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Fuel and energy metabolism in fasting humans[1–3]

Michael G Carlson, Wanda L Snead, and Peter J Campbell

ABSTRACT Fuel and energy homeostasis was examined in six male volunteers during a 60-h fast by using a combination of isotopic tracer techniques ([3-^{3}H]glucose, [$^2H_5$]glycerol, [1-^{14}C]palmitate, and L-[1-^{13}C]leucine) and indirect calorimetry. Plasma glucose concentration and hepatic glucose production decreased by 30% with fasting (5.2 ± 0.1 to 3.8 ± 0.2 mmol/L and 11.8 ± 0.5 to 8.2 ± 0.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively, both $P < 0.001$) and glucose oxidation declined ≈85% ($P < 0.01$). Lipolysis and primary (intraadipocyte) free fatty acid (FFA) reesterification increased 2.5-fold (1.7 ± 0.2 to 4.2 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and 1.5 ± 0.4 to 4.2 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively, both $P < 0.05$). This provided substrate for the increase in fat oxidation (from 2.7 ± 0.3 to 4.3 ± 0.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.01$), which contributed ≈75% of resting energy requirements after the 60-h fast and increased the supply of glycerol for gluconeogenesis. Proteolysis and protein oxidation increased ≈50% during fasting ($P < 0.01$ and $P < 0.05$, respectively). We conclude that the increase in FFA reesterification with fasting modulates FFA availability for oxidation and maximizes release of glycerol from triglyceride for gluconeogenesis.*Am J Clin Nutr* 1994;60:29–36.

KEY WORDS Fasting, lipolysis, glycerol, free fatty acids, proteolysis, gluconeogenesis

Introduction

The fasted state in humans is characterized by a shift from a carbohydrate-based to a fat-based fuel economy (1). This shift in energy supply with fasting results from the complex, interrelated changes in the hormonal milieu and in substrate metabolism that accompany food deprivation. Although several groups have examined carbohydrate (1–6), fat (7–11), and protein (6, 12–15) metabolism during fasting, none have examined the total metabolic response simultaneously in healthy humans. We believe that this examination would give the most information about the interaction between carbohydrate, fat, and protein kinetics during a short-term fast in humans.

Adipose tissue metabolism is a central component of the shift in fuel economy with fasting. Mobilization of free fatty acids (FFAs) from adipose tissue stores provides the majority of energy needs of peripheral tissues during fasting, sparing glucose for utilization by glucose-requiring tissues such as the brain (16). FFA availability for oxidation by peripheral tissues is determined not only by the lipolytic rate but also by the rate of FFA reesterification (17, 18). Furthermore, the release during lipolysis of glycerol, an important gluconeogenic substrate (19–21), could make an important contribution to glucose homeostasis during a fast, possibly reducing the requirement for gluconeogenic amino acids and sparing vital protein stores (1, 19).

In this study we examined the changes in carbohydrate, fat, and protein metabolism during a short-term fast in healthy human subjects by using a combination of isotopic tracer techniques and indirect calorimetry. A major purpose of the study was to evaluate the roles of lipolysis, glycerol release from adipose tissue, and FFA reesterification in fuel homeostasis during fasting.

Subjects and Methods

Subjects

Six lean male volunteers (aged 28 ± 3 y, weight 79.2 ± 3.9 kg, 22 ± 2% body fat) participated in the study. All subjects were in good health and were taking no medications. All subjects had normal glucose tolerance as judged by a standard oral-glucose-tolerance test. Body composition was determined from body density measured by underwater weighing (22). Informed written consent was obtained from each volunteer before participation in the study, after the nature, purpose, and risks of the study were explained. The study protocol was approved by the Vanderbilt University Committee for the Protection of Human Subjects.

Experimental design

Subjects were studied after 12 and 60 h of fasting. All subjects were given a standard weight-maintenance diet containing ≥200 g carbohydrate/d for 3 d before beginning the fast. The volunteers were admitted to the Vanderbilt University Clinical Research Center at 1800 on the evening before the initial study and were given a standard meal. After a 12-h overnight fast, an antecubital vein was cannulated with an 18-gauge catheter for infusion of isotopes. A contralateral hand vein was cannulated with a 20-gauge catheter and the hand was placed in a thermo-regulated box maintained at a minimum of 55 °C to permit sampling of

[1] From the Diabetes Research and Training Center, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN.

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[3] Address reprint requests to PJ Campbell, Diabetes Research and Training Center, Vanderbilt University School of Medicine, B-3307 Medical Center North, Nashville, TN 37232-2230.

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arterialized blood (23). Both catheters were kept patent by means of a continuous infusion of 0.9% saline. At 0600, a primed, continuous infusion of [1-^{13}C]leucine (0.62 mg/kg, 0.06 μmol·kg^{-1}·min^{-1}) was begun and continued for 4 h. A priming bolus of [1-^{13}C]sodium bicarbonate (0.087 mg/kg) was given at 0600 in order to prime the bicarbonate pool. Two-hour primed, continuous infusions of [3-^{3}H]glucose (1.1 MBq, 11.1 kBq/min; 30 μCi, 0.3 μCi/min), [$^{2}H_5$]glycerol (1.0 μmol/kg, 0.1 μmol·kg^{-1}·min^{-1}), and [1-^{14}C]palmitate (14.8 kBq/min; 0.4 μCi/min) were administered beginning at 0800. All tracers were infused through calibrated syringe pumps (Harvard Apparatus Co Inc, Natick, MA).

Arterialized blood samples were drawn immediately before beginning the isotope infusions and at 10-min intervals during the final 30 min of the tracer infusions for measurement of plasma substrate and hormone concentrations and substrate specific activity or enrichment. Indirect calorimetry was performed during the final 30 min of the isotope infusions in five of the volunteers for measurement of carbohydrate and fat oxidation. Expired air samples were collected before and during the final 30 min of the [^{13}C]leucine infusion for measurement of $^{13}CO_2$ enrichment to calculate rates of leucine oxidation. Urine was collected for 12 h beginning at 2200 the evening before study for determination of urinary nitrogen excretion.

