

## Basic disturbances in skeletal muscle fatty acid metabolism in obesity and type 2 diabetes mellitus

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The present article addresses the hypothesis that disturbances in skeletal muscle fatty acid handling in abdominal obesity and type 2 diabetes mellitus may play a role in the aetiology of increased adipose tissue stores, increased triacylglycerol storage in skeletal muscle and skeletal muscle insulin resistance. The uptake and/or oxidation of fatty acids have been shown to be impaired during post-absorptive conditions in abdominally-obese subjects and/or subjects with type 2 diabetes. Also, human studies have shown that muscle of subjects that are (abdominally) obese and/or have type 2 diabetes is characterized by an inability to increase fatty acid uptake and/or fatty acid oxidation during  $\beta$ -adrenergic stimulation and exercise. This disturbance in fat oxidation may promote, on one hand, the development of increased adipose tissue stores and obesity. On the other hand, fatty acids that are taken up by muscle and not oxidized may increase triacylglycerol storage in muscle, which has been associated with skeletal-muscle insulin resistance. Disturbances in the capacity to increase fat oxidation during post-absorptive conditions,  $\beta$ -adrenergic stimulation and exercise in subjects who are obese and/or have type 2 diabetes persist after weight reduction, indicating that the diminished fat oxidation may be a primary factor leading to the obese and/or insulin-resistant state rather than an adaptational response. Clearly, the precise sequence of events leading to an increased adiposity and insulin resistance in obesity and type 2 diabetes mellitus is not yet fully understood.

### Fatty acid metabolism: Skeletal muscle: Obesity: Type 2 diabetes

Obesity and type 2 diabetes mellitus frequently occur together, indicating that these conditions may share common pathological mechanisms. Both conditions are characterized by insulin resistance and are often accompanied by increased circulating concentrations of NEFA, an increased adipose tissue mass and disturbances in skeletal-muscle fatty acid handling. As it has a large total mass and increased energy expenditure during physical activity, skeletal muscle is one of the most important organs involved in NEFA metabolism in healthy human subjects (Dagenais *et al.* 1976). Recent investigations imply that the use of lipid is inefficient in skeletal muscle of subjects who are obese and/or have type 2 diabetes, a finding that links muscle metabolism to the development or maintenance of the obese state. Also, the potential of muscle to use carbohydrate or lipid fuels and the potential of substrate competition between these fuels have been a major topic of many investigations on muscle insulin resistance. The present article addresses the relationship between muscle lipid handling and obesity and insulin

resistance. It is hypothesized that inefficient muscle fatty acid utilization may promote fat storage above oxidation, leading to increased adipose tissue stores and triacylglycerol storage within skeletal muscle, thereby promoting obesity and insulin resistance.

### Skeletal muscle fatty acid utilization in obesity

Several observations in the past decade have suggested that obesity is associated with impaired skeletal muscle fatty acid handling. A diminished capacity to take up fatty acids has been reported in skeletal muscle of obese males during  $\beta$ -adrenergic stimulation (Blaak *et al.* 1994) and in viscerally-obese women during post-absorptive conditions (Colberg *et al.* 1995). This observation was made despite the fact that the obese subjects had neither lower plasma NEFA nor lower rates of systemic appearance of NEFA. Furthermore, skeletal muscle fatty acid oxidation has been shown to be impaired in leg muscle of viscerally-obese

subjects during post-absorptive conditions, whereas glucose uptake and glucose oxidation are increased (Colberg *et al.* 1995). Additionally, studies using intact muscle strips from obese subjects have shown a lowered fat oxidation (as measured by  $^{14}\text{CO}_2$  production from  $^{14}\text{C}$ -labelled fatty acids) in obese women (BMI 38.3 (SD 3.1) kg/m<sup>2</sup>; Jong-Yeon *et al.* 2002), which in a subsequent study has been suggested to be a characteristic of extremely-obese subjects (BMI 53.8 (SD 3.5) kg/m<sup>2</sup>; Hulver *et al.* 2003). Weight reduction does not improve the impaired capacity to utilize plasma fatty acids in obese subjects during  $\beta$ -adrenergic stimulation (Blaak *et al.* 1994) or during post-absorptive conditions (Kelley *et al.* 1999), suggesting that these defects may be important in the aetiology of obesity by promoting a positive fat balance.

