REVIEW

Effects of GH on protein metabolism during dietary restriction in man

Helene Nørrelund, Anne Lene Riis, and Niels Møller
Medical Department M (Endocrinology and Diabetes), Aarhus Kommunehospital, Aarhus, Denmark

Summary The metabolic response to dietary restriction involves a series of hormonal and metabolic adaptations leading to protein conservation. An increase in the serum level of growth hormone (GH) during fasting has been well substantiated. GH has potent protein anabolic actions, as evidenced by a significant decrease in lean body mass and muscle mass in chronic GH deficiency, and vice versa in patients with acromegaly. The present review outlines current knowledge about the role of GH in the metabolic response to fasting, with particular reference to the effects on protein metabolism. Physiological bursts of GH secretion seem to be of seminal importance for the regulation of protein conservation during fasting. Apart from the possible direct effects of GH on protein dynamics, a number of additional anabolic agents, such as insulin, insulin-like growth factor-I, and free fatty acids (FFAs), are activated. Taken together the effects of GH on protein metabolism seem to include both stimulation of protein synthesis and inhibition of breakdown, depending on the nature of GH administration, which tissues are being studied, and on the physiological conditions of the subjects.

BACKGROUND

Throughout evolution man has been exposed to periods of abundance or shortage of food, and series of endocrine and metabolic adaptations have enabled the human body to withstand starvation for relatively long periods. Cahill has estimated that a normally proportioned man metabolically relies on 10–15 kg triacylglycerol in adipose tissue (125 000 kcal), 6 kg mobilizable muscle protein (25 000 kcal), but only 0.3 kg of glycogen (1500 kcal).1 During a fast liver glycogen stores decreases rapidly,2 due to which the body becomes progressively dependent on gluconeogenesis to meet the demand for glucose.3,4 Studies of experimental underfeeding of volunteers have shown that muscle function becomes impaired by surprisingly small degrees of undernutrition.3 Approximately 1.75 g of muscle protein must be broken down to provide one gram of glucose, since not all amino acids can be converted to glucose; the brain alone requires 100–120 g of glucose per day.3 If no other adaptations took place, this would correspond to the breakdown of more than 150 g of protein per day, and the body’s store of protein in muscle would rapidly be depleted. This is prevented by a cascade of inter-related adaptations to starvation.

Classic studies by Benedict in 19154 and Cahill 1,5 have partly clarified the complex nature of these adaptations. The metabolic events are characterized initially by increased release of free fatty acids (FFAs) from endogenous triglyceride stores for use as fuel, and reduced production and oxidation of glucose. Successful metabolic adaptation to starvation depends, in great part, on the organism’s ability to generate de novo glucose for utilization in the brain, as hepatic glycogen stores are depleted and during short-term starvation the brain is not adapted to the use of...
Deficiency increases lean body mass and reduces fat anabolic effects of GH remain to be established. Years ago the mechanisms underlying the protein metabolism were viewed as beneficial when considering GH treatment in obesity.

The transition to fat fuel utilization was originally thought primarily to be mediated by the decrease in insulin levels that occurs during fasting. Subsequent studies have emphasized the direct role of ketone bodies per se on inhibition of muscle breakdown and the role of decreased serum T3 levels in decreasing urinary nitrogen excretion. The role of other hormones, including cortisol, glucagon, and growth hormone (GH) remains less clear. It is well known that GH levels increase during fasting and that GH administration is associated with stimulation of lipolysis, decreased oxidative glucose disposal and protein retention. As early as 1957 Russell summarized the known metabolic effects of GH and proposed "that growth hormone has a particular function in enabling nitrogen conservation whenever the supplies of protein or of carbohydrate are restricted, that is, between meals as it were. If this were true, one might imagine that either the activity of growth hormone or its rate of secretion could be increased to assist in the metabolic adjustments which the animal must make when the absence of food threatens its economy." In humans, Raben demonstrated that fasting values of FFAs increased after injection of GH, whereas glucose and food suppressed this effect. It was suggested that "growth hormone could control, at least in part, the rate of fat mobilization in the postabsorptive state and yet be present at all times to direct the available energy whether from food or fat depots, towards protein synthesis and sparing." In 1963 Rabinowitz and Zierler published their "feast-famine" hypothesis, suggesting that metabolism during the day is dominated alternately by the action of insulin and GH in a three-phased cycle: "Exposure to insulin in the immediate postprandial period encourages storage of carbohydrate and fat exposure to human growth hormone plus insulin in the delayed postprandial period encourages protein synthesis and exposure to human growth hormone in the remote postprandial phase encourages mobilization and peripheral oxidation of fat and retards translocation of glucose into muscle and adipose tissue." Although this was proposed more than 30 years ago the mechanisms underlying the protein anabolic effects of GH remain to be established.