After the above study was completed, all intravenous catheters were removed and the subjects continued to fast for an additional 48 h. Volunteers consumed only non-energy-containing beverages (without caffeine) during this period and were encouraged to drink water ad libitum. Urinary ketone concentrations were monitored at regular intervals (≈6 h) to document compliance with the fast. Serum potassium was measured after a total of ≈40 h of fasting. No subject developed hypokalemia and none required oral potassium supplements. After 60 h of fasting, the isotope infusion protocol was repeated exactly as described above.

Materials

HPLC-purified [3-^{3}H]glucose was obtained from New England Nuclear (Boston, MA). [$^{2}H_5$]Glycerol, L-[1-^{13}C]leucine, and [1-^{13}C]sodium bicarbonate (Tracer Technologies Inc, Newton, MA) were dissolved in sterile 0.9% saline. [1-^{14}C]Palmitate in ethanol (Research Products International, Mount Prospect, IL) was evaporated to dryness under nitrogen, resuspended in warmed 25% human serum albumin (Baxter Healthcare Corp, Glendale, CA), pasteurized at 60 °C for 10 h, and diluted in sterile 0.9% saline. Isotopes were passed through sterile 0.22-μm filters (Millipore Products Division, Bedford, MA) and checked for pyrogens and sterility before infusion.

Analytical techniques

Plasma glucose concentration was measured by a glucose oxidase method (Beckman glucose analyzer; Beckman, Fullerton, CA). Insulin (24), C-peptide (25), glucagon (26), growth hormone (27), and cortisol (Gamma Coat cortisol radioimmunoassay kit; Baxter Clinical Assays, Cambridge, MA) concentrations were determined by standard radioimmunoassays. Plasma epinephrine and norepinephrine were measured by HPLC (28). Plasma glycerol, lactate, alanine, and β-hydroxybutyrate concentrations were determined by automated fluorimetric enzymatic methods (29). Urinary nitrogen was measured by the Kjeldahl procedure (30). Respiratory gas exchange measurements were performed by computerized open-circuit indirect calorimetry (2900 Energy Measurement Module; SensorMedics, Anaheim, CA). Ventilation was measured by a mass flow meter. Oxygen was measured by a paramagnetic analyzer and carbon dioxide by an infrared analyzer.

Blood for glycerol and palmitate determination was collected in EDTA tubes and placed immediately on ice. The plasma was promptly separated by centrifugation and stored at −70 °C until analysis. Plasma palmitate concentration and specific activity were determined by HPLC (31) using [$^{2}H_{31}$]palmitate as the internal standard. The ratio of palmitate to total FFA concentration was determined by gas chromatography of the methyl esters after extraction and isolation of the plasma FFA by thin-layer chromatography (32). [$^{2}H_5$]Glycerol enrichment was determined by gas chromatography–mass spectrometry (GCMS) of the tributyldimethylsilyl derivatives using electron-impact ionization as previously described (17, 18). Ions were selectively monitored at mass per unit charge (m/e) 377 and 382.

Plasma [3-^{3}H]glucose radioactivity in the plasma was determined after deproteinization with perchloric acid and evaporation of the tritiated water under nitrogen. The residue was resuspended in distilled water and radioactivity was determined on a β-scintillation counter (Tri-Carb Liquid Scintillation Analyzer; Packard Co, Downers Grove, IL) after the addition of 10 mL scintillation cocktail (Ultima Gold; Packard Co, Meriden, CT).

Plasma [1-^{13}C]leucine and [1-^{13}C]α-ketoisocaproate ([1-^{13}C]KIC) enrichment were determined by GCMS (33). Expired air samples for $^{13}CO_2$ analysis were collected using a Douglas bag. Gas samples were transferred to non-silicone-coated vacuum tubes and $^{13}CO_2$ enrichment was determined by using isotope-ratio mass spectrometry (Metabolic Solutions, Acton, MA).

Calculations and statistics

The duration of the primed, continuous isotope infusions was chosen to ensure that all isotopic specific activities and enrichments had reached a steady state by the sampling times. Therefore, plasma glucose, palmitate, glycerol, and leucine rates of appearance (RAs) were calculated using the steady state equation (ie, isotope infusion rate divided by isotopic enrichment or specific activity). At steady state, the rate of disposal (RD) must equal the RA. Leucine RA was calculated from plasma [1-^{13}C]KIC enrichment according to the reciprocal pool model of Schwenk et al (34). The [$^{2}H_5$]glycerol and [1-^{13}C]leucine infusion rates were subtracted from total RA to yield the endogenous rates of appearance. FFA RA was calculated as the product of palmitate RA and the ratio of plasma FFA to palmitate concentration.

Plasma leucine oxidation was calculated using the formula:

$$\text{leucine oxidation} = (\text{IE } {}^{13}CO_2/\text{IE KIC}) \times (\dot{V}CO_2/0.81),$$

where IE $^{13}CO_2$ is the isotopic enrichment of expired $^{13}CO_2$, IE KIC is the isotopic enrichment of plasma KIC, and VCO_2 is the carbon dioxide production rate corrected by the factor of 0.81 for incomplete collection of expired $^{13}CO_2$ (35). Rates of proteolysis and protein oxidation were estimated from leucine RA and oxidation, assuming that leucine accounts for 8% by weight of total body proteins (33). Rates of fat and carbohydrate oxidation and energy expenditure were calculated from the measured rates of oxygen consumption and carbon dioxide production as described by Jéquier et al (36), but by substituting the estimate of protein

TABLE 1
Plasma substrate concentrations after 12-h and 60-h fasts[1]

	12-h Fast	60-h Fast
Glucose (mmol/L)	5.2 ± 0.1	3.8 ± 0.2[2]
Glycerol (μmol/L)	52 ± 7	129 ± 20[3]
Free fatty acids (μmol/L)	490 ± 57	1046 ± 72[2]
β-Hydroxybutyrate (μmol/L)	91 ± 51	2403 ± 532[3]
Alanine (μmol/L)	320 ± 35	206 ± 25[4]
Lactate (μmol/L)	1056 ± 101	973 ± 48

[1] $\bar{x}$ ± SEM, $n = 6$.
[2–4] Significantly different from 12-h fast: [2] $P < 0.001$, [3] $P < 0.01$, [4] $P < 0.05$.

oxidation derived from [1-^{13}C]leucine oxidation for that derived from urinary nitrogen excretion. Molar triglyceride oxidation was calculated from fatty acid oxidation, assuming a molecular weight of 861 for a typical triglyceride (palmitoyl-stearoyl-oleoyl-glycerol, $C_{55}H_{104}O_6$). Because each triglyceride molecule contains three fatty acid molecules, the value for fatty acid oxidation was multiplied by three to obtain molar FFA oxidation. Rates of nonoxidative disposal of glucose, leucine, and protein were derived from the differences between their RDs and oxidation.

