑, TG-derived FFA ↓, glucose oxidation ↑ Exercise: = Recovery: plasma-derived FFA ↑
(3) Obese IGT (fGlu 6.6) vs. obese NGT A-V over forearm; StI, forearm O ₂ and CO ₂ Fasting (overnight); postprandial (mixed meal with 60E% fat); WL in IGT subjects (-14 kg)	Fasting: = Postprandial: = IGT after WL, fasting: = IGT after WL, postprandial: =	Fasting: = Postprandial: no ↑ in forearm RQ IGT after WL, fasting: ↓ forearm RQ IGT after WL, postprandial: ↑ forearm RQ

*Fasting glucose or HbA1c not reported. '=' stands for 'comparable between groups'; '↑' stands for increased (change) relative to control group; '↓' stands for 'reduced (change) relative to control group'. A-V, arterio-venous differences; FFA, free fatty acids; fGlu, fasting glucose given as mmol/l, HbA1c given if available; IGT, impaired glucose tolerant subjects; n.a., not applicable; RQ, respiratory quotient; StI, Stable isotopes; T2D, type 2 diabetic patients; VO₂max, maximal oxygen uptake capacity; WL, weight loss, Wmax, maximal workload.

utilization with stable isotopes in subjects with insulin resistance and/or impaired glucose metabolism. It shows a complex range of results with many variables that vary widely between studies, such as the methods used, the unit studied (whole body or muscle), the degree of overweight and the types of subjects (lean, overweight, obese, T2D with low or high HbA1c). In general, the insulin resistant/glucose intolerant phenotype is related to a reduced utilization of fatty acids, and the impairments are shown most clearly in stimulated conditions. In healthy lean to obese women, low rates of leg muscle fat oxidation during fasting were related to decreased glucose storage during insulin stimulation (50). During beta-adrenergic stimulation, fatty acid uptake and oxidation showed a lack of increase in obese subjects when compared with lean controls (6). During insulin stimulation, the suppression of fat oxidation was impaired in obese men and women (4). In T2D and IGT, fuel switching was impaired after a meal (3,51), and a blunted increase in fat oxidation was found during exercise (8,9), and during beta-adrenergic stimulation (7). Some studies in T2D patients however show the opposite: an increased reliance on fat oxidation during fasting, during insulin stimulation (18) and during recovery from exercise (58). These type 2 diabetic patients are characterized by higher HbA1c and higher fasting glucose values. A likely explanation is that in the development towards T2D, fatty acid utilization is impaired, which may contribute to ectopic fat storage and insulin resistance. In long-standing T2D however, fat oxidation may compensate for reduced glucose availability. Also in type 1 diabetes mellitus patients with secondary insulin resistance, the reliance on fat oxidation was higher during fasting, and the rise in whole body RQ was blunted during a hyperinsulinemic euglycemic clamp (60). Substrate availability can be a strong determinant of metabolic flexibility under certain conditions. In T2D subjects, the role of

lower glucose availability was studied during a hyperinsulinemic euglycemic clamp (80 mU m⁻²) with almost complete suppression of plasma fatty acids (5). Metabolic flexibility, represented by the change in whole body RQ, was reduced in T2D subjects compared with obese, and was mainly explained by the glucose disposal rate, independent of diabetes status. Weight loss improved both glucose disposal and metabolic flexibility in T2D. Although total glucose disposal differed between groups and conditions, glucose disposal always largely exceeded glucose oxidation, and glucose partitioning seemed comparable between the obese subjects, the T2D subjects before weight loss and the T2D subjects after weight loss (~34% of glucose disposal was oxidative). During these clamp studies, the levels of plasma FFA were almost completely suppressed, also in the T2D subjects. The question rises what effect increased fatty acid concentrations during high glucose availability may have on metabolic flexibility. During a hyperglycaemic hyperinsulinemic clamp in healthy subjects, the effects of high levels of glucose and insulin in combination with high levels of FFA were studied (16). It appeared that in healthy subjects, glucose oxidation was stimulated and muscle fat oxidation was suppressed, despite maintenance of high FFA uptake. This could be explained by the observed increase in malonyl-CoA levels in skeletal muscle, which inhibits CPT-1 and thus fatty acid entry into mitochondria. This indicates that metabolic flexibility is not only dependent on substrate availability, but is additionally regulated by intracellular fuel-sensing molecules like malonyl-CoA. In Table 1, in about half of the studies/comparisons between fatty acid uptake and oxidation (6–9,18,50,51,58,61), an impaired substrate oxidation can be plausibly linked to an impaired substrate availability, whereas in the other studies/comparisons (3,4,18,51,56,58), additional factors are likely to play a more important role than substrate avail-

