

## Fatty Acids, Insulin Resistance, and Protein Metabolism

Satish C. Kalhan

Department of Pathobiology, Cleveland Clinic, Lerner College of Medicine of Case Western Reserve University, Cleveland, Ohio 44195

**A**cretion and maintenance of lean body mass, because of its fundamental role in health and disease, continues to be an area of intense investigation. By using sophisticated tracer isotopic methods, organ balance techniques, and molecular analysis of tissue samples in humans and animal models, a detailed analysis of the signaling pathway and metabolic fluxes has been possible. Despite these, understanding the regulatory mechanism of nitrogen/protein accretion and maintenance of lean body mass *in vivo* continues to be challenging. This is particularly difficult because of the adaptation of metabolism to various modifying influences, and because of the multiple levels of regulation *in vivo*. Yet, by careful studies, a number of investigators have systematically examined the influence of diverse modifiers on the physiology of protein synthesis and breakdown in humans *in vivo*.

In this issue of *JCEM*, Katsanos *et al.* have examined, in healthy humans, the impact of elevated plasma fatty acids levels, induced by iv infusion of intralipid and heparin, on the responsiveness of the skeletal muscle protein synthesis and balance to an enterally administered essential amino acid mixture. By using isotopic tracers in combination with arteriovenous balance measurements across the leg and by quantifying tracer incorporation into muscle proteins, Katsanos *et al.* (1) have presented a detailed analysis of protein kinetics in the skeletal muscle. As anticipated, increase in plasma free fatty acids resulted in the development of insulin resistance (2). Their data show that in the basal state, in the presence of elevated plasma fatty acids levels and insulin resistance, there was a decrease in the rate of appearance of phenylalanine across the leg, suggesting a lower rate of protein breakdown. The rate of disappearance of phenylalanine was not affected, resulting in an improved or less negative amino acid balance. Essential amino acid administration resulted in no change in protein breakdown and caused an increase in phenylalanine rate of disappearance (increase in protein synthesis) and an increase in the fractional rate of protein synthesis. Of significance, fatty acids and/or fatty acid-induced insulin resistance did

not impact the rate of protein synthesis by skeletal muscle in response to the amino acid load.

Fatty acids are critical oxidative substrates that are mobilized during fasting and result in sparing of glucose and amino acids. Elevated fatty acid levels during fasting are associated with decreased glucose uptake by skeletal muscle, decreased protein breakdown, and a lower rate of protein oxidation and urea synthesis (3). The cause and effect relating fatty acids to changes in fasting metabolism has been examined in a large number of studies. These studies have been done either during fasting when there is a spontaneous increase in plasma fatty acid concentration or by infusing intralipid with heparin, resulting in an increase in the plasma fatty acid levels in the high physiological range. Fatty acids could impact glucose and protein metabolism (protein synthesis and breakdown) either directly or via their effect on insulin secretion and/or insulin action (insulin sensitivity/resistance).

In studies in healthy humans, exogenously infused fatty acids have been shown to result in a decrease in the rate of protein breakdown by the skeletal muscle, evidenced by a decrease in the rate of net release of amino acids across the limb (4), and by a decrease in the rate of appearance of essential amino acids leucine and phenylalanine as measured by isotopic tracer methods (5, 6). Although the mechanism of the observed effect of fatty acids on protein breakdown has not been identified, it does not appear to be related to hepatic oxidation of fatty acids and consequent increase in ketone levels. In addition, fatty acids have been shown to cause a decrease in the rate of oxidation of leucine and of urea synthesis (protein sparing) (5, 6). The critical role of fatty acids in inhibiting protein breakdown was confirmed by Nørrelund *et al.* (7) in subjects who fasted for 37 h. Their data showed that acute suppression of lipolysis by acipimox resulted in an approximately 50% increase in muscle protein breakdown. Stoichiometric calculation of protein synthesis (synthesis = breakdown – loss) from these data suggests that fatty acid infusion resulted in a decrease in the rate of protein synthesis. The lower rate of protein synthesis could be the result of a decrease in

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Abbreviation: IRS, Insulin receptor substrate.

protein breakdown and, consequently, a decrease in available intracellular amino acid pool for protein synthesis. Whether the decrease in proteolysis in the skeletal muscle is the consequence of the fatty acids themselves, their chain length, or a product of fatty acid oxidation in the skeletal muscle has not been examined.

Although the data are not uniformly consistent, parenteral administration of fatty acids in humans enhances insulin secretion, which could influence protein turnover (8–10). In man, acute administration of insulin has consistently been shown to suppress protein breakdown. In addition, the stimulatory effects of insulin on protein synthesis and its molecular mechanism have been described extensively in *in vivo* and *in vitro* systems (reviewed in Ref. 11). The effect of fatty acids on protein breakdown is unlikely to be related to their synergistic effect on insulin secretion because the effects were observed in the fasting state when the insulin levels were low and there was relative insulin resistance in association with elevated fatty acids and GH levels (7).