Rates of lipolysis and FFA reesterification were calculated as described in detail previously (18). In summary, lipolysis was calculated by multiplying the glycerol RA by three. Triglycerides undergo complete hydrolysis in adipose tissue (37, 38) and mono- and diacylglyceride concentrations in human adipose tissue are very low (39). Significant glycerol metabolism within the adipocyte is impossible because human adipose tissue lacks the glycerol kinase enzyme that is necessary for phosphorylation of glycerol before its entry into the glycolytic or gluconeogenic pathways (40). Therefore, lipolysis in adipose tissue results in the release of one glycerol molecule and three FFA molecules. Glycolysis did not contribute to glycerol turnover in humans (41) and very-low-density lipoproteins accounted for < 4% of plasma glycerol turnover in rats (42). Previously, we estimated that intramuscular triglyceride made up < 2% of total body fat and that its contribution to glycerol and FFA turnover in resting humans would be minimal (18). Mesenteric lipolysis contributes < 10% to whole body flux and only one-quarter of that is cleared in the first pass through the liver (43, 44). Therefore, measurement of peripheral FFA turnover would miss < 3% of the total. Furthermore, there is no net release of glycerol from the gut in resting dogs (44) or humans (45), which is consistent with minimal mesenteric lipolysis in the resting state. Therefore, exact measurement of whole-body adipose tissue lipolysis and FFA turnover is not possible when using the described isotopic methods, but for the reasons outlined above we believe the potential errors are small.

The rate of FFA mobilization from adipose tissue is determined by the balance between the rates of lipolysis and primary (or intraadipocyte) reesterification, whereas FFA disposal occurs by two routes, oxidation and secondary (or peripheral) reesterification. Therefore, primary reesterification was calculated as the difference between lipolysis and FFA RA, whereas total FFA reesterification equals the difference between lipolysis and fat oxidation. Secondary reesterification was calculated as the difference between total and primary reesterification. The energy cost of FFA reesterification was calculated as 602 kJ/mol (144 kcal/mol) triglyceride reesterified, assuming that eight high-energy phosphate bonds (ATP to ADP) are consumed per mole triglyceride reesterified (10) and that the energy equivalent of each bond is 75 kJ (18 kcal) (46).

All values are expressed as $\bar{x}$ ± SEM. Statistical analyses were performed by using paired Student t tests or the Wilcoxon matched-pairs test for nonparametric data (CSS: Statistica software program; Statsoft, Tulsa, OK). All comparisons were two-tailed and $P < 0.05$ was considered significant.

Results

Substrate and hormone concentrations

Plasma glucose concentration decreased after the 60-h fast from 5.2 ± 0.1 to 3.8 ± 0.2 mmol/L ($P < 0.001$). Glycerol and FFA concentrations doubled with fasting, whereas β-hydroxybutyrate concentration increased 25-fold (**Table 1**). Plasma alanine concentration decreased by one-third ($P < 0.05$). Lactate concentrations were not different after the 12-h and 60-h fasts.

Plasma insulin concentration decreased by 50% after 60 h of fasting (from 53 ± 5 to 26 ± 2 pmol/L, $P < 0.001$), whereas C-peptide concentrations decreased by 75% ($P < 0.01$) (**Table 2**). Plasma concentrations of glucagon, epinephrine, and norepinephrine doubled with fasting. Growth hormone concentration increased nearly sixfold from 1.9 ± 0.3 to 11.0 ± 3.6 μg/L ($P < 0.05$). Plasma cortisol concentrations were not different after the 12- and 60-h fasts.

Carbohydrate metabolism

Rates of whole-body glucose utilization and hepatic glucose production decreased by one-third after 60 h of fasting (from 11.8 ± 0.5 to 8.2 ± 0.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.001$), which is similar to the decline in plasma glucose concentration (**Fig 1**). Glucose oxidation decreased by 85% from 8.3 ± 1.1 to 1.2 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.01$). Despite the decline in whole-body glucose utilization, nonoxidative glucose disposal doubled after the 60-h fast (from 3.5 ± 1.6 to 7.0 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$).

Fat metabolism

Plasma glycerol RA, which reflects adipose tissue lipolysis, increased 2.5-fold with fasting (from 1.7 ± 0.2 to 4.2 ± 0.2

TABLE 2
Plasma hormone concentrations after 12-h and 60-h fasts[1]

	12-h Fast	60-h Fast
Insulin (pmol/L)	53 ± 5	26 ± 2[2]
C-peptide (nmol/L)	0.34 ± 0.09	0.09 ± 0.04[3]
Glucagon (ng/L)	159 ± 25	299 ± 37[3]
Epinephrine (pmol/L)	143 ± 10	296 ± 46[3]
Norepinephrine (pmol/L)	601 ± 146	1219 ± 254[4]
Growth hormone (μg/L)	1.9 ± 0.3	11.0 ± 3.6[4]
Cortisol (nmol/L)	339 ± 50	412 ± 58

[1] $\bar{x}$ ± SEM, $n = 6$.
[2–4] Significantly different from 12-h fast: [2] $P < 0.001$, [3] $P < 0.01$, [4] $P < 0.05$.

