The Protein-Retaining Effects of Growth Hormone During Fasting Involve Inhibition of Muscle-Protein Breakdown

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The metabolic response to fasting 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. The present study was designed to test the hypothesis that GH may be a principal mediator of protein conservation during fasting and to assess the underlying mechanisms. Eight normal subjects were examined on four occasions: *1*) in the basal postabsorptive state (basal), *2*) after 40 h of fasting (fast), *3*) after 40 h of fasting with somatostatin suppression of GH (fast-GH), and *4*) after 40 h of fasting with suppression of GH and exogenous GH replacement (fast+GH). The two somatostatin experiments were identical in terms of hormone replacement (except for GH), meaning that somatostatin, insulin, glucagon and GH were administered for 28 h; during the last 4 h, substrate metabolism was investigated. Compared with the GH administration protocol, IGF-I and free IGF-I decreased 35 and 70%, respectively, during fasting without GH. Urinary urea excretion and serum urea increased when participants fasted without GH (urea excretion: basal 392 ± 44, fast 440 ± 32, fast-GH 609 ± 76, and fast+GH 408 ± 36 mmol/24 h, $P < 0.05$; serum urea: basal 4.6 ± 0.1, fast 6.2 ± 0.1, fast-GH 7.0 ± 0.2, and fast+GH 4.3 ± 0.2 mmol/l, $P < 0.01$). There was a net release of phenylalanine across the forearm, and the negative phenylalanine balance was higher during fasting with GH suppression (balance: basal 9 ± 3, fast 15 ± 6, fast-GH 17 ± 4, and fast+GH 11 ± 5 nmol/min, $P < 0.05$). Muscle-protein breakdown was increased among participants who fasted without GH (phenylalanine rate of appearance: basal 17 ± 4, fast 26 ± 9, fast-GH 33 ± 7, fast+GH 25 ± 6 nmol/min, $P < 0.05$). Levels of free fatty acids and oxidation of lipid decreased during fasting without GH ($P < 0.01$). In summary, we find that suppression of GH during fasting leads to a 50% increase in urea-nitrogen excretion, together with an increased net release and appearance rate of phenylalanine across the forearm. These results demonstrate that GH—possibly by maintenance of circulating concentrations of free IGF-I—is a decisive component of protein conservation during fasting and provide evidence that the underlying mechanism involves a decrease in muscle protein breakdown. ***Diabetes*** **50:96–104, 2001**

In humans, the metabolic response to fasting involves a series of hormonal and metabolic adaptations. After 2–3 weeks of fasting, urinary nitrogen excretion is decreased to nearly constant values in the presence of low and steady circulating levels of insulin and glucose and high free fatty acid (FFA) and ketones (1–3). Whole-body protein breakdown decreases (4,5), while plasma amino acid concentrations remain relatively constant, with a decrease in alanine levels and an increase in branched amino acid levels (1,5).

During the first days of fasting, urinary nitrogen excretion has been reported to be either unchanged (2,6) or increased (3), whereas whole-body protein breakdown has been reported to increase after 3 days of fasting (4,7). In addition, forearm net protein breakdown has been shown to increase after 30 and 60 h of fasting (8,9). Human and animal studies have yielded conflicting results regarding the effect of short-term fasting on leucine oxidation (4,7,10–13).

The transition to fat fuel utilization may in part be mediated by a decrease in insulin levels (2), and high concentrations of lipid intermediates together with decreased serum T_3 concentrations may inhibit protein catabolism (14,15). The role of other hormones, including cortisol, glucagon, and growth hormone (GH), remains unclear. It is well known that GH levels increase during fasting (16,17) and that GH administration is associated with stimulation of lipolysis, decreased oxidative glucose disposal, and protein retention (18–25). Most studies assessing the effects of GH on protein metabolism have used designs in which tissue exposure to GH has been pharmacological rather than physiological.

The present study was therefore designed to assess the physiological role of GH in the regulation of substrate metabolism during short-term fasting and, more specifically, to test the hypothesis that physiological hypersecretion of GH plays a pivotal role in protein conservation during fasting. In addition, we sought to identify the underlying mechanisms responsible for the protein conservation of GH. For this purpose, we studied eight normal subjects on four occasions (to define the

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fast, 40 h of fasting; fast-GH, 40 h of fasting with suppression of endogenous growth hormone secretion with somatostatin; fast+GH, 40 h of fasting with suppression of endogenous growth hormone secretion and replacement of growth hormone by infusion; FFA, free fatty acid; FFM, fat-free mass; GH, growth hormone; RER, respiratory exchange ratio; TBW, total body water; UNSR, urea nitrogen synthesis rate.

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specific effects of GH) with concomitant determination of substrate oxidation, urea excretion, and whole body and regional muscle fluxes of phenylalanine and tyrosine.

RESEARCH DESIGN AND METHODS

The study population comprised eight healthy males (mean ± SE age 23 ± 0.4 years, BMI 23 ± 0.8 kg/m^2), who were examined on four occasions. Before the study, subjects were on a weight-maintaining diet for at least 3 months. All subjects gave their written informed consent, and the study was approved by the regional ethics committee and conducted according to the Declaration of Helsinki.

