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MUSCLE TRIGLYCERIDE AND INSULIN RESISTANCE

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■ **Abstract** Skeletal muscle contains the majority of the body's glycogen stores and a similar amount of readily accessible energy as intramyocellular triglyceride (imTG). While a number of factors have been considered to contribute to the pathogenesis of insulin resistance (IR) in obesity and type 2 diabetes mellitus (DM), this review will focus on the potential role of skeletal muscle triglyceride content. In obesity and type 2 DM, there is an increased content of lipid within and around muscle fibers. Changes in muscle in fuel partitioning of lipid, between oxidation and storage of fat calories, almost certainly contribute to accumulation of imTG and to the pathogenesis of both obesity and type 2 DM. In metabolic health, skeletal muscle physiology is characterized by the capacity to utilize either lipid or carbohydrate fuels, and to effectively transition between these fuels. We will review recent findings that indicate that in type 2 DM and obesity, skeletal muscle manifests inflexibility in the transition between lipid and carbohydrate fuels. This inflexibility in fuel selection by skeletal muscle appears to be related to the accumulation of imTG and is an important aspect of IR of skeletal muscle in obesity and type 2 DM.

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INTRODUCTION

Skeletal muscle is a major player in whole-body nutrient balance. It accounts for the majority of insulin-stimulated glucose utilization and is therefore the major site of insulin resistance in obesity and type 2 diabetes mellitus (type 2 DM). In energetic terms it is the repository of the majority of the body's glycogen stores and contains a similar amount of readily accessible energy as intramyocellular triglyceride (imTG).

Although a number of factors have been considered to contribute to the pathogenesis of insulin resistance in obesity and type 2 DM, this review focuses on the potential role of skeletal muscle triglyceride content. It is important to note that we concentrate on techniques that allow identification of the triglyceride actually stored within the myocyte (imTG) as the metabolically critical lipid depot. Equally, obesity and type 2 DM affect the composition of skeletal muscle. In both of these disorders there is an increased lipid content within and around muscle fibers. We make the case that changes in the physiology and biochemistry of skeletal muscle lipid metabolism dispose to this muscle lipid accumulation. Indeed, these changes in muscle in fuel partitioning of lipid, between oxidation and storage of fat calories, almost certainly contribute strongly to the pathogenesis of both obesity and type 2 DM.

A consistent theme developed here is that skeletal muscle insulin resistance entails perturbations not only of glucose but also of fatty acid metabolism. In metabolic health, skeletal muscle physiology is characterized by the capacity to utilize either lipid or carbohydrate fuels and to effectively transition between these fuels. Resistance to the antilipolytic effects of insulin is undoubtedly a critical factor in insulin resistance. We review recent findings that indicate that in type 2 DM and obesity, skeletal muscle manifests inflexibility in the transition between lipid and carbohydrate fuels. This inflexibility in fuel selection by skeletal muscle appears to be an important aspect of insulin resistance of skeletal muscle in obesity and type 2 DM.

NONINVASIVE IMAGING OF SKELETAL MUSCLE LIPID CONTENT IN HUMANS

The ability to image intramyocellular lipid in vivo without disturbing the tissue is proving to be a major advance in our understanding of muscle metabolism. Whereas extensive data linking increased muscle lipid content with insulin resistance has been accumulated from both experimental animal and human studies (7, 18, 63, 78, 81), the ability to distinguish intra- versus extramyocellular lipid has clarified the extremely important role the former plays in muscle insulin resistance.

Magnetic resonance spectroscopy has recently been developed as a noninvasive imaging method for assessing muscle lipid content and appears to be able to distinguish between intramyocyte and extramyocyte lipid. Using magnetic resonance spectroscopy to exploit fairly subtle chemical shift differences in these two lipid depots, it is possible to identify peaks that correspond to the methylene carbon of triglyceride from intra- and extramyocyte triglyceride (8). The extramyocyte triglyceride is contained within adipocytes within muscle and the intramyocyte triglyceride (imTG) is located within muscle fibers. ImTG is increased in obesity and is correlated with the severity of insulin resistance (83). A number of studies assessing muscle attenuation characteristics on computed tomography imaging have also indicated increased lipid content in association with obesity and type 2 DM and as a determinant of insulin resistance (27, 29, 47, 78).

Increases of imTG have also been found in nonobese, first-degree relatives of individuals with type 2 DM and to relate to insulin resistance in this population (64). These data suggest that the regional deposition of fat within skeletal muscle may be an early body composition abnormality in relation to insulin resistance, obesity, and type 2 DM rather than arising only as a late complication of excess adiposity. The biochemical mechanisms that might contribute to the increased partitioning of fatty acids into muscle triglyceride are discussed below. The fact that lipid accumulation can be seen relatively early in the development of insulin resistance adds to the concept that perturbed lipid metabolism by skeletal muscle may have a pivotal role in the development of obesity and type 2 DM.

REGIONAL ADIPOSE TISSUE DISTRIBUTION ADJACENT TO SKELETAL MUSCLE

Another aspect of muscle composition concerns the distribution of adipose tissue outside skeletal muscle. There can be substantial subcutaneous adipose tissue located near muscle, and this is especially true with respect to the lower extremities. In general, adipose tissue located in the extremities has been regarded as benign with respect to insulin resistance. However, recent studies suggest that this perspective needs to be modified to account for adipose tissue distribution. In the lower extremities, the majority of adipose tissue is in a subcutaneous

location, above the muscle fascia. In computed tomography imaging of the midthigh, the fascial plane formed by the *fascia lata* can be discerned, and it has been found that adipose tissue located beneath the *fascia lata* is significantly negatively correlated with insulin sensitivity. In contrast, the much greater depot located above this fascia is not significantly related to insulin sensitivity in either men or women (28).