Support for this concept has come from findings that a decreased reliance on lipid oxidation is a risk factor for weight gain in Pima Indians in Arizona (Zurlo *et al.* 1990). Subjects with a high RQ (0.877) are 2.5 times more likely to gain  $\geq 5$  kg than those with a low RQ (0.822), even after adjustment for differences in energy balance, adiposity and gender. Additionally, post-obese women show decrements in postprandial and 24 h fat oxidation as compared with never-obese women, and the value of post-absorptive RQ is positively associated with weight gain after a period of low energy intake (Froidevaux *et al.* 1993). Also, in post-obese women it has been shown that fat oxidation during exercise (at 60–65%  $\dot{V}_{\text{O}_{2\text{max}}}$ ) is subnormal for their high circulating levels of NEFA (Ranneries *et al.* 1998; Ezell *et al.* 1999).

Wade *et al.* (1990) reported a positive relationship between percentage body fat and the RER during exercise in obese subjects, again indicating that the capacity to oxidize fat during exercise is reduced in obesity. However, this finding has been criticized because in this study most subjects were lean (percentage body fat <25). Also, in this study subjects exercised at a fixed workload of 100 W, which may have disturbed the relationship between fitness and fatness. Indeed, two subsequent studies did not confirm the results of the Wade *et al.* (1990) study (Geerling *et al.* 1994; Helge *et al.* 1999).

A number of recent studies have indicated that it is not only the total capacity to oxidize fat that is impaired during exercise, but that there may also be differences in the source of fatty acids used. Abdominally-obese women have been shown to have an increased utilization of triacylglycerol-derived fatty acids (derived from plasma or intramuscular triacylglycerols) during exercise as compared with normal-weight controls (Horowitz & Klein, 2000). It is possible that increased triacylglycerol-derived fatty acid oxidation during exercise in obese subjects is driven by the mass of muscle triacylglycerol stores, which have been reported to be increased in obese subjects (Pan *et al.* 1997; Kelley & Goodpaster, 2001).

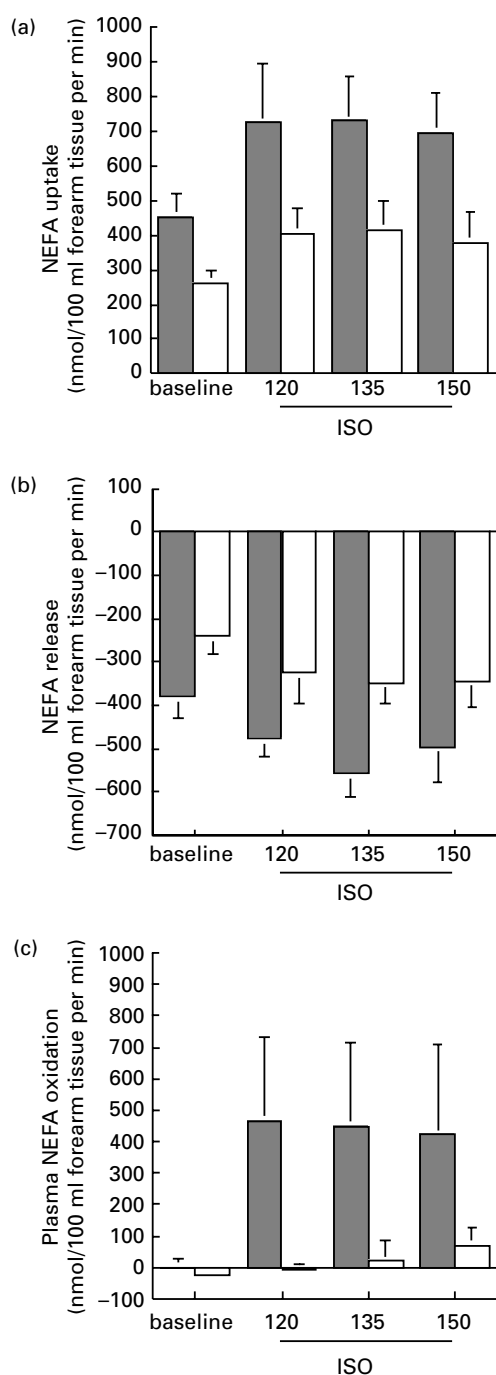
#### **Skeletal muscle fatty acid utilization in type 2 diabetes mellitus**

