

# Perspectives in Diabetes

## Fuel Selection in Human Skeletal Muscle in Insulin Resistance

### A Reexamination

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**For many years, the Randle glucose fatty acid cycle has been invoked to explain insulin resistance in skeletal muscle of patients with type 2 diabetes or obesity. Increased fat oxidation was hypothesized to reduce glucose metabolism. The results of a number of investigations have shown that artificially increasing fat oxidation by provision of excess lipid does decrease glucose oxidation in the whole body. However, results obtained with rodent or human systems that more directly examined muscle fuel selection have found that skeletal muscle in insulin resistance is accompanied by increased, rather than decreased, muscle glucose oxidation under basal conditions and decreased glucose oxidation under insulin-stimulated circumstances, producing a state of "metabolic inflexibility." Such a situation could contribute to the accumulation of triglyceride within the myocyte, as has been observed in insulin resistance. Recent knowledge of insulin receptor signaling indicates that the accumulation of lipid products in muscle can interfere with insulin signaling and produce insulin resistance. Therefore, although the Randle cycle is a valid physiological principle, it may not explain insulin resistance in skeletal muscle. *Diabetes* 49:677-683, 2000**

**I**n 1963, Sir Philip Randle outlined a principle that spawned an enormous amount of research over the following 4 decades. He performed a series of experiments that were designed to test the supposition that cardiac and skeletal muscle possessed mechanisms that allowed these tissues to shift readily back and forth between carbohydrate and fat as oxidative energy sources, depending primarily on the availability of free fatty acids (FFAs). In particular, these experiments eventually focused on the bio-

chemical mechanisms that are involved in the switch from carbohydrate to fat oxidation (1-3). The main features of the model that was developed were that increased fat oxidation in muscle would inhibit both pyruvate dehydrogenase (PDH) and phosphofructokinase by accumulation of acetyl CoA and citrate, respectively. These roadblocks placed in the glycolytic pathway would lead to increased glucose 6-phosphate concentration, inhibiting hexokinase and resulting in reduced glucose uptake and oxidation. This homeostatic mechanism became known as the glucose fatty acid cycle or the Randle cycle (Fig. 1).

Insulin-deficient rat models of diabetes, as well as people with type 1 diabetes, exhibit elevated rates of lipolysis, increased plasma FFAs and triglyceride concentrations, elevated blood ketone bodies, and decreased respiratory quotients (RQs). Thus, it was hypothesized that the glucose fatty acid cycle operated under these conditions to inhibit glucose metabolism and contribute to hyperglycemia (2). Later, as the extent of insulin resistance in obese and type 2 diabetic patients was discerned, investigators took note of the association between insulin resistance and increased plasma nonesterified fatty acids (4). It has been postulated that the Randle cycle might be responsible for insulin resistance in skeletal muscle. However, some earlier studies cast doubt on whether the glucose fatty acid cycle could explain insulin resistance in skeletal muscle. Experiments by Schonfeld and Kipnis (5) using rat diaphragm, Beatty and Bocek (6) using isolated sartorius muscle fibers from rhesus monkeys, and Ruderman et al. (7) using the perfused rat hindquarter failed to show that insulin-stimulated glucose uptake was decreased by addition of palmitate or oleate. However, these experiments used supraphysiological insulin concentrations, and an effect on insulin sensitivity could have been missed. Other studies demonstrated the operation of a glucose fatty acid cycle, but under selective circumstances, in some tissues but not others (8,9). The questions raised by these observations have led, over the last 30 years, to a substantial effort to determine 1) whether the glucose fatty acid cycle occurs in humans, and 2) whether increased fat oxidation in insulin-resistant conditions such as type 2 diabetes could be responsible for insulin resistance.