The mechanism(s) that accounts for the association of subfascial adipose tissue but not subcutaneous adipose tissue with insulin resistance are not well understood. Only speculations can be made. Potential factors might be related to effects of fatty acids released by adipocytes adjacent to muscle, or it is also possible that in a paracrine manner there are secreted products of this depot of adipocytes (e.g., cytokines) that influence insulin action within the adjacent skeletal muscle.

MICROSCOPY OF LIPID CONTENT IN MUSCLE FIBERS IN OBESITY

Another approach to the study of lipid content within skeletal muscle is to directly examine tissue obtained by muscle biopsy. Pan et al. extracted lipid from biopsy samples of *vastus lateralis* and found that triglyceride was greater in obesity and was directly related to the severity of insulin resistance (63). A potential limitation of performing lipid extraction from muscle biopsy samples is that the respective contributions of imTG and extramyocyte triglyceride cannot be determined, whereas direct visualization of muscle fibers using microscopy can address this issue (65). In situ staining of neutral lipid with oil red O has been used not only to ascertain that lipid droplets are contained within muscle fibers, but by the use of computer-assisted image analyses, this method can be used to obtain quantitative assessments. Several ultrastructural investigations of human skeletal muscle have shown that lipid droplets account for approximately 1% of cell volume within muscle cells in lean, healthy individuals (87). Goodpaster et al. (30) observed that the volume of lipid droplets in skeletal muscle is increased in obesity and type 2 DM. In their study the approximate volume of myocytes occupied by lipid droplets was 1.5% in lean volunteers, 3–4% in obesity, and slightly greater again in type 2 DM.

In another study using light microscopy and the oil red O method (55) it was noted that not only the volume, but also the cellular distribution, of lipid droplets differs in muscle from obese individuals. Muscle from obese individuals appeared to have a higher proportion of lipid droplets located more centrally within the muscle fiber. It might be speculated that a more central location of lipid droplets within myocytes sequesters lipid, rendering it less likely to be utilized for energy. However, it also needs to be considered that trained athletes have higher levels of imTG (38). The fact that they are not insulin resistant may reflect both the increased mitochrondial density and possibly the distribution and relationship of the lipid droplets to those mitochondria (i.e., increased ability to utilize the fats).

NUTRITIONAL AND OTHER FACTORS CONTROLLING INTRAMYOCELLULAR TRIGLYCERIDE STORES

There is only limited information on the nutritional regulation of imTG levels. The original observation of the relationship between skeletal muscle triglyceride levels and insulin resistance was gained in rats fed isocalorically on high-fat diets differing only in the fatty acid profile (81). This modest dietary intervention led to a wide (>2-fold) range of muscle triglyceride levels (and insulin resistance). Diets high in saturated fats resulted in more accumulation of muscle triglyceride, whereas diets equally high in total fat, but in which that fat was polyunsaturated (particularly of the n-3 variety), did not. Because, under these controlled feeding conditions, overall adiposity was not greatly altered between groups, these changes in muscle triglyceride were not significantly related to changes in body fatness. The original work did not quantitate only the imTG. However, subsequent work has confirmed these dietary effects specifically on imTG and their relation to muscle insulin resistance (H. Thomas & L. Storlien, unpublished). Mechanisms that underlie these observations have not been established, but there are now strong, if somewhat indirect, leads of great interest.

First, an influence of circulating fats on imTG is very likely. Recent studies have shown good relationships between circulating fatty acids and triglyceride and imTG [see, e.g., (52)]. That may explain why many publications have shown excellent relationships between circulating triglyceride levels and insulin resistance. There are many drivers of circulating lipid levels, but in the present nutritional context it is important to note that both fasting (66) and postprandial (88) triglyceride levels are markedly influenced by dietary fat profile. In pleasing harmony with the rodent data presented above, there is again the pattern of high basal levels and prandial triglyceride excursions with saturated fats, lower levels with monounsaturated and n-3 polyunsaturated fatty acids (PUFA), and the lowest with high n-3 PUFA content.

Second, there are now clear intracellular metabolic pathways that are differentially tuned by fatty acid subtypes. A prime example is sterol regulatory element binding proteins (SREBPs). These are important transcription factors that regulate fatty acid and cholesterol metabolism in a number of tissues. SREBP1, in its "1a" and "1c" isoforms, is a potent regulator of enzymes of fatty acid metabolism and triglyceride synthesis (39). Increased expression of SREBP1 increases transcription of key enzymes such as fatty acid synthase, glycerol-3phosphate acyltransferase, and acylCoA:diacylglycerol acyltransferase, leading to increased formation of triglyceride. SREBP1 exists in a number of tissues including skeletal muscle where it is clearly regulated by, for example, insulin (19). Fatty acids are potent regulators of SREBP1, and this has been studied mostly in liver and in cell lines such as HEK293, which has a kidney origin. It has been clearly shown that polyunsaturated fatty acids, particularly n-3 PUFAs, potently downregulate SREBP expression, whereas saturated and monounsaturated fatty acids have little effect (34). This is illustrated in Figure 1. These results show, again, a remarkable similarity to the data on dietary fat feeding where, as we have already