All subjects were instructed to maintain (for at least 3 days) their normal diet before admission; the participants were allocated to *1*) basal postabsorptive investigations (basal), *2*) 40 h of fasting (fast), *3*) 40 h of fasting with suppression of endogenous GH secretion with somatostatin (fast-GH), or *4*) 40 h of fasting with suppression of endogenous GH secretion and replacement of GH by infusion (fast+GH). During fasting with GH suppression, somatostatin, insulin, and glucagon were infused for the last 28 h. During fasting with GH replacement, GH was added to the protocol for the last 28 h. Somatostatin (200 µg/h) was infused together with insulin (Actrapid 100 I.E./ml, Novo Nordisk; infusion rate adjusted to maintain plasma glucose level between 3 and 5 mmol/l [0.15 and 0 mU · kg^{-1} · min^{-1}]) and glucagon (GlucaGen 1 mg/ml, Novo Nordisk; the infusion rate adjusted to maintain stable glucose levels [1.0–1.5 ng · kg^{-1} · min^{-1}]). The two somatostatin experiments were identical in terms of hormone replacement doses, except for GH (Norditropin 12 U/ml, Novo Nordisk; 4.5 U partly as bolus injections [0.375 U every fourth hour for 28 h], partly as continuous infusion [0.08 U/h]). To avoid hypoglycemia during fasting without GH, six participants temporarily received glucose intravenously (between 0.3 and 0.5 mg · kg^{-1} · min^{-1} for a period of 0.5–2.5 h); exactly the same amount of glucose was given in exactly the same manner in the experiment with GH replacement. Because potential hypoglycemia was anticipated during GH suppression, the fast-GH experiments were always conducted before fast+GH to allow for control of exogenous glucose. The subjects were blinded to this procedure. Otherwise, the sequence of experiments was random, with at least 6 weeks elapsing between each experiment. Somatostatin, insulin, glucagon, and GH infusions were continued for a total of 28 h. During the last 4 h, substrate metabolism was investigated. No participants received glucose during these last 4 h; glucose infusion was tapered at least 2 h before investigations of substrate metabolism were started. Blood was sampled frequently during the fast, and data are based on triplicate measurements within the last 30 min of the study. Urine for determination of urea was collected over a 16-h period in the postabsorptive state. In the three 40-h experiments, urine was collected from 0–24 h and while substrate metabolism was investigated (24–28 h). During fasting, only tap water was allowed.

Measurements. All studies were performed with the participants in the supine position. Catheters for measurements of forearm arteriovenous substrate balances were placed as previously described (26). In brief, one catheter was placed retrogradely in a deep antecubital vein, and one catheter was inserted retrogradely in a heated contralateral dorsal hand vein. Criteria for correct positioning were oxygen saturations <70% and >91%, respectively. A third catheter was placed antegradely in an antecubital vein of the heated hand for infusions. Before each deep venous sample, total ipsilateral forearm blood flow was determined by means of venous occlusion plethysmography (27). Hand blood flow was interrupted by a wrist cuff inflated to a pressure of 250 mmHg immediately before each blood flow determination and 1 min before each deep venous sample.

At 8:00 A.M., priming doses of [3-^{3}H]glucose (New England Nuclear, Boston, MA) (20 µCi), L-[^{15}N]phenylalanine (0.7 mg/kg), L-[$^2H_4$]tyrosine (0.5 mg/kg), and L-[^{15}N]tyrosine (0.3 mg/kg) (Cambridge Isotope Laboratories) were given to accomplish an early plateau. A continuous infusion of [3-^{3}H]glucose (20 µCi/h), L-[^{15}N]phenylalanine (0.7 mg · kg^{-1} · h^{-1}), and L-[$^2H_4$]tyrosine (0.5 mg · kg^{-1} · h^{-1}) was started and maintained for 4 h. The chemical, isotopic, and optical purity of the isotopes was tested before use. Solutions were prepared under sterile conditions and were shown to be free of bacteria and pyrogen before use. The specific activity of tritiated glucose was assayed as previously described (19). L-[^{15}N]phenylalanine, L-[$^2H_4$]tyrosine, and L-[^{15}N]tyrosine were measured as their t-butyldimethylsilyl ether derivatives under electron ionization conditions (28). Plasma concentrations of amino acids were determined by high-performance liquid chromatography (HP 1090 series 2 HPLC, 1046 fluorescence detector and cooling system) with precolumn *O*-phthalaldehyde derivatization (29). In addition, concentrations of phenylalanine and tyrosine were measured by mass spectrometry using β-methylphenylalanine and α-methyltyrosine, respectively, as internal standards (28). Fat-free mass (FFM), comprising both muscle tissue and nonmuscle fat-free tissue, was estimated by means of dual-energy X-ray absorptiometry scanning (Hologic QDR-1000/W, Waltham, MA). Plasma glucose levels were measured in duplicate immediately after sampling on a glucose analyzer (Beckman Instruments, Palo Alto, CA). A double monoclonal immunofluorometric assay (Delfia, Wallac, Finland) was used to measure serum GH. Plasma glucagon levels (30) and serum C-peptide (Immunoclear, Stillwater, MN) were measured by radioimmunoassay. Total and free IGF-I were determined as previously described (31,32). Insulin was determined by a commercial enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark). Catecholamines were measured by liquid chromatography (33). Urea excretion was determined by an indophenol method and serum urea by a commercial kit (Cobas Integra, Roche, Hvidovre, Denmark). Cortisol was measured by an automated chemiluminescence system (Chiron Diagnostics, Fernwald, Germany). FFAs were determined by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany). Total and free hormone concentrations of T_4 and T_3 were measured in serum, as previously described (34). Thyrotrophin was measured in a solid-phase, two-site chemiluminescent enzyme immunometric assay (Immulite, DPC, Los Angeles). Indirect calorimetry (Deltatrac monitor, Datex Instrumentarium, Helsinki, Finland) was performed for 30 min (10:30 to 11:00 A.M.), allowing measurements of respiratory exchange ratios (RERs). The initial 5 min of calorimetry was used for acclimatization, and calculations were based on mean values of 25 measurements of 1 min each. Likewise, estimated rates of lipid and glucose oxidation and total energy expenditure were obtained by indirect calorimetry after correction for protein oxidation, as estimated by urinary excretion of urea (35). Net nonoxidative glucose disposal was calculated by subtracting glucose oxidation from the isotopically assayed total glucose disposal. Urea accumulation was determined assuming immediate dispersion of urea from the blood to total body water (TBW), which was estimated by the formula (36):

$$TBW = (0.3625 \times BW) + (0.2239 \times BH) - (0.1387 \times Y) - 14.47$$

where Y is age (years), BW is body weight (kg), and BH is body height (cm). Urea nitrogen synthesis rate (UNSR) was determined as urinary excretion of urea corrected for accumulation of urea in total body water and for hydrolysis in gut (37). UNSR was calculated as urinary excretion rate (E), corrected for accumulation (A) in TBW and for the fractional intestinal loss (L):

$$UNSR = (E + A)/(1 - L)$$

where E = (urine flow, l/h) × (urinary urea nitrogen, mmol/l) and A = (change in blood urea nitrogen, mmol · l^{-1} · h^{-1}) × (TBW) (l). L (fractional intestinal loss) was estimated to be 0.14.

