Metabolic Flexibility and Insulin Resistance

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Abstract

Metabolic flexibility is the capacity for the organism to adapt fuel oxidation to fuel availability. The inability to modify fuel oxidation in response to changes in nutrient availability has been implicated in the accumulation of intramyocellular lipid and insulin resistance. The metabolic flexibility assessed by the ability to switch from fat to carbohydrate oxidation is usually impaired during a hyperinsulinemic clamp in insulin-resistant subjects; however, this “metabolic inflexibility” is mostly the consequence of impaired cellular glucose uptake. Indeed, after controlling for insulin-stimulated glucose disposal rate (amount of glucose available for oxidation), metabolic flexibility is not altered in obesity regardless of the presence of type 2 diabetes.

To understand how intramyocellular lipids accumulate and cause insulin resistance, the assessment of metabolic flexibility to high-fat diets is more relevant than metabolic flexibility during a hyperinsulinemic clamp. An impaired capacity to up-regulate muscle lipid oxidation in face of high lipid supply may lead to increased muscle fat accumulation and insulin resistance. Surprisingly, only very few studies have investigated the response to high-fat diets. This review, we discuss the role of glucose disposal rate, adipose tissue lipid storage and mitochondrial function on metabolic flexibility. Additionally, we emphasize the bias of using the change in respiratory quotient to calculate metabolic flexibility and propose novel approaches to assess metabolic flexibility.

Based on current evidence, one cannot conclude that impaired metabolic flexibility is responsible for the accumulation of intramyocellular lipid and insulin resistance. We propose to study metabolic flexibility in response to high-fat diets in

individuals having contrasting degree of insulin sensitivity and/or mitochondrial characteristics.

Keywords: Fuel selection, insulin sensitivity, mitochondria, lipid oxidation, skeletal muscle.

I. Introduction

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 triglycerides 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 the insulin receptor substrate 1, and/or reduced serine phosphorylation of PKB/AKt (38, 60) (Figure 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 & 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 RQ) during an overnight fast or in response to high-fat diets should also be part of the assessment of metabolic flexibility (Figure 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 which 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 when compared to oxidative fibers (30), which have high mitochondrial density and oxidative enzymes 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.

II. Metabolic flexibility and macronutrient oxidative regulation

During long-term energy balance, macronutrient oxidation has to eventually 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.

III. Metabolic flexibility in individuals with insulin resistance, obesity or a family history of type 2 diabetes

1. 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 inflexibility to lipid (Figure 2A). Several studies suggest that fasting RQ is elevated in skeletal muscle from obese insulin-resistant (24) and type 2 diabetic adults (28). Similar findings have been reported at whole-body level in obese adolescents (41) and subjects with a family history of type 2 diabetes (7, 32). Furthermore, obese insulin-resistant individuals subjected to a 16-week exercise and weight loss program enhanced their insulin sensitivity associated with a reduction in whole-body fasting RQ (17). The relationship between fasting RQ and insulin sensitivity has however not always been observed. For instance, no association was found between fasting RQ and insulin-stimulated glucose disposal rate in healthy volunteers (61, 62) and Blaak et al. (4) reported lower fasting RQ in obese compared to lean subjects.

Differences in energy balance and macronutrient dietary composition are key determinant factors of the variability in fasting RQ or fuel oxidation. Accordingly, the response to a one-day drastic increase in energy intake (~4500 Kcal/d) preceded and followed by underfeeding (500 Kcal/d) provided quite

different 24-h RQ values (0.80, 0.88 and 0.85, respectively) (54). Additionally, consumption of a eucaloric high-fat or high-carbohydrate diet for one week modified the fasting RQ according to the diet FQ (22, 34). Interestingly, when macronutrient intake and energy balance were carefully controlled, fasting RQ measured in a respiratory chamber for 48 h was similar between obese and lean subjects (64).

In addition to the influence of energy balance and diet composition, RQ is also affected by plasma substrate concentrations (e.g., glucose, FFA). Thus, as a result of hyperglycemia, glucose-dependent muscle glucose uptake can be increased leading to higher RQ. On the other hand, when ketone bodies are synthesized without further oxidation, then a reduction in RQ is expected (55). These factors can eventually explain part of the differences in fasting RQ, particularly in people with diabetes. Another aspect to take into account when measuring metabolic flexibility in insulin resistant vs. insulin sensitive people is the difference between whole-body and muscle RQ. Kelley et al. (23) observed that whole-body RQ was directly correlated with leg RQ in non-diabetic individuals but not in type 2 diabetic subjects.