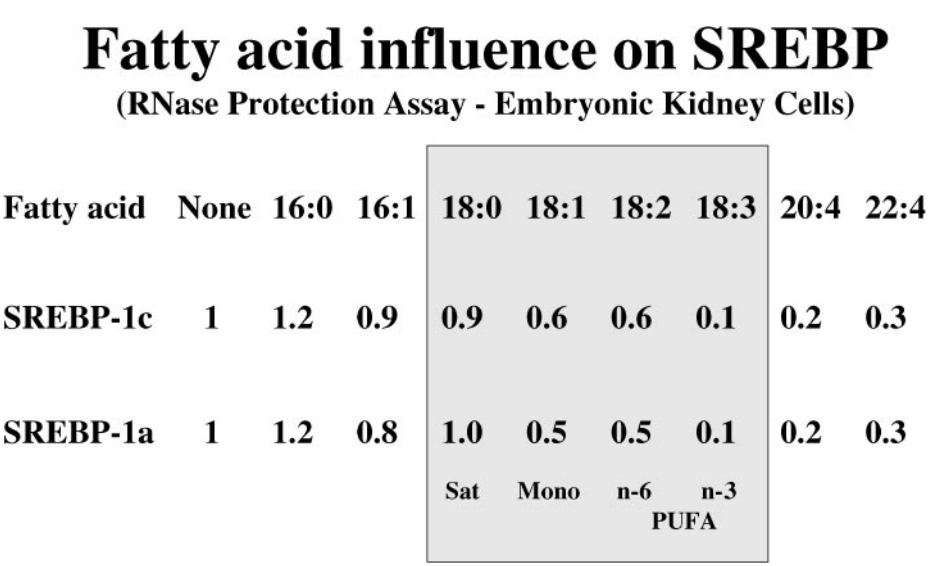

Figure 1 The effects of chain length and saturation of various fatty acids upon the expression of the transcription factors SREBP-1c and SREBP-1a are shown, as adapted from the data of Hannah et al. (34). SREBP, sterol regulatory element binding proteins; PUFA, polyunsaturated fatty acids.

noted, diets high in saturated and monounsaturated fatty acids resulted in high levels of muscle triglyceride (and insulin resistance); diets high in n-6 polyunsaturated fats were intermediary; and diets with a high n-3 PUFA had low muscle triglyceride. Although it is tempting to extrapolate these cell and liver results to muscle, direct confirmation of the role of fatty acid profile on SREBP and lipogenesis in skeletal muscle is still necessary. Finally, the level of accumulation of imTG, and likely its intracellular distribution, is influenced by regular physical activity. Diet also plays a role in these dynamics. There is evidence that imTG is an important source of energy for skeletal muscle during endurance exercise. The work of Hoppeler and co-workers has been outstanding on this issue in studies across experimental animal species and into humans (17). This study showed that trained athletes had higher levels of both imTG and intramyocellular glycogen than untrained, but normal weight, controls before exercise. The athletes also obtained more of their energy requirements from these intramyocellular stores during the course of 2 h of moderately intense exercise (50% VO_2max). The imTG levels were almost twice the rate in trained than untrained individuals but approximately the same in terms of percentage imTG depletion. Repletion of imTG in the 24-h period postexercise was regulated by nutritional status. Feeding of a low-fat (15% of calories) diet led to prolonged imTG depletion. In contrast, feeding of a high-fat (55% of calories) diet resulted in a "supercompensation" phenomenon with higher levels achieved than preexercise. Interestingly, in the trained athletes the imTG and intramyocellular glycogen levels were not only higher but were positively correlated, and both

measures also correlated positively with a measure of insulin sensitivity based on fasting values of insulin and glucose. These findings are in marked contrast to other work on obese and insulin-resistant populations noted elsewhere in this review. Clearly there is much to be learned from the training model about how imTG is regulated and how it does, or does not, lead to insulin resistance.

LEPTIN AND THE CONTROL OF INTRAMYOCELLULAR TRIGLYCERIDE

The role of leptin in control of intramyocellular triglyceride (imTG) is also an important, unfolding story with a complex plot. In many ways discussion of insulin resistance must now be broadened to include the concept of leptin resistance. Circulating leptin levels are influenced by diet composition (23), and leptin was shown some time ago to reduce imTG (71). It has a range of effects in skeletal muscle that include increased exogenous fatty acid oxidation, increased muscle triglyceride breakdown, and decreased triglyceride esterification (79). Effectively then, as these authors noted, leptin induces in skeletal muscle a repartitioning away from fatty acid esterification and toward oxidation. Feeding high-fat diets quickly leads to a failure of these effects of leptin. This leptin resistance is tightly coupled with insulin resistance (86). Equally, when leptin is moderately elevated by adenoviral delivery over a 6-day period in high-fat fed insulin-resistant rats, there is substantial correction of the insulin resistance that was nicely related to reduced muscle triglyceride levels (14). It can reasonably be hypothesized that this leptin effect on insulin resistance was subsequent to the imTG-lowering effect, as acute leptin administration actually decreases glycogen synthesis while increasing fatty acid oxidation (modulation of muscle fuel mix) (60). Regarding SREBPs, hyperleptinemia induced by adenoviral delivery has also been shown to greatly reduce SREBP1c in liver and pancreatic islets, which would be consistent with the antilipogenic effects of leptin (41). Such data for skeletal muscle do not, to our knowledge, exist, but it may be reasonable to assume that the effects would be in the same direction. Equally, leptin resistance may then involve a failure to downregulate SREBP1, suggesting enhanced muscle lipid accumulation.