ability alone. These data support the view that substrate uptake and substrate oxidation are related, but impairments do not always occur in parallel, as uptake and oxidation can be impaired independently from each other.

Evidence is increasing that defects in substrate switching cluster together with disturbances in mitochondrial content and/or function (62–65). Studies with micro-array techniques show that a whole cluster of genes under the control of peroxisome proliferator activator receptor (PPAR) gamma coactivator-1 α (PGC-1 α), involved in fat oxidation and mitochondrial biogenesis, was coordinately down-regulated in muscle biopsies from diabetic patients (66) and in some studies (67) but not all (68) also in healthy offspring of diabetic subjects. PGC-1 α is acutely up-regulated by insulin (69,70), but it is not clear how PGC-1 α gene expression is related to metabolic flexibility. A dysregulation between mitochondrial oxidative capacity, the capacity for glycolysis and the capacity for beta-oxidation can shift fuel preference towards glucose during fasting. A decrease in activity of oxidative enzymes (citrate synthase, cytochrome-c oxidase) was found in parallel with an increase in activity of glycolytic enzymes (phosphofructokinase, glyceraldehyde phosphate dehydrogenase, hexokinase) in skeletal muscle biopsies from subjects with T2D when compared with lean subjects (65). The ratio between glycolytic and oxidative enzyme activities within skeletal muscle, sampled during fasting, correlated negatively with insulin sensitivity (65).

Fatty acid storage

The degree to which an increased fatty acid supply leads to ectopic fat storage in muscle and whether ectopic fat storage leads to insulin resistance depends on adequate fatty acid handling in skeletal muscle. IMTG accumulation in sedentary individuals is associated with reduced insulin sensitivity, whereas in trained athletes, it is rather related to directly available energy storage and to increased insulin sensitivity, called the 'athletes paradox'. As trained athletes are markedly insulin sensitive, despite high IMTG content (71), it was suggested that the matching between mobilization and oxidation of IMTG-derived fatty acids is one of the main factors that reduces lipid intermediates and dissociates IMTG storage from muscle insulin resistance (72). This was supported by an acute increase in DAG acyltransferase activity in skeletal muscle by exercise, which converts DAG into TAG. The consequent channelling of fatty acid substrates into storage reduced DAG and ceramide levels, increased IMTG levels and prevented lipid-induced insulin resistance (73,74). Muscle IMTG accumulation is an important risk marker of insulin sensitivity, via lipid intermediates interfering with insulin signalling. In addition it is possible that when IMTG accumulation is established and lipid intermediates

accumulate, these may interfere with fuel-sensing and fuel selection. In situations when energy demand does not challenge the fat oxidative capacity of skeletal muscle, the dynamics of lipid oxidation (metabolic flexibility) and fine-tuning with fatty acid uptake and IMTG turnover may be very important to limit the accumulation of lipid intermediates.

