Metabolic flexibility and insulin resistance

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Submitted 2 July 2008; accepted in final form 22 August 2008

LIPID ACCUMULATION IN SKELETAL MUSCLE of sedentary people is associated with impaired insulin-stimulated glucose metabolism (31). A reduced capacity of oxidative tissues and organs to adjust lipid oxidation to lipid availability can lead to tissue accumulation of lipids as triglycerides. Excess lipid accretion and/or lower triglyceride turnover can induce lipotoxicity, as reflected by the cellular accumulation of ceramides and diglycerides (39). These lipid species ultimately impair insulin signaling through different mechanisms, either increased serine phosphorylation of the insulin receptor and insulin receptor substrate 1 and/or reduced serine phosphorylation of PKB/Akt (38, 60) (Fig. 1). Therefore, the ability to increase lipid oxidation as a function of their availability eventually reduces the formation of ceramides and diglycerides leading to improved insulin sensitivity.

In general, the ability of a system (i.e., whole organism, organ, tissue, or cell) to adjust fuel oxidation to fuel availability is known as metabolic flexibility. This term was coined by Kelley and Mandarino as “the capacity to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasting conditions to the suppression of lipid oxidation and increased glucose uptake, oxidation, and storage under insulin-stimulated conditions” (26). In line with the above definition, the switch from carbohydrate to lipid oxidation [drop in respiratory quotient (RQ)] during an overnight fast or in response to high-fat diets should also be part of the assessment of metabolic flexibility (Fig. 2).

There is now a growing interest to assess the influence of metabolic flexibility, particularly to dietary fat as a mechanism to explain how lipids can accumulate in skeletal muscle. The switch in fuel oxidation will depend on the type and amount of nutrient available for oxidation at the cellular level. In tissues and organs, fuel availability (glucose, fatty acids, and amino acids) is integrated at the cellular level by fuel sensors that activate or inhibit specific metabolic pathways (47, 50). In response to fuel oversupply, anabolic pathways are activated, whereas the activity of hydrolytic and lipolytic pathways is increased when fuel availability is restricted. In addition, the ability to change substrate oxidation in response to nutritional status will depend on the genetically determined balance between cellular oxidation and storage capacities.

For example, in skeletal muscle, white (glycolytic) or red (oxidative) muscle homogenates respond differently to a supply of fatty acids or glucose. Glycolytic fibers have low rates of
fat oxidation compared with oxidative fibers (30), which have high mitochondrial density and oxidative enzyme activities. As a consequence, the oxidative capacity of skeletal muscle may be of utmost importance to boost lipid oxidation to the level of lipid supply and therefore modulate insulin sensitivity. If skeletal muscle cannot match fat oxidation to lipid uptake, fat accumulation will ensue, which in turn will cause insulin resistance.

This review discusses the studies in which metabolic flexibility has been measured at the whole body level or specifically in skeletal muscle tissue or in muscle cells with particular emphasis on the comparison between insulin-resistant and insulin-sensitive individuals. In addition, we discuss the main determinants of metabolic flexibility, how metabolic flexibility should be measured, and which questions need to be answered to better understand the pathophysiology of insulin resistance.

Metabolic Flexibility and Macronutrient Oxidative Regulation

During long-term energy balance, macronutrient oxidation eventually has to match macronutrient intake such that no macronutrients are stored or lost (10). In other words, not only does 24-h energy expenditure have to be equal to 24-h energy intake, but 24-h RQ has to be equal to 24-h food quotient (FQ). The 24-h RQ corresponds to the mean proportion of macronutrient oxidized over a day, whereas 24-h FQ represents the proportion of daily dietary macronutrients available for oxidation (5). Many studies have shown that when people are in energy balance then 24-h RQ eventually matches 24-h FQ (6,
21, 44, 53, 56, 58). Increased availability of carbohydrate results in a rapid increase in carbohydrate oxidation associated with concomitant suppression of lipid oxidation. However, when dietary fat intake increases, one observes a much slower progressive increase in lipid oxidation paralleled by suppression of carbohydrate oxidation.