GH substitution in hypopituitary adults with GH deficiency increases lean body mass and reduces fat mass and studies in the postabsorptive state suggest that GH stimulates protein synthesis without altering protein breakdown. The fact that fasting constitutes a very robust stimulus for pituitary GH release suggests a physiological role of GH during this condition, but experimental data to substantiate this hypothesis are surprisingly few.

As stressed in the first paragraph the body is able to adapt well to starvation. It is therefore not surprising that the effectiveness of hypocaloric regimens in the treatment of obesity is modest: they are antagonised by physiological mechanisms which have evolved to minimize the consequences of starvation. As food intake decreases, the level of thyroid hormone falls and metabolic rate is lowered. In addition, as weight loss occurs, the lean body mass will decrease as well as the fat mass — this in itself will reduce the daily energy expenditure. In treatment of obesity with very low calorie diet (VLCD) protein loss presents a major obstacle for its use. However, diet restricted obese subjects treated with high-dose GH have been reported to conserve protein and to reduce fat mass. Moreover, the intrinsic ability of GH to increase energy expenditure and to enhance peripheral T3 generation may be viewed as beneficial when considering GH treatment in obesity.

Based on these considerations the aim of the present review is to outline current knowledge about the role of GH in the metabolic response to fasting and starvation, with particular reference to the effects on protein metabolism.

**THE EFFECT OF GH ON PROTEIN METABOLISM DURING SHORT-TERM FASTING IN NORMAL SUBJECTS**

Metabolic changes during fasting

Over 80 years ago Benedict fasted a normal volunteer for 30 days, and by indirect calorimetry and measurement of excreted nitrogen found carbohydrate to provide only a very small proportion of the body’s fuel demands. After a few days of fasting fat provided over 3/4 of the daily caloric loss and protein the remainder. Benedict was unaware of the obligatory requirement of the central nervous system for glucose as fuel and did not correlate nitrogen loss with gluconeogenesis. In 1967 Owen et al. reported displacement of a major share of man’s brain requirement for glucose by β-hydroxybutyrate and acetoacetate in three obese patients after 5–6 weeks of starvation.

The events precipitating the metabolic characteristics of fasting are not entirely understood.
subtle decrease in the concentration of glucose in plasma is accompanied by a small decrease in insulin/glucagon ratio. The capacity of insulin in high physiologic amounts to stimulate lipogenesis in adipose tissue is well known as is the capacity of insulin in low physiologic amounts to inhibit triglyceride lipolysis and FFA release from adipose tissue. Insulin is also directly capable of inhibiting the release of amino acids from muscle. Net proteolysis in muscle — resulting from the falling insulin concentration — leads to release of amino acids, mainly alanine and glutamine; the latter is partially converted to alanine in the intestine, and thus the liver receives an increased supply of this amino acid, and gluconeogenesis at this early stage of starvation is therefore proceeding largely at the expense of muscle protein. The elevation in FFA leads to a number of adaptations. Skeletal muscle will use FFA almost entirely in proceeding largely at the expense of muscle protein. The elevation in FFA leads to a number of adaptations. Skeletal muscle will use FFA almost entirely in preference to glucose. In the liver the rate of fatty acid esterification, usually stimulated by insulin, will decrease; fatty acids will be diverted into oxidation (glucagon stimulates this pathway). This diversion is mediated in part by a decrease in hepatic malonyl-CoA concentration, a result of the decrease in insulin concentration. Increased oxidation of fatty acids leads to increased production of the ketone bodies β-hydroxybutyrate and acetoacetate. These can be used as an oxidative fuel by many tissues, at a rate which largely depends on their concentration in the blood.