Effects of lifestyle on metabolic flexibility

Effect of weight loss

As described in the previous part, the insulin resistant state is characterized by an impaired *metabolic flexibility* of substrate oxidation (Fig. 1a). As insulin resistance and T2D are strongly associated with obesity, it seems reasonable to suggest that weight loss would improve insulin sensitivity and reduce concomitant impairments in metabolism. This is partly true. Weight loss improves insulin sensitivity and reduces fasting glucose, insulin and usually also FFA plasma concentrations in obese, insulin resistant and diabetic subjects. Weight loss also reduces IMTG content (75,76), although not always significantly (3,77). With regard to substrate oxidation, an improvement in the insulin-mediated suppression of fat oxidation was found in obese subjects after weight loss induced by energy restriction (–15 kg) (4) and after weight loss induced by a combined energy restriction and exercise programme (–7 kg body weight loss; +20% increase in maximal oxygen uptake) (78). A recent study shows that weight loss (–14 kg) in men with IGT, a prediabetic state, improved the capacity to switch from fat oxidation to carbohydrate oxidation after a meal, which indicates that the impairments in the regulation of fat oxidation in skeletal muscle are still reversible in obese, IGT men (3). This improvement in metabolic flexibility at skeletal muscle levels is illustrated in Fig. 1b, showing in parallel improvements in insulin sensitivity and a tendency for reduced IMTG content. However, previous studies report different findings on fasting fat oxidation. Fasting fat oxidation is part of the definition of metabolic flexibility, but in a broader sense can be regarded as the capacity to increase fat oxidation upon increased fatty acid availability. In the studies mentioned above, weight loss showed either an improvement (3,78) or no change (4) in fasting fat oxidation. Two other weight loss studies show a lack of change in muscle fat oxidation after weight loss (6) or in skeletal muscle markers of fat oxidation (79,80), despite improved insulin sensitivity. Furthermore, weight loss in diabetic patients (–15 kg) had no effect on plasma-derived fatty acid oxidation and whole body fat oxidation during fasting (61). This lack of improvement in skeletal muscle fat oxidation after weight loss was also observed during beta-adrenergic stimulation and exercise (6,61).

Altogether, there are clear indications that weight loss is able to partly reverse postprandial impairments in metabolic flexibility of substrate oxidation, whereas with respect to the fasting condition, improved regulation of fat oxidation could not be confirmed in all studies. It seems that the challenge of overnight fasting is less predictive for metabolic inflexibility compared with postprandial fuel switch or fuel choice during exercise.

Effect of a combined diet-and-exercise programme

In most lifestyle intervention studies, dietary advice, exercise intervention and/or energy restriction are combined. Goodpaster and co-workers performed a combined training and weight loss study in obese subjects. This programme did improve fat oxidation during fasting as well as the insulin-mediated switch from fat oxidation to carbohydrate oxidation. The improvements in fasting fat oxidation were strongly related to improvements in insulin sensitivity (78). Mensink and co-workers showed that a combined diet-exercise lifestyle intervention in overweight IGT subjects improved insulin sensitivity after 1 year of intervention, which was accompanied by a reduction in the muscle mRNA expression of acetyl-CoA carboxylase-2 (ACC2) and a tendency for increased hydroxyacyl-CoA dehydrogenase protein content (81). Furthermore, the IGT subjects that followed the lifestyle intervention were able to maintain or even slightly improve the capacity to oxidize fatty acids during exercise (owing to improved plasma-derived fatty acid oxidation), and thus maintained metabolic flexibility, whereas in IGT subjects of the control group fatty acid oxidation was reduced (82). Weight loss alone improved insulin sensitivity and reduced IMTG accumulation, but only weight loss combined with exercise also improved aerobic capacity, increased mitochondrial content and improved electron chain transport activity in skeletal muscle of sedentary obese subjects (80). It seems that weight loss alone is effective to improve insulin sensitivity, but is more likely to improve fasting fat oxidation and mitochondrial function if combined with exercise training.

