ONE HUNDRED YEARS AGO, Francis Benedict published his detailed monograph on a 31-day starvation of a single subject (4). Subsequently, many studies have investigated the metabolic response to starvation in humans and have detailed our physiological and biochemical understanding of the interaction of lipid and glucose metabolism (e.g., see Refs. 13, 14, 14a, 26, 33, 86, and 87). Specifically, the inhibition of glucose oxidation by fatty acids (FA) (32), the glucose-fatty acid (Randle) cycle (90), and inhibition of lipid oxidation by malonyl-CoA (72) have been of particular importance. Starvation and its associated changes in substrate fluxes have been studied extensively in humans with the use of isotopic tracer methods followed later by more genetic imaging and molecular approaches.

From an evolutionary point of view, the human organism has acquired tools to cope with the metabolic challenges induced by food deprivation for shorter or longer periods. The physiological adaptation that is induced by the fasting state includes increased lipolysis, lipid oxidation, ketone body synthesis, tailored endogenous glucose production (EGP) and uptake, and decreased glucose oxidation. These processes are crucial for survival and serve to protect the organism from excessive erosion of protein mass, which is the predominant supplier of carbon chains for synthesis of newly formed glucose (14, 26, 87, 99). The human organism has extensive fuel reserves that can meet energy demands for substantial periods and that are represented mainly by adipose tissue (14a). To enable this starvation response, profound changes occur in (neuro)endocrine homeostasis with counter-regulatory activities by growth hormone, cortisol, glucagon, and catecholamines (45, 47, 97) and changes in the gonadal and thyroid axes that are mediated partially via decreasing leptin levels (5, 8, 16, 18, 34, 63).

Most studies addressing this starvation response focus on total starvation for ~2–4 days (except for water) and define this as short-term fasting because the adaptive changes occur and become maximal within this time frame. With this in mind, the physiology of starvation has been studied as a model of semiacute lipid exposure but also, perhaps as a consequence, as a model to study the pathophysiological interference of lipids with glucose metabolism and associated insulin resistance.

In this review, we discuss the metabolic adaptation to starvation in humans with special focus on the interaction between lipid and glucose metabolism; short-term starvation demonstrates that the human organism has the intriguing physiological capability to selectively adapt insulin sensitivity to modulate in a tissue-specific manner the production, uptake,
TGest, triglyceride reesterification.

sufficient glucose for basal glucose-dependent processes. GGL, glycogenolysis; turnover rates in lipid metabolism during short-term starvation.

According to duration of starvation and, if applicable, clamp design. In general, rates of lipolysis under hyperinsulinemic conditions are close to zero. Main

Triglyceride reesterification

Insulin-suppressed lipolysis

Lipolysis

Studies that investigated subjects after both overnight and short-term starvation, expressed fluxes in μmol·kg⁻¹·min⁻¹ or mg·kg⁻¹·min⁻¹, assessed glycerol or total fatty acid turnover as measure for lipolysis, and used hyperinsulinemic clamps to test insulin sensitivity are included

<table>
<thead>
<tr>
<th>Process (Lipid Metabolism)</th>
<th>Postabsorptive</th>
<th>Starved</th>
<th>Starving Duration, h</th>
<th>%Difference</th>
<th>Duration and Insulin Dose</th>
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Values are approximates from original articles, and differences do not necessarily denote significance. BSA body surface area. Results should be interpreted according to duration of starvation and, if applicable, clamp design. In general, rates of lipolysis under hyperinsulinemic conditions are close to zero. Main turnover rates in lipid metabolism during short-term starvation.
factor-I (IGF-I) decreases during short-term starvation, most likely reflecting a state of GH resistance (81). At present, it is unclear to which extent these contrasting changes in GH and IGF-I levels modulate (muscle) insulin signaling.

Short-term starvation (~84 h) results in enhancement of adipose tissue responsiveness to epinephrine or β-adrenergic blockade-induced lipolysis in humans (59–61, 116). This may increase lipolytic response during muscle activity, which induces epinephrine secretion. In short, lipolysis is readily stimulated within the first days of starvation.