INTERACTION OF MUSCLE FIBER TYPE AND LIPID CONTENT IN OBESITY

One of the striking features that can be noted when examining the oil red O staining patterns in skeletal muscle, especially human skeletal muscle, is that there is considerable heterogeneity between muscle fibers in the amount of lipid staining. This heterogeneity is related to muscle fiber type. It is well known, based on prior work with rat skeletal muscle, that muscle fiber types differ in their lipid content (10, 13, 20). It is also known that fiber types differ greatly in their insulin sensitivity (40). In general, type 1 or slow-twitch, oxidative (endurance) fibers contain greater lipid than type 2 fibers and are the most insulin sensitive. Of the type 2 fibers,

fast-twitch oxidative (sprint) fibers (intermediate insulin sensitivity) contain more lipid than do fast-twitch glycolytic (intermediate) fibers (least insulin sensitive). Therefore, the question arises as to whether the increase in muscle triglyceride content in humans with obesity or type 2 DM reflects interdependence with muscle fiber–type distribution.

There have been several important studies that address whether the patterns of fiber-type distribution differ in obesity and whether this is related to the pathogenesis of insulin resistance. Several recent studies have reported that individuals with a high percentage of total body fat exhibit a low percentage of type 1 fibers in the *vastus lateralis* muscle (37, 54, 85). Wade et al. (85), on the basis of a rather small group of men ($N = 11$), suggested that at least 40% ($r = -0.65$) of the variability in fatness was related to variation in fiber type 1 proportion of *vastus lateralis* muscle. Similar results were obtained by Helge et al. (37), who also showed that a measure of trunk fat (central adiposity) obtained by dual energy x-ray absorptiometry was well related to the percentage of type 1 fibers ($r = -0.58$, $p < 0.01$, $n = 21$). This relationship was still significant ($p < 0.05$) when adjusted for VO_2max (J.W. Helge, personal communication). A more modest correlation coefficient ($r = -0.32$; $p < 0.01$) was reported between the fiber type 1 proportion of *vastus lateralis* muscle and percent body fat in the study of Lillioja et al. (54). In addition, Segal et al. found a higher proportion of type 2b fibers in *vastus lateralis* muscle of obese individuals (29% vs. 17%) among lean and obese subjects paired on the basis of fat-free mass and VO_2max (70). On the other hand, Krotkiewski et al. (51) have shown no significant relationship between the proportion of type 1 fibers and obesity, as did Simoneau & Bouchard (74) when this relationship was corrected for VO_2max.

On balance then, it is likely that there is a relationship between fiber type proportion and obesity (high type 1/low type 2b = less obesity), but the relationship is not striking when VO_2max is taken into account. Equally, oxidative capacity as assessed by enzyme capacity might actually be more important to analyze than fiber type in this context (we return to this issue below). Finally, there is the possibility that the relationship between fiber type and obesity/insulin resistance may relate back to alterations in membrane lipid composition. It has been demonstrated in humans that an increased percentage of type 1 fibers is associated with an increased proportion of n-3 PUFA in muscle membranes (75).

Turning to the issue of the potential interdependence of muscle fiber type and overall muscle lipid content in obesity and type 2 DM, He et al. (36) performed single-fiber analyses. They stained serial sections of a muscle biopsy sample for muscle lipid content (oil red O staining), muscle fiber type, muscle oxidative enzyme activity, and muscle glycolytic enzyme activity. By serially measuring these characteristics for each muscle fiber, an overall profile for each fiber type could be ascertained. They examined *vastus lateralis* muscle from lean, obese, and obese type 2 diabetic men and women. Lipid content was noted to be highest in type 1 fibers and lowest in type 2b fibers, with an intermediate value in type 2a fibers. This pattern was observed in all three groups of subjects, but skeletal muscle from obese and type 2 diabetic individuals was found to have increased lipid content regardless of fiber type. In each fiber type, muscle from obese individuals

had greater lipid content than that from lean individuals; this was also found for individuals with type 2 DM.

An additional and equally important finding from the study by He et al. (36) was that skeletal muscle from obese individuals and from those with type 2 DM had a reduced oxidative enzyme activity, as determined by standard histochemical methods. As would be expected, type 1 fibers had the highest oxidative enzyme activity, followed in order by types 2a and 2b. Within each fiber type, oxidative enzyme activity was lower in obesity and type 2 DM. He et al. also examined the ratio of oxidative enzyme activity to lipid content. In lean individuals this ratio was relatively consistent across fiber types, despite substantial differences between fiber types in content of lipid and oxidative enzyme activities. In muscle from obese or type 2 diabetic individuals, the ratio of lipid content to oxidative capacity was also relatively consistent across fiber types, but this ratio differed markedly from that found in lean individuals. This suggests that in obesity and type 2 DM, lipid storage is increased out of proportion to the capacity of these myocytes for substrate oxidation. We return below to the issue of oxidative capacity.

Another factor that could influence muscle triglyceride content is aging. Skeletal muscle capacity for oxidative metabolism, particularly to utilize fat, appears to decline with age. Although a number of studies show no major change in proportion between type 1 and type 2 fibers with age [see, e.g., (31)], there is one report in an Australian population of an age-related increase in type 2b ($r = 0.45$, $p = 0.01$) at the expense of type 2a fibers (49). In that study both body mass index and waist circumference were positively related to the percentage of type 2b fibers ($r = 0.44$ and 0.49, respectively; both $p = 0.01$). Interestingly, infants of age <2 years had extremely low levels of type 2b fibers (~6 vs. 20+% in adults). In contrast, a young adult population of obesity-prone Pima Indians were shown to have a much higher (approximately double) percentage of type 2b fibers than would have been predicted from the regression line of age vs. percent type 2b for the Australian subjects. From these studies it seems that fiber type changes over the life span to forms with less oxidative capacity and less ability to burn fat for fuel. In addition, the data from the Pima Indians suggests that there may be genetic predisposition either to higher proportions of type 2b fibers at birth or to an increased rate of transition to type 2b fibers with age. It is not clear whether an active or sedentary lifestyle contributes to the rate of change with aging, but certainly a relatively intense exercise program can shift the proportion of type 2b towards type 2a fiber type (38). This may suggest that habitual exercise will help to maintain skeletal muscle capacity for fat utilization across the life span.