Future clinical studies must therefore be designed to ensure that participants are in energy balance and fed standardized diets of similar nutrient composition. Particular attention must be given to RQ data interpretation when differences in plasma glucose and/or FFA concentrations are observed. Finally, the assessment of whole-body RQ as a marker of metabolic flexibility can lead to erroneous conclusion about skeletal muscle metabolism, at least in people with type 2 diabetes.

2. Acute metabolic flexibility in response to nutrients

i) Metabolic flexibility to high-carbohydrate meal

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 have usually lower insulin-stimulated glucose disposal rate during a hyperinsulinemic clamp, although it was not the case in this study. However, in response to a high-carbohydrate meal (1000 Kcal, 76% energy from carbohydrate), individuals with a family history of diabetes had higher plasma insulin concentration and similar increase in RQ when compared to control subjects.

ii) Metabolic flexibility during a hyperinsulinemic clamp

The increase in RQ during a euglycemic-hyperinsulinemic clamp (∆RQ) is the original and now common approach to evaluate the metabolic flexibility to carbohydrate (Figure 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 & 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). This data indicate that the increase in ∆RQ 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.

iii) 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 up-regulate skeletal muscle lipid oxidation therefore causing intracellular lipid accumulation and insulin resistance. Although the rationale for metabolic inflexibility to lipid may appear obvious, only few studies investigated so far metabolic flexibility to lipid in individuals with contrasting degrees of insulin sensitivity.

Kelley & Simoneau (28) compared the leg RQ in the postprandial state of non-diabetic vs. weight-matched diabetic subjects in response to a high-fat meal (737 Kcal, 62% energy from fat). During post-absorptive conditions, diabetic individuals had higher RQ throughout the 6 hours following the high-fat meal when compared to non-diabetic 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. non-diabetic subjects likely drove the difference in leg RQ.

At the whole-body level, the drop in RQ after a high-fat meal (1000 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 history of diabetes had larger decrease in RQ when compared to 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 approximately 50% of their daily energy requirement as fat, showed that insulin resistance measured by HOMA-IR was associated with increased postprandial fat oxidation (as percent of energy expenditure) after controlling for confounding variables such as fat mass, fasting fat oxidation, gender and physical activity (4).

3. Prolonged metabolic flexibility in response to diets

i) 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 (Figure 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 similar increase in RQ in response to the dietary challenge. Using a similar approach, Freymond et al. compared children offspring from obese and non-obese parents (12). Offspring from obese parents were heavier and tended to be fatter than offspring from non-obese parents. Both groups were evaluated under eucaloric conditions and after 3 days of progressive overfeeding with a mixed diet (two-fold energy excess on the third 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.

ii) Metabolic flexibility to high-fat diets

Metabolic flexibility to lipid has seldom been evaluated in the context of insulin resistance (Figure 2D). Ukropcova et al. 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 family history of type 2 diabetes, but similar insulin-stimulated glucose disposal rates. After 3 days of high-fat diet, both groups had similar decrease in 24-h RQ and therefore similar increase in fat oxidation. However, a lesser decrease in RQ was observed during the sleeping period in the offspring from diabetic parents when compared to controls. No association between sleep RQ and insulin sensitivity was observed, but a negative association with muscle mitochondrial content was found (62).

4. 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 mostly determined by genetic and/or epigenetic factors controlling metabolic pathways. Ukropcova et al. investigated whether substrate switching is preserved in cultured myotubes and reflects the metabolic characteristics of the donors (61). The authors investigated the suppression of palmitate oxidation by glucose in absence of insulin, which was termed *in vitro* “suppressibility”. Contrary to the 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 while 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 and 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 non-diabetic controls (16). Together, these data suggest that intrinsic defects in fat oxidation are present in the skeletal muscle of insulin resistance subjects. This is somehow

consistent with studies showing reduced mitochondrial CPT-I activity in the muscle of sedentary obese with and without type 2 diabetes (29, 57). On the other hand, such impaired mitochondrial lipid oxidation could be simply 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 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.

IV. 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., ΔRQ). Below, we discuss the main variables to take into account when metabolic flexibility is assessed.