Falling insulin levels enable lipolysis during fasting. Insulin potently inhibits lipolysis via phosphodiesterase-3B stimulation, resulting in degradation of cyclic AMP, thereby inactivating protein kinase A and reducing phosphorylation of HSL and perilipins (65). In addition to decreasing insulin concentrations during progressive starvation, there is substantial evidence that lipolysis is less sensitive to inhibition by insulin starvation, thereby enabling increased lipolysis. Insulin tolerance tests (ITT) after 67 h of starvation show less absolute suppression of FA levels compared with after an overnight fast (36, 55). Oral glucose tolerance tests (OGTT) after 6 days of starvation are unable to lower plasma FA to the same absolute level as seen after an overnight fast (13, 37). This is also true in a more physiological setting; meal-induced insulin secretion after 72 h of starvation does not suppress FA to postabsorptive levels (49). In accord with this, clamp studies employing low insulin rates show that the lipolytic rates (palmitate release) after 84 h of starvation are higher compared with the postabsorptive state (53). In hyperinsulinemic conditions, both low [13C]palmitic acid enrichment and high plasma FA levels demonstrate that there is ongoing lipolysis after 36, 48, and 72 h of fasting compared with an overnight fast, reflecting insulin resistance of lipolysis (Table 1) (6, 114). Unfortunately, there are no human data on signaling events at the subcellular level in white adipose tissue during starvation. Moreover, there is some time delay to restore insulin sensitivity of lipolysis to postabsorptive levels upon meal ingestion. This delay is seen in virtually all other adaptations to starvation as well, which we will show later. Gradual, but opposite, changes in glucose and lipid metabolism permit a smooth transition from the fed to the starved state to ensure fuel availability while simultaneously sparing glucose.

Released glycerol and FAs are not fully oxidized: cycling and storage. The rate of appearance of FA in plasma exceeds oxidative requirements by approximately twice the amount that would be required to cover the resting metabolic rate (see Figs. 1 and 2) (15, 25, 59, 116). This “excess” plasma FA flux illustrates that free fatty acids (FFA) are disposed otherwise. Triglyceride reesterification (triglyceride cycle) increases ~2.6-fold after 87 h of starvation, which was shown using a combined glycerol and palmitic acid tracer approach (Table 1). (116). Comparable rates of triglyceride cycling were found after 84 and 96 h of starvation with glycerol tracers and indirect calorimetry to assess whole body lipid oxidation (59). Analogous to this, the energetic cost of triglyceride cycling increases as well during starvation (25). Much insight in starvation-induced lipolysis and reesterification of TG came from Jensen et al. (52), who showed that there are regional differences in FA reesterification after 60 h of starvation. Intriguingly, splanchnic output of FA in triglycerides is much lower than total FA reesterification, suggesting that nonhepatic sites of FA reesterification are important for FA and glycerol uptake during starvation (52). These studies confirmed previous studies, which documented that hepatic glycerol uptake and hepatic FA reesterification are actually low (66). In this regard, it was suggested that the ratio between white adipose tissue lipolysis and FA reesterification modulates glycerol availability for glucose production and FA oxidation (15).

Imaging studies subsequently assessed this extrahepatic FA reesterification. Proton magnetic resonance spectroscopy (MRS) demonstrated that intramuscular triglyceride concentrations double during ~60 h of starvation in leg muscles (55, 107, 112). Similarly, biopsy studies reveal that the mean intramuscular triglyceride area fraction doubles after 60 h of fasting (46). In addition, myocardial triglyceride content doubles during starvation, as shown by MRS studies (40, 91). This increase in triglyceride storage during starvation also occurs in
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the liver, but to a much lesser degree. Hepatic FA uptake is followed by triglyceride output, as shown after 3 days of starvation (33). Indeed, hepatic MRS imaging confirms the concept of predominantly extrahepatic FA reesterification during starvation (12, 40, 76, 91). These studies investigating 24 and 72 h of starvation show that the increase in hepatic triglyceride content is limited compared with muscle, a larger compartment, if present at all (Fig. 2).

Increased triglyceride storage in muscle is only modestly paralleled by increased expression of genes that are involved in the uptake and trafficking of FA. Twenty hours of fasting induces lipoprotein lipase expression (88). Tunstall et al. (111) investigated the expression levels of a variety of genes but did not find significant increases in expression levels of FA binding protein or HSL expression after ~40 h of fasting nor of diacylglycerol acyltransferase that catalyzes the formation of triglycerides from diacylglycerol and acyl-CoA. Sterol-responsive element-binding protein-1c regulates lipogenic gene expression and decreases during starvation, underscoring the difference between de novo lipogenesis by chain elongation and triglyceride reesterification from (acyl)glycerol and acyl-CoA (110).

In addition to oxidation or storage as triglycerides, FAs may enter other biosynthetic or metabolic pathways. Although intramuscular triglyceride stores have been invariably suggested to result from lipid overflow and to cause insulin resistance in obesity and type 2 diabetes mellitus (44, 48, 58, 77, 96), only to result from lipid overflow and to cause insulin resistance in exchange (acyl)glycerol and acyl-CoA. Lipogenesis by chain elongation and triglyceride reesterification may not immediately serve general energy requirements. In summary, lipolysis exceeds oxidation during starvation by far, and reesterification and storage of FA occurs in extrahepatic tissues (Fig. 2). It has been argued that these reesterification cycles enhance the ability of substrate metabolism to increase or decrease rapidly when needed (15). Indeed, the lipolytic response in white adipose tissue and subsequent transport to target tissues may take some time and therefore may not immediately serve general energy requirements. Moreover, the reesterification of FA yielding triglyceride may be preferred over storage as bioactive lipid intermediates (e.g., ceramide, diacylglycerol, acylcarnitines) (96).