Phenylalanine kinetics. For measurements of whole body phenylalanine kinetics, the equations of Thompson et al. (38) were used. For regional phenylalanine kinetics, our previously described equations (28) were used. Phenylalanine flux (Q_p) and tyrosine flux (Q_t) were calculated as follows:

$$Q_{flux} = i[(E_i/E_p) - 1]$$

in which i is the rate of tracer infusion (mmol · kg^{-1} · h^{-1}), and E_i and E_p are enrichment of the tracer infused and plasma enrichment of the tracer at isotopic plateau, respectively.

The rate of phenylalanine conversion to tyrosine (I_{pt}) was calculated as follows:

$$I_{pt} = Q_t \times ([^{15}N]Tyr_{ei} / [^{15}N]Phe_{ei}) \times (Q_p / (I_p+Q_p))$$

where $[^{15}N]Tyr_{ei}$ and $[^{15}N]Phe_{ei}$ are the isotopic enrichments of the respective tracers in plasma and I_p is the infusion rate of [^{15}N]phenylalanine (mmol · kg^{-1} · h^{-1}).

Phenylalanine incorporation into protein was calculated by subtracting I_{pt} from Q_p, because phenylalanine is irreversibly lost either by conversion into tyrosine or by incorporation into protein.

In the forearm study, phenylalanine balance (PheBal) was calculated as follows:

$$PheBal = (Phe_A - Phe_V) \times F$$

in which Phe_A and Phe_V are phenylalanine concentrations in arteries and veins and F is blood flow.

Regional protein breakdown represented by phenylalanine rate of appearance (Ra Phe) was calculated as follows (22):

$$Ra\ Phe = Phe_A\ [(Phe_{EA}/Phe_{EV}) - 1] \times F$$

in which Phe_{EA} and Phe_{EV} represent phenylalanine isotopic enrichment in arteries and veins, respectively.

Local rate of disappearance was calculated as: Rd Phe = Phe Bal + Ra Phe.

Statistics. Results are expressed as means ± SE. The Kolmogorov-Smirnov test was used to test for normal distribution. Statistical comparisons between the study periods (basal, fast, fast-GH, fast+GH) were assessed by a two-way analysis

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TABLE 1
Serum and plasma concentration of hormones and metabolites

	16-h fast	40-h fast	40-h fast with GH suppression	40-h fast with GH substitution	*P* (ANOVA)
GH (μg/l)	0.34 ± 0.13*	2.36 ± 0.38	1.26 ± 0.24*	2.09 ± 0.19	<0.01
Total IGF-I (μg/l)	256 ± 22	275 ± 32	173 ± 22*	246 ± 25	<0.05
Free IGF-I (μg/l)	0.68 ± 0.10*	0.34 ± 0.08	0.10 ± 0.03*	0.28 ± 0.09	<0.01
Insulin (pmol/l)	19.3 ± 1.5*	11.1 ± 1.0*	5.1 ± 1.4	7.8 ± 1.2	<0.01
Glucagon (pg/ml)	53.1 ± 8.9*	178.3 ± 25.1*	86.5 ± 8.7	101.3 ± 4.8	<0.01
C-peptide (pmol/l)	383 ± 37*	163 ± 12*	64 ± 15	109 ± 25	<0.01
Cortisol (nmol/l)	252 ± 16*	356 ± 20	381 ± 30	345 ± 14	<0.01
Norepinephrine (pg/ml)	142 ± 12	181 ± 15	131 ± 15	141 ± 15	0.08
Epinephrine (pg/ml)	29.5 ± 3.4	51.1 ± 5.6*	31.6 ± 2.5	32.1 ± 5.2	<0.01
Free T_3 (pmol/l)	5.0 ± 0.5*	3.7 ± 0.2	3.3 ± 0.3	3.2 ± 0.3	<0.05
Total T_3 (nmol/l)	2.05 ± 0.08*	1.53 ± 0.04	1.37 ± 0.07	1.43 ± 0.06	<0.01
TSH (mU/l)	1.41 ± 0.15*	0.53 ± 0.09	0.24 ± 0.03	0.23 ± 0.03	<0.01
FFA (mmol/l)	0.47 ± 0.04*	1.58 ± 0.11	1.27 ± 0.09*	1.51 ± 0.19	<0.01
Glucose (mmol/l)	5.1 ± 0.1	3.6 ± 0.1*	4.5 ± 0.1*	5.3 ± 0.2	<0.01

Data are means ± SE. *Vs. other groups. ANOVA, analysis of variance; TSH, thyrotrophin.

of variance. If this test was positive, the Student-Newman-Keuls test was used for post hoc analysis. A *P*-value below 0.05 was considered significant. Unless specified otherwise, data referred to below were obtained with arterialized blood based on triplicate measurements during the last 30 min of the experiments.

RESULTS

Circulating hormones and metabolites. The level of GH was significantly lower postabsorptively and after 40 h of fasting with GH suppression than after the plain fasting and GH replacement situations (Table 1). Notably, both total IGF-I and free IGF-I concentrations were comparable during fasting and during GH replacement, whereas IGF-I and free IGF-I decreased 35 and 70%, respectively, during fasting without GH. Insulin and C-peptide levels were slightly lower during somatostatin infusions. Glucagon and cortisol were significantly increased during fasting. Epinephrine increased after 40 h of fasting and thyroid parameters decreased, but no difference was found with or without GH substitution.