Both day-to-day variations in energy/macronutrient intake and day-to-day changes in energy expenditure lead to either slightly positive or negative energy balance. In response to these short-term variations in energy balance, carbohydrate and protein stores are closely regulated by an adjustment of oxidation to intake. Consequently, positive or negative energy balances are mostly buffered by changes in fat stores as evidenced by the tight correlation between fat storage and energy balance (1, 9). It is therefore only after short periods of time (one to a few days) that a difference in metabolic flexibility (switch from one type of fuel to another) can be observed. This situation can be particularly relevant in individuals exposed to an obesogenic environment (i.e., high-energy density diets and low physical activity). Whether the capacity to adapt fuel oxidation to fuel availability is preserved or impaired in states of insulin resistance is discussed below.

Metabolic Flexibility in Individuals with Insulin Resistance, Obesity or Family History of Type 2 Diabetes

Metabolic flexibility in response to fasting. Fatty acids are the main readily available energy sources during the transition from the fed to the overnight-fasted condition, as indicated by the progressive fall in RQ. An impaired drop in RQ during an overnight fast (high fasting RQ) may be defined as metabolic resistance. Although the rationale for metabolic resistance is discussed below.

Metabolic flexibility in response to high-carbohydrate meals. Metabolic flexibility in response to high-carbohydrate meals has not been frequently compared in subjects with different insulin sensitivity. Recently, the change in whole body RQ in subjects with and without a family history of type 2 diabetes was assessed (20). Individuals with a family history of diabetes usually have a lower insulin-stimulated glucose disposal rate during a hyperinsulinemic clamp, although it was not the case in that study. However, in response to a high-carbohydrate meal (1,000 kcal, 76% energy from carbohydrate), individuals with a family history of diabetes had a higher plasma insulin concentration and a similar increase in RQ compared with control subjects.

Metabolic flexibility during a hyperinsulinemic clamp. The increase in RQ during a euglycemic-hyperinsulinemic clamp (ΔRQ) is the original and now common approach to evaluate the metabolic flexibility to carbohydrate (Fig. 2B). The advantage of the clamp procedure is that plasma glucose and insulin concentrations are carefully matched among subjects, even when they have different nutritional and/or metabolic conditions. Kelley and Mandarino (26) and others (14, 24, 65) described an impaired capacity to increase muscle and whole body glucose oxidation and storage during the clamp in insulin-resistant subjects. Additionally, when insulin sensitivity was improved after weight loss, a concomitant enhancement in metabolic flexibility and glucose disposal were observed (14, 35). These data indicate that the increase in ΔRQ is reflecting the amount of glucose entering the cells and being available for oxidation and storage (see below and Ref. 14).

Despite a number of studies that have shown impaired metabolic flexibility during a clamp in insulin-resistant vs. insulin-sensitive individuals, studies are lacking to identify the mechanisms linking metabolic inflexibility to glucose and insulin resistance.

Metabolic flexibility to high-fat meal. High-fat diets usually lead to positive energy balance, since fat-rich foods are more palatable and have higher energy density than other foods (49). Fat overload may represent a metabolic challenge for many individuals, even in energy balance conditions, who may indeed fail to appropriately upregulate skeletal muscle lipid oxidation, therefore causing intracellular lipid accumulation and insulin resistance. Although the rationale for metabolic
inflexibility to lipid may appear obvious, few studies have so far investigated metabolic flexibility to lipid in individuals with contrasting degrees of insulin sensitivity.

Kelley and Simoneau (28) compared leg RQ in the postprandial state of nondiabetic vs. weight-matched diabetic subjects in response to a high-fat meal (737 kcal, 62% energy from fat). During postabsorptive conditions, diabetic individuals had higher RQ throughout the 6 h following the high-fat meal compared with nondiabetic subjects. Apparently, mitochondrial function was preserved, since key skeletal muscle mitochondrial markers such as citrate synthase (mitochondrial number), cytochrome c oxidase (electron transport chain activity), and 3-hydroxyacyl-CoA dehydrogenase activities were similar in both groups. On the other hand, the large difference in postprandial glycemia (~2-fold) and lower skeletal muscle FFA uptake observed in diabetic vs. nondiabetic subjects likely drove the difference in leg RQ.