As indicated earlier, obesity and type 2 diabetes mellitus may share common pathological mechanisms. Indeed, in obese subjects with type 2 diabetes mellitus similar

disturbances in skeletal muscle fatty acid handling have been reported to those present in obese subjects. Obese patients with type 2 diabetes also have reduced uptake rates of plasma NEFA by skeletal muscle. This result has been found by using both limb balance studies (Kelley & Simoneau, 1994; Blaak *et al.* 2000b; Blaak & Wagenmakers, 2002) and positron emission tomography to monitor the uptake of fatty acids in the lower extremity muscles of individuals with impaired glucose tolerance (Turpeinen *et al.* 1999).

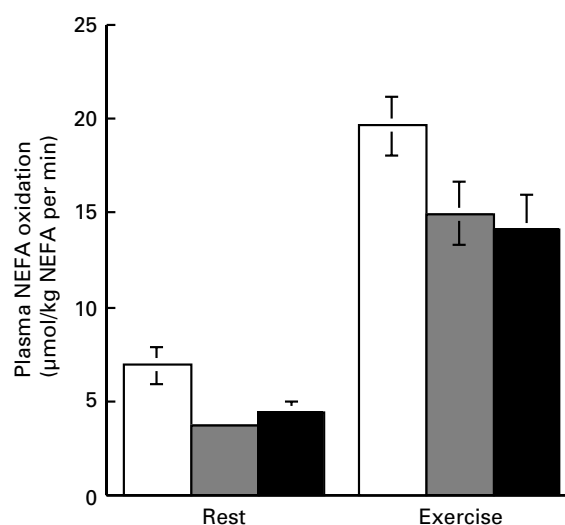
Studies using the forearm balance technique in combination with infusion of the stable-isotope tracer [U- $^{13}\text{C}$ ] palmitate have shown that the uptake and oxidation of NEFA are diminished in skeletal muscle of obese subjects with type 2 diabetes as compared with healthy controls during post-absorptive conditions and during  $\beta$ -adrenergic stimulation (Blaak *et al.* 2000b; Blaak & Wagenmakers, 2002). In addition, in skeletal muscle of control subjects there is a marked release of oxidation products from [ $^{13}\text{C}$ ] palmitate in the form of  $^{13}\text{CO}_2$  (15% of the  $^{13}\text{C}$  uptake from labelled palmitate), from which it can be calculated that about 50% of the fatty acids taken up by forearm muscle are directly oxidized during  $\beta$ -adrenergic stimulation. In subjects with type 2 diabetes there is no detectable release of  $^{13}\text{CO}_2$ , despite a marked uptake of the  $^{13}\text{C}$  label as [U- $^{13}\text{C}$ ]palmitate (60% of the control value; Fig. 1). These data indicate that the fatty acids taken up by muscle are not converted to oxidation products in muscles of subjects with type 2 diabetes. The most likely alternative fate of the tracer in subjects with type 2 diabetes is synthesis of triacylglycerols and incorporation into the muscle triacylglycerol stores. As an increased glycerol release has also been found in these subjects both during baseline conditions and during  $\beta$ -adrenergic stimulation (Blaak *et al.* 2000b), the implication is that the triacylglycerol turnover rate is higher in the diabetic subjects. Thus, it is also possible that the [U- $^{13}\text{C}$ ]palmitate tracer is diluted to a larger extent in the intramuscular triacylglycerol and NEFA pool in subjects with type 2 diabetes as compared with controls. This difference implies that the diabetic subjects and healthy controls differ in the fat sources that are oxidized in these conditions.

