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Commentary

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Hungry for your alanine: when liver depends on muscle proteolysis

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Fasting requires complex endocrine and metabolic interorgan crosstalk, which involves shifting from glucose to fatty acid oxidation, derived from adipose tissue lipolysis, in order to preserve glucose for the brain. The glucose-alanine (Cahill) cycle is critical for regenerating glucose. In this issue of *JCI*, Petersen et al. report on their use of an innovative stable isotope tracer method to show that skeletal muscle–derived alanine becomes rate controlling for hepatic mitochondrial oxidation and, in turn, for glucose production during prolonged fasting. These results provide new insight into skeletal muscle–liver metabolic crosstalk during the fed-to-fasting transition in humans.

Adaptive response to fasting

Adaptation to fasting is a fascinating physiological phenomenon allowing organisms to maintain energy supply to tissues despite declining energy stores. During evolution, multiple mechanisms evolved to counter the threat of starvation. Periods of famine and starvation have likely selected genotypes featuring adaptive responses, such as hepatic insulin resistance, e.g., by insulin receptor mutation in cave-dwelling *Astyanax mexicanus* fish (1) or insulin resistant subtypes of type 2 diabetes prone to nonalcoholic fatty liver disease (NAFLD) (2). On the other hand, various concepts of dietary restriction, e.g., interval/intermittent fasting (3) or very low caloric diets (4), may help to combat the current obesity and type 2 diabetes epidemic.

The liver plays the key role in maintaining blood glucose concentrations for obligate glucose utilizers (central nervous system, red blood cells, renal medulla) (5). During the transition from the fed to the early fasted state, the liver switches from glycogen storage to glucose production by glycogen breakdown as well as by gluconeogenesis from noncarbohydrate precursors, such as lactate, glycerol, and branched-chain amino acids (6). Prolonged fasting requires the liver to shift from carbohydrate oxidation to β-oxidation of free fatty acids (FFAs) so that ketone bodies become the main energy source (7).

In a previous study, the researchers developed a positional isotopomer nuclear magnetic resonance tracer analysis (PINTA) to elucidate the interaction between adipose tissue and liver crosstalk during starvation in rodents (8). During starvation, the decline in hepatic glycogenolysis results in a fall of plasma leptin, which stimulates the hypothalamic-pituitary-adrenal axis (HPA) and, in turn, adipose tissue lipolysis with release of FFA and glycerol (Figure 1). In the liver, the increase in FFA levels stimulates hepatic β-oxidation and the acetyl-CoA pool, which allosterically activates pyruvate carboxylase flux (V_{PC}), and which, together with glycerol as substrate, maintains the rates of hepatic gluconeogenesis and endogenous glucose production (V_{EGP}) (8).

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Liver–skeletal muscle metabolic crosstalk

Other metabolic pathways are also known to connect skeletal muscle and liver. The Cori cycle describes the shuttling of lactate derived from skeletal muscle anaerobic glycolysis to the liver to feed gluconeogenesis upon intensive exercising. In addition, skeletal muscle contributes to fasting metabolism, not only by glycogenolysis and glycolysis yielding pyruvate, but also by protein breakdown yielding amino acids (Figure 1). These pathways converge via alanine transaminase (ALT), which transfers amino groups from amino acids to pyruvate to form and release alanine and thereby prevent skeletal muscle from rapidly accumulating toxic ammonium (9). The latter glucose-alanine cycle, also known as the Cahill cycle, allows glucose to regenerate from alanine in the liver by a series of reactions (7). Although this interorgan communication is fairly productive, yielding 2 mol ATP per 1 mol glucose oxidized in muscle and yielding 2 mol of carbon-3 glucose precursors from alanine, energetic efficiency decreases with gluconeogenesis and urea synthesis (Figure 1). As a result, the transition from the fed to the fasted state shifts the control of energy metabolism and glucose production from the liver to adipose tissue and skeletal muscle, and alanine may become an important substrate, maintaining glucose homeostasis and regulating hepatic energy metabolism.

Alanine-to-glucose conversion during fasting in humans

In humans, examining the metabolic pathways of interorgan crosstalk has been limited by several factors. Measurements of hepatic metabolite concentrations or flux