At the whole body level, the drop in RQ after a high-fat meal (1,000 kcal, 76% energy from fat) was examined in individuals with similar insulin sensitivity but with positive or negative family history of type 2 diabetes (20). In both groups, the changes in plasma glucose, FFA, and insulin concentrations were similar; however, individuals without a history of diabetes had larger decrease in RQ compared with offspring from diabetic parents. The lower reliance on fat oxidation for energy supply in offspring from diabetic parents suggests that impaired fat oxidation might precede insulin resistance.

In contrast, a study in 113 lean and 701 obese subjects who ate ~50% of their daily energy requirement as fat, showed that insulin resistance measured by homeostasis model assessment of insulin resistance (HOMA-IR) was associated with increased postprandial fat oxidation (as percentage of energy expenditure) after controlling for confounding variables such as fat mass, fasting fat oxidation, sex, and physical activity (4).

Prolonged metabolic flexibility in response to diets. METABOLIC FLEXIBILITY TO HIGH-CARBOHYDRATE DIETS. Few studies have used the approach of prolonged dietary carbohydrate supplementation to evaluate the metabolic flexibility to carbohydrate, and none of them have evaluated muscle fuel metabolism (Fig. 2C). A carefully controlled study assessed the increase in 24-h RQ in lean and obese subjects who received a mixed diet for 3 days (50% energy from carbohydrate) containing twice the daily energy requirement (64). Surprisingly, both groups experienced a similar increase in RQ in response to the dietary challenge. Using a similar approach, Freymond et al. (12) compared children of obese and nonobese parents. Children of obese parents were heavier and tended to be fatter than children of nonobese parents. Both groups were evaluated under eucaloric conditions and after 3 days of progressive overfeeding with a mixed diet (2-fold energy excess on the 3rd day). There was no difference in the change in the 24-h RQ between groups after the 3-day overfeeding period. Both studies suggest similar metabolic flexibility to carbohydrate-enriched diets in obese and lean individuals.

METABOLIC FLEXIBILITY TO HIGH-FAT DIETS. Metabolic flexibility to lipid has seldom been evaluated in the context of insulin resistance (Fig. 2D). Ukropcova et al. (62) assessed the body’s ability to adjust 24-h fat oxidation to a 3-day eucaloric high-fat diet (50% energy from fat) in subjects with or without a family history of type 2 diabetes but similar insulin-stimulated glucose disposal rates. After 3 days of a high-fat diet, both groups had a similar decrease in 24-h RQ and therefore a similar increase in fat oxidation. However, a lesser decrease in RQ was observed during the sleeping period in the offspring of diabetic parents compared with controls. No association between sleep RQ and insulin sensitivity was observed, but a negative association with muscle mitochondrial content was found (62).

Metabolic flexibility in vitro. Metabolic flexibility in vitro has been assessed in cultures of human myotubes. Unlike in vivo skeletal muscle, cultured myotubes are not influenced by the physiological milieu, which is kept constant. Therefore, the variability in the response to FFA, glucose, or insulin is determined mostly by genetic and/or epigenetic factors controlling metabolic pathways. Ukropcova et al. (61) investigated whether substrate switching is preserved in cultured myotubes and reflects the metabolic characteristics of the donors. Those authors investigated the suppression of palmitate oxidation by glucose in the absence of insulin, which was termed in vitro “suppressibility”. Contrary to expectations, in vitro suppressibility of fat oxidation was lower in subjects with higher whole body insulin sensitivity and higher metabolic flexibility to glucose. Since the in vivo measurement was done under insulin-stimulated conditions whereas the in vitro assay was performed without insulin, it may be inappropriate to compare these findings.