Glucose Turnover During Starvation

Glucose production. Glucose metabolism has also been studied extensively using tracer methodology and imaging studies. The changes in gluoregulatory hormones during starvation enable maintenance of a low rate of glucose production (11, 47). Notably, falling insulin levels lead to diminished activation of the insulin receptor (IR), which deactivates the downstream insulin receptor substrates 1 and 2 and protein kinase B (Akt). This stimulates glycogenolysis and gluconeogenesis (GNG) while halting de novo lipogenesis (96). Additionally, increased secretion of the pancreatic peptide hormone glucagon stimulates glucose production through the glucagon receptor (47, 89). Here, binding of glucagon results in conformational changes that increase intracellular cAMP levels, that subsequently potentiate glycogenolysis and gluconeogenesis (54).

Plasma glucose levels decrease during fasting, which is a normal physiological finding (20, 60, 73). These decreasing glucose levels have been suggested to result from the slowly decreasing EGP (20, 60) that correlates very well with the plasma glucose level in the postabsorptive state (31). EGP decreases ~12% after 38 h and ~30% after 62 h of starvation compared with after an overnight fast (see also Table 2) (98, 100–103). The fall in EGP in the course of early starvation is explained largely by the decreased contribution of glycogenolysis to EGP (Fig. 1). Early liver biopsy studies show that after 24 h of fasting ~15% of liver glycogen remains (80). In accord with these findings, the slope of the decrease in liver glycogen during the 1st day of starvation as assessed by MRS is steeper than thereafter (94). The loss of glycogen and associated water is reflected in a decrease in liver volume of ~40%. Different isotopic tracer methods have been used to assess the ratio of glycogenolysis and gluconeogenesis during starvation (e.g., labeled glycerol, lactate, and deuterated water). For instance, Landau et al. (67) showed that the relative contribution of GNG to glucose production increases from ~67% after 22 h to 93% after 42 h of starvation (67). Additionally, the kidney contributes >20–25% of total GNG after 60 h of starvation (24, 35).

Triglyceride-derived glycerol and FA in relation to glucose production. Lactate/pyruvate and specific gluconeogenic amino acids such as alanine and glutamine serve as precursors for gluconeogenesis (14a, 26, 41). In addition, part of the starvation-induced glycerol flux is utilized for GNG (51). Catheterization studies (using uniformly labeled [13C]glycerol and measuring splanchnic blood flow) in 60-h-starved men showed that glycerol accounts for ~15% of glucose production (66). Although the contribution of glycerol to gluconeogenesis is apparently small, it may be relevant because acute blockage of lipolysis by nicotinic acid after 86 h of fasting lowers GNG, and this is prevented by concomitant glycerol infusion (50). Moreover, during progressive starvation the contribution of glycerol to glucose production becomes larger (10).

The role of FAs in the regulation of EGP in fasting humans is more enigmatic (9). Using nicotinic acid to block HSL and deuterated water to analyze gluconeogenesis, Chen et al. (19) showed that blocking lipolysis in the early adaptation to starvation decreases the relative contribution of GNG to EGP. Moreover it was suggested that the rebound increase of FA after nicotinic acid withdrawal increased GNG again. Acute inhibition of lipolysis by intravenous administration of nicotinic acid for only ~2 h after 86 h of fasting decreased GNG, although this decrease was insufficient to lower plasma glucose levels (50). In contrast, Féty et al. (28) demonstrated that FFA lowering by nicotinic acid during ~6–8 h in glycerogen-depleted subjects (after 104 h of starvation) increases GNG and urea excretion, reflecting proteolysis. Concomitant β-hydroxybutyrate infusion partially prevents these changes, reflecting the need for alternative oxidative fuel during FA depletion, i.e., the reliance on FAs as oxidative fuel during starvation.

The different results obtained in the above-mentioned studies may be due to different durations of starvation and nicotinic acid administration; an initial reduction in FA may be followed by a decrease in EGP, whereas prolonged antilipolysis results
in increased EGP using amino acid carbon, which is required to cover energy requirements, but leads to deleterious protein loss. Generally, both lipolysis-derived glycerol and FAs may function to meet energy demands. FAs are major substrates for the generation of energy, but this may be less obvious for glycerol, which may be oxidized but is also used as a precursor for gluconeogenesis (51, 52). The resulting glucose is in prolonged starvation not oxidized exclusively but is also used for biosynthetic purposes such as anaplerosis (85, 87, 99). Insulin resistance may mediate this effect.