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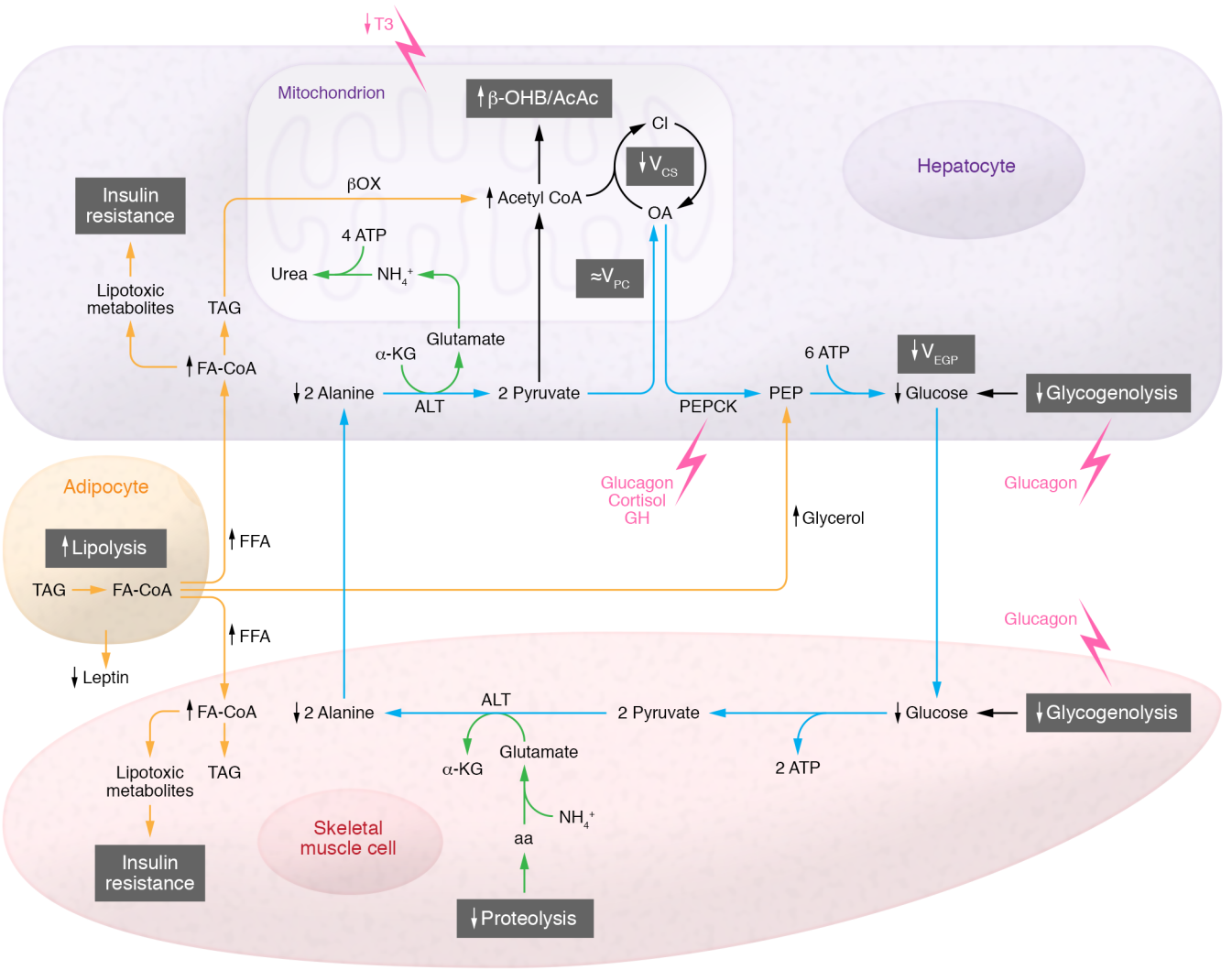

Figure 1. Liver–skeletal muscle crosstalk fuels metabolism in starvation. The Cahill cycle allows for recycling of hepatic glucose from skeletal muscle alanine via ALT and for detoxification of ammonium ions (NH_4^+) from proteolysis via the hepatic urea cycle. In 60-hour fasted humans, the nearly unchanged gluconeogenesis, as assessed from V_{PC}, indicates that reduced hepatic glycogenolysis accounts for the decrease in V_{EGP}. The decrease in V_{CS} occurred in parallel to a rise in the β-hydroxy-butyrate/acetoacetate ratio (β-OHB/AcAc) suggesting that the redox potential regulates V_{CS}. Of note, alanine infusion partially reversed these alterations under conditions of already stimulated hepatic mitochondrial oxidation resulting from substrate supply and endocrine stimulation. CI, citrate; FA-CoA, fatty acyl–coenzyme A; GH, growth hormone; α-KG, α-ketoglutarate; βOX, β-oxidation; OA, oxaloacetate; PEP, phosphoenolpyruvate; PEPCK, PEP carboxykinase; TAG, triglycerides; T3, triiodothyronine.

rates cannot be performed invasively due to ethical considerations precluding liver biopsies for physiological studies. Noninvasive in vivo magnetic resonance spectroscopy is expensive, not generally available, and confined to certain metabolites. Petersen and colleagues combined minimal invasive techniques to examine the glucose-alanine cycle during short-term (12 hour) and prolonged (60 hour) fasting in healthy humans (10). They applied their recently described PINTA method and infused three stable-isotope–labeled substrates that allowed for simultaneous measurement of V_{EGP}, V_{PC}, and hepatic mitochondrial oxidation from citrate synthase flux (V_{CS}) (11). In addition, they assessed systemic alanine turnover using [3-^{13}C]alanine infusion as well as the hepatic mitochondrial redox state (NADH:NAD$^+$) from the ratio of plasma β-hydroxybutyrate/acetoacetate concentrations.