MARKERS OF CAPACITY OF FATTY ACID OXIDATION IN SKELETAL MUSCLE IN OBESITY AND TYPE 2 DIABETES MELLITUS

As noted above, oxidative capacity is generally higher in type 1, intermediate in type 2a, and lowest in type 2b muscle fibers. However, there is considerable overlap between fiber type and oxidative capacity, suggesting that it may be more

appropriate to relate obesity and insulin resistance to measures of oxidative capacity. This is the approach pioneered by Simoneau and Kelley (15, 42, 46, 75). Overall these workers, and others, have consistently found that the activities of several marker enzymes of oxidative and glycolytic capacity are altered in obesity, and relationships with body fatness are better than when fiber type distribution, appropriately corrected for VO_2max, is employed.

For example, citrate synthase (CS), an enzyme of the Krebs cycle activity and a strong marker of oxidative capacity, is negatively correlated with visceral obesity ($r = -0.51$, $p < 0.05$) (78). This was confirmed in an entirely different population (Pima Indians) both for central adiposity and for percent total body fat ($r = -0.43$, $p < 0.01$) (50). Conversely, CS activity was positively correlated with rates of lipid oxidation across the leg during fasting conditions and with both whole-body insulin action and rates of glucose uptake during insulin stimulated conditions (15, 75). These data indicate that oxidative capacity, as exemplified by CS activity, influences both postabsorptive utilization of free fatty acids (FFA) and insulin sensitivity. Conversely, glycolytic potential of muscle, as reflected by activity of phosphofructokinase, a regulatory enzyme in the glycolytic pathway, is increased in individuals with visceral obesity. In particular, the ratio of phosphofructokinase/CS activity is a strong marker of insulin resistance.

Several clinical investigations suggest that the capacity for lipid oxidation is reduced in human skeletal muscle in obese subjects. Ferraro et al. found that skeletal muscle lipoprotein lipase activity was decreased in obesity and that this was related to a decreased reliance on fat oxidation, as measured in a whole-body calorimetry chamber (26). Zurlo et al., using a similar approach, found that marker enzymes of the β-oxidation pathway are reduced in obesity (96). Further, Simoneau found reduced activity of carnitine palmitoyl transferase 1 (CPT1, the enzyme responsible for fatty acid transport into mitochondria, permitting β-oxidation) in skeletal muscle in obesity (77). The reduction in skeletal muscle CPT1 activity was of approximately the same proportion as the decrease in activity of other marker enzymes of mitochondria, such as citrate synthase for the TCA cycle and cytochrome C oxidase of the electron transport chain (77). This suggests a decrease in mitochondria number or function, or both. Interestingly, following weight loss the subjects in the study by Simoneau did not show improvement in the capacity for fat oxidation as measured by activities of CPT1, CS, and cytochrome C oxidase. In that same study an increased content of cytosolic fatty acid binding protein was found in obesity, with an additional gender-related effect of higher concentrations in women (77). However, other groups have found diminished content of cytosolic fatty acid binding protein in skeletal muscle in obese individuals with type 2 DM (5).

Another line of investigation into the capacity of skeletal muscle for lipid oxidation concerns malonyl CoA. Although skeletal muscle has a relatively limited capacity for de novo lipogenesis, it does synthesize malonyl CoA. Ruderman and colleagues have found that muscle content of malonyl CoA in rodents increases in response to insulin and glucose and can be increased in insulin resistance and following denervation (68). Similar studies by Winder and colleagues indicate that increased muscle glucose metabolism in skeletal muscle of the rat led to an

increased malonyl CoA concentration (89). This is potentially germane to the capacity for lipid oxidation because an increase in malonyl CoA can inhibit CPT1. The muscle isoform of CPT1 is particularly sensitive to inhibition by malonyl CoA (59). Several groups have studied the regulation of acetyl CoA carboxylase, the enzyme responsible for synthesizing malonyl CoA from carbohydrate (90, 91). The thrust of this line of investigation is that conditions may prevail in obesity and glucose intolerance, as well as inactivity, for accumulation of malonyl CoA in skeletal muscle and thus lead to allosteric inhibition of CPT1 and consequently, an inhibition of lipid oxidation. Certainly, prolonged inhibition of CPT1 has been shown to increase intramyocellular triglyceride in studies of rodents (18). Thus, excess triglyceride or FFA in muscle might lead to increased long chain acyl-CoA concentrations (21, 24, 62), which, in turn, might lead to further insulin resistance. We return to this issue below.