Disturbed muscle lipid handling in diabetes has also been demonstrated by Kelley & Simoneau (1994), who have shown diminished fat oxidation in the post-absorptive state in subjects with type 2 diabetes mellitus. Furthermore, despite the reduced fat oxidation in the post-absorptive state, obese individuals and those with type 2 diabetes mellitus manifest an impaired suppression of NEFA uptake (Kelley & Simoneau, 1994) and a higher lipid oxidation in leg muscle during insulin-stimulated conditions (Kelley & Mandarino, 2000). Healthy muscle switches from predominantly lipid oxidation in fasting conditions to an increased glucose uptake and oxidation in postprandial conditions, with a suppression of lipid oxidation. On the basis of their findings, Kelley & Mandarino (2000) have hypothesized that the capacity of muscle to switch between fuels is lost in insulin resistance (metabolic inflexibility) and speculated that this mechanism may play an important role in the lipid accumulation within the insulin-resistant muscle.



**Fig. 1.** Skeletal muscle fatty acid (NEFA) uptake (a), release (b) and plasma NEFA oxidation (c) in skeletal muscle of eight subjects with type 2 diabetes (□) and eight control subjects (■) during baseline conditions and during infusion of the non-selective  $\beta$ -agonist isoprenaline (ISO). Values are means with their standard errors represented by vertical bars. (Adapted from Blaak *et al.* 2000b.)

It has been shown that obese subjects with type 2 diabetes have a diminished plasma NEFA oxidation and a higher triacylglycerol-derived fatty acid oxidation as compared with obese control subjects during moderate-intensity exercise ( $50\% V_{O_{2max}}$ ; Blaak *et al.* 2000a). This



**Fig. 2.** Plasma fatty acid oxidation in seven obese subjects (□), seven obese subjects with impaired glucose tolerance (■) and seven obese subjects with type 2 diabetes (■) at rest and during exercise. Values are means with their standard errors represented by vertical bars. At rest the differences between groups were significant ( $P < 0.01$ ). During exercise the differences between groups tended to be significant ( $P = 0.07$ ). (Adapted from Mensink *et al.* 2001.)

finding indicates that the disturbed fatty acid utilization in the post-absorptive state and  $\beta$ -adrenergically-stimulated conditions also extends to the condition of physical exercise. It should be noted that in this study the lowered plasma-derived fatty acid oxidation occurred in the presence of an unchanged total fat oxidation and a slightly increased triacylglycerol-derived fatty acid oxidation. This finding appears to be consistent with those observed in abdominally-obese subjects during exercise, showing that while the capacity for plasma fatty acid oxidation seems to be reduced, total fat oxidation may be normal or even increased (Horowitz & Klein, 2000), possibly as a result of a mass action effect of increased NEFA delivery from intramuscular triacylglycerol sources or plasma triacylglycerols.