Adaptive insulin sensitivity during starvation. In insulin-stimulated circumstances, glucose disposal occurs mainly in skeletal muscle (22) and is mediated via the insulin-signaling cascade. Insulin binds to the insulin receptor to induce tyrosine kinase activity, followed by phosphorylation of IRS-1 on tyrosine residues, allowing for the recruitment of the p85/p110 phosphoinositide 3-kinase (96). More downstream, protein kinase B (Akt) is activated, which in turn will increase activity of both glycogen synthase kinase (GSK) and Akt substrate of 160 kDa (AS160). This latter step is required for the translocation of GLUT4 to the plasma membrane. AS160 can also be phosphorylated independently from Akt (64).

There is no doubt that insulin-mediated glucose uptake decreases during short-term fasting, as shown by ITT studies (13, 82), OGTT/intravenous glucose tolerance test (IVGTT) and meal studies (2, 27, 37, 49, 55), and hyperinsulinemic euglycemic clamp studies (6, 21, 46, 69, 79, 101, 112, 114) (Table 2). Notwithstanding different study designs, the above clamp studies show on average an ~44% reduction in glucose uptake after starvation, emphasizing the magnitude of the decreased glucose uptake. Moreover, earlier OGTT studies showed that it takes ~48 h of refeeding to reverse these adaptive changes in glucose metabolism (2). Hyperinsulinemia-mediated suppression of glucose production is not altered after starvation (6, 21), although no true liver clamps have been performed. Therefore, the lower glucose flux reported by the above ITT, OGTT, and meal studies seems to be explained largely by the starvation effect on muscle tissue.

Effects of starvation on insulin signaling in muscle (during hyperinsulinemia) have not been unequivocal (6, 101, 110, 112). Bergman et al. (6) showed lower insulin receptor phosphorylation after 48 h of starvation but no changes in phosphorylation of insulin receptor substrates or the downstream located Akt. We, on the other hand, found both lower phosphorylated Akt in relation to total Akt and the downstream AS160 after 62 h of starvation (101). Although Vendelbo et al. (112) did not support lower Akt phosphorylation, phosphorylation at specific sites of AS160 did decrease during insulin administration after starvation. In addition, data on GLUT4 are variable. In one study, lower GLUT4 expression and protein levels were found after 48 h of starvation (82), but this was not found in other studies. Pilegaard et al. (88) found no change in GLUT4 expression after ~20–24 h of starvation. Likewise, Vendelbo et al. (112) did not detect differences in GLUT4 protein after ~72 h of starvation. In the study by Norton et al. (82), 24 h of refeeding increases GLUT4 expression again.

| Table 2. Studies that investigated subjects after both overnight and short-term starvation, expressed fluxes in μmol·kg⁻¹·min⁻¹ or mg·kg⁻¹·min⁻¹, and used hyperinsulinemic clamps to test insulin sensitivity are included |

<table>
<thead>
<tr>
<th>Process (Glucose Metabolism)</th>
<th>Postabsorptive, μmol·kg⁻¹·min⁻¹</th>
<th>Starved, μmol·kg⁻¹·min⁻¹</th>
<th>Starving Duration, h</th>
<th>%Difference</th>
<th>Duration and Insulin Dose</th>
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<tr>
<td>Glucose production</td>
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<td></td>
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<tr>
<td>Ref. 108</td>
<td>9.4</td>
<td>7.2</td>
<td>72</td>
<td>−24</td>
<td>Ref. 108, 120 min, 0.8 mU·kg⁻¹·min⁻¹</td>
</tr>
<tr>
<td>Ref. 11</td>
<td>11.1</td>
<td>8.3</td>
<td>48</td>
<td>−25</td>
<td>Ref. 20, 150 min, 0.2 mU·BSA⁻¹·min⁻¹</td>
</tr>
<tr>
<td>Ref. 45</td>
<td>11.3</td>
<td>7.6</td>
<td>60</td>
<td>−33</td>
<td>Ref. 6, 180 min, 40 mU·m²·min⁻¹</td>
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<td>Ref. 20</td>
<td>12.0</td>
<td>9.0</td>
<td>48</td>
<td>−30</td>
<td>Ref. 45, 800 min, 100 mU·m²·min⁻¹</td>
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<tr>
<td>Refs. 94 and 97</td>
<td>11.8</td>
<td>8.3</td>
<td>62</td>
<td>−30</td>
<td>Ref. 108, 60 min, 0.8 mU·kg⁻¹·min⁻¹</td>
</tr>
<tr>
<td>Refs. 94 and 97</td>
<td>8.9</td>
<td>6.7</td>
<td>48</td>
<td>−25</td>
<td>Ref. 108, 100 min, 0.8 mU·kg⁻¹·min⁻¹</td>
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<td>Insulin-suppressed glucose production</td>
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<td>1.1</td>
<td>48</td>
<td>0</td>
<td>Ref. 108, 120 min, 0.8 mU·kg⁻¹·min⁻¹</td>
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<tr>
<td>Insulin-mediated glucose uptake</td>
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<td>3.0</td>
<td>48</td>
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<td>150</td>
<td>Ref. 108, 60 min, 0.8 mU·kg⁻¹·min⁻¹</td>
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<tr>
<td>Insulin-stimulated nonoxidative glucose disposal</td>
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<td>112</td>
<td>Ref. 14, 60 min, 0.8 mU·kg⁻¹·min⁻¹</td>
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Values are approximates from original articles, and differences do not necessarily denote significance. Results should be interpreted according duration of starvation and, if applicable, clamp design. In general, rates of glucose production under hyperinsulinemic conditions are close to zero. Main turnover rates in glucose metabolism during short-term starvation.
Although these studies do not agree in every aspect, they provide clues that starvation decreases insulin signaling.