1. Baseline respiratory quotient

The difference between baseline and stimulated RQ (∆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 (Figure 3A). Fasting RQ is highly sensitive to differences in energy balance and diet composition within the few days preceding the measure (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 ∆RQ in response to energy macronutrients. The following example shows the influence of the baseline RQ on metabolic flexibility assessed by the ∆RQ (see Table 1). *"Two well-matched individuals (A and B) are maintained under energy balance conditions in a metabolic chamber. They are fed for one week with diets having different FQs (subject A = 0.92 and subject B = 0.82). Then, for another week both receive a diet with 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 ∆RQ as an index of metabolic flexibility, subject A will be characterized as flexible (∆RQ = -0.17) and subject B as inflexible (∆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 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 in order 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 ($\Delta RQ_{50\%}$). Such a $\Delta RQ_{50\%}$ 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.

2. 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) (Figure 3B). We recently described that insulin-stimulated glucose disposal rate is the main determinant of the change in RQ during a clamp, explaining approximately 50% of its variance (14). 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 non-oxidative 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 one-year 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 4-fold higher glucose disposal rate, non-oxidative glucose disposal rate and increased steady-state RQ when 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 non-oxidative glucose disposal rate and steady-state RQ between groups (“J.E. Galgani et al, unpublished observation”; 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 non-oxidative metabolism.

3. 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 an excess energy as fat, lipodystrophic subjects have higher fat oxidation compared to control individuals (51). Accordingly, a lesser decrease in RQ after a fat overload might be the result of enhanced adipose tissue lipid storage capacity rather than impaired lipid oxidative capacity.

4. Mitochondrial oxidative capacity

It is known that mitochondrial content and activity are determining fatty acid oxidation in response to lipids. 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 to 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 if the extent at 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 history of type 2 diabetes (62). The former group had on average 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 simple study has not been performed yet. Alternatively, in elderly individuals matched for body mass and fat content to young volunteers showed on average 26% lower maximal aerobic consumption (VO_2 max) vs. young individuals (6). Since VO_2 max 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.

On the other hand, fuel oxidation may not be necessarily 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 the impaired mitochondrial ATP synthesis (13). Similarly, mice with muscle-specific PGC1α 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 vs. 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 Krebs cycle, generating excessive amount 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 a decrease in insulin sensitivity (18), possibly as a consequence of increased partially oxidized lipid intermediates.

Taken together, the literature does not support that impaired mitochondrial function lead to lower metabolic flexibility to lipid. In fact, it remains largely unknown if muscle mitochondrial abnormalities described in insulin-resistant individuals are sufficient to affect metabolic flexibility to lipid.

5. Other determinant factors of metabolic flexibility

In a recent study, we identified other factors determining metabolic flexibility to glucose in humans (14). As expected, the metabolic flexibility to glucose during a clamp was related to surrogate markers of insulin resistance such as fasting plasma glucose, FFA and insulin concentrations. In addition, we observed that metabolic flexibility to glucose was directly related to plasma adiponectin concentration. Since most of these variables are interrelated, we performed a stepwise multiple regression analysis including these variables. Only glucose disposal rate and steady-state plasma FFA concentration –the latter explained 3% of the variance in metabolic flexibility– were independent determinants of the

change in RQ (ΔRQ) during a euglycemic-hyperinsulinemic clamp accounting for half of its variance. It is now needed to assess more deeply the cellular determinants of metabolic flexibility to lipid in order to improve our understanding of the underlying causes of impaired metabolic flexibility to lipid.

V. Conclusions

Most of the research on metabolic flexibility has focused on the capacity to metabolize glucose in response to an overload of carbohydrate during a euglycemic-hyperinsulinemic clamp. However, the difference in metabolic flexibility during the clamp in individuals with varying insulin sensitivity is mostly the consequence of variability in insulin-stimulated glucose disposal rate (14). Furthermore the mitochondrial capacity to oxidize acetyl-CoA coming from glucose is conserved in insulin-resistant subjects even if mitochondrial defects have been reported in those individuals (3, 25, 36, 37, 40, 42, 43, 48, 59). These findings should not negate the potential role of mitochondrial defect in insulin resistance, since during a euglycemic-hyperinsulinemic clamp the lipid demand as an energy source is mostly suppressed.