The impaired capacity to oxidize plasma fatty acids during exercise persists after weight reduction in subjects with type 2 diabetes (Blaak *et al.* 2001) and is already present in obese subjects with impaired glucose tolerance (Turpeinen *et al.* 1999; Mensink *et al.* 2001; Fig. 2), indicating that this lowered fat utilization during post-absorptive conditions or during exercise may play a primary role in the transition of impaired glucose tolerance into type 2 diabetes mellitus. It has been shown in a recent study that lifestyle intervention (a combined diet-physical activity intervention) can prevent a further deterioration of disturbances in plasma NEFA oxidation in subjects with impaired glucose tolerance (M Mensink, E Blaak, W Saris and A Wagenmakers, unpublished results), suggesting that the inclusion of physical activity into the programme is able to compensate for the impaired ability to oxidize fat. It can be speculated that this compensation process may be one of the mechanisms underlying the previously-reported beneficial effects of this type of lifestyle intervention

programme, addressing diet and exercise, on glucose tolerance and insulin resistance (Tuomilehto *et al.* 2001; Mensink *et al.* 2003b).

In summary, both (visceral) obesity and type 2 diabetes are characterized by disturbances in skeletal muscle fatty acid handling. During post-absorptive conditions,  $\beta$ -adrenergic stimulation and exercise fatty acid uptake and/or oxidation appear to be diminished, whereas during insulin-mediated (postprandial) conditions there are indications of an impaired ability to suppress muscle fatty acid uptake and muscle fatty acid oxidation. Weight reduction does not seem to improve skeletal muscle fatty acid oxidation during  $\beta$ -adrenergic stimulation, post-absorptive conditions or exercise in subjects who are obese and/or have type 2 diabetes, indicating that the blunted capacity to increase fat oxidation under conditions with a relatively high lipolytic rate may be a primary impairment in the aetiology of obesity and insulin resistance rather than an adaptational response.