It remains speculative how these changes in insulin signaling and glucose uptake in muscle are mediated. FAs and their derivatives have gained much attention because they may exert inhibitory roles on insulin signaling in insulin-responsive tissues (lipotoxicity) (44, 48, 77, 96). Although the data on accumulation of intramuscular triglycerides during starvation are very robust (see Released glycerol and FAs are not fully oxidized: cycling and storage) (40, 55, 91, 106, 112), this is less clear for accumulation of different specific lipid species. Intracellular ceramide synthesis from palmitate is one of the mechanisms by which palmitate may interfere negatively with insulin-stimulated phosphorylation of Akt (48, 109). Muscle ceramide tends to increase only after 62 h of starvation (101). Although it has been argued that small changes in ceramide may have biological consequences (such as inhibiting glucose uptake) (48, 109), the role of muscle ceramide as inhibitor of insulin signaling during starvation remains uncertain (101). In accord with the notion of lipotoxicity, it has been suggested that starvation may increase lipid peroxidation and oxidative stress. Studies from our department show that starvation decreases erythrocyte glutathione concentrations despite a similar fractional synthetic rate. These lower GSH concentrations are not accompanied by changes in soluble tumor necrosis factor receptors II/II, interleukin-6, or C-reactive protein (21). Moreover, during starvation, markers of oxidative stress such as urinary 8-isoprostaglandin-F2α and malondialdehyde show a reduction of lipid peroxidation products (68). This does not provide evidence that short-term starvation leads to increased oxidative stress or inflammation.

Altogether, the above studies show that starvation decreases insulin sensitivity with a pronounced decrease in muscle glucose uptake. Although it can be suggested that this is mediated by decreased insulin signaling and GLUT4 expression, the underlying mechanisms are not fully elucidated.

Glycogen and nonoxidative glucose disposal during starvation. Nonoxidative glucose disposal consists of glycogen synthesis, anaplerosis, and other biosynthetic purposes in which glucose is involved (43, 85, 87, 99). Glycogen metabolism specifically has a complex regulation via glycogen synthase and GSK-3 (92). Moreover, liver and muscle glycogen metabolism specifically has a complex regulation via glycogen synthase and GSK-3 (92). Furthermore, during starvation, markers of oxidative stress such as urinary 8-isoprostaglandin-F2α and malondialdehyde show a reduction of lipid peroxidation products (68). This does not provide evidence that short-term starvation leads to increased oxidative stress or inflammation.

Flexible interaction between lipid and glucose during starvation. The primary goal of the changes in substrates metabolism is to spare carbon from protein stores to meet anaplerotic and other biosynthetic purposes (14, 26, 85, 87, 99). Glucose is used partially by the central nervous system as an oxidative fuel, but the quantity that will be fully oxidized is only very small (86). Moreover, the central nervous system relies on ketone bodies as alternative fuel and thereby, indirectly, on FAs. Specifically, during long-term starvation, terminal glucose oxidation in the tricarboxylic acid (TCA) cycle and respiratory chain accounts for the loss of only ~24 g glucose (86) (Table 2). Glucose oxidation drops significantly during the first 24 h of starvation, in line with the increase in lipolysis and FA oxidation (Fig. 1) (60, 93). As expected, the respiratory quotient (RQ) decreases significantly and continues to fall until the organism is fully adapted (6, 98, 113).