Glycolytic cells and tissues such as erythrocytes and the renal medulla will still rely on glucose utilization. Glycolysis in these tissues, however, leads to the release of lactate which is returned to the liver and avidly reconverted into glucose. By these mechanisms the need to produce glucose from muscle protein is reduced, and the loss of nitrogen decreases. However, with insulin concentration decreasing, the net stimulus would seem to be for increasing muscle protein breakdown. How then is the sparing of muscle protein accomplished? The onset of starvation is marked by a decrease in the level of the active thyroid hormone triiodothyronine (T3) in the blood. The effect of a fall in T3 concentration is to reduce overall metabolic rate, and possibly to reduce the rate of proteolysis in muscle. Other possible mechanisms include a rise in epinephrine, a rise in ketone bodies, a decrease in energy expenditure, a rise in FFA, or a rise in GH. The constellation of enhanced fat utilization, diminished carbohydrate consumption and restriction of protein catabolism observed during fasting, is consistent with the known effects of exogenous GH.

During the first days of fasting, urinary nitrogen production has been reported to be unchanged or increased whereas the urea-N synthesis rate (UNSR) increases after 40 h of fasting. Since total fasting is a combination of absent dietary carbohydrate, leading to a low insulin, high glucagon state, and absent dietary protein, the N losses and changes in whole body protein turnover could potentially represent the combined effects of different mechanisms of protein wasting. These mechanisms have been explored by several groups using tracers of leucine, lysine or phenylalanine to model whole body protein kinetics. The results indicate an increase in whole body protein breakdown after 3 days of fasting. Human and animal studies have yielded conflicting results as regards the effect of short term fasting on leucine oxidation. After 3 days of fasting increased leucine oxidation has been demonstrated in normal subjects as well as in rodent models, both in vivo and in vitro. Other animal studies have demonstrated decreased leucine oxidation during short-term fasting. We assessed the effects of short-term fasting on substrate metabolism in eight healthy adults and after 40 h of fasting we did not observe any change in phenylalanine degradation. The apparently conflicting results probably reflect differences in duration of fasting.

Forearm proteolysis has been shown to increase after 30 and 60 h of fasting and isotopic turnover methods have shown that this effect after 60 h of fasting is mediated by accelerated local rates of amino acids appearance (Ra), with no reduction in rates of disposal (Rd). In the study of Pozefsky, the increase in calculated net amino acid release was due to an increase in forearm blood flow and not to an increase in the amino acid arteriovenous concentration gradients. We found a tendency towards increased muscle breakdown after 40 h of fasting, and the increase in net amino acid release was due to increased arteriovenous concentration gradients.

Increases in branched-chain amino acid levels and decreases in other amino acids during short-term fasting have been reported previously. The increase in branched-chain amino acid levels (leucine, isoleucine, valine) is consistent with the increased proteolysis. The reduction in some of the other amino acids may be related to reduced amino acid synthesis (in case of nonessential amino acids) or increased utilization of amino acids for gluconeogenesis (especially alanine). Alanine stands out as the principal amino acid released from muscle and extracted by liver, consistent with its role as a gluconeogenic precursor. The decrease in plasma alanine concentration during fasting is the result of decreased muscle release and to some extent perhaps increased hepatic clearance. Infusion of alanine to fasted man results in an increase in...
blood glucose concentration supporting the hypothesis that one rate-limiting step for gluconeogenesis in this metabolic setting is the rate of alanine release from muscle rather than some enzymatic step within the liver itself.