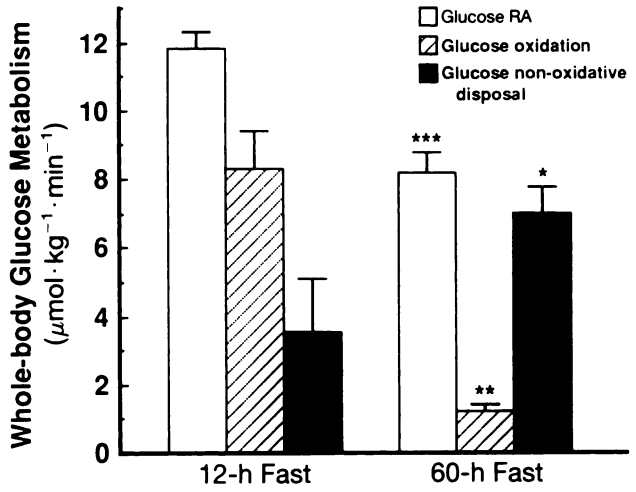

FIG 1. Rates of hepatic glucose appearance (RA), glucose oxidation, and nonoxidative glucose disposal after 12-h and 60-h fasts. Bars represent $\bar{x} \pm$ SEM; $n = 6$. Significantly different from 12-h fast: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

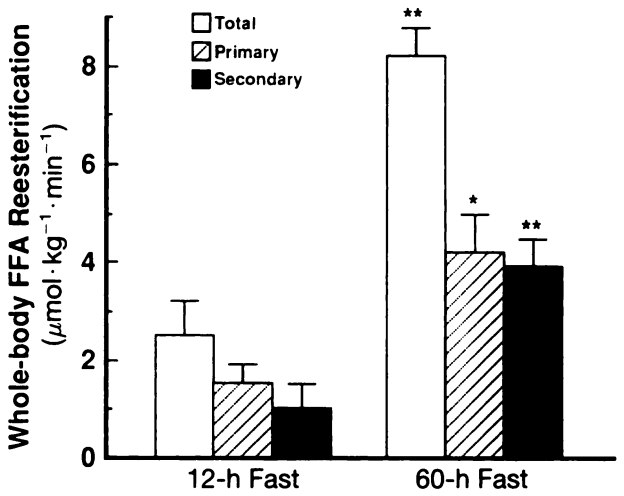

FIG 3. Rates of total, primary or intraadipocyte, and secondary or peripheral free fatty acid (FFA) reesterification after 12-h and 60-h fasts. Bars represent $\bar{x} \pm$ SEM; $n = 6$. Significantly different from 12-h fast: * $P < 0.05$, ** $P < 0.01$.

μmol·kg^{-1}·min^{-1}, $P < 0.05$), whereas FFA RA increased from 3.6 ± 0.5 to 8.3 ± 0.6 μmol·kg^{-1}·min^{-1} ($P < 0.001$) (**Fig 2**). Fat oxidation increased less than FFA RA, increasing from 2.7 ± 0.3 μmol·kg^{-1}·min^{-1} in the postabsorptive state to 4.3 ± 0.1 μmol·kg^{-1}·min^{-1} after the 60-h fast ($P < 0.01$).

Significant increases in FFA reesterification occurred after 60 h of fasting (**Fig 3**). Total FFA reesterification increased from 2.5 ± 0.7 to 8.2 ± 0.6 μmol·kg^{-1}·min^{-1} ($P < 0.01$). Primary FFA reesterification increased more than 2.5-fold with fasting (from 1.5 ± 0.4 to 4.2 ± 0.8 μmol·kg^{-1}·min^{-1}, $P < 0.05$), whereas secondary FFA reesterification increased from 1.0 ± 0.5 to 3.9 ± 0.6 μmol·kg^{-1}·min^{-1} ($P < 0.01$).

Protein metabolism

Plasma leucine rate of appearance, an index of skeletal muscle proteolysis, increased ≈50% after the 60-h fast (from 1.5 ± 0.1 to 2.2 ± 0.1 μmol·kg^{-1}·min^{-1}, $P < 0.01$) (**Fig 4**). Leucine oxidation, measured from $^{13}CO_2$ production in the expired air, rose from 0.27 ± 0.02 to 0.40 ± 0.05 μmol·kg^{-1}·min^{-1} ($P < 0.05$), which is similar to the increase in plasma leucine RA. Urinary nitrogen excretion, however, was the same after the 12- and 60-h fasts (0.7 ± 0.1 and 0.7 ± 0.1 g/h, respectively, NS). Nonoxidative leucine disposal increased 40% with fasting. Proteolysis increased from 2.5 ± 0.2 to 3.7 ± 0.2 mg·kg^{-1}·min^{-1} ($P < 0.01$) and protein oxidation from 0.45 ± 0.04 to 0.66 ± 0.08 mg·kg^{-1}·min^{-1} ($P < 0.05$). Nonoxidative protein disposal, an index of whole-body protein synthesis, increased by 40% with fasting (from 2.1 ± 0.2 to 3.0 ± 0.2 mg·kg^{-1}·min^{-1}, $P < 0.01$).

Energy metabolism

The respiratory quotient (RQ) fell from 0.83 ± 0.02 to 0.74 ± 0.02 with fasting ($P < 0.01$). Resting energy expenditure

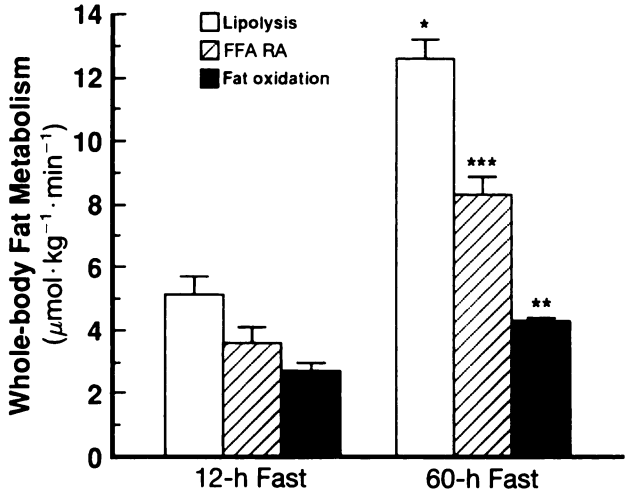

FIG 2. Rates of adipose tissue lipolysis, plasma free fatty acid rate of appearance (FFA RA), and fat oxidation after 12-h and 60-h fasts. Bars represent $\bar{x} \pm$ SEM; $n = 6$. Significantly different from 12-h fast: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