Similar studies have been designed to evaluate the capacity of myotubes to oxidize fat in response to lipid exposure (15). The increase in fat oxidation in response to palmitate called in vitro “adaptability” was positively related to in vivo insulin sensitivity and metabolic flexibility. In addition, in vitro adaptability was positively related to aerobic capacity, suggesting that myotubes established from lean, fit, insulin-sensitive subjects are more metabolically flexible in response to palmitate exposure in vitro. Similarly, palmitate oxidation has been shown to be lower in myotubes established from type 2 diabetic vs. matched nondiabetic controls (16). Together, these data suggest that intrinsic defects in fat oxidation are present in the skeletal muscle of insulin-resistant subjects. This is somehow consistent with studies showing reduced mitochondrial carnitine palmitoyltransferase I (CPT I) activity in the muscle of sedentary obese subjects with and without type 2 diabetes (29, 57). On the other hand, such impaired mitochondrial lipid oxidation could simply be the result of reduced energy demand and not necessarily due to impaired substrate switching. Indeed, the suppression of glucose oxidation in response to palmitate in the presence of insulin was similar in myotubes established from lean, obese, and type 2 diabetic donors (15).

Together, the above results are difficult to interpret in the context of mitochondrial dysfunction, metabolic flexibility, and insulin resistance. In fact, none of these studies have reported whether differences in mitochondrial density and/or activity may be the underlying mechanism explaining the variability in muscle lipid oxidation and/or lipid accumulation between insulin-resistant and insulin-sensitive individuals.

Determinant Factors of Metabolic Flexibility

Significant differences in mitochondrial number, structure and function have been described between insulin-resistant and insulin-sensitive subjects (3, 25, 36, 37, 40, 42, 43, 48, 59). The hypothesis that mitochondrial abnormalities may be a primary cause of metabolic inflexibility and insulin resistance has been
raised but the causal link between the two still remains to be established (38). However, metabolic flexibility also depends on the rate at which nutrients are available to the cells, the ability of adipose tissue to handle fatty acids and even the method used for its calculation (i.e., \( \Delta \text{RQ} \)). Below, we discuss the main variables to take into account when metabolic flexibility is assessed.

**Baseline RQ.** The difference between baseline and stimulated RQ (\( \Delta \text{RQ} \)) is the usual way to estimate metabolic flexibility in response to meals, diets or euglycemic-hyperinsulinemic clamps. Depending on the testing paradigm, baseline RQ corresponds to the fasting RQ (hyperinsulinemic clamp or meal) or the initial 24-h RQ (diet). Accordingly, impaired metabolic flexibility may result from both a lower stimulated RQ and/or an elevated baseline RQ. In fact, fasting RQ is inversely related to metabolic flexibility to carbohydrate during a clamp (Fig. 3A). Fasting RQ is highly sensitive to differences in energy balance and diet composition within the few days preceding the measurement (22, 34, 54). Therefore, differences in metabolic flexibility may be explained by insufficient control of such variables. Subjects in negative energy balance or fed a high-fat diet have lower baseline RQ, increasing the potential to influence \( \Delta \text{RQ} \) in response to energy macronutrients. The following example shows the influence of the baseline RQ on metabolic flexibility assessed by the \( \Delta \text{RQ} \) (Table 1).

Two well-matched individuals (A and B) are maintained under energy balance conditions in a metabolic chamber. They are fed for 1 wk with diets having different FQs (subject A = 0.92 and subject B = 0.82). Then, for another week, both receive a diet with an FQ of 0.75. Since both subjects are in energy balance, 24-h RQ is expected to match FQ at the end of each week. Using the \( \Delta \text{RQ} \) as an index of metabolic flexibility, subject A will be characterized as flexible (\( \Delta \text{RQ} = -0.17 \)) and subject B as inflexible (\( \Delta \text{RQ} = -0.07 \)). Since both subjects were able to match the 24-h RQ to FQ at the end of each week, both subjects should be considered similarly flexible.