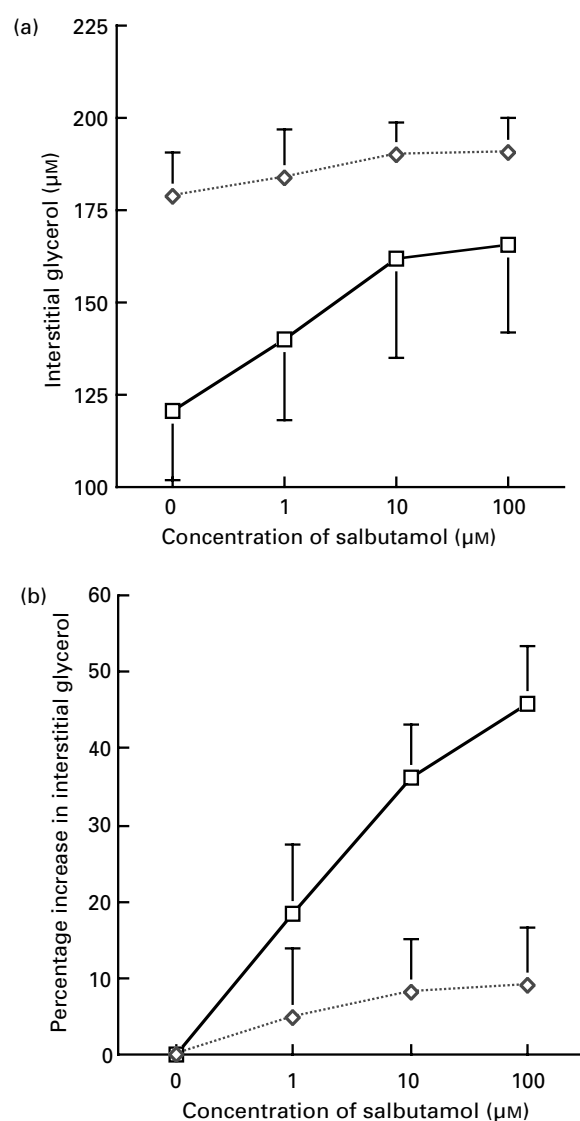
### Skeletal muscle lipolysis

In addition to the lowered capacity to oxidize fatty acids, disturbances in the regulation of skeletal muscle lipolysis may also contribute to the increased lipid accumulation in skeletal muscle. So far, little is known about the regulation of skeletal muscle lipolysis. It has been shown that hormone-sensitive lipase is also expressed in myocytes (Langfort *et al.* 1999) and that hormone-sensitive lipase can be activated by adrenaline and by muscle contraction (Langfort *et al.* 1998) and may be regulated in parallel by  $\beta$ -adrenergic stimulation and contraction-related factors during exercise (Watt *et al.* 2003). Although it seems plausible that the key mechanisms involved in the regulation of the catecholamine-mediated lipolysis in skeletal muscle are the same as those in adipocytes, there are some recent data indicating that interstitial glycerol, as studied by the microdialysis technique, is regulated differently in adipose tissue as compared with muscle by both insulin and  $\beta$ -adrenergic stimulation (Stumvoll *et al.* 2000; Hagstrom-Toft *et al.* 2001). Recent studies (E Blaak, S Schiffelers, W Saris, M Mensink and E Kooi, unpublished results) have indicated that obesity is accompanied by a blunted capacity to increase muscle lipolysis (as reflected by an increase in interstitial glycerol; Fig. 3) during *in situ*  $\beta_2$ -adrenergic stimulation, suggesting a role for defects in the regulation of skeletal muscle lipolysis in the excessive muscle triacylglycerol storage. So far, it is not known whether this disturbed muscle lipolysis also extends to type 2 diabetes.

### Underlying mechanisms for disturbed muscle fatty acid oxidation

#### Fatty acid uptake

As indicated earlier, obese subjects and subjects with type 2 diabetes may have reduced efficiency for plasma fatty acid uptake under conditions with a high lipolytic rate (post-absorptive conditions,  $\beta$ -adrenergic stimulation and exercise), which may be a contributing factor to a reduced



**Fig. 3.** Effect of local administration of the  $\beta_2$ -adrenoceptor agonist salbutamol on interstitial glycerol concentration (as studied by microdialysis; a) and percentage increase in glycerol (b) in skeletal muscle (gastrocnemius) of eight obese (□—□) and eight lean (◇····◇) subjects. Values are means with their standard errors represented by vertical bars. Two-way repeated measures ANOVA: repeated measures  $P < 0.001$ ; group  $P < 0.05$ .

fatty acid oxidation. Plasma concentrations of NEFA play an important role in determining the rate of NEFA uptake into tissues, but are not the only factor. Fatty acid uptake by skeletal muscle most probably occurs via both diffusion and protein-mediated transport of NEFA (Glatz *et al.* 2001a,b). A number of proteins have been identified as putative fatty acid transporters in skeletal muscle, i.e. cytosolic fatty acid-binding protein (FABPc), fatty acid translocase (FAT)/CD36, plasma membrane fatty acid-binding protein and fatty acid transport protein, but their role in the regulation of fat metabolism remains unclear. In a study by Simoneau *et al.* (1999), the content of FABPc has been found to be normal and that of sarcolemmal fatty acid-binding protein increased in muscle biopsies from

obese subjects with normal glucose tolerance as compared with healthy controls. Furthermore, studies by Blaak *et al.* (2000b) have shown a lowered NEFA uptake and lowered FABPc protein content in muscle of subjects with type 2 diabetes as compared with that of lean control subjects. However, it has been shown that FABPc is present in relative excess in muscle and plays a substantial but merely permissive role in fatty acid uptake by skeletal muscles (Glatz *et al.* 2003), indicating that a diminished content of FABPc is probably not rate limiting for plasma NEFA utilization by the muscle. Interestingly, a mutation in the *FAT/CD36* gene has been associated with a defective fatty acid metabolism and hypertriacylglycerolaemia in the spontaneously-hypertensive rat (Aitman *et al.* 1999), and *FAT/CD36* deficiency has been associated with functionally important impairment of fatty acid transport in muscle and adipose tissue (Coburn *et al.* 2000; Hajri *et al.* 2001). Support for an important role for fatty acid transport proteins in disturbed fatty acid handling has come from a recent study in which it was shown that the protein-mediated component of fatty acid transport is reduced in human diabetic muscle cells and can be improved by thiazolidinedione treatment (rosiglitazone, troglitazone and pioglitazone) in concert with an increased *FAT/CD36* expression (Wilmsen *et al.* 2003). Unfortunately, in this study no distinction is made between the *FAT/CD36* present in the sarcolemma and in an intracellular (endosomal) pool; *FAT/CD36* may be redistributed towards the sarcolemma as a result of muscle contraction (Luiken *et al.* 2003) or insulin stimulation (Luiken *et al.* 2002b) with a concomitant increase in the rate of muscular fatty acid uptake.