Levels of FFAs increased during fasting, but fasting without GH caused a relative decrease. The level of plasma glucose was significantly lower during ordinary fasting and fasting with GH suppression.

Creatinine levels remained identical in all four situations (basal 78 ± 5, fast 83 ± 4, fast-GH 82 ± 7, and fast+GH 83 ± 3 μmol/l; $P > 0.05$).

Whole-body protein metabolism. Plasma concentrations of amino acids are shown in Table 2. From the postabsorptive state to 40 h of fasting, there was a significant increase in plasma concentrations of valine, isoleucine, and leucine. Serine decreased during fasting with GH suppression, whereas tyrosine, valine,

TABLE 2
Plasma concentration of amino acids

Plasma concentration (μmol/l)	16-h fast	40-h fast	40-h fast with GH suppression	40-h fast with GH substitution	*P* (ANOVA)
Aspartate	257 ± 21	246 ± 26	211 ± 16	225 ± 22	—
Glutamate	134 ± 14	133 ± 10	126 ± 9	131 ± 8	—
Serine	155 ± 11	118 ± 7*	131 ± 5	151 ± 10	<0.05
Glutamine	901 ± 70	833 ± 102	883 ± 76	927 ± 74	—
Histidine	86 ± 9	100 ± 11	108 ± 12	109 ±11	—
Glycine	414 ± 38	303 ± 29	298 ± 24	359 ± 40	—
Threonine	198 ± 21	163 ± 14	182 ± 15	216 ± 22	—
Alanine	299 ± 23	208 ± 13*	249 ± 16	269 ± 21	<0.05
Arginine	292 ± 29	306 ± 31	277 ± 17	261 ± 28	—
Tyrosine	55 ± 6	47 ± 4*	72 ± 4*	61 ± 5	<0.01
Valine	217 ± 14*	391 ± 38	445 ± 36	351 ± 32	<0.01
Methionine	Below detection limit	Below detection limit	38 ± 15	Below detection limit	—
Phenylalanine	50 ± 3	49 ± 2	61 ± 2*	54 ± 3	<0.01
Isoleucine	54 ± 5*	128 ± 10	152 ± 10	111 ± 9	<0.01
Leucine	124 ± 11*	295 ± 27	352 ± 29	254 ± 29	<0.01
Lysine	214 ± 21	148 ± 23	189 ± 15	185 ± 22	—
Total	3,476 ± 224	3,498 ± 281	3,744 ± 208	3,674 ± 233	—

Data are means ± SE. *Vs. other groups. ANOVA, analysis of variance.

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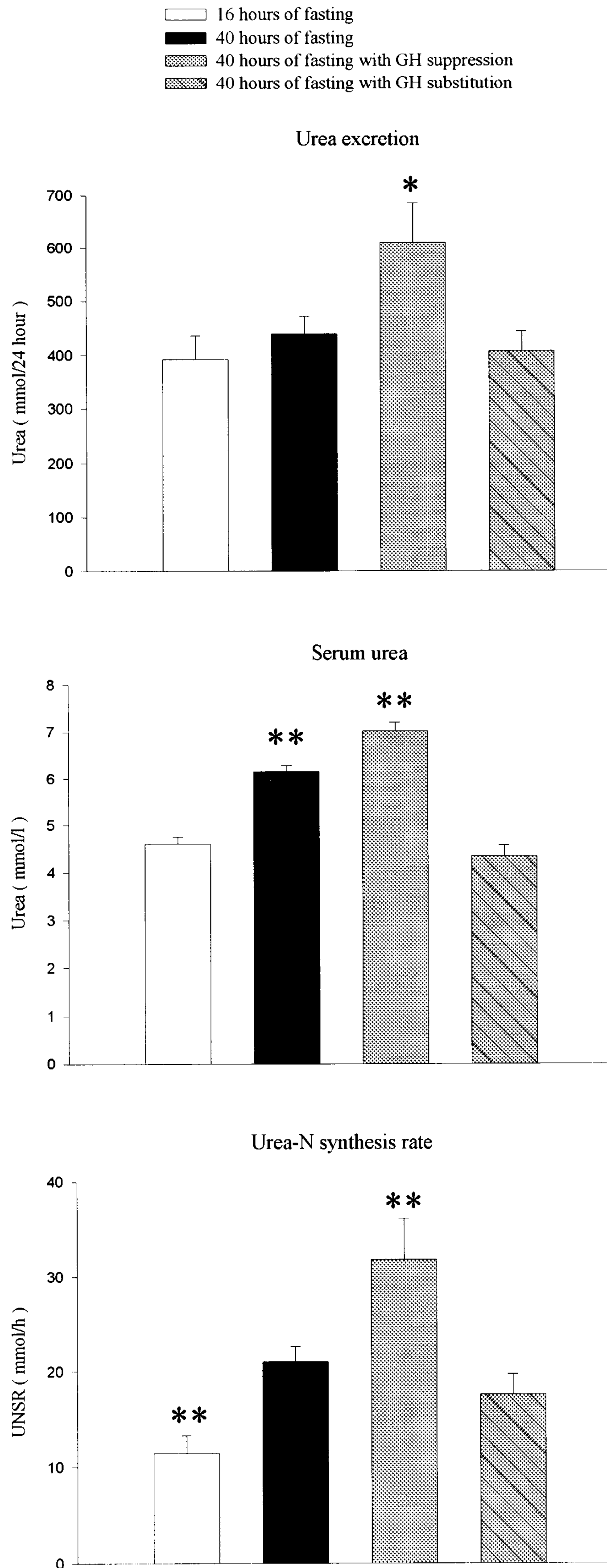

FIG. 1. Urea excretion, serum urea, and UNSR (means ± SE). $^*P < 0.05$ vs. other groups; $^{**}P < 0.01$ vs. other groups.

phenylalanine, isoleucine, and leucine increased compared with fasting with GH.

