Muscle insulin resistance: A case of fat overconsumption, not mitochondrial dysfunction

Edward W. Kraegen*, Gregory J. Cooney, and Nigel Turner

Diabetes and Obesity Program, Garvan Institute of Medical Research, Sydney NSW 2010, Australia

Insulin resistance—an impairment in the ability of insulin to appropriately regulate glucose and lipid metabolism—is an early and key defect associated with weight gain (obesity), type 2 diabetes (T2D), and other disorders of metabolism. The etiology of insulin resistance is still not completely understood, but it is now clear that a strong association exists between insulin resistance and excess lipid accumulation in nonadipose tissues, particularly muscle and liver. Whether muscle cytosolic lipid accumulation causes insulin resistance still requires definitive proof, but highly plausible mechanisms have been elucidated supporting this possibility. These mechanisms involve links between metabolically active cytosolic lipid intermediates, such as diacylglycerols and ceramides, and specific steps in insulin signaling (1) (see blue shaded box in Fig. 1). Certainly we know that excess dietary fat intake in animals over the course of several weeks (or acute pharmacological elevation of circulating fatty acids over several hours) can cause muscle insulin resistance and that both conditions are accompanied by cytosolic lipid accumulation. Tissue accumulation of lipids could occur as a result of increased fatty acid uptake, a decreased rate of fatty acid utilization, or a combination of both. Accordingly, there has been considerable interest in recent reports that have used a variety of techniques (e.g., gene expression, ^{31}P-magnetic resonance spectroscopy, histological analyses) to indicate that insulin-resistant, obese, or T2D subjects have a reduced mitochondrial content and/or impaired mitochondrial function (2, 3). The conclusion from many of these studies is that mitochondrial dysfunction could be a major factor contributing to muscle lipid accumulation and insulin resistance. However, an important study by Hancock *et al.* (4) in this issue of PNAS shows that insulin resistance can develop in animals in response to the feeding of high-fat diets, despite a significant increase in mitochondrial content.

The Hancock *et al.* study (4) is significant at a number of levels. First, it clearly shows that a high-fat diet can lead to increased muscle mitochondrial content at the level of mitochondrial protein expression and mitochondrial DNA copy number. These data are compelling, particularly when combined with the other findings from this study, which show (*i*) an increase in the *in vitro* capacity of muscle from these animals to oxidize palmitate and (*ii*) an increase in the content of the transcription factor peroxisome proliferator-activated receptor (PPAR)δ and the PPARγ coactivator 1α (PGC1α), key elements in regulating mitochondrial biogenesis. As pointed out above, the question of whether high fat feeding can lead to mitochondrial dysfunction or defective fat oxidation has been controversial, with the literature fairly evenly divided on this issue. Hancock *et al.* provide strong and complementary data to support recent findings (5) demonstrating that skeletal muscle from mice fed a high-fat diet for 5 or 20 weeks has increased mitochondrial fatty acid oxidative capacity, higher activity of oxidative enzymes, and elevated protein expression of PGC1α and mitochondrial respiratory chain subunits. It makes sense that animals, and presumably humans, can adapt to variations in dietary nutrient balance and increase their ability to metabolize and gain energy from the most prevalent fuel in the diet. Hancock *et al.*'s findings are, therefore, in accord with studies demonstrating that upstream steps in muscle lipid metabolism, such as expression of fatty acid transport proteins and activity of acyl-CoA synthase, are also enhanced by fat feeding (6, 7).