#### ATTEMPTS TO CONFIRM THE GLUCOSE FATTY ACID CYCLE IN HUMANS

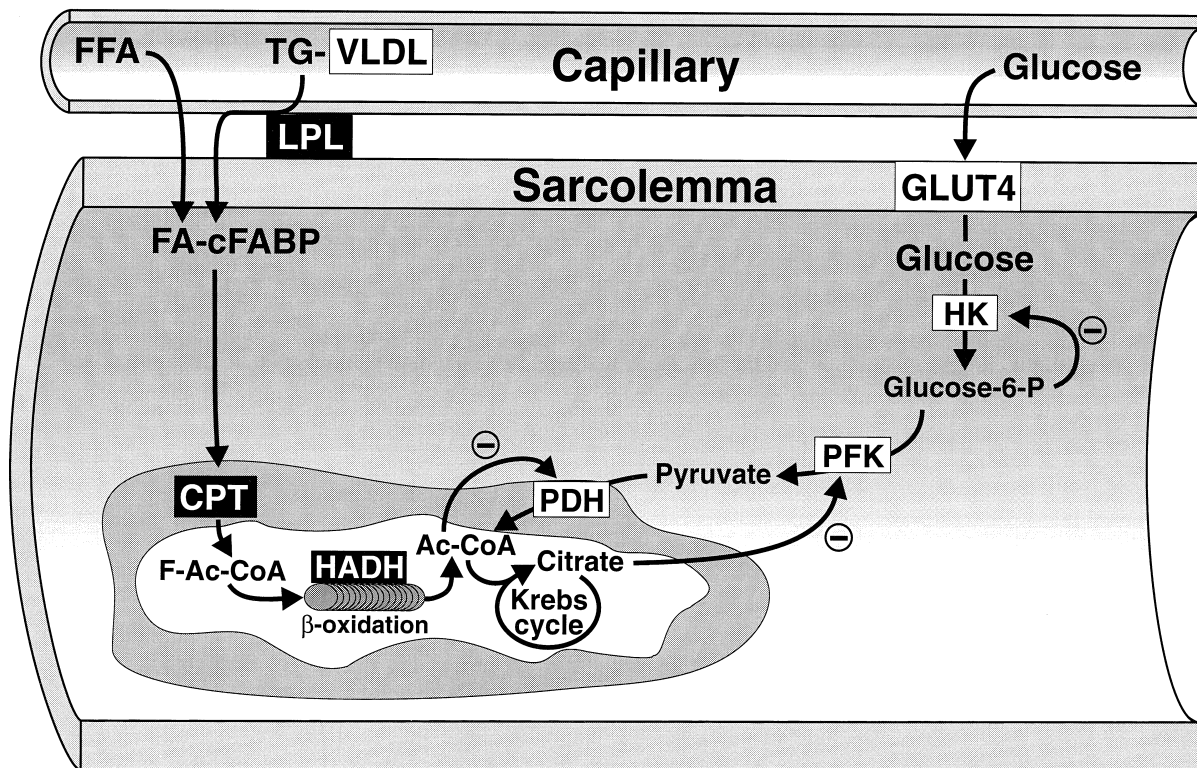
With increasing knowledge of the importance of insulin resistance and lipid abnormalities in the development of type 2 dia-

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CPT, carnitine palmitoyl transferase; DAG, diacylglycerol; FABP, fatty acyl binding protein; FFA, free fatty acid; IRS, insulin receptor substrate; PDH, pyruvate dehydrogenase; PI, phosphatidylinositol; PKC, protein kinase C; RQ, respiratory quotient; UCP2, uncoupling protein 2.



**FIG. 1.** Features of the glucose fatty acid cycle. Fatty acids are taken up from the plasma either as the FFA or by action of lipoprotein lipase (LPL) and carried to the mitochondria by FABPs. The fatty acids are transported into the mitochondria by the CPT system, where they undergo  $\beta$ -oxidation to produce acetyl CoA that enters the Krebs cycle. Accumulation of acetyl CoA and citrate inhibits PDH and phosphofructokinase (PFK), respectively. This leads to a buildup of glucose-6-phosphate (Glucose-6-P) and inhibition of hexokinase (HK), resulting in reduced glucose uptake. cFABP, cytosolic fatty acid binding protein; F-Ac, fatty acyl; HADH, hydroxyacyl-CoA dehydrogenase; TG, triglyceride.

betes, a host of investigators attempted to verify the operation of the Randle cycle in humans. One of the earliest attempts was by Felber and Vanotti (10), who administered glucose tolerance tests with and without an infusion of a fat emulsion and found that glucose tolerance was decreased. Other early investigators reached similar conclusions using a variety of techniques (11,12). The advent of the euglycemic-hyperinsulinemic clamp allowed an explosion of studies of how infusion of lipid alters insulin-stimulated glucose metabolism systemically (13–18) and in forearm (19,20) or leg muscle (21). Essentially all of these studies showed that maintaining or increasing plasma FFA concentrations during an insulin infusion inhibits insulin-stimulated glucose uptake, as would be predicted by the glucose fatty acid cycle.