Impact of GH during fasting

Administration of 30 IU of GH daily in normal subjects during dietary restriction or 0.06 IU/kg GH daily during a hyponitrogenous diet results in a fall in serum urea level and urinary urea excretion. In a design which allowed for control of insulin and glucagon (by infusion of somatostatin) we tested the hypothesis that GH is the principal mediator of protein conservation during fasting. The participants fasted for 40 h and during that period GH (4.5 IU) was given partly as bolus injections, partly as continuous infusion. Urinary urea excretion and serum urea increased by 50% when participants fasted without GH. Experiments in hypophysectomized rats have provided evidence that GH may act on the liver to decrease UNSR, and, in parallel, increase glutamine release, thereby diminishing hepto-renal clearance of the circulating nitrogen pool. In our study, UNSR was further increased by GH suppression. In the postabsorptive state, Wolthers et al. have shown unchanged UNSR during short-term GH exposure (GH infusion for 12 h, 1 IU/h) and a decrease with more prolonged administration (subcutaneous GH for 2 days (8 IU/day), and sc GH for 4 days (0.1 IU/kg/day)), suggesting that part of the established anabolic effect of GH on whole body protein metabolism in normal subjects postabsorptively could be exerted through effects on peripheral protein conversion rather than on liver metabolism.

Previous studies assessing the impact of GH (0.3 IU/kg/day for 7 days) on protein metabolism postabsorptively have shown that GH primarily increases protein synthesis at the whole body level, and there is evidence that acute exposure to GH (0.003 IU/kg/h via the brachial artery for 6 h) may directly increase muscle protein synthesis. Furthermore it has been reported that 6 weeks of high-dose GH treatment (15 IU thrice weekly) to malnourished hemodialysis patients resulted in stimulation of muscle protein synthesis without any effects on muscle protein degradation. On the other hand some studies have failed to detect any effect of GH on muscle protein synthesis. In one of these studies Cope et al. infused somatostatin and replacement doses of insulin, glucagon, and GH (initially 0.0015 IU/kg/h, followed by 0.006 IU/kg/h for the last 3.5 h) for a 7-h period. They did, however, find that isotopically measured muscle protein breakdown across the leg was relatively lower after acute GH exposure with borderline P-values of 0.05 for phenylalanine and 0.09 for leucine. In our study, muscle protein breakdown increased by 25% among participants fasted without GH, and forearm phenylalanine release increased by 40%. It should be noted that the studies assessing muscle protein metabolism have employed exposure to very high levels of GH due to which the more prolonged studies in all likelihood have increased the existing lean body mass, which in itself increases total protein turnover.

A decrease in plasma glucose during fasting is well known and it has been shown that GH infusions (0.006–0.012 IU/kg/h for 12 h; 0.006–0.024 IU/kg/h for 1 h) induce acute insulin resistance characterized by impaired suppression of hepatic glucose production and decreased insulin-dependent glucose disposal. Though the mechanism remains unclear, the finding of an increase in forearm blood flow in response to fasting (36–72 h) has been reported previously. Likewise, it is unclear what causes the increase in energy expenditure (EE) observed after short-term starvation, although several studies have shown that GH (0.008 IU/kg/h for 5 h; 0.1 IU/kg/day for 4 days) may stimulate EE independent of body composition.

It is well known that lipid oxidation increases during fasting and during GH infusion. In addition the increased lipolytic responsiveness to GH (0.4 IU GH as an intravenous bolus injection after 36 h of fasting) may be increased during energy restriction. Episodic GH exposure (0.2–1.1 IU GH as an intravenous bolus) results in marked stimulation of lipolysis, reaching a peak after 2–3 h. In young healthy subjects the nocturnal mean peak of GH precedes that of FFAs by 2 h, a time span comparable to that found after GH bolus administration, suggesting that GH may act as an important regulator of diurnal fluctuations in fuel supply and consumption.