The assessment of metabolic flexibility to lipid will probably unravel defects in lipid oxidative capacity. Surprisingly, there are no studies evaluating metabolic flexibility to lipid in subjects with contrasting degree of insulin resistance. Furthermore, most studies have used fasting RQ as a marker of metabolic flexibility to lipid. However, fasting RQ is not a reliable indicator of lipid oxidation capacity, since RQ under fasting condition is mostly influenced by energy balance and dietary macronutrient composition (22, 34, 54). In addition, under resting

conditions, energy demand and concomitant fat oxidation requirement is hardly a metabolic challenge for muscle mitochondria. Consequently, an eventual defect in fat oxidation is unlikely to be evidenced in resting conditions.

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 to assess 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 quickly increase fat oxidation in response to day-to-day changes in fat intake will eventually have lower muscle fat accumulation and be less prone to insulin resistance when compared to slower adapters. Based on the RQ profile for metabolically flexible and inflexible subjects shown in Figure 2D and considering an energy intake of 2500 kcal/day (protein intake 20% of total energy), the metabolically inflexible individuals would accumulate ~200 grams more fat than flexible subjects after one week of high-fat diet (60% of total energy).

We propose that using the resting condition to test metabolic flexibility, particularly at the skeletal muscle level, is not appropriate since the absolute lipid oxidation rate is minimal. Energy metabolism under exercise conditions provides a paradigm requiring a highly coordinated regulation between fuel supply and the oxidative machinery. This approach in combination with muscle lipid and glycogen

content determination may be useful to assess the role of mitochondrial density/function on metabolic flexibility to lipid.

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 require further studies. Only this kind of data will allow us to establish a causal link between impaired capacity to metabolize fat, muscle lipotoxicity and insulin resistance.

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Disclosures

None.

Figure legend

Figure 1. Model for fat-induced insulin resistance. This model describes how a failure to appropriately store lipids into subcutaneous adipose tissue (quantitatively predominant), will lead to ectopic lipid deposition into visceral fat, and insulin-sensitive tissues such as liver and skeletal muscle. These tissues will progressively develop a state of lipotoxicity altering insulin signaling and action, and contributing to whole-body insulin resistance and deterioration of glucose tolerance.

Figure 2. Different features of metabolic flexibility. A: Metabolic flexibility during overnight fasting. B: Metabolic flexibility during a hyperinsulinemic clamp. C: Metabolic flexibility in response to a high-carbohydrate diet. D: Metabolic flexibility in response to a high-fat diet. Metabolically flexible (closed circle) and inflexible (open circle) subjects.

Figure 3. Correlation between metabolic flexibility (steady-state respiratory quotient (RQ) – fasting RQ = Δ RQ) and fasting respiratory quotient (Panel A), and insulin-stimulated glucose disposal rate (Panel B).

Table legend

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

Table 2. Mean $\pm$ SE. *$p<0.05$. $^{\dagger}p>0.44$ after controlling for gender, race, presence of type 2 diabetes, age and insulin-stimulated glucose disposal rate ($p<0.02$ when values are not controlled for glucose disposal rate).