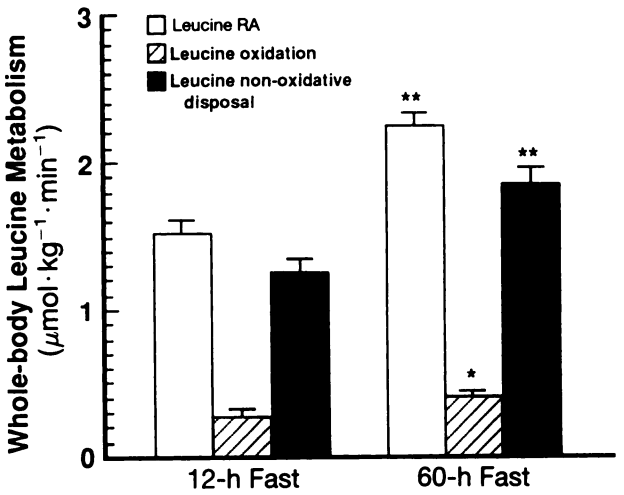

FIG 4. Rates of plasma leucine rate of appearance (RA), leucine oxidation, and leucine nonoxidative disposal after 12-h and 60-h fasts. Bars represent $\bar{x} \pm$ SEM; $n = 6$. Significantly different from 12-h fast: * $P < 0.05$, ** $P < 0.01$.

(REE) was not different after the 12-h and 60-h fasts (314 ± 17 and 309 ± 13 kJ/h, respectively, NS) (**Table 3**). However, significant changes occurred in the contributions of carbohydrate, fat, and protein oxidation to basal energy requirements. Carbohydrate oxidation accounted for 41 ± 6% of REE in the postabsorptive state, but only 6 ± 1% after the 60-h fast ($P < 0.01$). Fat oxidation, which accounted for 46 ± 5% of basal energy needs, supplied 75 ± 2% of energy requirements after the 60-h fast ($P < 0.01$). The contribution of protein oxidation to REE also increased during the fast (from 13 ± 1 to 19 ± 2%, $P < 0.05$), but was less than the contribution of fat oxidation ($P < 0.05$).

The daily energy cost of the threefold increase in total FFA reesterification with fasting rose from 54 ± 13 kJ/d after the overnight fast to 188 ± 21 kJ/d after 60 h of fasting ($P < 0.01$). Thus, the energy cost of whole-body FFA reesterification accounted for only 0.8% of REE in the postabsorptive state but rose to 2.5% of REE after the 60-h fast ($P < 0.01$).

TABLE 3
Changes in energy metabolism with fasting[1]

	12-h Fast	60-h Fast
Resting energy expenditure (kJ/h)	314 ± 17	309 ± 3
Carbohydrate oxidation		
(kJ/h)	125 ± 17	21 ± 4[2]
(% REE)	41 ± 6	6 ± 1[2]
Fat oxidation		
(kJ/h)	146 ± 21	234 ± 13[2]
(% REE)	46 ± 5	75 ± 2[2]
Protein oxidation		
(kJ/h)	42 ± 4	59 ± 4[3]
(% REE)	13 ± 1	19 ± 2[3]
Energy mobilized from fat stores		
(kJ/h)	192 ± 21	447 ± 17[4]
(% REE)	63 ± 9	144 ± 9[4]
Potential energy from lipolysis[5]		
(kJ/h)	280 ± 25	685 ± 17[3]
(% REE)	90 ± 10	218 ± 11[3]
Energy cost of FFA reesterification		
(kJ/d)	54 ± 13	188 ± 21[2]
(% REE)	0.8 ± 0.2	2.5 ± 0.2[2]

[1] $\bar{x}$ ± SEM, $n = 6$. REE, resting energy expenditure; FFA, free fatty acid.
[2-4] Significantly different from 12-h fast: [2] $P < 0.01$, [3] $P < 0.05$, [4] $P < 0.001$.
[5] Potential energy available from free fatty acids generated by lipolysis if no primary reesterification had occurred.

Discussion

This study represents the first simultaneous assessment of the changes in energy metabolism and fat, carbohydrate, and protein kinetics during fasting in humans. After a 60-h fast, plasma glucose concentrations decreased by 30% (from 5.2 to 3.8 mmol/L, $P < 0.001$) and glucose oxidation decreased by 85% (from 8.3 ± 1 to 1.2 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.01$) (Fig 1). The decrease in glucose concentration was accompanied by a 50% decrease in plasma insulin concentrations (from 53 ± 5 to 26 ± 2 pmol/L, $P < 0.001$), whereas glucagon, epinephrine, and norepinephrine concentrations doubled and growth hormone increased sixfold (Table 2). Adipose tissue lipolysis increased 2.5-fold and fat oxidation nearly doubled (Fig 3), which resulted in a dramatic increase in ketogenesis as shown by the 25-fold rise in plasma β-hydroxybutyrate concentration (Table 1). Rates of total and intraadipocyte (primary) FFA reesterification tripled after 60 h of fasting (Fig 3), which has also been shown by others (8–10). Proteolysis and protein oxidation increased by ≈50% with fasting (Fig 4) despite no change in urinary nitrogen excretion. We hypothesize that the difference between these estimates of protein oxidation reflects a greater precision and sensitivity of the measurement of leucine oxidation compared with measurement of urinary nitrogen excretion. Nair et al (6) also noted this discrepancy between leucine oxidation and urinary nitrogen excretion in fasting humans. Wolfe et al (47) noted a similar discrepancy between leucine oxidation and urea production in exercising humans and concluded that the production of urea, the major component of urinary nitrogen, did not accurately reflect protein catabolism during exercise. Because we believed that the increase in protein oxidation during fasting, which was estimated from leucine oxidation, was a more credible measurement than the lack of difference found by using urinary nitrogen excretion, we used the former measure in our indirect calorimetry calculations. However, the difference between the measures was small and did not affect the values for energy expenditure, fat, and carbohydrate oxidation significantly. REE was not different after the overnight and 60-h fasts because the increase in fat oxidation, and to a lesser degree protein oxidation, compensated for the decline in carbohydrate oxidation with fasting (Table 3).