Still another line of inquiry concerns uncoupling proteins. Uncoupling proteins are postulated to influence thermogenesis, as is well supported by the role of UCP1 in brown adipose tissue and suggested for homologues UCP2 and -3. These findings have potential implications for the pathogenesis of obesity, but results have been inconsistent. Nordfors et al. (61) reported decreased expression of UCP2 in skeletal muscle of human obese subjects. In contrast, Simoneau et al. found increased protein content UCP2 in human skeletal muscle from obese individuals and examined the potential relation to energy expenditure and substrate oxidation, as well as to insulin resistance, which is common in obesity (76). The higher skeletal muscle UCP2 content in obesity was positively correlated with percent of fat mass ($r = 0.60$; $p < 0.05$). However, UCP2 content in muscle was not correlated with basal energy expenditure ($r = 0.03$; $p = 0.93$) or with insulin stimulated rates of glucose metabolism Rd ($r = -0.21$; $p = 0.47$) (76), throwing doubt on any role for UCP2 in development of obesity via changes in energy expenditure or in the etiology of insulin resistance. There was a significant correlation between muscle UCP2 and patterns of macronutrient substrate oxidation within skeletal muscle, such that increased UCP2 was associated with higher fasting values of leg respiratory quotient (RQ) ($r = 0.57$; $p < 0.05$), suggesting that UCP2 may help to regulate nutrient partitioning within muscle and favor carbohydrate oxidation. Its role in the etiology of obesity may then occur via modulating the relative contribution of lipid and carbohydrate for energy production. In relation to UCP3, several lines of evidence suggest that its expression is related to fatty acid availability (12), but fasting patterns of substrate oxidation within muscle did not change in that study. There is clearly much still to be learned about the true role of UCPs in skeletal muscle metabolism.

STRUCTURAL AND STORAGE LIPID SUBTYPES AND LIPID SIGNALING IN OBESITY

Lipids are not only an energy source. They form the major structural components of plasma and organelle membranes and act as potent metabolic intermediates in cellular signaling. These actions are strongly dependent on the fatty acid subtypes

involved. The fatty acid composition of structural and storage lipid in skeletal muscle is influenced both by genetic predisposition and by environment (particularly dietary fatty acid profile). There is now considerable evidence linking obesity and skeletal muscle insulin resistance to the fatty acid composition of both phospholipid, the major membrane structural lipid (11, 63, 84) and stored triglyceride (56). These studies show that an increased proportion of saturated fatty acids in both lipid compartments relate directly and positively to impaired insulin action and to various measures of regional and total adiposity. A number of mechanisms have been postulated that might contribute singly, or in combination, to explain these observations.

First, increased saturation of membrane lipids may decrease metabolic rate. Because saturated fats lack double bonds, their carbon backbones are very flexible. This results in a denser "packing" of phospholipids in the lipid bilayer. This, in turn, decreases membrane fluidity and leakiness to ions and protons. Because ion and proton pumping contributes substantially to cell energy requirements, it will decrease metabolic rate or, conversely, increase energy expenditure with increasing incorporation of PUFAs [see (80)]. Interestingly, increased membrane unsaturation improves intrinsic activity of ion transporters, thus providing the conditions, in concert, to allow maintenance of ion homeostasis (22). Equally, it has been demonstrated that beta adrenergic receptor affinity is decreased with dietary treatment emphasizing saturated fat intake (58), an observation that is also consistent with decreased metabolic rate.

The second area where changed cellular fatty acid composition might affect muscle metabolism overall, and insulin resistance specifically, is in the multiple roles that lipids play in cellular intermediary metabolism. Saturated fatty acids such as palmitate specifically inhibit the insulin signaling components PKB/Akt activation and, concomitantly, insulin-stimulated glucose uptake in primary myocyte cultures (82). Equally, PKB activation has been shown to inhibit insulin stimulation of glycogen synthase kinase-3, thus impairing cellular capacity for glycogen synthesis (32, 33). It has long been established that the relative failure of insulin to increase glycogen synthase activity, and hence glycogen synthesis rate, in skeletal muscle is the key defect in insulin resistance (9). Both of these observations are likely to involve ceramide production, which is a sphingolipid derivative of palmitate, being the condensation product of serine and palmitoyl CoA. Ceramides are on the synthetic pathway for, and are also formed from the breakdown of, sphingomyelins. Sphingomyelin concentrations in adipose tissue and plasma are positively related to obesity (93, 94). Cytokines such as tumor necrosis factor alpha increase breakdown of sphingomyelins. Interestingly, salicylates (aspirin is acetylsalicylate) inhibit sphingomyelin-generated ceramide production, and high doses have been shown to reduce obesity and improve insulin action (48, 92), although other mechanisms are also likely to be important in explaining this observation.

Additional mechanisms of fatty acid modulation of muscle lipid metabolism are likely to occur via influences on the sterol regulatory element binding proteins (SREBPs). As noted above, activation, particularly of SREBP1c, induces a number

of enzymes in the pathways of de novo fatty acid synthesis and through to triglyceride formation [see (39)]. However, as we saw, whereas palmitate slightly induces SREBP1 expression in cell systems, more unsaturated fatty acids are powerfully inhibitory (35). Equally, it has been shown that diacylglycerol accumulation, from an increase in fatty acyl-CoA availability, specifically from palmitate, impairs insulin-stimulated glucose uptake in primary myocyte cultures (82). Diacylglycerols activate a number of isoforms of protein kinase C, and it is likely that a mechanism linking diacylglycerol accumulation from palmitate to impaired insulin action involves the known protein kinase C inhibition of glucose transporter translocation. Finally, as recently reviewed by Shulman, serine phosphorylation of IRS and activation of isoforms of protein kinase C could inhibit PI 3-kinase stimulation of glucose transport, and thus mediate insulin resistance (72).

Taken together, these data indicate that it is likely not triglyceride per se that is chiefly responsible for lipid-induced insulin resistance within skeletal muscle in obesity and type 2 DM. Rather, lipid metabolites, such as long chain acyl CoA, diacylglycerol, and ceramides, elevated in concert with the increased imTG, are likely to be the effectors that more directly mediate insulin resistance.