After 60 hours of fasting, V_{EGP} decreased by more than 20% despite largely unchanged V_{PC}, indicating that the reduction in glucose production was mainly due to decreased net glycogenolysis (Figure 1). Hepatic V_{CS} and endogenous alanine turnover decreased by approximately 50% and approximately 30%, respectively. Next, they infused alanine intravenously in 60-hour fasted humans to match the higher alanine turnover observed after 12 hours of fasting, which raised V_{EGP} and V_{PC} and markedly stimulated V_{CS}, by approximately 70%. The alanine-stimulated gluconeogenesis (V_{PC}) occurred under conditions of supposedly maximal stimulation by glucagon and FFA from adipose tissue lipolysis. Of note, the rise of V_{PC} correlated with mitochondrial oxidation, which indicates an important role of skeletal muscle–derived alanine as rate controlling for hepatic mitochondrial oxidation and, in turn,

gluconeogenesis and glucose production in starving humans (10). At present, these results cannot yet be generalized because the study exclusively compared short- with long-term fasted healthy lean young men. Furthermore, hormones, aging, sarcopenia, obesity, and diabetes mellitus can affect protein turnover and possibly influence the contribution of the glucose-alanine cycle to liver metabolism (12, 13). It would also be of interest to quantify the proportion of gluconeogenesis by the renal medulla, which significantly increases in starving humans (14) and in mice with liver-specific knockdown of pyruvate carboxylase (15).

Hepatic mitochondrial function and insulin resistance

Another important observation was that starvation and alanine-induced changes in V_{CS} were associated with opposite changes in redox potential, as assessed from the β-hydroxy-butyrate/acetoacetate ratio (Figure 1). Based on this result, the authors suggested that alanine-mediated changes in the redox state may regulate hepatic V_{CS} in a manner similar to that shown in previous studies in rodents (8, 16). In agreement, the extracellular redox state modulates mitochondrial function, gluconeogenesis, and glycogen synthesis in murine hepatocytes (17). While this is a reasonable explanation, other mechanisms cannot be ruled out. After 60 hours of fasting, V_{PC} clearly increased during the subsequent alanine infusion, suggesting that replenishing citric acid (Krebs) cycle intermediates could have accelerated $V_{CS.}$ Humans with inborn V_{PC} deficiency show severe depletion of anaplerotic flux (18), and liver-specific knockdown of pyruvate carboxylase decreases mitochondrial oxidation. Also, thyroid hormones can markedly affect hepatic oxidation, and the observation of lower serum thyroid-stimulating hormone and unchanged total thyroid hormone levels does not completely preclude any effect of the active free hormone or its metabolites (19). Likewise, changes in cortisol and catecholamines may directly affect gluconeogenesis, glycogenolysis lipolysis, or protein catabolism, while other hormones, such as ghrelin/growth hormone (20) and leptin, can also participate via the central nervous system in the fed-to-fasting transition (8). In this context, the complementary rodent study by Petersen et al. demonstrated that the hypoleptinemia-induced glucose-FFA cycle during a 48-hour fast is indeed mediated by an increase in glucocorticoids, which stimulates adipose lipolysis to increase hepatic acetyl-CoA content and allosterically activate V_{PC} flux (8).

Modulation of hepatic mitochondrial oxidation is not only involved in the adaption to fasting, but also during the development of insulin resistance, obesity, and type 2 diabetes. Obese persons show increased hepatic oxidative capacity along with abnormal mitochondrial efficiency (21), whereas persons with type 2 diabetes have reduced hepatic ATP levels and synthase flux, suggesting impaired hepatic mitochondrial function (21, 22). Interestingly, branched-chain amino acids are not only elevated in several insulin-resistant states, but also interfere with insulin signaling (21). These findings underpin the idea that amino acids, similarly to lipid metabolites, play an important role in human insulin resistance. They also place hepatic energy metabolism at the center of the connection among fasting, insulin resistance, and NAFLD. Indeed, novel mild mitochondrial uncouplers identified hepatic energy metabolism as a target for treating obesity, type 2 diabetes, and NAFLD (23, 24).

Future research needed

Petersen and colleagues raise several questions and open the door for new studies on hepatic and systemic energy metabolism. First, it would be important to assess the relative contributions of lipolysis, lactate, and alanine to hepatic V_{CS} and V_{PC} during the early postprandial-to-postabsorptive condition. Second, one might be interested in examining the roles of hormones, such as cortisol, thyroid hormones, catecholamines, leptin, and ghrelin/growth hormone, in the direct or indirect regulation of these fluxes. Third, future studies should aim at combining the elegant PINTA method with independent direct measures of hepatic metabolism to monitor hepatic glycogen turnover and ATP/ADP ratios. Finally, this study also demonstrated that V_{CS} varies considerably (between 100 and 600 μmol/min after 12-hour fasting), suggesting that genetic or other factors may regulate hepatic mitochondrial oxidation, results that will have important implications for metabolic dysfunction in obesity, type 2 diabetes, and NAFLD (2).

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