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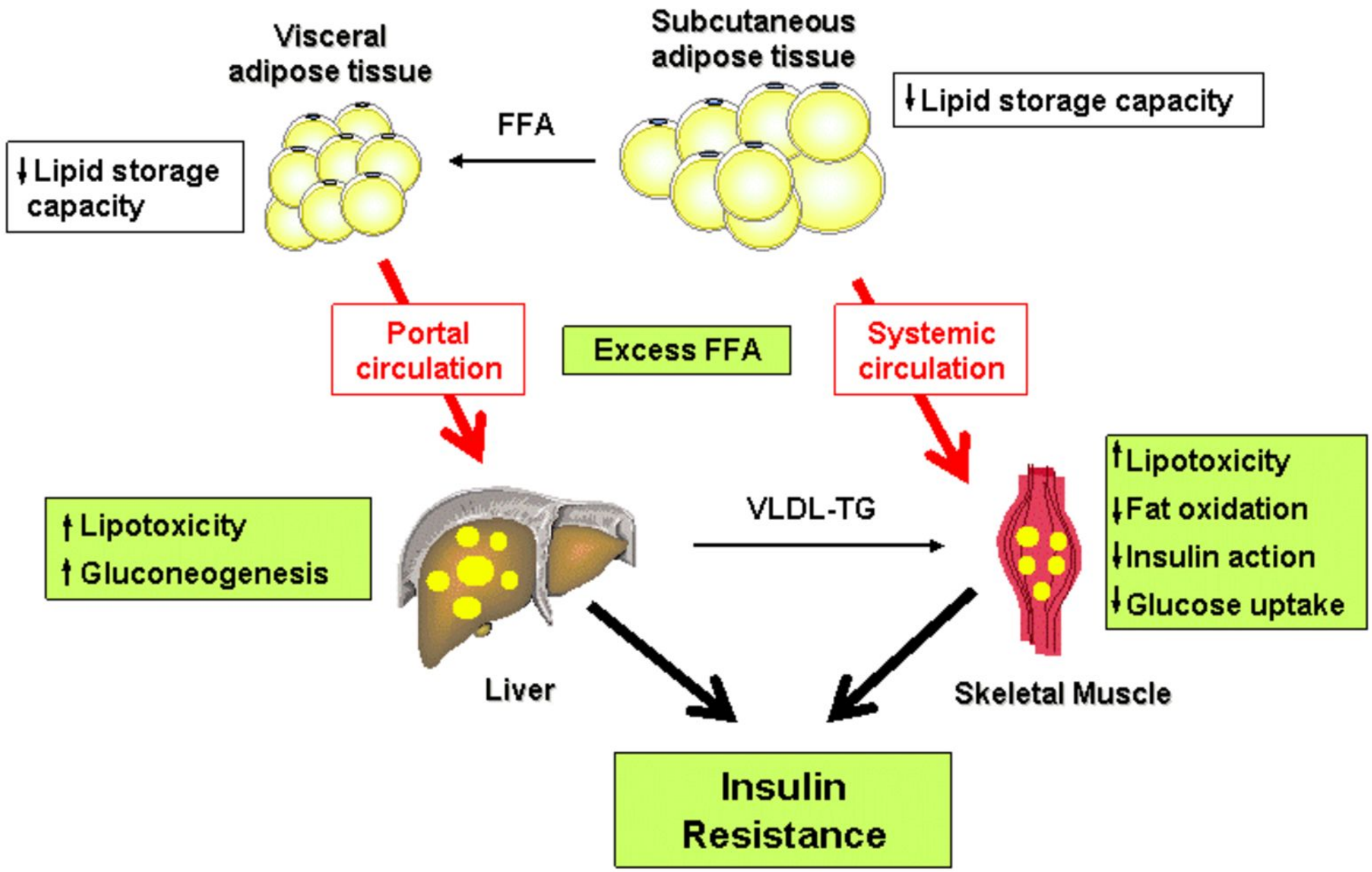
Visceral adipose tissue
Subcutaneous adipose tissue
↓Lipid storage capacity
FFA
↓Lipid storage capacity
Portal circulation
Excess FFA
Systemic circulation