POSTABSORPTIVE PATTERNS OF FATTY ACID USE BY SKELETAL MUSCLE

Skeletal muscle can oxidize either lipid or carbohydrate to yield energy. During postabsorptive conditions, as occur after an overnight fast, skeletal muscle predominantly relies upon lipid oxidation. This is reflected in an RQ across the forearm in lean individuals of approximately 0.71–0.82 (2, 3, 16). There is also a high rate of extraction of plasma FFA by skeletal muscle during fasting conditions of approximately 40% (16). Oxidation of plasma FFA taken up by muscle, if these were to be completely oxidized, would account for nearly 80% of resting oxygen consumption by muscle. Thus, it is clear that skeletal muscle can play an important role in systemic patterns of fatty acid utilization, especially during postabsorptive metabolism.

It is important to inquire about the mechanisms that could account for increased skeletal muscle lipid deposition in obesity and type 2 DM. Rates of de novo lipogenesis are low in skeletal muscle (69). Accordingly, skeletal muscle accretion of triglyceride in obesity and type 2 DM would seem to arise as a consequence of an imbalance between uptake of plasma fatty acids and rates of fatty acid oxidation. Such a putative imbalance might result from increased fatty acid uptake, perhaps driven by increased plasma concentrations of fatty acids. Recent work from Boden and co-workers (6, 7) has provided strong support for this possibility. In healthy, young volunteers they increased FFA levels by a combination of intralipid and heparin during a prolonged hyperinsulinemic, euglycemic clamp. They then measured both insulin action and accumulation of imTG by nuclear magnetic resonance NMR spectroscopy. Even within 3–4 hours there was significant accumulation of

imTG, which related significantly to both the magnitude of elevation of the plasma FFA levels and, importantly, to the FFA-induced insulin resistance (7). Alternatively (or additionally), the excess accumulation of skeletal muscle triglyceride in obesity might arise from diminished rates of fat oxidation. Given the normal high reliance of skeletal muscle on lipid oxidation during postabsorptive conditions, it would seem logical to inquire whether any defect in lipid oxidation in obesity is evident in this physiological context.

During the past decade and before, a number of studies have begun to address whether patterns of lipid utilization by skeletal muscle differ in obesity and type 2 DM. Ravussin and colleagues found that obesity is associated with an impaired capacity for oxidation of fat calories (26, 95). These studies included data that suggest that higher values for RQ predict weight gain over several subsequent years (95). Two of the principal areas of investigation of the Kelley laboratory, especially in those studies carried out in collaboration with the late Jean-Aime Simoneau, has been to examine whether skeletal muscle capacity for lipid oxidation is reduced in obesity and type 2 DM.

Hyperglycemic, obese individuals with type 2 DM have lower rates of fatty acid oxidation by skeletal muscle during clinical investigations using leg balance methods (43). Subsequently, reduced fatty acid uptake by muscle was also observed during fasting conditions despite the elevated circulating concentrations of plasma fatty acids (46). Induction of hyperglycemia in healthy volunteers produced a similar pattern, and this effect was potentiated in obesity (44, 57). Colberg et al. (15) carried out a study of fasting patterns of muscle fatty acid uptake and oxidation in healthy young women who had a range of body mass index from 19 to 39 kg/m^2. A key finding was that postabsorptive rates of FFA utilization by muscle were diminished in relation to visceral obesity. Women with increased visceral fat had neither lower plasma FFA nor lower rates for systemic appearance of FFA, yet manifested a reduced rate of plasma FFA uptake across the leg. The study also provided initial data that defects of lipid utilization by skeletal muscle and defects of insulin-stimulated glucose utilization might occur together. This was suggested by the correlation between fasting rates of lipid oxidation in muscle and insulin-stimulated rates of glucose storage in muscle ($r = 0.61$, $p < 0.05$). Studies by Blaak et al. yielded similar data (4).

METABOLIC INFLEXIBILITY OF SUBSTRATE UTILIZATION BY SKELETAL MUSCLE IN OBESITY

Recently, Kelley et al. (42) examined fasting patterns of lipid metabolism to test the hypothesis that reliance on lipid oxidation is reduced in obesity and associated with insulin resistance in obesity. Volunteers for this study were approximately 60 healthy, young adults. One-third of the group was lean. The rest were overweight or obese with body mass indexes from 25 to 40 kg/m^2. Leg balance measurements (product of arterio-venous differences and blood flow) for glucose and FFA uptake

(based on the fractional extraction of 9, 10 ^{3}H oleate) were carried out during fasting and insulin-stimulated conditions. Also, indirect calorimetry across the leg was performed to estimate substrate oxidation during fasting and insulin-stimulated conditions. The constant infusion of labeled oleate permitted measurement of uptake of plasma FFA across the leg despite the negative net balance of plasma FFA that occurs during postabsorptive conditions.

During fasting conditions there was robust fractional extraction of labeled FFA across the leg (~40%), indicative of the uptake of plasma FFA by leg tissues; the fractional extraction was similar in lean and obese subjects. Despite similar rates of FFA uptake across the leg, rates of fat oxidation across the leg during fasting conditions were less in obesity. Obese subjects had an elevated leg RQ (0.83 ± 0.02 vs. 0.90 ± 0.01; $p < 0.01$). The value for the respiratory quotient across the leg in obesity denoted a reduced reliance upon lipid oxidation, such that only a third of energy production was accounted for by fat oxidation, whereas nearly twice this proportion was found in muscle of lean volunteers. In both lean and obese volunteers the fasting rates of fatty acid uptake across the leg were greater than the fasting rates of lipid oxidation, indicating a modest net surplus of fatty acid uptake, as has been well described in animal studies (13, 20). The rates of "net storage" of fatty acids were greater in obesity. Thus, a paradigm suggested by these findings is that in obesity skeletal muscle accrues triglyceride owing to a reduced rate of lipid oxidation in the face of rates of fatty acid uptake that are equivalent to those of lean individuals.