By combining the glucose clamp technique with indirect calorimetry, some of these investigators were able to partition glucose uptake into glucose oxidation and storage (presumably as glycogen). When these techniques were combined with lipid infusion, the expected result from the Randle hypothesis would have been a primary decrease in glucose oxidation and glycolysis. Although most investigators found that lipid infusion did produce a decrease in insulin-stimulated glucose oxidation that was associated with decreased PDH activity (22), there was a greater decrease in glycogen synthesis associated with decreased glycogen synthase activity (22). This result would not have been predicted by the mechanisms used to explain the glucose fatty acid cycle. An often cited reference in those studies was work showing that, at least in liver, glycogen synthase activity was decreased by

palmitoyl-CoA (23), suggesting that increased fat oxidation and the Randle glucose fatty acid cycle might not be the only mechanism operating during a lipid infusion. In fact, the findings of Boden et al. (14) that infusion of lipid produced insulin resistance in glucose disposal only several hours after it had already decreased glucose oxidation suggested that the glucose fatty acid cycle may not be responsible for insulin resistance.

#### **RANDLE IN REVERSE: GLUCOSE COMPETITION WITH FAT**

At a time when the results of many studies were providing evidence that increased fatty acid oxidation decreased insulin-stimulated glucose oxidation, other investigators were exploring the possibility that provision of excess glucose could also inhibit oxidation of lipid. These studies were spurred, in part, by results that indicated that hyperglycemia prevented a lipid-induced decrease in glucose metabolism (24). As discussed above, 1 of the original projections of the glucose fatty acid cycle was that increased lipid availability in diabetes would interfere with muscle glucose metabolism. Even though this was originally envisioned in the context of insulin deficiency, it was extended to insulin-resistant states. Therefore, it was somewhat surprising when Kelley and Mandarino (21), using the leg balance technique, found that glucose oxidation was increased in leg muscle of type 2 diabetic subjects studied postabsorptively under conditions of fasting hyperglycemia. In fact, leg RQs in individuals with diabetes averaged 0.92 under basal conditions. Furthermore, when

glycemia was reduced to normal levels by a low-dose insulin infusion designed to suppress hepatic glucose output in people with type 2 diabetes, leg glucose oxidation decreased and fat oxidation increased (21). These studies called into question the idea that the traditional glucose fatty acid cycle was responsible for altered basal or insulin-stimulated glucose metabolism in type 2 diabetes. The conclusions were not surprising in light of the fact that muscle of lean healthy subjects predominantly uses lipid as an oxidative fuel (25,26). Kelley and Simoneau (27) extended these results to include uncomplicated obesity (28) and Ivy and colleagues (29,30) showed that obese insulin-resistant rats displayed increased glucose oxidation in skeletal muscle. The studies that used local indirect calorimetry and carbohydrate oxidation under postabsorptive conditions in type 2 diabetes and obesity seem to contradict other studies using systemic indirect calorimetry that indicated either decreased or unchanged glucose oxidation in insulin resistance. The explanation for this apparent discrepancy is likely to result from the fact that resting muscle under postabsorptive conditions contributes only a small fraction of whole-body substrate oxidation. Therefore, the small contribution of muscle to whole-body oxidative metabolism is overwhelmed by fuel oxidation in other tissues, such as the liver, which have a need to be metabolically active in the postabsorptive state.