Effect of physical activity alone

Weight loss combined with exercise training has been shown to improve metabolic flexibility, and the question remains what can be attributed to exercise training. Metabolic flexibility is defined as the capacity to switch from fat to carbohydrate oxidation after insulin stimulation, and to increase fat oxidation during fasting, which is a way to reflect the capacity to increase fat oxidation in general. The capacity to increase fat oxidation during exercise may also reflect the capacity to increase fat oxidation. Physical activity may improve insulin sensitivity and postprandial meta-

bolic flexibility through changes in lipid intermediates like DAG or saturated DAG species (83), by changes in the malonyl-CoA fuel-sensing system (84), and via mitochondrial function (83) [for review, see (85)]. Exercise can improve insulin sensitivity and metabolic flexibility both through acute and chronic mechanisms. Studies in impaired glucose tolerant and diabetic subjects show that fatty acid utilization is impaired during exercise (8,9). During 1 h cycling at 50% of maximal aerobic capacity, the acute response in fatty acid oxidation was comparable between obese, IGT and T2D subjects, but in IGT and T2D subjects, the utilization of plasma-derived fatty acids was reduced (8,9). In young, healthy, moderately trained men, an acute exercise bout (1 h at 65% of maximal aerobic capacity) stimulated α 2-AMP-activated protein kinase activation and ACC inhibition by phosphorylation. Malonyl-CoA and acetyl-CoA concentrations in skeletal muscle decreased and fat oxidation in leg muscle increased (86). A decreased malonyl-CoA production, by reduced ACC-2 activity and/or increased malonyl-CoA decarboxylase-1 activity, is beneficial for fatty acid uptake into the mitochondria via disinhibition of CPT-1, and stimulates fat oxidation (Fig. 3). In addition, a single bout of exercise prevented fatty acid-induced insulin resistance in healthy women. Protein expression of the enzymes DAG acyltransferase, mitochondrial glycerol-3-phosphate acyltransferase and Δ 9-desaturase were increased. Channelling of fatty acid substrates into storage reduced levels of DAG and ceramide, but increased IMTG levels (74). Chronic, low-intensity training (40% VO_2max , 12 weeks) tended to increase total fat oxidation during exercise, although not during fasting, in healthy trained subjects (87) and in obese men (88). This could not be accounted for by plasma-derived FFA oxidation, but most likely by an increased oxidation of IMTG-derived or plasma TG-derived fatty acids. In the healthy men, a decrease (-36%) in ACC mRNA was observed after training, in favour of fat oxidation. In elderly subjects (~ 74 years), of whom it is known that they have a reduced rate of fat oxidation, endurance training for 16 weeks increased the rate of fat oxidation during exercise (89). Interestingly, chronic exercise training reduced IMTG content to levels found in lean subjects, and improved whole body fat oxidation during exercise, but failed to completely restore insulin sensitivity in diabetic subjects when compared with lean controls with corresponding IMTG content (90). On the other hand, endurance training in obese individuals improved CPT-1 activity and reduced sensitivity of CPT-1 for malonyl-CoA, but did not change IMTG content, although DAG and saturated DAG species tended to be reduced (-15% , $P = 0.06$ and -27% , $P = 0.06$ respectively) (83). A similar lack of change in IMTG content after training was found previously in overweight to obese men, nevertheless showing an improved fasting fat oxidation and insulin sensitivity (91). A likely explanation is that an

improved (skeletal muscle) fat oxidation restores the balance between fatty acid uptake and oxidation, reducing the level of lipid intermediates (fatty acyl-CoA, DAG, ceramide) and improving insulin sensitivity. In conclusion, chronic exercise training improves the capacity of skeletal muscle to utilize fatty acids for fuel during exercise, and in some cases also improves fasting fat oxidation, which indicates that metabolic flexibility is improved. Metabolic flexibility improves fine-tuning of the balance between fatty acid uptake, oxidation and IMTG turnover in skeletal muscle, reducing lipid intermediates and thereby improving insulin sensitivity.