Several factors may contribute to the observed increase in adipose tissue lipolysis with fasting. Insulin is a potent inhibitor of lipolysis in vivo (18, 20) and the decrease in plasma insulin concentration during a fast has been suggested as the principal stimulus for the increase in lipolysis (1). Others have shown that the increase in catecholamines also contributes to the stimulation of lipolysis and FFA mobilization during fasting (8, 9, 11). The plasma glucose concentration independently regulates lipolysis in humans (17) such that the decrease in glucose may exert a stimulatory effect on lipolysis independently of the associated changes in glucoregulatory hormones. In addition, we have recently demonstrated an in vivo lipolytic effect of glucagon in male volunteers at physiologic concentrations that are similar to those observed in the present study after 60 h of fasting (48). Finally, the observed sixfold increase in growth hormone with fasting may also contribute to the accelerated mobilization of fat stores during food deprivation because growth hormone regulates nocturnal lipolysis in humans (49).

The significant fasting-associated increases in FFA reesterification (or triglyceride-fatty acid cycling) may initially appear paradoxical, given the increased demand for FFA for oxidation by peripheral tissues and the reduced plasma insulin concentrations with fasting. Such substrate cycles have been proposed to enhance the responsiveness of substrate mobilization to hormonal or other stimuli (46). Hence, modulation of FFA availability to meet changing energy requirements could be facilitated through alterations in the rate of FFA reesterification in addition to changes in lipolytic rate. The impact of the increase in primary (or intraadipocyte) and secondary (or peripheral) FFA reesterification on energy metabolism is illustrated in Table 3. The energy mobilized from fat stores after the 60-h fast (ie, via FFA RA) equalled ≈150% of resting energy requirements, but would have equalled ≈220% of REE had no primary reesterification

occurred. Furthermore, because of the increase in secondary FFA reesterification (ie, reesterification of FFA released into the plasma), the actual energy contribution from fatty acid oxidation represented only ≈75% of REE after 60 h of fasting, rather than the ≈150% of REE if all FFA mobilized from adipose tissue stores had undergone oxidation. The cause of the increases in primary and secondary reesterification with fasting is unknown, but may be related to a mass action effect from the increase in substrate (ie, FFA) availability or possibly to increased β-adrenergic stimulation during fasting (9).

The changes in FFA reesterification with fasting are also likely to be important in maintaining glucose homeostasis when glycogen stores are depleted. The increase in primary FFA reesterification, coupled with the accelerated rate of adipose tissue lipolysis, serves to modulate FFA availability for oxidation while maximizing adipose tissue release of glycerol, an important gluconeogenic substrate (19–21). In the postabsorptive state, gluconeogenesis from glycerol accounts for only 3% of hepatic glucose production (HGP) (19, 20). As glycogen stores are depleted during a fast, the contribution of gluconeogenesis increases to > 95% of HGP (21). A comparison of the rates of HGP (8.2 ± 0.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and glycerol (4.2 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) release from adipose tissue after 60 h of fasting in our subjects reveals that gluconeogenesis from glycerol could account for up to 25% of HGP with fasting, an eightfold increase over the contribution from glycerol in the postabsorptive state. This would overestimate gluconeogenesis from glycerol if a portion of the glycerol was oxidized, but because carbohydrate oxidation after the fast was small, we believe that the overestimate would be minimal. Furthermore, this estimate is similar to that of Bortz et al (19), who measured incorporation of [U-^{14}C]glycerol into glucose in lean, fasted subjects and calculated that glycerol gluconeogenesis accounted for 20% of glucose production after a short-term fast.

Nonoxidative disposal of glucose and leucine also increased with fasting. The fasting-associated decrease in whole-body glucose utilization was accompanied by a greater decrease in glucose oxidation, such that the rate of glucose disposal by nonoxidative pathways actually increased twofold after the 60-h fast (from 3.5 ± 1.6 to 7.0 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$). Under hyperinsulinemic conditions such as in the fed state, nonoxidative glucose disposal probably represents glycogen synthesis (50). However, in the fasted state, characterized by hypoinsulinemia and glycogen depletion, glycogen synthesis is probably minimal. One-third of the increase in nonoxidative glucose disposal (≈1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) could be attributed to the production of glycerol-3-phosphate that is necessary for the increase in total FFA reesterification (from 2.5 to 8.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). An increase in the Cori cycling of glucose to lactate could explain much of the remainder (≈2.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Such an increase in lactate production with fasting would not result in changes in plasma lactate concentration as long as lactate disposal also increased by the same rate. Gluconeogenesis probably accounted for most of the increase in lactate disposal, because carbohydrate oxidation was negligible after the 60-h fast. The increase in gluconeogenesis from lactate after the fast could account for approximately one-third of HGP and could make an even larger contribution than glycerol. Except under conditions of prolonged starvation when the compensatory increase in ketone body utilization by the brain reduces its demand for glucose (16), the obligatory glucose requirement of the brain is 100–150 g/24 h (≈6–8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (51), which closely approximates the calculated rate of nonoxidative glucose disposal in our subjects after a 60-h fast. Thus, the increase in nonoxidative glucose metabolism with fasting probably reflects the increase in FFA reesterification and Cori cycling. The increased supply of lactate and glycerol that is available for gluconeogenesis during fasting compensates for the depletion of glycogen stores and helps to provide the obligatory glucose requirement of the brain.

Accumulation and excretion of ketone bodies during fasting would lower the RQ and would produce an overestimate of fat oxidation and an underestimate of carbohydrate oxidation. Schutz and Ravussin (52), using the data of Balasse (53) for ketone body production rates and urinary loss in fasting obese humans, estimated that ketogenesis during a 3-d fast lowered the RQ by 4%. Ketogenesis would probably be less in our lean volunteers who fasted for 2 d, so the underestimate of RQ in our experiments would be < 4%. Nevertheless, a 4% error in the RQ of 0.74 found after the 60-h fast in our subjects would produce a 12% error in fat oxidation and a 50% error in carbohydrate oxidation, but would not affect the measurement of total energy expenditure by indirect calorimetry (54). However, because carbohydrate oxidation after the 60-h fast was minimal, even a 50% error would have had a negligible effect on the absolute value of carbohydrate oxidation and would not affect our conclusions.