It has been shown that after 40–48 h of starvation, increased lipid oxidation yields acetyl-CoA that activates muscle PDK4. This inhibits pyruvate dehydrogenase activation, thereby lowering terminal glucose oxidation (105, 110). These data actually show how glucose FA substrate competition (Randle cycling) regulates glucose oxidation (32, 90, 108). Very recently, it has been argued that carnitine acetyltransferase, the enzyme that converts acetyl-CoA to its membrane permeant acetylcarnitine ester, plays an important role in determining cellular acetyl-CoA availability and metabolic flexibility (77, 78). Muscle and plasma acetylcarnitine increase during starvation, reflecting an increase in FA-derived acetyl-CoA (98). In vivo experiments with increased FA oxidation (FAO), one would expect increased expression of carnitine palmitoyltransferase I (CPT I). This was confirmed in one study (88), but two other studies yielded inconclusive results (110, 111). Alternatively, activation of AMP-activated kinase during lower glucose availability in starvation is thought to lower malonyl-CoA levels, which stimulates FAO (72). This could not be confirmed in muscle after 72 h of fasting (112).

When glucose and insulin are reintroduced after starvation, the aforementioned changes are reversed. Insulin and glucose have a direct inhibiting effect on pyruvate dehydrogenase kinase 4 (108). Although refeeding after 20 h of starvation does not inhibit PDK4 expression acutely (88), refeeding for 24 h after 48 h of starvation decreases PDK4 expression and increases pyruvate dehydrogenase complex activity (110). Glucose oxidation and acetyl-CoA formation yield malonyl-CoA, acting as a starting point for de novo lipidogenesis and, in turn, inhibit CPT I (72).

As a consequence of refeeding (by IVGTT/OGTT or clamp) the RQ increases, and this can be interpreted as a measurement of the organism to adapt to different substrates, reflecting metabolic flexibility (57). Starvation decreases this metabolic flexibility (6, 69, 112, 114). Although in these studies different starvation periods and clamp designs were used, the increase in RQ is on average ~13.9% after an overnight fast as opposed to ~6.6% after short-term starvation. This ongoing lipid oxidation is evidenced by intramuscular long-chain acylcarnitines...
that are suppressed after a hyperinsulinemic clamp after 14 but not after 62 h of starvation (98).

In summary, there is a major switch from glucose to FAO during starvation that needs some time to become apparent. A similar duration of time is required for reversal of these fasting-induced changes upon refeeding. These observations point to a notion that a gradual transition during starvation and refeeding guarantees substrate availability. One might hypothesize that this is due partly to the time required to restore or to deplete glycogen stores. This may be a consequence of the fact that enzyme activity is dependent not only on substrate availability but also on activators or inhibitors that have to be switched on or off.

Energy expenditure during starvation: whole body and muscle. Conceptually, one would expect that starvation decreases resting energy expenditure (EE). Both decreased and unaltered EE as measured by indirect calorimetry have been reported during starvation (15, 25, 27, 46, 79, 115) (Table 3). However, some studies have also provided data that EE increases during the first stages of starvation, with the average increase in energy expenditure being ~5.5%, which is difficult to reconcile with energy saving (6, 69, 98). Even when the energy costs of FA recycling, glucose storage, and GNG are added, they may not account for this increase in EE. Another contributor to increased EE may be the rise in sympathetic nervous system activity and plasma catecholamine levels (47). Chan et al. (17) showed that after 72-h starvation, 24-h urinary norepinephrine and dopamine levels and heart rate increased, but not cardiac vagal modulation.

Intriguingly, Hoeks and Herpen (46) have shown that mitochondrial state 3 respiration is reduced ~17% after 60 h of starvation independently of the substrates used; by uncoupling mitochondrial oxidative phosphorylation, respiration was reduced by ~23% but was still higher than state 3 respiration. This points toward an adaptive reduction of both electron transport chain capacity and oxidative phosphorylation in starvation. Iodothyronine deiodinase type 2 (D2) is the enzyme responsible for thyroid hormone activation (deiodination of thyroxin to iodothyronine) and has been implicated in EE and insulin sensitivity (42). As might be expected, D2 expression decreases after 62 h of starvation and increases after insulin administration. It is uncertain whether resting muscle contributes substantially to changes in EE during starvation. This is different during exercise when starving, as described below.

Liver fatty acid oxidation: KB. Owen et al. (86) described the important role of KB for brain metabolism during starvation in the late 1960s. Ketogenesis becomes progressively important as liver glycogens stores fall (Fig. 1) (13, 14a, 30). KB synthesis is controlled by white adipose tissue lipolysis, entry of FAs into hepatic mitochondria, and mitochondrial 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase that is responsible for the irreversible first step of KB synthesis (30). The circulating [glucagon]/[insulin] ratio is an important stimulating factor for ketogenesis (71). Insulin has a strong inhibitory effect on KB and high circulating glucagon levels are necessary for effective ketogenesis.