Moreover, studies with streptozotocin-induced diabetic rats showed that fatty acid transport (as determined with giant vesicles) and plasma membrane *FAT/CD36* expression are increased rather than diminished (Luiken *et al.* 2002a). Additionally, there are indications that fatty acid transport is up regulated in skeletal muscle of obese Zucker rats (Luiken *et al.* 2001) and that *FAT/CD36* translocation to the plasma membrane may be increased in muscle strips of obese subjects and subjects with diabetes (A Bonen, personal communication). These findings may suggest that the lowered uptake of plasma fatty acids observed in obese subjects and obese subjects with type 2 diabetes in *in vivo* limb balance studies (Blaak *et al.* 1994, 2000b; Kelley & Simoneau, 1994; Colberg *et al.* 1995; Blaak & Wagenmakers, 2002) and using positron emission tomography scanning (Turpeinen *et al.* 1999) cannot be explained by a diminished content or translocation of fatty acid transporters, but may be rather secondary to other mechanisms that control the exchange of fatty acids across the muscle membrane.

In this context, it has been shown that the concentration gradient of NEFA between blood and muscle is one of the primary determinants of plasma NEFA uptake and oxidation (van der Vusse *et al.* 2002). A higher forearm lipolysis has been observed (as indicated by glycerol release) in the muscle of subjects with type 2 diabetes during post-absorptive conditions and during  $\beta$ -adrenergic stimulation, whereas NEFA release is reduced (Blaak *et al.* 2000b). It has, therefore, been hypothesized that an increase in

forearm lipolysis may lead to an increase in the muscle NEFA concentration and thus reduce the blood-tissue NEFA concentration gradient, thereby inhibiting NEFA uptake. To date, it is not known whether intramuscular NEFA concentrations are increased in obesity and diabetes.

There are increasing indications that a reduced fractional extraction is not the only mechanism that determines fat oxidation. Other factors intrinsic to muscle may also contribute to the decreased fatty acid oxidation and increased storage of fat within muscle.