At the same time, studies by Winder et al. (31) began to point out that increased muscle glucose metabolism in skeletal muscle of the rat led to an increased malonyl CoA concentration. The increase in malonyl CoA inhibited carnitine palmitoyl transferase (CPT)-I and blocked FFA entry into mitochondria (31). Witters et al. (32,33) as well as Winder et al. (34) and Winder and Hardie (35) character-

ized the regulation of acetyl CoA carboxylase, the enzyme responsible for synthesizing malonyl CoA from carbohydrate. Based on these results, it was proposed that if CPT1 was inhibited by increased malonyl CoA derived from glucose, then excess triglyceride or FFA in muscle in insulin-resistant states might lead to increased long-chain acyl CoA concentrations (36,37). An increase in fatty acyl CoAs can lead to increased diacylglycerol (DAG) concentrations, which could also result from partial lipolysis of intracellular triglyceride. DAG, in turn, activates many isoforms of protein kinase C (PKC), including PKC $_{\alpha}$  and PKC $_{\beta}$ . PKC, a serine kinase, can phosphorylate and inhibit tyrosine kinase activity of the insulin receptor as well as tyrosine phosphorylation of insulin receptor substrate (IRS)-1 (38-42). Other fatty acid derivatives have been implicated in altered insulin signaling. For example, ceramide, a sphingolipid derivative of palmitate, inhibits insulin stimulation of glycogen synthase kinase 3 and protein kinase B in a manner similar to that produced by palmitate itself (43).

Interestingly, in that study, neither palmitate nor ceramide inhibited insulin stimulation of the association of phosphatidylinositol (PI) 3-kinase with IRS-1, but acted on more distal steps (43). However, in the only study performed to date in vivo in humans, infusion of lipid to increase FFA concentrations inhibited insulin stimulation of IRS-1-associated PI 3-kinase (44). In yet another proximal step in glucose metabolism, acyl-CoA synthetase-1 is associated with GLUT4-containing vesicles in adipocytes, and fatty acyl CoAs play a role in budding and fusion in membrane trafficking (45). The possibility of lipid-induced abnormalities in the glucose transport system should also be seriously considered. Thus, there is growing evidence that it is not increased fat oxidation that

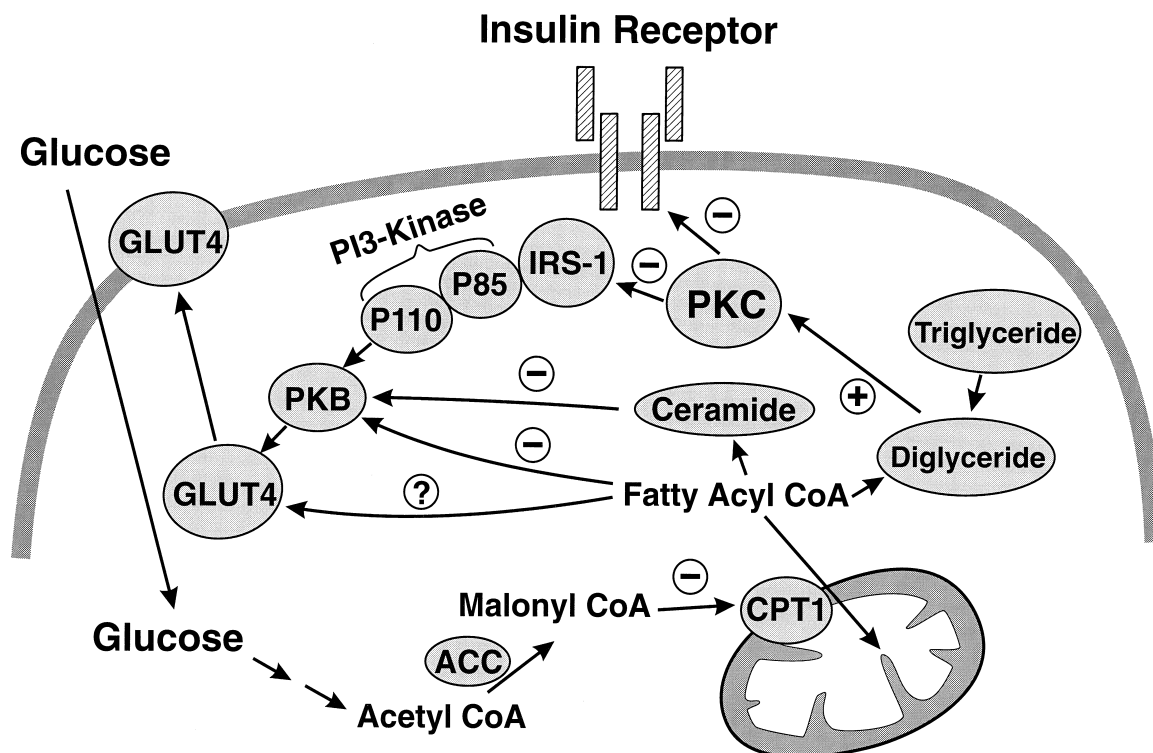


FIG. 2. Potential interactions between lipids and insulin signaling. -, Potential inhibitors; +, potential activators. ACC, acetyl-CoA carboxylase; PKB, protein kinase B.

produces insulin resistance, but instead, that abnormalities in insulin action may arise as a result of an overaccumulation of various lipid species in skeletal muscle cells. These potential mechanisms are depicted in Fig. 2.