A further key objective of the study of Kelley et al. (42) was to address the potential relation between insulin resistant glucose metabolism and patterns of fatty acid uptake and oxidation during both fasting and insulin-stimulated conditions. Considering all subjects, lean and obese, a decreased reliance on lipid oxidation during fasting conditions was associated with resistance to insulin-stimulation of glucose metabolism. Fasting values for leg RQ were negatively correlated with insulin sensitivity ($r = -0.57$; $p < 0.001$). This observation extends the perception of what constitutes the phenotype of skeletal muscle insulin resistance because it reveals metabolic defects beyond those of insulin-stimulated metabolism.

The more classic concept of substrate competition in relation to insulin resistance is that excessive lipid oxidation reduces glucose utilization by skeletal muscle. It is appropriate to ask whether the finding that reduced fat oxidation during fasting conditions is related to insulin resistance of obesity is a contradiction to the classic Randle hypothesis of substrate competition and insulin resistance. Several recent studies indicate that glucose inhibits fat oxidation (57, 73), a so-called reverse Randle cycle, which could be pertinent to the observation that insulin-resistant skeletal muscle in animal models of obesity has increased malonyl CoA (68) and that inhibition of acetyl CoA carboxylase 2, which could act to decrease malonyl CoA, results both in lower body fatness and improved glucose tolerance in mice (1).

In the study by Kelley et al. (42), insulin-stimulated conditions were examined and therefore, the role of fat oxidation during this physiological context was

assessed in obesity and in relation to insulin-stimulated glucose metabolism. Under the stimulation of insulin, utilization of fatty acids by skeletal muscle is normally suppressed (45), though this can be disturbed by increased availability of plasma fatty acids (6, 44). In lean subjects in these studies (42), infusion of insulin stimulated a significant increase in leg RQ, whereas in obese subjects the insulin-stimulated values did not differ from fasting values of leg RQ. Insulin-stimulated values for leg RQ were significantly greater in lean compared with obese subjects (0.99 ± 0.03 vs. 0.91 ± 0.02; $p < 0.01$). Thus, during insulin-stimulated conditions, obese subjects manifested a failure to suppress lipid oxidation, and rates of lipid oxidation were unchanged from fasting conditions.

During insulin infusions, rates of leg lipid oxidation were negatively correlated to insulin sensitivity ($r = -0.45$, $p < 0.001$). That is, greater lipid oxidation during insulin-stimulated conditions predicted insulin-resistant glucose metabolism, whereas during postabsorptive conditions lower rates of lipid oxidation predicted insulin-resistant glucose metabolism. These findings are not disparate but are interconnected pieces of the puzzle of how insulin resistance is manifest within skeletal muscle in obesity. The concept that links these two findings is one of metabolic flexibility as a component of insulin sensitivity in lean individuals and metabolic inflexibility as a component of insulin resistance in obesity. Obese subjects had less change in leg RQ in response to insulin infusion than did lean subjects. Across the entire cohort the amplitude of insulin-stimulated change in leg RQ (Δ leg RQ = insulin-stimulated leg RQ − fasting leg RQ) correlated significantly with insulin-stimulated increases in glucose metabolism ($r = 0.66$, $p < 0.001$). This indicates that responsiveness to insulin in modulation of leg RQ is related to capacity to respond to insulin stimulation of glucose uptake. In obesity the effect of insulin to suppress lipid oxidation was blunted, as has been previously reported (25, 53), and this clearly fits with the classic concept of fatty acid–induced insulin resistance (67).

However, these observations do not indicate that fatty acid oxidation within insulin-resistant muscle is persistently increased. Although insulin infusion did not suppress muscle lipid oxidation in obesity (compared with strong suppression in lean individuals), these rates of fat oxidation were unchanged from fasting conditions. During fasting conditions, rates of fat oxidation in skeletal muscle were lower in obese than lean individuals. Thus, in regard to the nature of substrate competition, muscle in obesity manifested a severe inflexibility in the modulation of fatty acid oxidation, with neither suppression by insulin infusion nor an appropriate enhancement in response to an overnight fast.

SUMMARY AND CONCLUSIONS

The composition and biochemistry of skeletal muscle is altered in obesity and type 2 DM as compared with nonobese, nondiabetic individuals. An important characteristic of skeletal muscle in obesity and type 2 DM is an increased content of triglyceride. Accretion of fat within muscle tissues appears to strongly correlate with insulin resistance. This fat accretion within muscle of obese individuals may

not be simply a passive process, paralleling fat storage in other tissues. Instead, and of particular metabolic interest, is the emerging concept that biochemical characteristics of skeletal muscle in obesity and type 2 DM dispose to fat accumulation in this tissue. The derangements are likely to be genetic in origin but exacerbated by lifestyle variables including physical inactivity and, importantly, the fatty acid profile of the diet. These biochemical characteristics of muscle in obesity and type 2 DM are recognized to include insulin resistance in pathways of glucose metabolism, but more recent studies indicate a "metabolic inflexibility" in the handling of fat calories. The task at present is to more precisely define the nature of the defects within the pathways of fat metabolism and utilize these insights to develop effective treatment strategies. An effort to modify skeletal muscle in obesity and type 2 DM so that its capacity for fat oxidation is improved should be considered as a potential goal of treatment.

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