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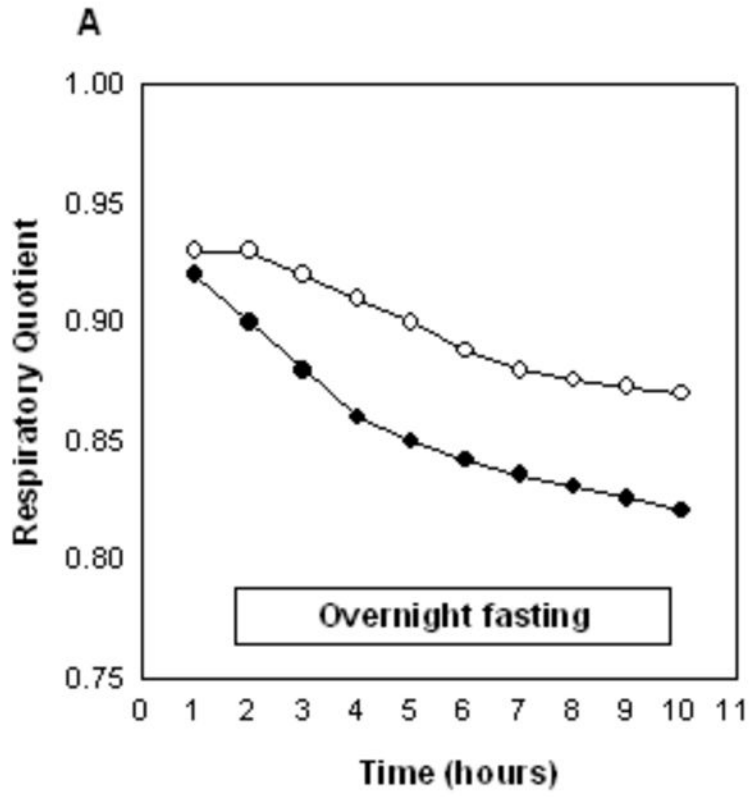
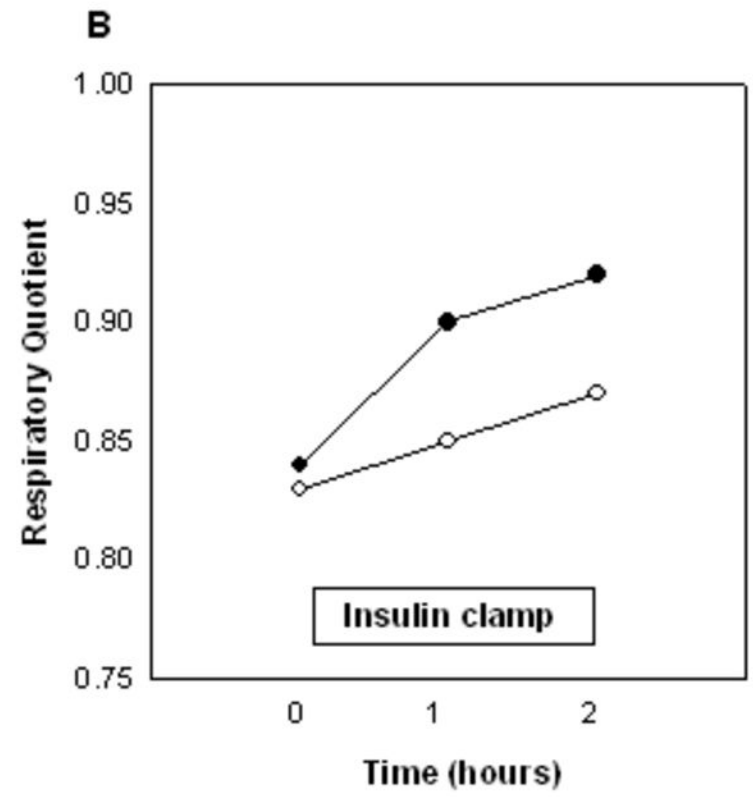
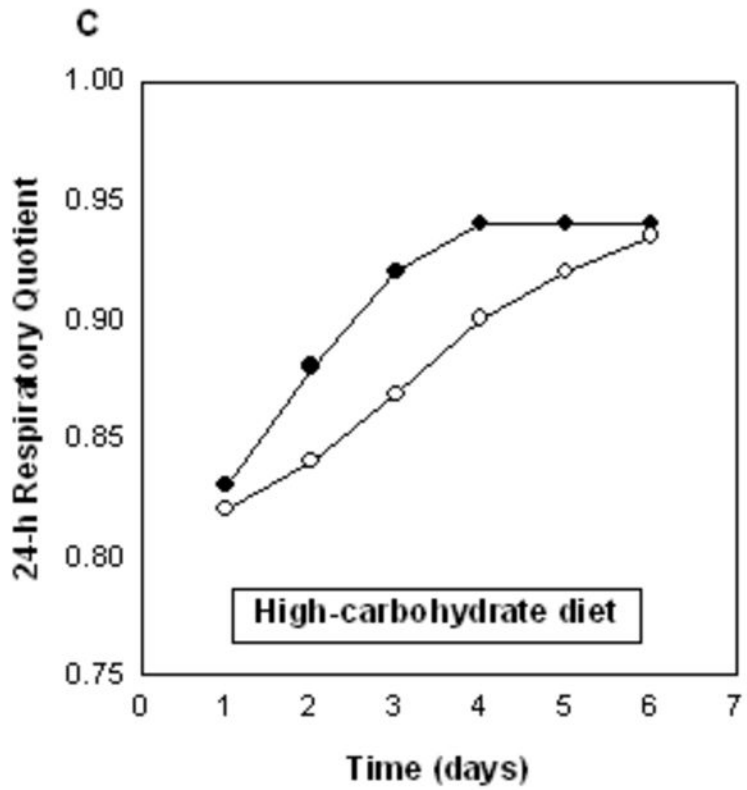
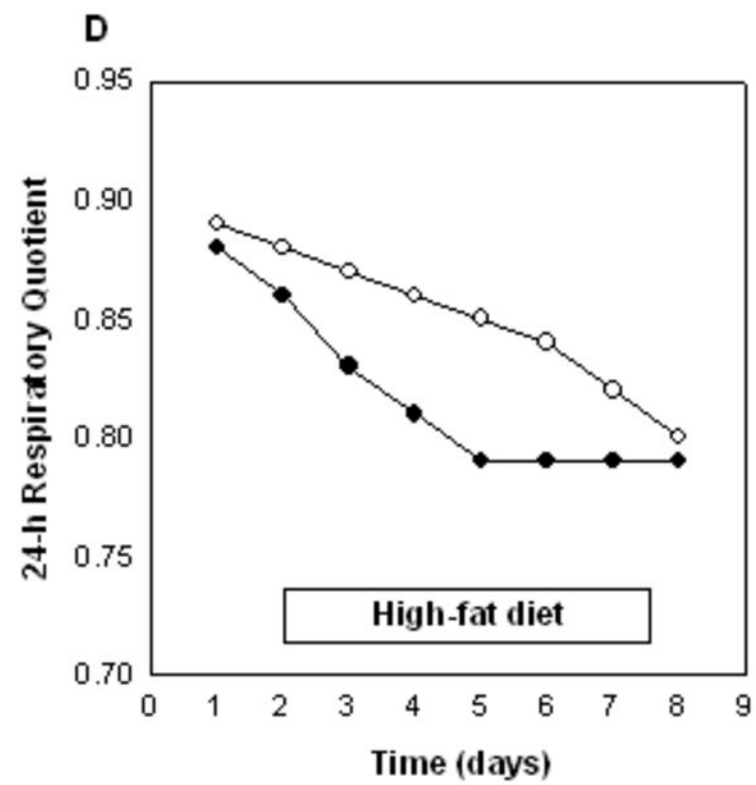
↓Insulin action
↓Glucose uptake
Liver
Skeletal Muscle
Insulin Resistance