### Table 3. Studies that investigated subjects after both overnight and short-term starvation, expressed fluxes in $\mu$mol·kg$^{-1}·min^{-1}$ or mg·kg$^{-1}·min^{-1}$ and energy expenditure in MJ/day or kcal·h$^{-1}·day^{-1}$, and used hyperinsulinemic clamps to assess insulin sensitivity are included

<table>
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<tr>
<th>Process</th>
<th>Postabsorptive</th>
<th>Starved</th>
<th>Starving Duration, h</th>
<th>%Difference</th>
<th>Duration and Insulin Dose</th>
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<td>115 min, 100 mU·BSA$^{-1}·min^{-1}$</td>
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Values are approximates from original articles, and differences do not necessarily denote significance. Results should be interpreted according to duration of starvation and, if applicable, clamp design. Main processes in energy metabolism during short-term starvation.
iting effect on ketogenesis via phosphodiesterase-3B dephosphorylation in white adipose tissue (65). In liver, insulin stimulates glucose uptake, resulting in increased malonyl-CoA levels and inhibition of both FAO and ketogenesis (30). Additionally, glucagon increases liver HMG-CoA synthase expression (30). There are tight relationships between KB production that are quantified using stable isotope-labeled D-3-hydroxybutyrate as a tracer, FA levels, and insulin concentrations after 38 h of fasting (102).

In addition to their role as fuels, KBs have biological effects. Ketolysis produces acetyl-CoA, which will, like FA derived acetyl-CoA, inhibit terminal glucose oxidation via pyruvate dehydrogenase (108). In addition, KB infusion reduces insulin-stimulated glucose uptake, suggesting that KBs or downstream intermediates like acetyl-CoA interfere with insulin signaling (113). KBs are in fact taken up by muscle tissue, where they can be oxidized or stored as a carnitine ester (104). This D-3-hydroxybutyrylcarnitine or “ketocarnitine” is synthesized via an acyl-CoA synthetase reaction, and its muscle concentrations correlate well with whole body ketone body production (38, 104). Ketocarnitine was identified as a possible culprit in the induction of insulin resistance (1). Analogous to the acetylarnitine accumulation, ketocarnitine increases ~30-fold during 62 h of starvation, but this does not correlate with changes in insulin-stimulated glucose uptake (unpublished data from Refs. 98 and 104).

Increasing energy demands during exercise: closing the circle. So far, we have discussed adaptive changes to starvation under resting circumstances. From an evolutionary perspective, however, activity (“exercise”) under starving conditions has been necessary to search or hunt for food. Therefore, maintaining and replenishing oxidative fuel stores in muscle to meet caloric demands during activity/stress is of utmost importance during starvation. In general, short-term fasting studies are carried out in ambulatory conditions, with measurements during several hours in supine positions. These studies cannot be used to assess the effects of ambulatory conditions on the response to starvation, since similar conditions were used for assessment of the postabsorptive state. This is important because an immobilized state can induce some of the changes in substrate metabolism, as described above (7).

Only a few studies have examined the effects of exercise during starvation. Nevertheless, these studies enable us to understand the metabolic response to exercise during starvation. At an exercise intensity of ~50% of \( V_{\text{O}_{2\text{max}}} \), the contribution of carbohydrates to energy production is small (62). Furthermore, during exercise after 3.5 days of starvation, the utilization of carbohydrates is lower than endogenous glucose production, partially explaining the observed rise in glucose levels (62). Lower terminal glucose oxidation via increased PDK4-induced inhibition of pyruvate dehydrogenase also explains increased levels of lactate and pyruvate (62, 95, 105). Muscle glycogen levels during exercise decrease less during starvation compared with the postabsorptive state, supporting lower carbohydrate oxidation (62). Likewise, ingested glucose during exercise after 67 h of starvation is less oxidized compared with after an overnight fast (95). Older studies investigating exercise at higher intensity (~70% of \( V_{\text{O}_{2\text{max}}} \)) also show higher plasma glucose and lactate levels during exercise after 23 and 36 h of starvation (23, 70). Higher-intensity exercise after glycogen depletion results in diminished exercise tolerance as opposed to lower intensity (36, 62, 70). Here, lack of glucose and glycogen as substrates results in diminished carbohydrate oxidation that is required to perform high-intensity exercise.

As described above, it is evident that glucose oxidation clearly does not meet the increased energy demand of active muscle during starvation. The same is true for KB oxidation. Although there is an abrupt increase in KB concentrations during exercise in starving conditions, KB levels soon plateau in contrast to FA levels (3). Older forearm studies show that KB uptake is actually very low during exercise in starvation (39). More intriguingly, muscle may be a site of KB production under these circumstances (39).