Urinary urea excretion (basal 392 ± 44, fast 440 ± 32, fast-GH 609 ± 76, and fast+GH 408 ± 36 mmol/24 h; $P < 0.05$) and serum urea (basal 4.6 ± 0.1, fast 6.2 ± 0.1, fast-GH 7.0 ± 0.2, fast+GH 4.3 ± 0.2 mmol/l; $P < 0.01$) were significantly increased when participants fasted without GH. UNSR increased after 40 h of fasting and was further increased by GH suppression (basal 11.4 ± 1.9, fast 21.0 ± 1.6, fast-GH 31.8 ± 4.3, fast+GH 17.6 ± 2.1 mmol/h; $P < 0.01$) (Fig. 1). Nitrogen excretion was significantly increased accordingly during fasting with GH suppression (basal 12.2 ± 1.4, fast 13.7 ± 1.0, fast-GH 19.0 ± 2.4, fast+GH 12.7 ± 1.1 g/24 h; $P < 0.01$); in addition, nitrogen excretion was increased during fasting with GH suppression in the preceding 24 h (fast 12.5 ± 1.0, fast-GH 14.3 ± 0.6, fast+GH 10.4 ± 0.8 g/24 h; $P < 0.01$). During fasting, a rise in nitrogen excretion was seen ($P < 0.05$).

Phenylalanine flux (Fig. 2) was increased after 40 h of fasting compared with the postabsorptive state (basal 50.9 ± 2.2, fast 59.0 ± 2.7, fast-GH 61.8 ± 1.6, fast+GH 60.5 ± 1.8 mmol · kg $FFM^{-1} \cdot h^{-1}$; $P < 0.01$), whereas tyrosine flux remained unaffected. Phenylalanine conversion to tyrosine did not change during fasting (basal 5.2 ± 0.8, fast 3.8 ± 0.3, fast-GH 4.6 ± 0.3, fast+GH 4.4 ± 0.4 mmol · kg $FFM^{-1} \cdot h^{-1}$; $P > 0.05$), whereas protein synthesis (phenylalanine disposal not accounted for by phenylalanine conversion to tyrosine) was higher (basal 45.7 ± 2.0, fast 55.2 ± 2.9, fast-GH 57.2 ± 1.7, fast+GH 56.0 ± 2.1 mmol · kg $FFM^{-1} \cdot h^{-1}$; $P < 0.01$). Phenylalanine clearance rate was decreased after fasting (basal 100 ± 7, fast 72 ± 5, fast-GH 78 ± 6, fast+GH 83 ± 8 ml/min; $P < 0.01$).

Forearm protein metabolism. Forearm blood flow increased after 40 h of fasting (basal 1.8 ± 0.1, fast 3.8 ± 0.3, fast-GH 3.7 ± 0.3, fast+GH 3.3 ± 0.3 ml · 100 $ml^{-1} \cdot min^{-1}$; $P < 0.01$) (Fig. 3). Forearm muscle exhibited a net release of phenylalanine, and the negative phenylalanine balance was higher during fasting with GH suppression than during fasting with GH replacement and fasting alone (basal 9 ± 3, fast 15 ± 6, fast-GH 17 ± 4, fast+GH 11 ± 5 nmol/min; $P < 0.05$). Muscle protein breakdown, represented by phenylalanine rate of appearance, was highest among participants fasted without GH (basal 17 ± 4, fast 26 ± 9, fast-GH 33 ± 7, fast+GH 25 ± 6 nmol/min; $P < 0.05$). Incorporation of phenylalanine into muscle, represented by phenylalanine rate of disappearance, was increased after fasting (basal 8 ± 2, fast 11 ± 6, fast-GH 16 ± 4, fast+GH 14 ± 3 nmol/min; $P < 0.05$). In general, forearm release of amino acids tended to be higher during fasting without GH (sum of forearm differences: basal 384 ± 267, fast –292 ± 318, fast-GH 733 ± 275, fast+GH, 329 ± 261 µmol/l; $P = 0.11$).

Indirect calorimetry and glucose turnover. Energy expenditure, measured by indirect calorimetry, increased after 40 h of fasting (basal 1,713 ± 59, fast 1,944 ± 44, fast-GH 1,916 ± 78, fast+GH 1,879 ± 51 kcal/24 h; $P < 0.05$) (Fig. 4). The RER decreased with fasting (basal 0.84 ± 0.01, fast 0.77 ± 0.01, fast-GH 0.79 ± 0.01, fast+GH 0.78 ± 0.01; $P < 0.01$). Protein oxidation was higher during GH suppression (basal 0.70 ± 0.08, fast 0.81 ± 0.08, fast-GH 1.10 ± 0.15, fast+GH 0.74 ± 0.08 mg · $kg^{-1} \cdot min^{-1}$; $P < 0.01$), whereas lipid oxidation was significantly lower (basal 0.65 ± 0.05, fast 1.16 ± 0.05, fast-GH 0.89 ± 0.07, fast+GH 1.05 ± 0.07 mg · $kg^{-1} \cdot min^{-1}$; $P < 0.01$) (Fig. 4). Glucose turnover (basal 1.98 ± 0.08, fast 1.46 ± 0.06, fast-GH 1.44 ± 0.07, fast+GH 1.49 ± 0.07 mg · $kg^{-1} \cdot min^{-1}$; $P <$ 0.01) and glucose oxidation (basal 1.57 ± 0.13, fast 0.79 ± 0.13,