This example clearly shows the relevance of having similar baseline RQ values when metabolic flexibility is assessed by the change in RQ. In addition, this example raises the concept of the time required to achieve a new equilibrium, since after 7–10 days it is generally expected that all individuals will be in equilibrium between 24-h RQ and FQ. However, subjects with higher metabolic flexibility will reach this equilibrium faster.

Consequently, the assessment of metabolic flexibility should take into account the differences in baseline RQ and the timing required to match 24-h RQ to FQ. One alternative to control for the baseline RQ is to include this factor as a covariate in a regression analysis model. Such an approach requires large sample sizes, something often difficult to afford in clinical studies. Undoubtedly, the best option to reduce the variability in baseline RQ is to maintain individuals for a period of time on a given diet under energy balance conditions to match 24-h RQ to FQ. The next step is to evaluate the differences in the timing required to reach the equilibrium between fuel oxidation and availability. To do that, one can measure the day-to-day change in RQ in a respiratory chamber and calculate the number of days necessary to reach 50% of the expected change in RQ (\( \Delta \text{RQ}_{50\%} \)). Such a \( \Delta \text{RQ}_{50\%} \) may be in the range of 1–2 days in response to high-carbohydrate diets and in the range of 3–5 days in response to high-fat diets.

**Glucose disposal rate.** Metabolic inflexibility to glucose and impaired glucose storage during a hyperinsulinemic clamp is consistently reported in insulin-resistant subjects. In fact, a direct relationship between metabolic flexibility to glucose and glucose disposal rate is described (14, 24, 61, 62) (Fig. 3B). We (14) recently described that insulin-stimulated glucose disposal rate is the main determinant of the change in RQ during a clamp, explaining ~50% of its variance. The commonly reported metabolic inflexibility and impaired glucose storage in insulin-resistant individuals is expected, since cellular glucose uptake is decreased and cellular glucose available for oxidation and storage is low (8). As a consequence, data corrected for glucose disposal rate, a mechanism proximal to glucose oxidation, indicates no difference in metabolic flexibility and nonoxidative glucose disposal rate between subjects with or without type 2 diabetes and matched for body mass index, sex, and race. Furthermore, after controlling for glucose disposal rate, no improvement in metabolic flexibility is observed after
weight loss in type 2 diabetic individuals subjected to a 1-yr intensive lifestyle intervention including energy restriction and increased physical activity (14). When subjects were divided into quartiles of insulin sensitivity, the insulin-sensitive group (upper quartile; n = 25) had about a fourfold higher glucose disposal rate, nonoxidative glucose disposal rate, and increased steady-state RQ compared with the insulin-resistant group (lower quartile; n = 25). However, after adjustment for differences in glucose disposal rates, there was no longer any difference in nonoxidative glucose disposal rate and steady-state RQ between groups (Ref. 14; Table 2). Further support for these findings comes from studies in which glucose disposal rates were matched by increasing the glucose or insulin infusion rates (27, 66). Together, the results indicate that the impaired metabolic flexibility and glucose storage so often observed during a clamp in insulin-resistant individuals are the consequence of impaired glucose transport rather than a defective cellular glucose oxidative and nonoxidative metabolism.

Adipose tissue lipid storage capacity and plasma FFA concentration. Just as intracellular glucose availability influences metabolic flexibility, higher plasma lipid concentration also drives fuel oxidation by increasing fat oxidation (14, 33, 46, 52). Additionally, high plasma FFA concentration impairs insulin-stimulated glucose disposal rate and decreases intracellular glucose, which in turn reduces glucose oxidation and consequently enhances lipid oxidation (2, 8).