FAs are the major fuel of muscle during exercise under starved conditions. This is shown by their oxidation, as evidenced by the decrease in RQ (29, 36, 62). More importantly, Green et al. (36) actually showed that intramyocellular triglyceride (IMTG) levels may be dependent on activity during starvation. Starved humans were subjected to an exercise regimen (80 min on ~50% \( V_{\text{O}_{2\text{max}}} \) on the 2nd and 3rd days) during 72 h of starvation, followed by proton MRS studies that showed the typical starvation-induced increase in IMTG to be absent. Starvation-induced insulin resistance still occurred despite the fact that exercise prevents IMTG accumulation (36). This may question the role of IMTG and other lipid mediators in the induction of insulin resistance during starvation. Exercise during starvation relies almost exclusively on the increased IMTG stored for FAO, whereas glucose and KB oxidation are restrained.

Conclusion

Starvation is a physiological adaptation that has been used by many investigators as a paradigm to modulate lipid and glucose metabolism. The reciprocal changes in lipid and glucose metabolism during starvation occur gradually to guarantee substrate availability at all times and can be summarized as follows (Fig. 1). Terminal glucose losses are decreased by changes in glucose and lipid fluxes, uptake, and oxidation. This may spare glucose for the brain to a limited degree, but mainly conserves carbon for biosynthetic purposes and anaplerosis (85–87, 99). FAs are the major fuel to yield energy (60, 93). Quite interestingly, triglyceride storage during starvation occurs in muscle and to a much lesser degree in liver (Fig. 2) (52, 55, 76, 112). The latter organ oxidizes FAs for its own energy needs but also produces KB that are oxidized mainly by the central nervous system (86). Cycling of substrates and the formation of substrate depots may serve to enable prompt responsiveness of substrate metabolism upon changing nutritional conditions and demands like exercise. At the molecular level, the regulation of glucose and lipid oxidation is quite well understood (32, 72, 90, 105, 108), but this is less the case for the mechanisms that regulate cellular glucose uptake. It is unlikely that one single lipid metabolite induces insulin resistance in view of the fact that, e.g., KB also induce insulin resistance (113). Although exercise prevents the starvation-induced rise in IMTG, exercising subjects nevertheless exhibit reduced muscle uptake of glucose during starvation. If there is a role for lipid metabolism in mediating insulin resistance during starvation, other metabolites such as acetyl-CoA may be considered as candidate mediators (32, 77, 90).
Obviously, starvation per se does not really have a direct clinical relevance in Western society, obesity, diabetes, or metabolic disease. However, the metabolic fundamentals of the starvation response by which the adaptive reciprocity of lipid and glucose metabolism interferes with insulin sensitivity are relevant for these other conditions; the available knowledge of physiological starvation-induced changes in lipid and glucose metabolism may be helpful to study lipotoxicity and insulin resistance in prevalent nonfasting conditions such as obesity and type 2 diabetes mellitus. In starvation, insulin resistance enables survival, but in obesity and type 2 diabetes chronic insulin resistance causes progressive problems and complications. In these pathophysiological conditions, the differences in liver and muscle metabolism with those observed during starvation are of particular interest. In starvation, muscle becomes severely insulin resistant while storing excess triglycerides, presumably to allow muscle activity when required. Liver, on the other hand, stores less triglycerides and produces substantial amounts of KB, which are utilized predominantly in the central nervous system. The fact that liver does not preferentially store lipid during starvation is of interest in conditions like obesity and insulin resistance-induced liver disease (i.e., nonalcoholic fatty liver disease) (96). It may be the case that, compared with muscle, liver is more sensitive to lipid overload in these pathological conditions.

Moreover, the starvation response shows that the adaptation to energy deficit is very effective and coordinated with multiple adaptations in different organs. Short-term starvation forces the organism to make an enormous switch in substrate utilization to finally rely, directly or indirectly, on FAs as a fuel source while saving glucose. From an evolutionary perspective, this physiological lipid-induced effect on glucose oxidation and uptake is very strong, and therefore, it may help to understand why insulin resistance due to lipid overload in obesity and type 2 diabetes mellitus is so difficult to treat. Most of the currently available antidiabetic drugs merely treat the symptom of hyperglycemia but do not act as disease-modifying drugs by addressing the fundamentals of metabolic pathophysiology. The importance of reciprocity in lipid and glucose metabolism during human starvation should be taken into account when studying lipid and glucose metabolism in general and in pathophysiological conditions in particular.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.R.S. and P.B.S. did the conception and design of the research; M.R.S. analyzed the data; M.R.S. prepared the figures; M.R.S. drafted the manuscript; M.R.S., P.B.S., M.G.S., S.M.H., and J.A.R. edited and revised the manuscript; M.R.S., P.B.S., M.G.S., S.M.H., and J.A.R. approved the final version of the manuscript.

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