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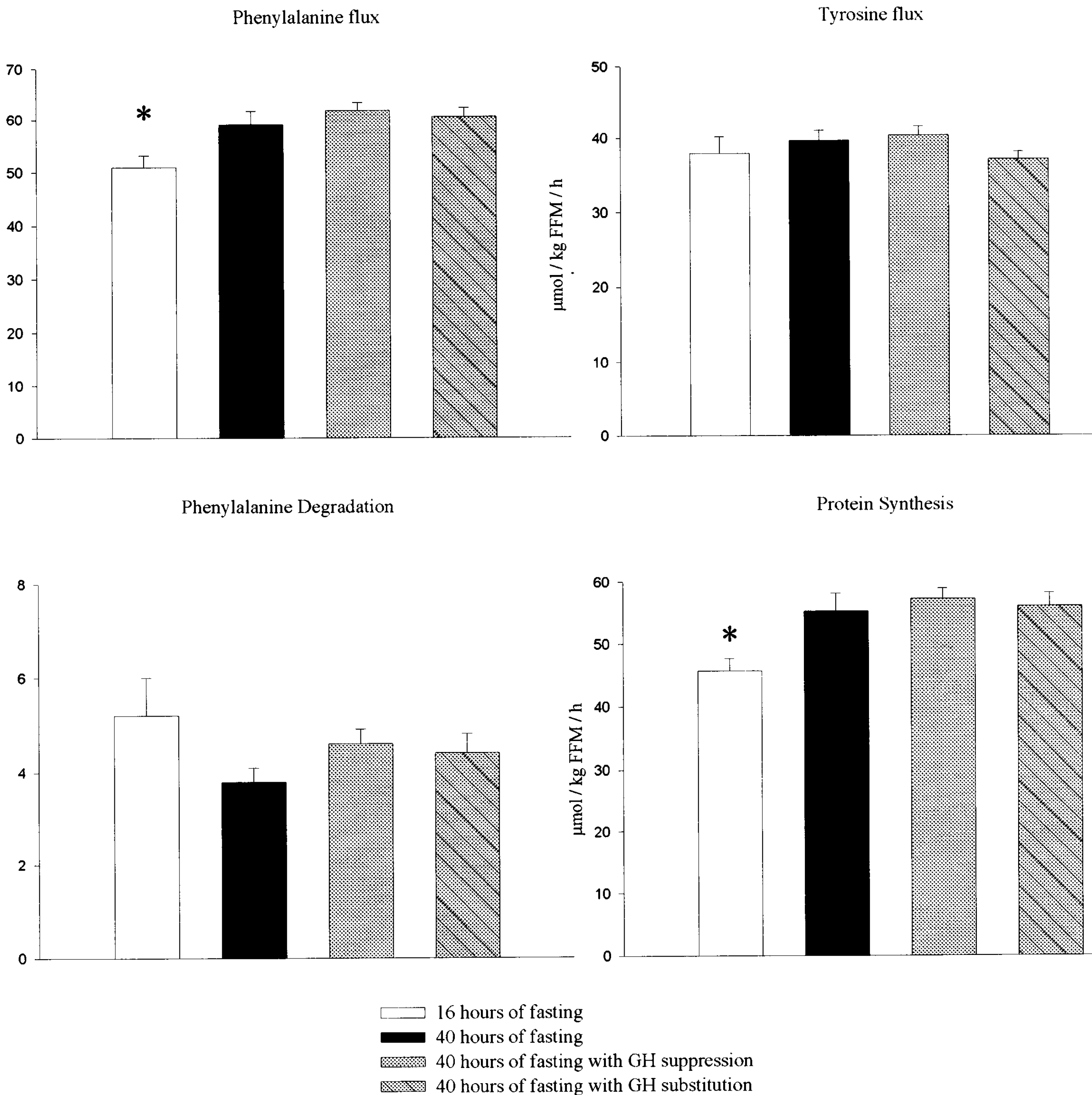

FIG. 2. Whole-body protein dynamics (means ± SE). *P < 0.01 vs. other groups.

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fast-GH 0.91 ± 0.18, fast+GH 0.95 ± 0.11 mg · kg^{-1} · min^{-1}; $P < 0.01$) decreased during fasting. The ratio between glucose oxidation and total glucose turnover was also significantly increased postabsorptively (basal 0.79 ± 0.06, fast 0.54 ± 0.09, fast-GH 0.63 ± 0.1, fast+GH 0.64 ± 0.07; $P < 0.05$) (Fig. 4).

DISCUSSION

The present study was designed to assess the effects of short-term fasting on substrate metabolism with particular reference to the effects of GH. The main findings of the study are that *1*) GH deprivation during a 40-h fast increases production of urea nitrogen by 50%; *2*) GH replacement restores normal urea nitrogen generation; and *3*) the underlying mechanisms involve GH-mediated inhibition of muscle protein breakdown.

As is the case with all whole-body studies using somatostatin, it is impracticable to faithfully mimic original hormonal conditions for insulin and glucagon patterns. Obviously, this limitation impedes direct comparison between conditions with and without somatostatin. For this reason, we included an experimental situation in which we reproduced GH patterns (circulating GH concentrations and free and total IGF-I) to allow isolation of the effects of GH. Thus, unless somatostatin per se profoundly changes the protein kinetic actions of GH (of which there is no evidence), we are confident that our findings are both qualitatively and quantitatively tenable in a physiological context.

It is impressive that even a modest 40–50% reduction in GH levels during somatostatin infusion was associated with substantial effects on the metabolic adaptation to short-term fasting. A moderate dose of somatostatin (200 µg/h) was