Since adipose tissue is the main source of plasma FFA, the capacity to store and release FFA from adipose tissue may influence metabolic flexibility (11). For instance, patients with lipodystrophy (partial or total absence of subcutaneous adipose tissue) have similar fat oxidation than control individuals when fed a eucaloric, mixed diet (51). However, in response to tissue) have similar fat oxidation than control individuals when fed a eucaloric, mixed diet (51). However, in response to

Table 1. Influence of baseline RQ on metabolic flexibility assessed by the change in RQ (ΔRQ)

<table>
<thead>
<tr>
<th>Subject</th>
<th>FQ</th>
<th>24-h RQ*</th>
<th>FQ</th>
<th>24-h RQ*</th>
<th>Δ24-h RQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.92</td>
<td>0.92</td>
<td>0.75</td>
<td>0.75</td>
<td>-0.17</td>
</tr>
<tr>
<td>B</td>
<td>0.82</td>
<td>0.82</td>
<td>0.75</td>
<td>0.75</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

Subjects A and B are two well-matched individuals maintained under energy balance conditions in a metabolic chamber. RQ, respiratory quotient. They were fed for 1 wk with diets having different food quotients (FQ). Then, for another week they received a diet with an FQ of 0.75. *Value at the end of the respective week.

Mitochondrial oxidative capacity. It is known that mitochondrial content and activity determine fatty acid oxidation in response to lipid. This has been shown by comparing fatty acid oxidation rates between homogenates of red (oxidative) and white (glycolytic) skeletal muscles (30). These data and the evidence indicating multiple mitochondrial abnormalities in type 2 diabetic and insulin-resistant individuals (3, 25, 36, 37, 40, 42, 43, 48, 59) led to the hypothesis that lower mitochondrial capacity is associated with reduced resting lipid oxidation and therefore increased muscle lipid accumulation. Such muscle mitochondrial dysfunction was suggested to be an intrinsic characteristic of muscle cells, since fat oxidation in response to palmitate was lower in myotubes from insulin-resistant vs. insulin-sensitive individuals (16, 61). However, comparisons among obese, diabetic, and lean individuals have failed to appropriately match the groups for physical fitness, opening up the possibility that mitochondrial dysfunction may be related to lower physical activity in metabolically inflexible subjects (45).

Independently of the origin of these mitochondrial abnormalities, it is not clear whether the extent to which muscle mitochondria are impaired is sufficient to influence metabolic flexibility to lipid, especially in resting conditions. Some suggestion comes from the comparison between subjects with and without a history of type 2 diabetes (62). The former group had on average a 22% less mitochondrial DNA copy number (marker of mitochondrial number) than control subjects. Increased mitochondrial number was related to a higher decrease in sleeping RQ in response to a 3-day isoenergetic high-fat diet. However, when mitochondrial number was related to 24-h RQ after the high-fat diet period, no association between mitochondrial number and the change in 24-h RQ was observed. Sleeping RQ may be relevant, because under this condition most of the energy comes from lipid. However, since energy expenditure is minimal in resting conditions, the absolute lipid oxidative demand is hardly a metabolic challenge for muscle mitochondria. As indicated in the previous section, a higher decrease in RQ after fat overload may well be a consequence of reduced adipose tissue lipid storage capacity.

Another interesting experimental paradigm is to assess the metabolic flexibility to lipids (e.g., meals or diets) between athletes and sedentary individuals. Clearly, one knows that athletes have better muscle oxidative capacity (i.e., mitochondrial number and activity); however, such a simple study has not been performed yet. Alternatively, elderly individuals matched for body mass and fat content to young volunteers showed on average 26% lower maximal aerobic consumption (VO2max) vs. young individuals (6). Since VO2max has been shown to be directly related to muscle mitochondrial density (62, 63), one could expect an improved metabolic flexibility in young vs. elderly individuals. However, after 4 days of intervention, both groups were similarly able to match whole body fuel oxidation to fuel intake.