The increase in nonoxidative leucine disposal with fasting was also found by Nair et al (6), although this has not been a consistent finding (14, 15). Because leucine disposal occurs by either oxidation or by incorporation into protein (33), nonoxidative leucine disposal represents an index of whole-body protein synthesis. Thus, our results suggest that a modest increase in protein synthesis occurs during a short-term fast, despite the reduced insulin concentrations and accelerated rate of proteolysis, which has also been reported in uncontrolled insulin-dependent diabetes mellitus (55). Because plasma concentrations of leucine and other branched-chain amino acids (BCAAs) are increased during fasting (12) and plasma BCAAs are reported to have a regulatory role in protein synthesis in vivo (56, 57), an increase in protein synthesis with fasting may be a consequence of the increase in circulating concentrations of BCAAs. The concomitant increase in proteolysis and protein synthesis during food deprivation represents another example of a substrate cycle and may serve to enhance the responsiveness of amino acid mobilization during the changing requirements for oxidative and gluconeogenic substrates.

In summary, the present study illustrates the precise regulation of energy metabolism during a short-term fast in lean, healthy humans. Our results demonstrate that the fasted state in human subjects is characterized by an increase in lipolysis and proteolysis, which supply substrates for oxidation and for gluconeogenesis and compensate for the decline in carbohydrate oxidation and glycogenolysis. The increase in FFA reesterification with fasting serves to modulate FFA availability for oxidation, while maximizing the release of the gluconeogenic substrate glycerol from triglyceride stores.

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References

1. Cahill GF Jr, Herrera MG, Morgan AP, et al. Hormone-fuel interrelationships during fasting. J Clin Invest 1966;45:1751–69.
2. Wahren J, Efendic S, Luft R, Hagenfeldt L, Bjorkman O, Felig P. Influence of somatostatin on splanchnic glucose metabolism in postabsorptive and 60-hour fasted humans. J Clin Invest 1977;59:299–307.
3. Haymond MW, Karl IE, Clarke WL, Pagliara AS, Santiago JV. Differences in circulating gluconeogenic substrates during short-term fasting in men, women, and children. Metabolism 1982;31:33–42.
4. Boyle PJ, Shah SD, Cryer PE. Insulin, glucagon, and catecholamines in prevention of hypoglycemia during fasting. Am J Physiol 1989;256:E651–61.
5. Clore JN, Helm ST, Nestler JE, Blackard WG. Impaired modulation of hepatic glucose output overnight after a 72-h fast in normal man. J Clin Endocrinol Metab 1990;70:865–8.
6. Nair KS, Woolf PD, Welle SL, Matthews DE. Leucine, glucose, and energy metabolism after 3 days of fasting in healthy human subjects. Am J Clin Nutr 1987;46:557–62.
7. Klein S, Young VR, Blackburn GL, Bistrian BR, Wolfe RR. Palmitate and glycerol kinetics during brief starvation in normal weight young adult and elderly subjects. J Clin Invest 1986;78:928–33.
8. Wolfe RR, Peters EJ, Klein S, Holland OB, Rosenblatt J, Gray H. Effect of short-term fasting on lipolytic responsiveness in normal and obese human subjects. Am J Physiol 1987;252:E189–96.
9. Klein S, Peters EJ, Holland OB, Wolfe RR. Effect of short- and long-term β-adrenergic blockade on lipolysis during fasting in humans. Am J Physiol 1989;257:E65–73.
10. Elia M, Zed C, Neale G, Livesey G. The energy cost of triglyceride-fatty acid recycling in non-obese subjects after an overnight fast and four days of starvation. Metabolism 1987;36:251–5.
11. Jensen MD, Haymond MW, Gerich JE, Cryer PE, Miles JM. Lipolysis during fasting: decreased suppression by insulin and increased stimulation by epinephrine. J Clin Invest 1987;79:207–13.
12. Felig P, Owen OE, Wahren J, Cahill GF Jr. Amino acid metabolism during prolonged starvation. J Clin Invest 1969;48:584–94.
13. Tsalikian E, Howard C, Gerich JE, Haymond MW. Increased leucine flux in short-term fasted human subjects: evidence for increased proteolysis. Am J Physiol 1984;247:E323–7.
14. Frexes-Steed M, Warner ML, Bulus N, Flakoll P, Abumrad NN. Role of insulin and branched-chain amino acids in regulating protein metabolism during fasting. Am J Physiol 1990;258:E907–17.
15. Fryberg DA, Barrett EJ, Louard RJ, Gelfand RA. Effect of starvation on human muscle protein metabolism and its response to insulin. Am J Physiol 1990;259:E477–82.
16. Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill FG Jr. Brain metabolism during fasting. J Clin Invest 1967;46:1589–95.
17. Carlson MG, Snead WL, Hill JO, Nurjhan N, Campbell PJ. Glucose regulation of lipid metabolism in man. Am J Physiol 1991;261:E815–20.
18. Campbell PJ, Carlson MG, Hill JO, Nurjhan N. Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. Am J Physiol 1992;263:E1063–9.
19. Bortz WM, Paul P, Haff AC, Holmes WL. Glycerol turnover and oxidation in man. J Clin Invest 1972;51:1537–46.
20. Nurjhan N, Campbell P, Kennedy FP, Miles JM, Gerich JE. Insulin dose-response characteristics for suppression of glycerol release and conversion to glucose in humans. Diabetes 1986;35:1326–31.
21. Consoli A, Kennedy F, Miles J, Gerich J. Determination of Krebs cycle metabolic carbon exchange in vivo and its use to estimate the individual contributions of gluconeogenesis and glycogenolysis to overall glucose output in man. J Clin Invest 1987;80:1303–10.
22. Goldman RF, Buskirk ER. A method for underwater weighing and the determination of body density. In: Brozek J, Herschel A, eds. Techniques for measuring body composition. Washington, DC: National Academy Press, 1961:78–106.
23. Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. Metabolism 1981;30:936–40.
24. Morgan CR, Lazarow AL. Immunoassay of insulin: two antibody systems. Diabetes 1963;12:115–26.
25. Faber O, Binder C, Markussen J, et al. Characterization of seven C-peptide antisera. Diabetes 1978;27:170–7.
26. Faloona G, Unger RH. Glucagon. In: Jaffe BM, Behrman HR, eds. Methods of hormone radioimmunoassay. New York: Academic Press, 1974:317–30.
27. Schalch D, Parker M. A sensitive double antibody radioimmunoassay for growth hormone in plasma. Nature 1964;203:1141–2.
28. Causon RC, Carruthers ME, Rodnight R. Assay of plasma catecholamines by liquid chromatography with electrochemical detection. Anal Biochem 1981;116:223–6.
29. Lloyd B, Burrin J, Smythe P, Alberti KGMM. Enzymic fluorometric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol, and 3-hydroxybutyrate. Clin Chem 1978;24:1724–9.
30. Hawk PB. Kjeldahl method. In: Practical physiological chemistry. 12th ed. Toronto: Blakiston, 1947:814–22.
31. Miles JM, Ellman MG, McClean KL, Jensen MD. Validation of a new method for determination of free fatty acid turnover. Am J Physiol 1987;252:E431–8.
32. McDonald-Gibson RG, Young M. The use of an automatic solids injection system for quantitative determination of plasma long chain non-esterified fatty acids by gas-liquid chromatography. Clin Chim Acta 1974;53:117–26.
33. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C] leucine. Am J Physiol 1980;238:E473–9.
34. Schwenk WF, Beaufrère B, Haymond MW. Use of reciprocal pool specific activities to model leucine metabolism in humans. Am J Physiol 1985;249:E646–50.
35. Wolfe RR, Goodenough RD, Wolfe MH, Royal GT, Nadel ER. Isotopic analysis of leucine and urea metabolism in exercising humans. J Appl Physiol 1982;52:458–66.
36. Jéquier E, Acheson K, Schutz Y. Assessment of energy expenditure and fuel utilization in man. Annu Rev Nutr 1987;7:187–208.
37. Brooks B, Arch JRS, Newsholme EA. Effects of hormones on the rate of the triacylglycerol/fatty acid substrate cycle in adipocytes and epididymal fat pads. FEBS Lett 1982;146:327–30.
38. Vaughan M, Steinberg D. Effect of hormones on lipolysis and esterification of free fatty acids during incubation of adipose tissue in vitro. J Lipid Res 1963;4:193–9.
39. Arner P, Ostman J. Mono- and diacylglycerols in human adipose tissue. Biochim Biophys Acta 1974;369:209–21.
40. Shapiro B, Chowers I, Rose G. Fatty acid uptake and esterification in adipose tissue. Biochim Biophys Acta 1957;23:115–120.
41. Nurjhan N, Kennedy F, Consoli A, Martin C, Miles J, Gerich J. Quantification of the glycolytic origin of plasma glycerol: implications for the use of the rate of appearance of plasma glycerol as an index of lipolysis in vivo. Metab Clin Exp 1988;37:386–9.
42. Wolfe RR, Durkot MJ. Role of very low density lipoproteins in the energy metabolism of the rat. J Lipid Res 1985;26:210–7.
43. Basso LV, Havel RJ. Hepatic metabolism of free fatty acids in normal and diabetic dogs. J Clin Invest 1970;49:537–47.