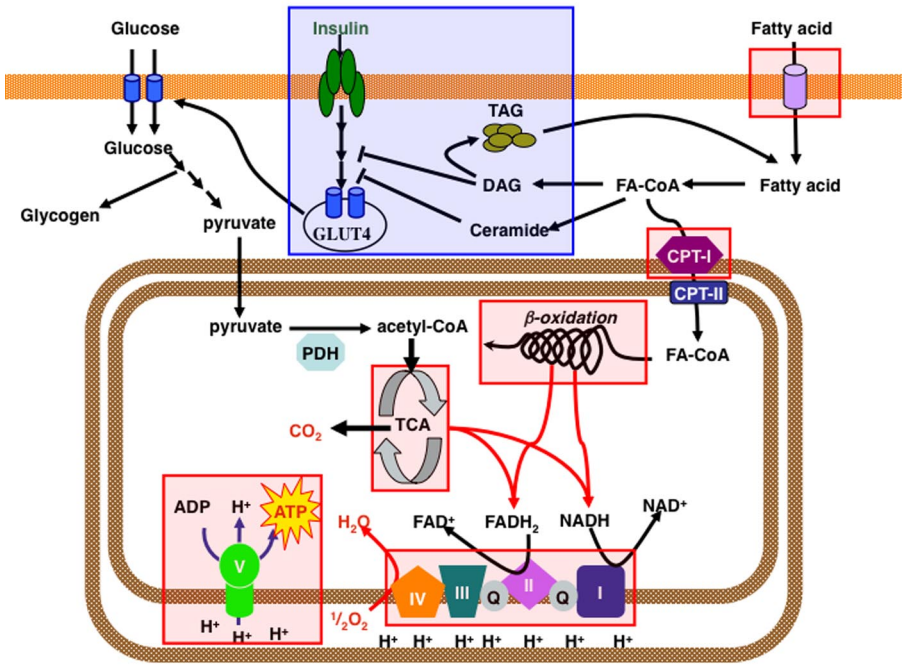

Fig. 1. Interrelationship of energy metabolism and insulin action in skeletal muscle. Fatty acids and glucose are both substrates for energy metabolism in skeletal muscle. Cytosolic accumulation of lipid species such as diacylglycerol and ceramide are thought to decrease insulin action by inhibiting the insulin signaling pathway (blue shaded box). Metabolism of fatty acids by muscle cells is subject to regulation at the level of uptake and activation of fatty acids, the entry of activated fatty acids into the mitochondrion (CPT-1) and the capacity of the β-oxidation pathway, the tricarboxylic acid (TCA) cycle, electron transport chain (complex I–IV), and ATP synthesis (complex V) (red boxes). The balance between uptake and utilization of fatty acids will ultimately determine the magnitude of lipid accumulation in muscle cells.

Hancock *et al.* (4) also provide evidence of a mechanism for a fatty acid-induced increase in mitochondrial biogenesis involving the muscle transcription factor PPARδ. Their findings draw on the

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*To whom correspondence should be addressed at: Diabetes and Obesity Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney NSW 2010, Australia. E-mail: e.kraegen@garvan.org.au.

pivotal work of the Evans group (8), which found that fatty acids are endogenous ligands for PPARδ and that increased expression or activation of PPARδ can induce mitochondrial biogenesis. Hancock *et al.* observed an increase in PPARδ expression and activation in their high-fat-fed rats but reasoned that this increase would be insufficient to fully mediate the mitochondrial biogenic program, which would require coordinated activity of a number of transcription factors and both the nuclear and mitochondrial genomes. A key observation in interpreting their findings was the gradual increase in PGC1α content in the absence of any change in PGC1α mRNA. PGC1α is crucial for regulating mitochondrial biogenesis (9), and the study results suggested a possible posttranslational regulatory link between PPARδ activation and PGC1α content. Such a link was confirmed by additional studies using *in vivo* muscle DNA electroporation to specifically overexpress PPARδ, resulting in a similar increase in PGC1α protein but not mRNA content. The exact nature of the regulation of PGC1α protein by PPARδ activation will need further investigation, but it is in accord with other studies that show that the stability and activity of PGC1α can be affected by posttranslational modifications such as phosphorylation and acetylation (10, 11). The data are also a clear example of how a distorted view could, perhaps all too often, be obtained by studies of mRNA levels without corresponding estimates of protein content, protein activity, or some other downstream or functional output of the process under investigation.