Effect of dietary fat quantity

As dietary fat is the most energy-dense macronutrient, an increase in dietary fat intake dramatically increases energy intake and leads unnoticed to a positive energy balance (92). The capacity to increase fat oxidation on a high-fat diet may contribute to weight maintenance, or may predispose to obesity if this capacity is impaired (for mechanisms see (93,94)). It was reported that habitual high fat consumers had a higher fat oxidation during fasting and demonstrated a relatively higher fat oxidation in response to a high fat load than habitual low fat consumers (95). A high body mass index was associated with an impaired ability to increase fat oxidation after a fat load (95 energy % fat), mainly in obese subjects with a relatively low fasting fat oxidation (96). The inability to increase fat oxidation on a high fat diet may be related to the inability to increase fat oxidation during fasting, and may be a primary characteristic of impaired metabolic flexibility of substrate oxidation (97). A mismatch between fat intake and fat oxidation, owing to the impaired capacity to regulate fat oxidation, may promote a positive fat and energy balance and fat storage in other tissues than adipose tissue and may thereby enhance obesity and insulin resistance.

Effect of dietary fat quality

Dietary fat quality has been related to insulin sensitivity (98), and to fatty acid partitioning, which is the direction of dietary and endogenous fatty acids towards storage or oxidation. Differences in fatty acid partitioning between the various type of fatty acids were demonstrated in cultured human myotubes (99). The uptake of oleic and palmitic acid were comparable, but oleic acid accumulated rather as intracellular FFAs, whereas palmitic acid was more directed towards storage as DAG and IMTG. In the myotubes established from T2D subjects, palmitic acid oxidation was intrinsically reduced compared with myotubes from lean. Recent data from the Dutch Study on Lifestyle intervention and Impaired glucose tolerance (SLIM study) (100) showed that improved insulin resistance is typically associated with

a reduction in serum cholesteryl ester fractions of myristic acid (C14:0), palmitoleic acid (C16:1 n-7), γ -linolenic acid (C18:3 n-6) and dihomo- γ -linolenic acid (C20:3 n-6) fractions, and an increase in oleic acid (C18:1 n-9) and arachidonic acid (C20:4 n-6), which is consistent with previous reports (101–104). Further, improved insulin sensitivity was characterized by a decrease in Δ 9-desaturase and Δ 6-desaturase activities and an increase in Δ 5-desaturase activity, which were estimated with product-to-precursor ratios (100). Desaturase enzymes (mainly present in the liver and adipose tissue) regulate the degree of unsaturation of lipids throughout the body, including serum fatty acid profiles. Stearyl CoA Desaturase, or Δ 9-desaturase, catalyses the conversion of palmitic and stearic acid into palmitoleic and oleic acid respectively (105). Increased physical activity was associated with an increased estimated Δ 5-desaturase activity in skeletal muscle phospholipids (106,107). Δ 5-desaturase activity contributes to the production of highly unsaturated fatty acids (105) which are ligands for transcription factors like PPARs, hepatocyte nuclear factors 4, nuclear factor kappa B, and sterol regulatory element-binding protein, involved in lipogenesis and fatty acid oxidation (108). With regard to n-3 polyunsaturated fatty acids (PUFAs), very little is known in relation to metabolic flexibility. Although from rodent studies it has been suggested that n-3 PUFAs are related to insulin sensitivity, most human studies using a euglycemic hyperinsulinemic clamp have found no effect of on insulin sensitivity in healthy or diabetic subjects (109). Based on rodent studies it was speculated that the reduction in circulating triglycerides by n-3 PUFAs is partly due to an increase in beta-oxidation (110,111). Human data on substrate utilization or metabolic flexibility to confirm this are currently lacking.