To summarize, there is a great deal of evidence that in vivo in healthy humans, infusion of lipid increases fat oxidation and decreases glucose oxidation, providing evidence for the existence of the classical Randle glucose fatty acid cycle. During an insulin infusion, infusion of lipid concomitantly reduces glucose uptake and insulin-stimulated glycogen synthesis. However, there is no evidence in humans that it is actually the increase in fat oxidation that produces insulin resistance. In fact, there is now evidence that in skeletal muscle from insulin-resistant subjects, fat oxidation is actually decreased under postabsorptive conditions, rather than increased. Furthermore, there is a growing body of evidence that long-chain fatty acyl-CoAs themselves may produce insulin resistance. It should be noted that in a recent update of his immense contributions, Randle (46) has incorporated many of these new ideas into an overall theory of glucose and fat competition.

#### EVIDENCE FOR INCREASED LIPID CONCENTRATIONS IN SKELETAL MUSCLE IN INSULIN RESISTANCE

One indicator of an altered pattern of fatty acid metabolism by skeletal muscle in obesity and type 2 diabetes is an increased content of triglyceride within muscle fibers. In human skeletal muscle in obesity, increased triglyceride has been reported on the basis of biochemical extraction of lipids from biopsies of vastus lateralis muscle (47), histological staining with Oil Red O (48), with electron microscopy (49), and by several noninvasive imaging methods, including computed tomography (50–52) and magnetic resonance spectroscopy (53,54), this last method offering the potential to identify the intracellular content of lipid. In animal models, a high-fat diet can induce increased muscle lipid content and this appears to relate to both the temporal development of insulin resistance as well as its severity (55,56). Similarly, in human studies, muscle lipid content is correlated with the severity of insulin resistance, even after adjusting for visceral adiposity (47,52).

Although these findings strongly suggest that lipid accumulation within muscle fibers can be associated with insulin resistance, there is also the paradox that increased triglyceride content can be found within muscle of highly trained athletes. Strenuous exercise can transiently deplete muscle triglyceride, and metabolic studies indicate the importance of this fuel depot for sustained aerobic exercise (57). Because highly trained athletes have normal or enhanced insulin sensitivity, it is apparent that increased lipid content within muscle does not always denote insulin resistance. Therefore, muscle lipid content should be appraised within a context of other markers of metabolic capacity. One such marker is likely to be the oxidative enzyme capacity of skeletal muscle, which is increased in trained athletes, yet diminished in sedentary and obese individuals. In accord with these principles, type 1 muscle fibers generally have a higher lipid content, yet also higher oxidative enzyme capacity, higher rates of uptake of fatty acids, and greater insulin sensitivity for glucose transport than do type 2 muscle fibers (58,59). Exercise training can enhance capacity for fatty acid uptake, including muscle content of fatty acid binding proteins (26,60). These findings suggest that muscle lipid content may

not be adverse if it is occurring within muscle that has a metabolic capacity for efficient lipid utilization. Perhaps another aspect of this is whether there is periodic depletion and repletion of muscle triglyceride. However, these precepts do not appear to apply to skeletal muscle in sedentary and insulin-resistant individuals.

#### MECHANISMS LIMITING FAT USE IN HUMAN MUSCLE

Despite the findings that skeletal muscle in type 2 diabetes or obesity may have reduced efficiency in the uptake of fatty acids from plasma (21,27,61), this reduction does not seem to be the mechanism that limits fat oxidation. Rates of fatty acid uptake were observed to be more than sufficient to account for rates of energy expenditure had the oxidized substrate been exclusively lipid. Moreover, the findings of increased triglyceride accumulation within muscle indicate that the balance between uptake and oxidation favors net accumulation of stored lipid.