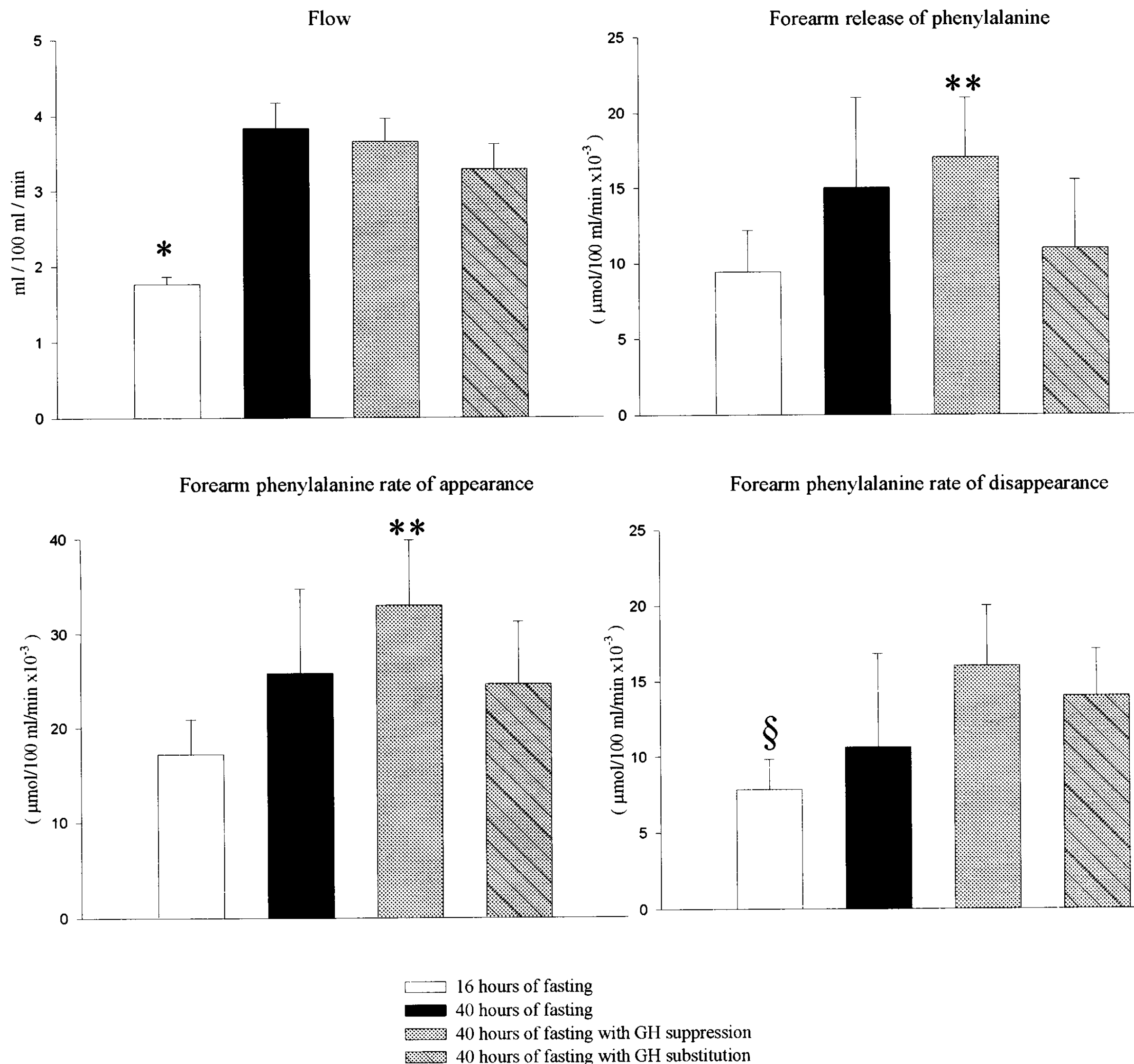

FIG. 3. Forearm protein dynamics (means ± SE). *P < 0.01 vs. other groups; **P < 0.05 vs. basal and fast+GH groups; §P < 0.05 vs. fast-GH and fast+GH groups.

used to avoid gastrointestinal discomfort during the 28-h infusion. Nevertheless, plasma GH concentrations were significantly decreased compared with ordinary fasting and GH substitution. Furthermore, total IGF-I levels decreased 30–40% and free IGF-I decreased 60–70% during GH suppression, but were almost identical during ordinary fasting and GH substitution. This finding indicates that a significant degree of GH suppression was accomplished and that the GH replacement experiments in these terms matched normal fasting conditions. GH levels during GH suppression tended to be higher than in the basal state, suggesting that the observed effects of GH suppression represent a conservative estimate and that the impact might have been even more pronounced with more complete suppression of GH secretion.

The metabolic effects of GH are complex and involve increased lipolysis, hyperinsulinemia, and stimulation of IGF-I activity, all of which have protein anabolic properties (15,39–43). In addition, GH has direct anabolic effects (44) and may—as observed presently—induce hyperglycemia and low circulating concentrations of tyrosine and phenylalanine. Of these potential secondary mediators, lipid intermediates have been shown to stimulate protein synthesis (15,39,40), insulin to inhibit breakdown (41,45), and IGF-I to act through both mechanisms (42–46). Furthermore, hyperglycemia may be protein sparing (47).

Previous studies assessing the impact of GH on protein metabolism at the whole-body level have in general shown that GH primarily increases protein synthesis (48,49), and there is evidence that acute exposure to high levels of GH may increase muscle protein synthesis (50,51). Furthermore, it has been reported that 6 weeks of high-dose GH treatment to malnourished hemodialysis patients resulted in stimulation of muscle protein synthesis without any effects on muscle protein breakdown (52). In contrast, some studies have failed to detect any effect of GH on muscle protein synthesis (22,53). In one of these studies, Copeland and Nair (22) found that isotopically measured muscle protein breakdown across the leg was relatively lower after acute GH exposure, with bor-