Table 2. Steady-state RQ between subjects with high and low insulin-stimulated glucose disposal rate

<table>
<thead>
<tr>
<th>Highest Insulin Sensitivity</th>
<th>Lowest Insulin Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male</td>
<td>19/6</td>
</tr>
<tr>
<td>DM/non-DM</td>
<td>4/21</td>
</tr>
<tr>
<td>White/black/other</td>
<td>15/10/0</td>
</tr>
<tr>
<td>Age, yr</td>
<td>54.3 ±1.4</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>32.9 ±0.5</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>39 ±1.5</td>
</tr>
<tr>
<td>GDR, mg·kg·FFM⁻¹·min⁻¹</td>
<td>11.8 ±0.4</td>
</tr>
<tr>
<td>Nonoxidative GDR, mg·kg·FFM⁻¹·min⁻¹</td>
<td>8.5 ±0.5</td>
</tr>
<tr>
<td>Steady-state RQ</td>
<td>0.94 ±0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, †P > 0.44 after controlling for sex, race, presence of type 2 diabetes mellitus (DM), age, and insulin-stimulated glucose disposal rate (GDR; P < 0.02 when values are not controlled for GDR).

AJP-Endocrinol Metab • VOL 295 • NOVEMBER 2008 • www.ajpendo.org
On the other hand, fuel oxidation may not necessarily be reduced in presence of mitochondrial dysfunction. For instance, frank mitochondrial dysfunction in humans (e.g., myopathies with impaired electron transport chain activity) is accompanied by increased fuel oxidation to compensate for the impaired mitochondrial ATP synthesis (13). Similarly, mice with muscle-specific PGC1α (PPARγ coactivator-1α) deletion have defective mitochondrial function (e.g., lower staining for cytochrome c oxidase and succinate dehydrogenase); however, they have increased metabolic rate, lower RQ, and improved insulin-stimulated glucose uptake in skeletal muscle compared with wild-type animals (19). Therefore, mitochondrial dysfunction may well be associated with high fuel oxidation and enhanced insulin sensitivity.

Another hypothesis has recently been proposed by Koves et al. (30), stating that lipid oversupply can promote excessive lipid oxidation, which will disconnect the coupling between β-oxidation and the TCA cycle, generating excessive amounts of incompletely oxidized acyl-carnitine intermediates. The latter lipid species may interfere with insulin signaling and glucose transport through currently unknown mechanisms. This is in line with the finding that high-fat diets increase mitochondrial biogenesis and fat oxidation in parallel with currently unknown mechanisms. The rate at which fat oxidation adjusts to high fat intake is variable among individuals (21, 53), but only scarce evidence exists about which factors determine this variability. However, this adaptation cannot take much more time than that required to deplete the glycogen stores, i.e., a few days to a week. The question then becomes: is there any importance in assessing the metabolic flexibility to lipid, since eventually fat oxidation will match fat intake in all individuals? The answer is yes, since the speed of the adaptation will probably impact the amount of lipid accumulation in the muscle and therefore impact insulin sensitivity. Individuals able to increase fat oxidation quickly in response to day-to-day changes in fat intake will eventually have lower muscle fat accumulation and be less prone to insulin resistance compared with slower adapters. On the basis of the RQ profile for metabolically flexible and inflexible subjects shown in Fig. 2D, and considering an energy intake of 2,500 kcal/day (protein intake 20% of total energy), the metabolically inflexible individuals would accumulate ~200 g more fat than flexible subjects after 1 wk of a high-fat diet (60% of total energy).

In conclusion, evidence indicating mitochondrial defects as a driving factor of metabolic inflexibility and insulin resistance are far from conclusive or even unavailable. It will be important to test whether whole body or skeletal muscle metabolic flexibility to lipid is affected by muscle mitochondrial characteristics such as density, morphology, and activity. In addition, the role of metabolic flexibility in muscle lipid accumulation and the development of insulin resistance requires further studies. Only this kind of data will allow us to establish a causal link among impaired capacity to metabolize fat, muscle lipotoxicity, and insulin resistance.
ACKNOWLEDGMENTS

We acknowledge Dr. Darcy Johannsen and Stacy Carling for their valuable help editing the manuscript.

GRANTS

J. Galgani is supported by a fellowship from The International Nutrition Foundation/Ellison Medical Foundation. E. Ravussin is supported by National Institutes of Health grants U01-AG-020478 and RO1-DK-60412.

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