The third significant aspect of the Hancock *et al.* study (4) is that muscle insulin resistance can develop despite increased mitochondrial content and an enhanced capacity for fat oxidation in high-fat-fed rats. This finding is interesting, although there are some caveats. Insulin resistance was not investigated in depth but was based on *ex vivo* glucose transport measurements in muscle strips. However, from other extensive studies in the literature that have used this and similar models, it is highly likely that *in vivo* muscle insulin resistance was present in these animals and was associated with increased levels of lipid intermediates. Another problem is that we do not have a complete picture of relevant *in vivo* lipid metabolism in these rats. Even though muscle may have an increased capacity for fat metabolism, as demonstrated here by using *ex vivo* methodologies, it is not possible to predict the *in vivo* rates from the data presented. Muscle fatty acid metabolism is subject to extensive *in vivo* regulation, for example, by control of fatty acid entry into the cell, transfer of fatty acid into the mitochondria (regulated by malonyl CoA at CPT-1), the capacity of β-oxidation and the tricarboxylic acid cycle, the electron transport chain capacity, and the degree of mitochondrial coupling (see red boxes in Fig. 1). Although there are many potential points of control, ultimately the rates of endogenous fatty acid oxidation are likely to be dictated by the need of the muscle for energy, rather than by its total capacity for oxidation. Respirometry data and levels of lipid intermediates in muscle would have been informative in this study to indicate the degree of enhancement of fat oxidation and tissue lipid accumulation under *in vivo* conditions in these animals. However, even under conditions of enhanced mitochondrial fatty acid utilization, it is still quite possible that this rate would be insufficient, in the face of increased tissue fatty acid uptake, to prevent the cytosolic lipid accumulation that leads to the development of insulin resistance. Alternatively, it is also possible that enhanced fatty acid oxidation in mitochondria may have a more direct role in reducing insulin action, given that fatty acids have been shown to promote the production of reactive oxygen species (12), which are linked with insulin resistance (13). Furthermore, elevated fatty acid oxidation during high fat feeding has been proposed to result in a buildup of potentially toxic incomplete lipid oxidation products that may contribute to reduced insulin sensitivity (14). Thus, further quantitative functional studies of *in vivo* lipid metabolism in rats under similar study conditions will be needed before implications for insulin sensitivity can be completely understood.

Although Hancock *et al.* (4) demonstrate insulin resistance in the presence of improved mitochondrial function, they provide no evidence for, or against, previous findings that mitochondrial content or function is reduced in insulin-resistant humans. However, if the study results are taken at face value, it would appear unlikely that reduced muscle mitochondrial content in insulin-resistant states such as obesity or T2D is a direct result of an excessive dietary intake of fat. Indeed, when related to findings in insulin-resistant relatives of type 2 diabetics (15), the present study supports an argument that impaired mitochondrial function may be a primary (diabetogenic) defect or, as suggested (4), may be mainly due to a sedentary lifestyle, based on evidence that physical activity has a positive association with mitochondria content (16). Hancock *et al.*, however, question the physiological impact of reduced mitochondrial content and present an excellent argument that even a 30% reduction in mitochondrial number would hardly be expected to significantly affect overall rates of muscle fatty acid oxidation during basal or normal free-living conditions. Although more substantial mitochondrial dysfunction may still impair muscle oxidation, the authors' data stress the need for more quantitative studies of muscle lipid fluxes and their controlling factors in insulin-resistant states.

Overall, the insightful article by Hancock *et al.* (4) has provided important new information regarding the proposed link between mitochondrial metabolism and insulin sensitivity. Their finding of a dissociation between muscle mitochondrial function and insulin resistance complements other recent studies (5, 17, 18) and highlights the need for further research to determine the role of mitochondrial metabolism in the etiology of insulin resistance in muscle.

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