44. Wasserman DH, Lacy DB, Goldstein RE, Williams PE, Cherrington AD. Exercise-induced fall in insulin and increase in fat metabolism during prolonged muscular work. Diabetes 1989;38:484–90.
45. Bjorkman O, Eriksson LS, Nyberg B, Wahren J. Gut exchange of glucose and lactate in basal state and after oral glucose ingestion in postoperative patients. Diabetes 1990;39:747–51.
46. Newsholme EA, Crabtree B. Substrate cycles in metabolic regulation and in heat generation. Biochem Soc Symp 1976;41:61–109.
47. Wolfe RR, Goodenough RD, Wolfe MH, Royle GT, Nadel ER. Isotopic analysis of leucine and urea metabolism in exercising humans. Am J Physiol 1982;52:458–66.
48. Carlson MG, Snead WL, Campbell PJ. Regulation of free fatty acid metabolism by glucagon. J Clin Endocrinol Metab 1993;77:11–5.
49. Boyle PJ, Avogaro A, Smith L, et al. Role of GH in regulating nocturnal rates of lipolysis and plasma mevalonate levels in normal and diabetic humans. Am J Physiol 1992;263: E168–72.
50. Kelley DE, Reilly JP, Veneman T, Mandarino LJ. Effects of insulin on skeletal muscle glucose storage, oxidation, and glycolysis in humans. Am J Physiol 1990;258:E923–9.
51. Sokoloff L. Metabolism of the central nervous system in vivo. In: Handbook of physiology, Section I. Neurophysiology. Baltimore: Waverly Press, 1959:1843.
52. Schutz Y, Ravussin E. Respiratory quotients lower than 0.70 in ketogenic diets. Am J Clin Nutr 1980;33:1317–9.
53. Balasse EO. Kinetics of ketone body metabolism in fasting humans. Metab Clin Exp 1979;28:41–50.
54. Livesey G, Elia M. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. Am J Clin Nutr 1988;47:608–28.
55. Nair KS, Garrow JS, Ford C, Mahler RF, Halliday D. Effect of poor diabetic control and obesity on whole body protein metabolism in man. Diabetalogia 1983;25:400–3.
56. Fukagawa NK, Minaker KL, Young VR, Mathews DE, Bier DM, Rowe JW. Leucine metabolism in aging humans: effect of insulin and substrate availability. Am J Physiol 1988;256:E288–94.
57. Tessari P, Inchiostro S, Biolo G, et al. Differential effects of hyperinsulinemia and hyperaminoacidemia on leucine-carbon metabolism in vivo. Role of substrate availability on estimates of whole body protein synthesis. J Clin Invest 1987;79:1062–9.