Conclusion

Altogether, there are clear indications that lifestyle changes affect metabolic flexibility in a positive way. Weight loss is able to partly reverse postprandial impairments in metabolic flexibility of substrate oxidation, whereas with respect to the fasting condition, improved regulation of fat oxidation could not be confirmed in all studies. Chronic exercise training improves the capacity of skeletal muscle to utilize fatty acids for fuel during exercise, and in some cases also improves fasting fat oxidation. This may be mediated by changes in the malonyl-CoA 'fuel-sensing' pathway. In general, all these changes improve the capacity of skeletal muscle to regulate the oxidation of fatty acids and thus improve metabolic flexibility. Whether this is related to a decrease in IMTG depends on the type of intervention. Weight loss usually reduced IMTG content whereas there are indications that exercise changes the physical properties of IMTG and IMTG metabolism rather than reducing

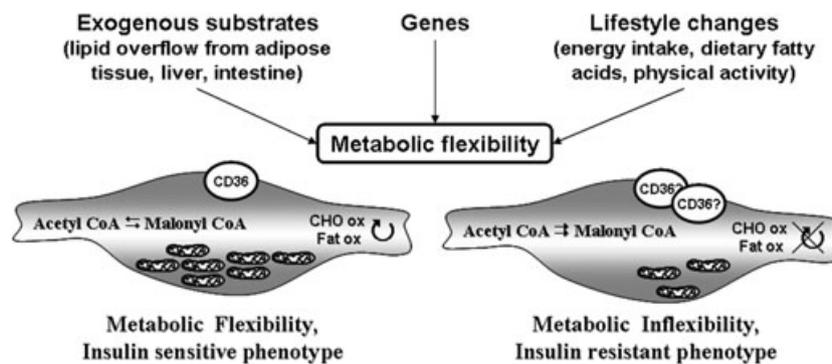


Figure 4 Factors involved in metabolic flexibility and insulin resistance. Increased adipose tissue mass and impaired adipose tissue buffering function can lead to lipid overflow, together with triglyceride fluxes from liver and intestine. Lifestyle factors modulate metabolic flexibility, e.g. physical activity improves oxidative capacity and IMTG metabolism, an increased energy intake leads to overweight and may enhance lipid overflow, and dietary fatty acids may change metabolic flexibility via the amount or via differences in fatty acid partitioning of specific types of fatty acids. Factors at skeletal muscle level that contribute are: a primary impaired metabolic flexibility related to genetic background; impaired regulation of fatty acid transport (CD36) which may enhance the fractional extraction of lipids from plasma; impaired sensitivity of the fuel-sensing (malonyl-CoA) for extracellular substrates; and impaired mitochondrial content/function which may reduce the capacity to increase fat oxidation during fasting or physical activity. CHO ox, carbohydrate oxidation; fat ox, lipid oxidation.

the quantity of IMTG. Also the amount and type of fatty acids (ingested or endogenously produced by desaturation enzymes) may be relevant. Unsaturated fatty acids may improve metabolic flexibility and redirect nutrient partitioning towards less lipid accumulation in muscle. A schematic representation of the factors that mediated the effects of lifestyle on metabolic flexibility and insulin resistance is given in Fig. 4, including lipid overflow from other tissues, fatty acid transporter proteins, the malonyl-CoA fuel-sensing system, mitochondrial content and/or function, and genetic background. Note that substrate uptake and substrate oxidation are related but impairments do not always occur in parallel. In our view, a primary, genetically reduced (regulation of) lipid oxidation combined with lipid overflow (obesity, high fat intake, fasting) may lead to a relatively high FFA uptake and IMTG accumulation. Thereafter, when insulin resistance develops, FFA uptake may become impaired, contributing to secondary impaired lipid oxidation and maintenance of IMTG and whole body lipid storage. In long-standing T2D, the cell may need to compensate glucose deficiency with increased FFA uptake and oxidation to maintain its level of metabolism. In general, an improved metabolic flexibility may contribute to restore the balance between fatty acid uptake, fat oxidation and IMTG turnover in the obese, insulin resistant phenotype, and thereby contribute to insulin sensitivity and the prevention or improvement of type 2 diabetes.

Conflicts of Interest Statement

EC none, EEB none, WHMS no conflict of interest related to the research and topic of the manuscript.

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