THE EFFECT OF GH ON PROTEIN METABOLISM DURING SHORT-TERM FASTING IN GH-DEFICIENT PATIENTS

The effect of GH on protein kinetics

The fact that fasting constitutes a very robust stimulus for pituitary GH release implies a physiological role of GH during this condition, and hyposomatotropinemia during fasting in GH-deficient adults has recently been shown to be associated with increased whole body protein loss, accounted for by a net reduction in
protein synthesis. A study of protein turnover in GH-deficient adults have demonstrated reduced rates of protein synthesis and breakdown and subsequent normal net protein loss compared to normal controls in line with earlier observations of the effect of chronic GH deficiency on protein metabolism. An initial decline in LBM may be the consequence of GH insufficiency but clinical experience suggests that LBM stabilizes at a reduced level and this adaptation may explain the development of stable, albeit reduced, protein and LBM in GH deficiency. GH substitution (0.018 IU/kg/day for 1 month followed by 0.036 IU/kg/day for 8 weeks in GH-deficient adults revealed increased net protein synthesis and unaltered total protein turnover, in line with a recent dose–response study (GH 0.01 IU/kg/day for 1 week). Furthermore a significant decrease in plasma concentration of amino acids was observed during short-term fasting (36 h) with GH substitution (1.3 IU/day). The effect of substantial hypoaminoacidemia on protein kinetics is unknown, but intravenous infusion of amino acids in healthy adults exerts a protein anabolic effect by stimulation of protein synthesis at the whole body level and inhibition of endogenous protein degradation, and a dose-dependent relationship between amino acid concentration and leucine kinetics has been demonstrated.

The effect of GH on substrate metabolism

A significant increase in fasting glucose level with therapy (GH dose 0.02–0.1 IU/kg/day for 6–12 months) has been demonstrated repeatedly. Hypoglycemia during fasting is a regular occurrence in untreated GH-deficient children and hypopituitary children have been shown to have decreased fasting glucose production and utilization. Extrahepatic insulin resistance may be concealed in hypopituitary children treated with GH (3 IU for 2–4 weeks), as suggested by their normal glycemic response to exogenous insulin. This could relate to the fact that skeletal muscle contributes to a much lesser extent to glucose utilization in children, who have limited muscle mass compared to brain.

Several lines of evidence support the notion that GH stimulates energy expenditure. GH deficiency is associated with subnormal EE and GH administration in GH-deficient adults is accompanied by a significant increase in EE.

Stimulation of lipid metabolism during GH substitution has been demonstrated repeatedly. During fasting with GH we found an increase in oxidation of lipids and circulating lipid fuel substrates, and in line with this observation, an anecdotal study of short-term fasting (48 h) in a pituitary dwarf has demonstrated that GH substitution (12 IU/day) during fasting accelerates mobilization of fatty acids to normal values.

THE EFFECT OF GH ON PROTEIN METABOLISM DURING LONGER-TERM SEMIFASTING

Protein metabolism follows a characteristic pattern during longer term energy deprivation. Immediately after the introduction of a hypocaloric diet, protein wasting is considerable, but after 2–3 weeks urinary N-excretion is decreased to nearly constant values in the presence of stably decreased circulating insulin and glucose levels and increased FFA and ketone body concentrations. Whole body protein breakdown becomes reduced, while leucine oxidation decreases while plasma amino acid concentrations are relatively constant apart from a decrease in alanine levels and increase in branched amino acid levels. The moderation of protein loss is partly accounted for by the lower protein and energy requirement of the reduced residual lean tissue mass. However, specific metabolic adaptations act to increase retention of dietary protein and to maximize the reutilization of released amino acids. A specific mechanism suggested to promote amino acid reutilization during energy deficiency is a diminished rate of protein breakdown.