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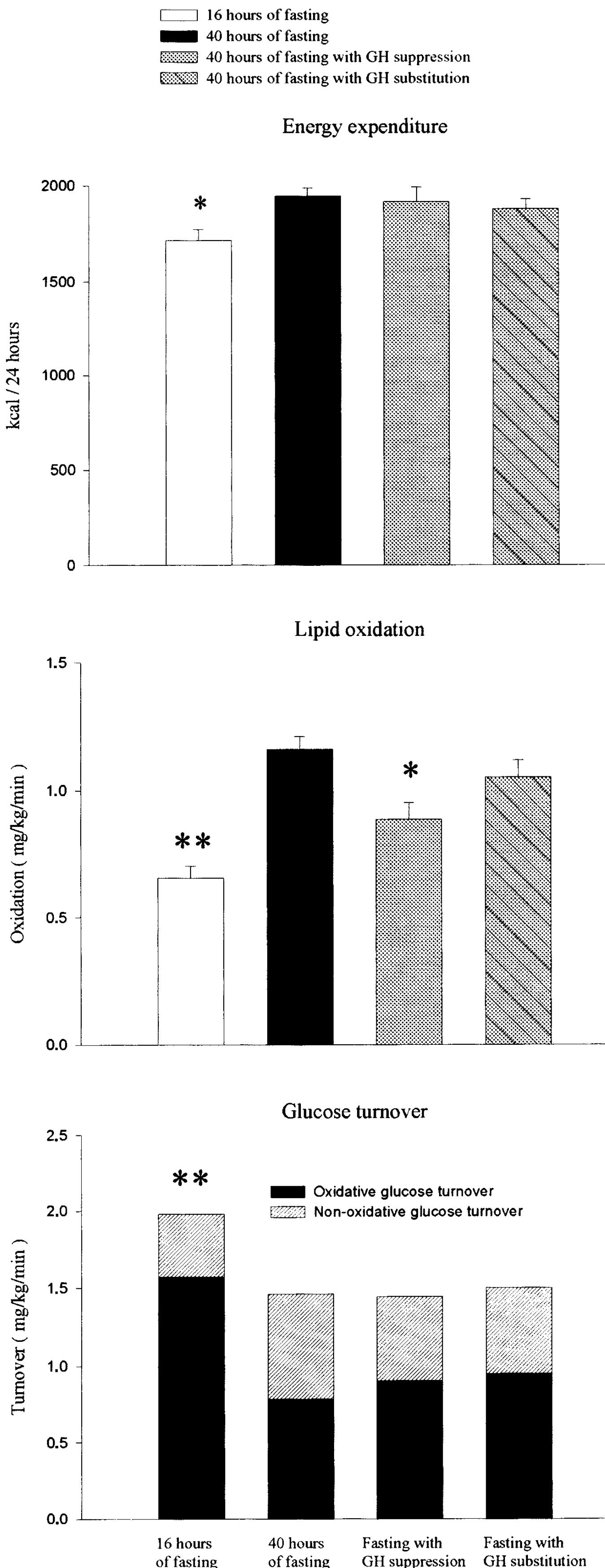

FIG. 4. Energy expenditure and lipid and glucose metabolism (means ± SE). *P < 0.05 vs. other groups; **P < 0.01 vs. other groups.

derline P-values of 0.05 for phenylalanine and 0.09 for leucine. It should be noted that the majority of studies assessing muscle protein metabolism have employed exposure to very high levels of GH. The present study strongly suggests that under conditions of physiological fasting, even small increments in circulating concentrations of GH have substantial preserving effects on whole body nitrogen balance and that these effects are due in part to inhibition of muscle protein breakdown. The apparent complexity of the impact of GH on protein metabolism is perhaps predictable, considering the widespread metabolic actions of GH mentioned above. In this context, it should also be underlined that GH has been reported to specifically suppress hepatic ureagenesis (54,55). One of the most striking effects of GH deprivation in the present study was a 60–70% decrease in circulating free IGF-I. The magnitude of this suppression indicates that free IGF-I may be an important mediator of the protein-conserving effects of GH during brief fasting. This notion is in line with the study in malnourished hemodialysis patients, which reported a strong correlation between GH-induced reduction in protein catabolism and increments in circulating free (but not total) IGF-I (52). Interestingly, IGF-I, when perfused across the human forearm, has been shown to inhibit muscle protein breakdown (46); it is thus tempting to suggest that the observed decrease in muscle protein catabolism could be generated by free IGF-I. It is also possible that increased FFA concentrations and subtle hyperinsulinemia may have participated. In the present study, FFA concentrations were increased and insulin and C-peptide concentrations ascended slightly (P > 0.05) during GH infusion.

Because phenylalanine hydroxylation (a catabolic step) may reflect catabolism of amino acids in general, the degradation at steady state should ideally correspond to urea-nitrogen synthesis (56). Presently, phenylalanine degradation did not change during fasting, although the urea-nitrogen synthesis rate increased in accordance with other studies (21,57,58). A strict comparison between urinary nitrogen excretion and labeled isotope turnover cannot be made, because several variables have to be taken into account. First, the urea pool is large and equilibrates slowly, meaning that calculated urea synthesis and excretion rates to a large extent are products of events occurring in the time period preceding the final 4-h fasting period during which urine was collected and circulating concentrations measured. Second, it has been suggested that the magnitude of urinary nitrogen loss in the postabsorptive state reflects protein intake the previous days, that postabsorptive protein oxidation is therefore predominantly generated by antecedent surplus protein ingestion, and that only hence forward are structural proteins being extensively degraded (7,59). Finally, the pattern of phenylalanine degradation does not necessarily reflect degradation of all amino acids. Taken together, our results are compatible with the concept that when studied while fasting, the subjects were in a transitional phase from early fasting with high ureagenesis and nitrogen loss (as evidenced by high urea-nitrogen synthesis) to a later stage with protein conservation (as evidenced by low whole-body phenylalanine degradation).

The present study demonstrates that phenylalanine flux (reflecting proteolysis) increases in healthy young men after 40 h of fasting. Tyrosine flux did not change, presumably because tyrosine flux represents protein breakdown as well as tyrosine

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appearance from phenylalanine hydroxylation. Our phenylalanine flux results support reports of an increase in leucine flux after 1.25 days of fasting in healthy subjects (9) and an even more pronounced increase after 3 days of fasting (4,41).

In conclusion, our results provide new evidence that physiological increments in GH secretion are a crucial adaptive response to fasting to achieve protein conservation, as evidenced by the 50% increase in ureagenesis during GH deprivation. The observed decrease in muscle protein breakdown during GH substitution—possibly mediated by the sustainment of circulating concentrations of free IGF-I—contributes to this overall anticatabolic impact.

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