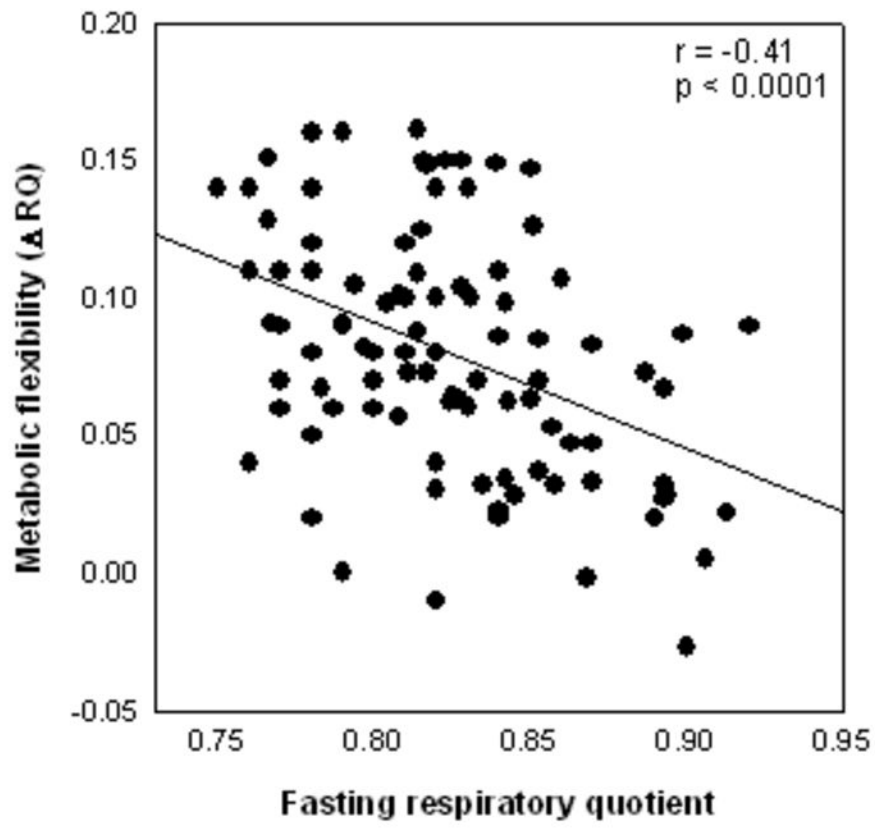

A
1.00
0.95
0.90
0.85
0.80
0.75
Respiratory Quotient
Overnight fasting
0 1 2 3 4 5 6 7 8 9 10 11
Time (hours)