Before oxidation within mitochondria, long-chain fatty acids must be activated to long-chain acyl CoA, then translocated into mitochondrial matrix by the enzyme complex, CPT. The muscle isoform of CPT-I is quite sensitive to allosteric inhibition by malonyl CoA, the precursor of fatty acid synthesis (62). Insulin and glucose augment skeletal muscle content of malonyl CoA, consistent with a role in regulating substrate oxidation (63). In animal models of insulin resistance, Ruderman et al. (36) have found increased skeletal muscle content of malonyl CoA during postabsorptive conditions, suggesting potential inhibition of fat oxidation. Anapleurotic surfeit of citrate may be one of the key mechanisms contributing to elevated malonyl CoA concentration (64), but more knowledge concerning binding or compartmentalization of malonyl CoA is needed since concentrations in muscle homogenate, even in insulin-sensitive animals, would be anticipated to yield complete inhibition of CPT-I. Moreover, rigorous testing of the hypothesis that malonyl CoA is increased in skeletal muscle in human volunteers with obesity and type 2 diabetes has not been performed.

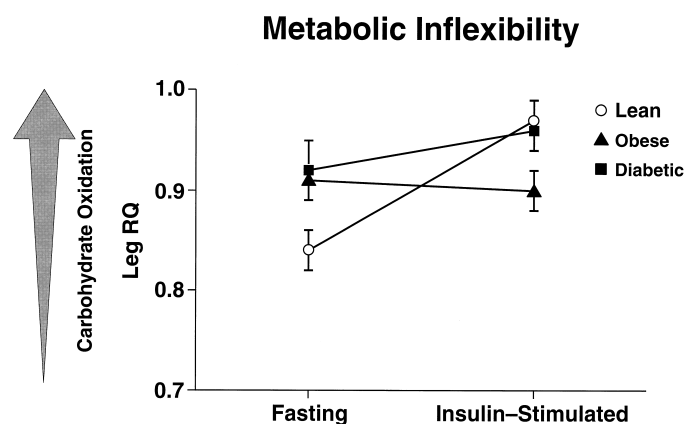
Simoneau et al. (65) found that human vastus lateralis muscle has reduced CPT activity in insulin-resistant obese volunteers who also manifested increased fasting values for RQ across the leg (28). The reduction in CPT activity was proportional to an overall reduction in activity of the oxidative enzymes citrate synthase, cytochrome C oxidase, and hydroxyacyl dehydrogenase; marker enzymes of the Krebs cycle, electron transport, and  $\beta$ -oxidation, respectively (65). Reduced oxidative enzyme activity has also been associated with insulin-resistant glucose metabolism (66–68). Thus, the reduction in CPT activity may reflect reduced mitochondrial content or function rather than a specific impairment for fatty acid oxidation. Some additional evidence pertinent to skeletal muscle mitochondrial metabolism is the finding of increased content of uncoupling protein 2 (UCP2) in obesity and an association between elevated postabsorptive values for RQ across the leg with UCP2 content (69). On the other hand, in these studies of human skeletal muscle, neither the content of cytosolic fatty acid transport protein nor that of the sarcolemmal fatty acyl binding protein (FABP) was diminished in obesity (65). Because the role of FABP is to facilitate movement of fatty acids and acyl CoA, dynamic studies of FABP function are needed to more critically understand the roles of these abundantly expressed proteins in muscle lipid

metabolism. Certainly, considerably more research is needed to delineate regulation of pathways of fatty acid utilization in obesity and type 2 diabetes to understand the mechanisms that lead to lipid accumulation and in relation to insulin-resistant glucose metabolism. Nevertheless, the pioneering studies by the late Jean-Aime Simoneau point to impediments centered at mitochondria and portray that skeletal muscle in obesity and insulin resistance is disposed toward lipid esterification rather than lipid oxidation.