The effect of GH on protein kinetics

The metabolic response to GH during prolonged fasting (5–6 weeks) in obese subjects was studied 25 years ago by Felig et al. Supraphysiologic doses were employed (30 IU/day from the 35th day of the fast and continued for 3 days) and a significant reduction in urinary urea could be demonstrated, implying that GH reduces protein catabolic processes, primarily in the liver. GH treatment in combination with a hypocaloric diet has been studied by Snyder and Clemmons. Twenty obese subjects received 75 kJ/kg IBW for 13 weeks in combination with either GH (0.3 IU/kg ideal body weight (IBW) every other day during weeks 2–12) or saline. Nitrogen balance was significantly more positive in the GH group, but the effect vanished after 33 days. No significant acceleration of fat loss could be demonstrated, as estimated by underwater weighing. During dietary restriction, Tagliaferri et al. evaluated the effects of administration of GH (1 IU/kg IBW/week for 4 weeks) and found significant preservation of FFM in the GH-treated participants. Under experimental conditions similar to these we examined whether GH administration (0.03–0.08 IU/IBW for 4
weeks) preserves FFM and protein stores in obese women during well-defined hypocaloric regimens. In line with the observation of Tagliaferri we found reduced loss of fat free mass among GH-treated patients, compatible with a more pronounced decrease in urine urea excretion and serum urea. Protein breakdown decreased in both groups during the VLCD, but phenylalanine degradation in relation to phenylalanine concentration, representing an index of phenylalanine hydroxylase activity independent of circulating phenylalanine, decreased by 9% in the GH group, whereas an increase of 8% was observed in the placebo group. In vitro data have shown that phenylalanine itself is both substrate and an essential activating factor for phenylalanine hydroxylase. This is supported by a study of the relationship between phenylalanine degradation and phenylalanine concentration, in which a direct linear correlation between phenylalanine concentration and its hydroxylation rate was demonstrated. A suppressive effect of GH on phenylalanine hydroxylase activity in the liver seems a plausible explanation and generally coexistence of increased levels of amino acids and decreased serum urea level and urine urea excretion in the GH group suggest a direct suppressive effect of GH on hepatic ureagenesis (UNSR) as proposed earlier. In earlier studies a positive correlation between the concentration of leucine in plasma and its oxidation have also been demonstrated, supporting the concept that factors that minimize the size of the free amino acid pool (the balance of protein breakdown and synthesis) will work together to diminish oxidation.

The effect of GH on substrate metabolism

The concept of using GH in obese patients is obvious and several clinical trials have been conducted. After treating middle aged, obese men for 9 months Johannsson et al. administered GH (0.03 IU/kg), demonstrated a reduction in abdominal fat mass, improvement of glucose and lipoprotein metabolism and reduction in diastolic blood pressure. Jørgensen al. administered GH (0.09 IU/IBW) to obese women for 5 weeks and showed an increase in EE, increased rates of lipid oxidation, suppression of oxidative glucose disposal and markedly impaired insulin sensitivity. In the same study a significant reduction in total fat mass and LPL activity was demonstrated. Svensson et al. treated obese subjects with the oral GH secretagogue MK-677 for two months and showed a sustained increase in fat-free mass and a transient increase in basal metabolic rate.

SUMMARY AND CONCLUSION

On the whole it appears that the effects of GH on protein metabolism include both stimulation of protein synthesis and inhibition of breakdown depending

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on the nature of GH administration, which tissues are being studied, and on the physiological conditions of the subjects being studied.

The apparent complexity of the impact of GH on protein metabolism is perhaps predictable, when considering the widespread metabolic actions of GH. Table 1 summarizes the changes observed in our studies. Stimulation of lipolysis (and ketogenesis), hyperinsulinemia and activation of IGF-I all have protein anabolic properties. A striking effect of GH deprivation is a substantial decrease in circulating free IGF-I during short-term fasting in normal subjects. The magnitude of this suppression suggests that free IGF-I may be an important mediator of the protein conserving effects of GH during a brief fast. IGF-I has been shown to act through both stimulation of protein synthesis and inhibition of muscle protein breakdown. Lipid intermediates stimulate protein synthesis and insulin inhibits proteolysis. In addition GH has direct anabolic effects and may induce hyperglycemia and low circulating levels of amino acids. Administration of small amounts of glucose during fasting as well as hyperglycemia may be protein sparing and the concentration of amino acids may affect protein loss as hyperaminoacidemia has been shown to stimulate protein synthesis and to inhibit breakdown.

In conclusion, physiological increments in GH secretion seem to be of importance for the regulation of protein conservation during fasting. These phenomena add weight to the concept of GH substitution in protein metabolism during fasting. These phenomena add weight to the concept of GH substitution in protein metabolism during fasting. These phenomena add weight to the concept of GH substitution in protein metabolism during fasting.

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