B
1.00
0.95
0.90
0.85
0.80
0.75
Respiratory Quotient
Insulin clamp
0 1 2
Time (hours)


C
1.00
0.95
0.90
0.85
0.80
0.75
24-h Respiratory Quotient
High-carbohydrate diet
0 1 2 3 4 5 6 7
Time (days)


D
0.95
0.90
0.85
0.80
0.75
0.70
24-h Respiratory Quotient
High-fat diet
0 1 2 3 4 5 6 7 8 9
Time (days)

A


r = -0.41
p < 0.0001
Metabolic flexibility (Δ RQ)
0.20
0.15
0.10
0.05
0.00
-0.05
0.75
0.80
0.85
0.90
0.95
Fasting respiratory quotient

B

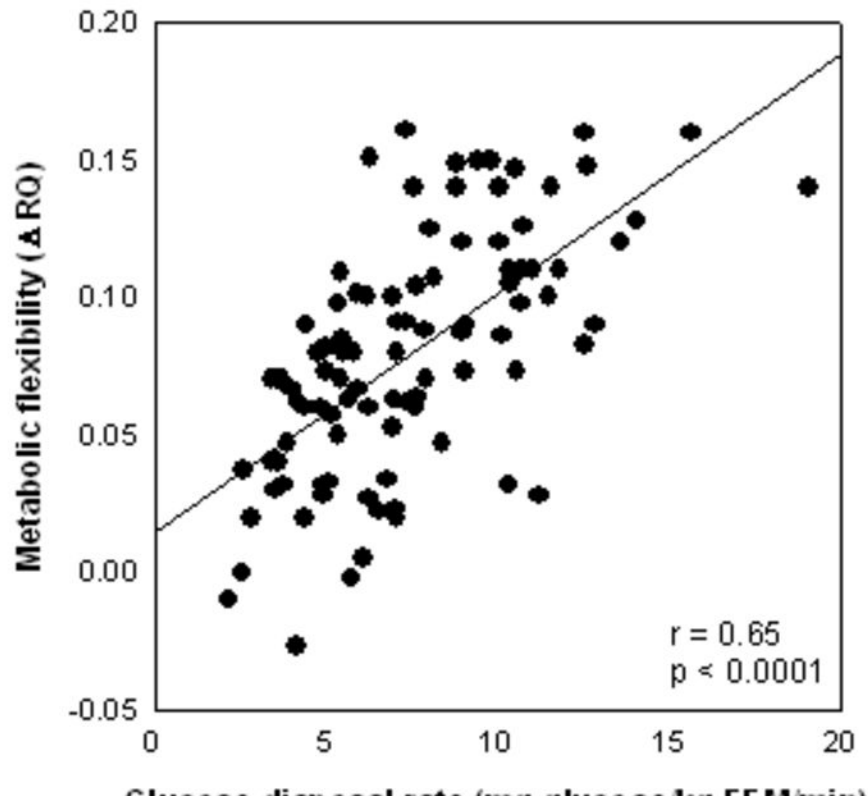

r = 0.65
p < 0.0001
Metabolic flexibility (Δ RQ)
0.20
0.15
0.10
0.05
0.00
-0.05
0
5
10
15
20
Glucose disposal rate (mg glucose/kg FFM/min)

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

Subject	1st week		2nd week		Δ 24h-RQ
	FQ	24-h RQ*	FQ	24-h RQ*	(2nd – 1st week)
A	0.92	0.92	0.75	0.75	- 0.17
B	0.82	0.82	0.75	0.75	- 0.07

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

	Highest insulin sensitivity	Lowest insulin sensitivity
Female/male	19/6	12/13*
DM/non-DM	4/21	23/2*
White/Black/Other	15/10/0	23/0/2*
Age (y)	54.3 ± 1.4	60.1 ± 1.6*
BMI (kg/m^2)	32.9 ± 0.5	33.0 ± 0.5
Body fat (%)	39.0 ± 1.5	35.3 ± 1.2
Glucose disposal rate (mg/kg FFM/min)	11.8 ± 0.4	4.0 ± 0.2
Non-oxidative glucose disposal rate (mg/kg FFM/min)	8.5 ± 0.5	2.3 ± 0.2†
Steady-state RQ	0.94 ± 0.01	0.87 ± 0.01†