#### METABOLIC INFLEXIBILITY OF FATTY ACID UTILIZATION IN INSULIN RESISTANCE

In lean healthy individuals, skeletal muscle displays substantial metabolic flexibility (70), with the capacity to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasting conditions (25) to the suppression of lipid oxidation and increased glucose uptake, oxidation, and storage under insulin-stimulated conditions (71). Insulin resistance is most clearly characterized as a limited response of muscle to stimulate glucose metabolism. One aspect of this includes resistance to the suppression of lipid oxidation, and, as previously cited, obese and type 2 diabetic patients manifest higher lipid oxidation during insulin-stimulated conditions (72). Therefore, the question arises as to how the seemingly disparate findings of increased lipid oxidation during insulin-stimulated conditions in obesity and type 2 diabetes can be reconciled with the reports that, in these disorders, there are diminished rates of lipid oxidation during fasting conditions. The way in which these 2 seemingly opposite observations can be reconciled is to reemphasize that a key aspect of metabolic fitness in skeletal muscle is its capacity to switch between fuels and that this capacity may be lost in insulin resistance.

In recent studies in insulin-sensitive and obese insulin-resistant subjects, studied during fasting and insulin-stimulated conditions with limb balance methods to examine rates of substrate uptake and oxidation (28), obese subjects had reduced fasting rates of lipid oxidation, yet, during insulin infusions, rates of lipid oxidation by muscle were greater than in lean subjects. As shown in Fig. 3, in lean subjects, there was a sharp transition from a predominant reliance on lipid oxidation during fasting to predominantly glucose oxidation during insulin infusions, accompanied by sharp changes in the respective rates of lipid and glucose oxidation. In contrast, in obese subjects, there was metabolic inflexibility. In obesity, there was not modulation in the relative reliance of lipid and glucose oxidation in comparing fasting and insulin-stimulated conditions. Thus, obese subjects manifested less lipid oxidation during fasting conditions and greater lipid oxidation during insulin-stimulated conditions relative to the lean volunteers, but the absolute rates of lipid oxidation remained fixed in obese subjects. The key point is that the "higher" rate of lipid oxidation during insulin-stimulated conditions does not denote that lipid oxidation is increased in all conditions, but instead is part of an inflexibility in response to either insulin or fasting in the modulation of substrate oxidation. Many chronic illnesses are characterized by the loss of physiologic reserve, and in this context, the pattern of lipid oxidation within skeletal muscle in insulin resistance of obesity manifests disturbances both in adaptation to fasting (by failing to increase) and to the effect of insulin (by failing to suppress). The failure to augment lipid oxidation during fasting conditions likely is a key mechanism leading to lipid accumulation within skeletal muscle, whereas



**FIG. 3. Metabolic inflexibility of oxidative fuel selection in skeletal muscle of insulin-resistant obese and type 2 diabetic subjects. Illustration of the inflexibility in altering fuel choice between carbohydrate and lipid. Leg RQ was determined by indirect calorimetry in lean control subjects (○), obese subjects without diabetes (▲), and subjects with type 2 diabetes (■) after an overnight fast or at the end of a hyperinsulinemic glucose clamp (insulin-stimulated). Leg RQ of the control subjects during fasting of ~0.8 indicated a predominant use of lipid, which switched to carbohydrate during insulin infusion. In contrast, fasting leg RQs in the obese and diabetic subjects were elevated, indicating a predominance of carbohydrate as fuel, and remained unaltered by insulin infusion. Adapted from Kelley and Mandarino (21) and Kelley et al. (28).**

the increased lipid stores that accumulate in muscle may, in turn, contribute to patterns of insulin-resistant glucose metabolism through processes of substrate competition and other mechanisms.

Another important component of the metabolic inflexibility and perturbed patterns of fatty acid oxidation in obesity was the observation that a poor reliance on fatty acid oxidation by skeletal muscle during fasting conditions significantly predicted the severity of insulin-resistant glucose metabolism, as shown in Fig. 3. This observation is complementary to prior observations that "elevated" lipid oxidation during insulin-stimulated conditions is correlated with insulin-resistant glucose metabolism. Again, from our perspective, these are not contradictory observations, quite the opposite; the fasting and insulin-stimulated data are consistent with a formulation of insulin resistance in skeletal muscle that is characterized by metabolic inflexibility. These observations, though only associative in nature rather than truly mechanistic, are useful to extend the "phenotype of insulin resistance" in skeletal muscle beyond defects of insulin-regulated metabolism to a broader concept of poor adaptations to fasting conditions as well.

#### ACKNOWLEDGMENTS

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