#### *Mitochondrial transport*

A step that may possibly be rate limiting for (long-chain) fatty acid oxidation is the transport of fatty acids into the mitochondria by means of carnitine palmitoyltransferase 1 (CPT1). An increased content of malonyl-CoA, as a result of dysregulation by acetyl-CoA carboxylase and malonyl-CoA decarboxylase, can lead to inhibition of CPT1 and subsequently lower fatty acid oxidation. An increased content of malonyl-CoA has been reported in rodent models of obesity and insulin resistance in conjunction with hyperglycaemia and hyperinsulinaemia and reduced lipid oxidation (Saha *et al.* 1997). Interestingly, a concept has emerged that substrate competition might not only operate in the direction of lipid-induced insulin resistance (as originally described by Randle, 1998), but that a subnormal fat oxidation may result from the glucose-induced inhibition of fatty acid oxidation (a reverse Randle cycle, in which the intracellular glucose availability regulates fatty acid oxidation; Sidossis & Wolfe, 1996). Superimposed on this regulatory effect of glucose availability may be the mass action effect of NEFA availability. Indeed, skeletal muscle glucose oxidation has been reported to be higher in obese insulin-resistant subjects and subjects with type 2 diabetes during post-absorptive conditions (Kelley *et al.* 1999) and during  $\beta$ -adrenergic stimulation (Blaak *et al.* 1994), and this situation does not change as a result of weight loss (Blaak *et al.* 1994; Kelley *et al.* 1999). In addition, Mandarino *et al.* (1996) have shown that at comparable arterial glucose concentration and higher NEFA concentrations leg glucose uptake and oxidation are higher in obese subjects compared with lean subjects. Interestingly, a recent study in human skeletal muscle has shown that a combination of hyperglycaemia and hyperinsulinaemia increases malonyl-CoA, inhibits functional CPT1 activity and shunts long-chain fatty acids away from oxidation and towards storage in human muscle (Rasmussen *et al.* 2002), indicating that this proposed concept of 'reverse' substrate competition may be operative in human muscle. Additionally, two recent studies have shown that an improvement in fat oxidation as a result of training (Schrauwen *et al.* 2002) or lifestyle intervention (Mensink *et al.* 2003a) is accompanied by decreased acetyl-CoA carboxylase mRNA expression, suggesting that a lowered inhibition of mitochondrial fatty acid transport through malonyl-CoA may have contributed to the improved fat oxidative capacity. Thus, there are strong indications that a lowered mitochondrial transport through CPT1 may contribute to the reduced fat oxidation in the muscle of subjects who are obese and/or have type 2 diabetes.

A recent study looking at fat oxidation in muscle strips of obese and lean subjects has shown that, in addition to defects at CPT1, disturbances downstream of CPT1 (such as mitochondrial content) also contribute to the reduced reliance on lipid oxidation in human skeletal muscle in obesity (Kim *et al.* 2000).

### Conclusion

The role of skeletal muscle glucose and lipid substrate competition in the development of insulin resistance has been the topic of many investigations. Several years ago, Randle (1998) hypothesized that excessive fat oxidation in skeletal muscle interferes with insulin-mediated glucose uptake by muscle cells through an inhibition of pyruvate dehydrogenase. Studies of several investigators support the concept of the effect of elevated NEFA concentration on glucose metabolism during insulin-mediated conditions, although the post-receptor mechanisms involved may argue against the Randle cycle, as originally proposed (Roden *et al.* 1996; Boden *et al.* 2001). At present, there is increasing evidence that insulin resistance of muscle in obesity and type 2 diabetes mellitus is characterized by a lowered ability to oxidize fatty acids taken up during post-absorptive conditions, during exercise or during catecholamine stimulation, i.e. during conditions with a relatively high lipolytic rate. Weight reduction does not seem to improve skeletal muscle fatty acid oxidation during  $\beta$ -adrenergic stimulation (Blaak *et al.* 1994) or post-absorptive conditions in obese subjects (Kelley *et al.* 1999) or during exercise in obese subjects with type 2 diabetes (Blaak *et al.* 2001). Thus, this disturbance in fat oxidation may be a primary factor leading to the development of increased adipose tissue stores and obesity. Fatty acids that are taken up by muscle and not oxidized may also increase triacylglycerol storage in muscle, which has been associated with skeletal muscle insulin resistance (Pan *et al.* 1997; Kelley & Goodpaster, 2001). It is proposed that underlying mechanisms for the reduced muscle fat oxidation during post-absorptive conditions and catecholamine-stimulated conditions ( $\beta$ -adrenergic stimulation, exercise) may be related to disturbances in fatty acid transport capacity, a glucose-induced inhibition of mitochondrial fatty acid transport (through an inhibition of CPT1) and/or disturbances downstream of CPT1 (such as a reduced mitochondrial content).

Based on these observations an important direction for future research is to obtain more information on the capacity of muscle for lipid utilization in obesity and type 2 diabetes, how this capacity is affected by body fat distribution and the extent of adiposity, and to what extent there is compensation for this defect once the obese and/or diabetic state has developed.

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