Fatty acids could impact protein metabolism by their effect on the insulin signaling pathway, resulting in insulin resistance (12). A number of physiological and pathological states are accompanied by the appearance of insulin resistance, defined as a decrease in uptake of glucose, primarily by the skeletal muscle, in response to prevailing insulin levels. Insulin resistance is considered to play a major role in the development of type 2 diabetes. Although a number of studies have examined the impact of insulin resistance on carbohydrate and lipid metabolism, there is a paucity of data in relation to its effect on protein metabolism. Increased nitrogen accretion in physiological states such as puberty, pregnancy, and possibly growing infants, and during pharmacological intervention by GH, is characterized by the development of insulin resistance by diverse mechanisms (13–16). The development of insulin resistance during pregnancy is a critical physiological response for the growth of the fetus. The birth weight of the neonate is positively correlated with the magnitude of insulin resistance in the mother (13, 14). Pathological states such as chronic renal failure, cirrhosis of the liver, and sepsis are also associated with insulin resistance (15, 16). The biological role of insulin resistance in these conditions is unclear, but it may be aimed at accretion and preservation of lean body mass. The relative contribution of other factors that can cause insulin resistance, such as counterregulatory hormones like glucagon, catecholamines, GH, or the pregnancy-related hormones, and cytokines such as TNF $\alpha$ , may vary in these conditions. Nevertheless, in several of these states, an elevated concentration of plasma fatty acids is consistently seen. More than 35 yr ago, Randle (17) and colleagues, based upon a series of studies on skeletal muscle *in vitro*, had proposed a competition between oxidative substrates, *i.e.* fatty acid and glucose. The mechanism of this so-called glucose-fatty acid cycle or substrate competition has been an area of intense scrutiny and now has been related to impaired insulin action. As proposed by Shulman (12) and colleagues, based upon extensive data in humans and in animal models, increased delivery of fatty acids to the muscle (fatty acid overload) leads to an increase in the products of fatty acid metabolism, *e.g.* long chain fatty acyl coenzyme A, diacylglycerol, ceramides, *etc.* These metabolites, in particular diacylglycerol, activate serine-threonine phosphorylation cascades initiated by

protein kinase C, leading to phosphorylation of the serine/threonine site on insulin receptor substrates (IRS1 and IRS2). This, in turn, reduces the ability of IRS to activate phosphoinositide 3-kinase. Phosphoinositide 3-kinase is considered the key branch point for a number of downstream signaling pathways for protein synthesis, for cell growth, and for glycogen synthesis. Our understanding of the regulation and control of these signaling pathways continues to grow exponentially, leading to identification of isoforms of these signaling proteins and their differential regulation in response to various signals (18, 19). A decrease in the activity of the insulin signaling cascade as a result of fatty acid load would be expected to attenuate the insulin-induced response to protein synthesis via the downstream protein kinase B (also known as AKt) and target of rapamycin signaling pathway. However, the data of Katsanos *et al.* (1) showed that even in the presence of fatty acid-induced insulin resistance, the protein synthesis response to amino acid load was not impaired. Studies in healthy humans have shown that amino acids stimulate skeletal muscle protein synthesis through an AKt-independent pathway (11, 20). This effect is more evident with branched-chain amino acids, specifically with leucine. The stimulation of protein synthesis has been identified to be via activation of target of rapamycin, which in turn activates p70<sup>S6</sup> kinase and dephosphorylates 4EBP-1 (eIF4E-binding protein 1), leading to translation initiation, protein synthesis, and protein metabolism. Additional downstream effects of amino acids independent of insulin signaling have been suggested (11). However, the stimulatory effect of amino acids is not seen in the total absence of insulin *in vivo*, due in part to the counterregulatory responses to the lack of insulin.

Elevated fatty acids, by their effect on protein metabolism and by causing insulin resistance, may provide a distinct biological advantage to the organism. By decreasing protein breakdown via mechanisms still to be identified, fatty acids contribute to the conservation of skeletal muscle protein mass. On the other hand, the lack of any effect of fatty acids, and possibly of fatty acid-induced insulin resistance on stimulation of protein synthesis by amino acids, contributes to accretion of nitrogen during growth and to the maintenance of lean body mass in mature organisms. These effects of fatty acids may be mitigated by other modifiers such as cytokines and counterregulatory hormones and drugs in different pathophysiological states. The interaction between the decrease in insulin-mediated glucose uptake (insulin resistance) and protein synthesis and breakdown remains to be determined.

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Address all correspondence and requests for reprints to: Satish Kalhan, M.D., Department of Pathobiology, Cleveland Clinic, 9500 Euclid Avenue, NE40, Cleveland, Ohio 44195. E-mail: sck@case.edu.

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