Am J Physiol Endocrinol Metab 293: E1580–E1589, 2007.
First published September 18, 2007; doi:10.1152/ajpendo.00169.2007.

The effect of total starvation and very low energy diet in lean men on kinetics of whole body protein and five hepatic secretory proteins

Paul R. Afolabi,[1] Farook Jahoor,[2] Alan A. Jackson,[1] James Stubbs,[3] Alexander M. Johnstone,[3] Peter Faber,[3] Gerald Lobley,[3] Eileen Gibney,[4] and Marinos Elia[1]

[1]*Institute of Human Nutrition, University of Southampton, Southampton General Hospital, Southampton, United Kingdom;* [2]*Department of Pediatrics, Childrens' Nutrition Research Center, Baylor College of Medicine, Houston, Texas;* [3]*Rowett Research Institute, Bucksburn, Aberdeen, United Kingdom;* [4]*School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Dublin, United Kingdom*

Submitted 15 March 2007; accepted in final form 9 September 2007

Afolabi PR, Jahoor F, Jackson AA, Stubbs J, Johnstone AM, Faber P, Lobley G, Gibney E, Elia M. The effect of total starvation and very low energy diet in lean men on kinetics of whole body protein and five hepatic secretory proteins. *Am J Physiol Endocrinol Metab* 293: E1580–E1589, 2007. First published September 18, 2007; doi:10.1152/ajpendo.00169.2007.—It is unclear whether the rate of weight loss, independent of magnitude, affects whole body protein metabolism and the synthesis and plasma concentrations of specific hepatic secretory proteins. We examined *1*) whether lean men losing weight rapidly (starvation) show greater changes in whole body protein kinetics, synthesis, and circulating concentrations of selected hepatic secretory proteins than those losing the same amount of weight more slowly [very low energy diet (VLED)]; and *2*) whether plasma concentrations and synthetic rates of these proteins are related. Whole body protein kinetics were measured using [1-^{13}C]leucine in 11 lean men (6 starvation, 5 VLED). Fractional and absolute synthetic rates of HDL-apolipoprotein A1 (apoA1), retinol binding protein, transthyretin, α_1-antitrypsin (α_1-AT), and transferrin were measured using a prime-constant intravenous infusion of [$^{13}C_2$]glycine. Compared with VLED group, the starvation group showed greater increases (at a 5% weight loss) in whole body protein oxidation ($P < 0.05$); fractional synthetic rates of HDL-apoA1 (25.3 vs. −1.52%; $P = 0.003$) and retinol binding protein (30.6 vs. 7.1%; $P = 0.007$); absolute synthetic rates of HDL-apoA1 (7.1 vs. −3.8 mg·kg^{-1}·day^{-1}; $P = 0.003$) and α_1-AT (17.8 vs. 3.6 mg·kg^{-1}·day^{-1}; $P = 0.02$); and plasma concentration of α_1-AT ($P = 0.025$). Relationships between synthetic rates and plasma concentrations varied between the secreted proteins. It is concluded that synthetic rates of hepatic secreted proteins in lean men are more closely related to the rate than the magnitude of weight loss. Changes in concentration of these secreted proteins can occur independently of changes in synthetic rates, and vice versa.

protein turnover; plasma proteins; very low energy diet

ALTHOUGH MANY OF THE METABOLIC CHANGES associated with weight loss are well described, not all are consistent. For example, basal metabolic rate has been reported to be significantly increased (18, 21, 48, 76), decreased (21, 30, 43, 44), or no significant change (21, 23). Similarly, studies of whole body protein kinetics have reported variable responses in synthesis, breakdown, and oxidation. While this may be due to differences in the tracers employed or the initial body composition, other factors such as the extent and rate of diet-dependent weight loss may also be important. For example, plasma retinol binding protein (RBP) and prealbumin [transthyretin (TTR)], both advanced as markers of nutritional status, decrease during experimental and therapeutic starvation (no macronutrient intake) and during weight loss associated with inflammatory diseases, but are often normal in patients with severe anorexia nervosa (62). Little attention appears to have been given to the possibility that the rate of weight loss, determined by the extent of dietary restriction, may have major effects on physiology and metabolism, independent of the magnitude and composition of weight loss. Such a consideration may explain some of the discordance between literature reports.

Recently, it has been proposed that physical, psychological, and metabolic functions are affected to a greater extent when the rate of weight loss is rapid than when the same amount of weight loss occurs more slowly in both lean (19, 29) and obese subjects (24). Evidence is limited, however, partly because experimental data are difficult to interpret due to the confounding effects of a variety of other factors. For example, in clinical practice, rapid weight loss suggests greater risk of malnutrition and worsened outcome, but this is often confounded by the presence of disease with variable severity and/or treatments that produce a number of unwanted side effects. Better experimental control exists for animal studies, e.g., turpentine-induced abscesses in rats healed more slowly and the acute phase protein response (measured using α_2-macroglobulin, which is a major acute phase protein in the rat) was more attenuated after rapid as opposed to slow, but similar, weight loss (37, 39). This occurred even if the abscesses were induced at the end of a period of weight loss and followed immediately by maintenance intake.

Experimental studies in humans have generally not controlled for both the rate and extent of weight loss, nor for the independent effect of initial adiposity, known to influence the metabolic response to starvation and semistarvation (18). One reason is the difficulty of recruiting lean subjects, within the desirable range of weight, to lose 5–10% of their body weight. In this study, we examined the effect of 5% loss of body weight induced either rapidly (5 days) through total starvation (water only), or more slowly (9 days) with a very low energy diet (VLED; 2.5 MJ/day), on whole body protein kinetics and the synthesis of specific proteins secreted from the liver. These secreted proteins were selected because they fulfill a range of functions, including transport of minerals [iron, transferrin (TR)], vita-

Address for reprint requests and other correspondence: M. Elia, Institute of Human Nutrition, Univ. of Southampton, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD United Kingdom (e-mail: elia@soton.ac.uk).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

mins (vitamin A, RBP), hormones (thyroxine, TTR), lipids [cholesterol, high-density lipoprotein (HDL)-apolipoprotein A1 (apoA1)], and participation in the acute-phase protein response to inflammatory stimuli [e.g., α_1-antitrypsin (α_1-AT)]. In addition, the inflammatory response can also be modulated by anti-inflammatory properties of HDL-apoA1 (42). Finally, animal experiments have implicated RBP in insulin sensitivity (75), but evidence for this in humans has been lacking (72).

For the VLED intervention, the study was extended up to 21 days to induce greater weight loss and to distinguish the effect of the magnitude of weight loss from the rate of weight loss.

METHODS

Subjects. Approval was obtained from the Cambridge Local Research Ethics Committee (Addenbrookes Hospital, Cambridge, UK) and the Ethics Committee (Dunn Clinical Nutrition Centre, Cambridge, UK). All subjects gave written, informed consent for participation after the nature of the protocol was fully explained, and an honorarium was provided for their participation. Eleven lean men (body mass index: 18.1–26.2 kg/m^2) who were in good health and weight stable within the previous 2 mo (±1.5 kg) were studied. Subjects were within a limited age range, nonsmokers, and not taking any medication. The physical characteristics of the subjects are shown in Table 1.

Study design. The subjects were studied during weight loss induced either by starvation (rapid weight loss; $n = 6$) or semistarvation with a VLED (slower weight loss; $n = 5$). All subjects were admitted to a residential metabolic facility (at the Dunn Clinical Nutrition Centre, Cambridge, UK) and given a weight maintenance diet for a period of 7 days (see section on dietary intervention below). Six subjects were then starved (water only) for a period of 5 days, with the aim of achieving a 5% weight loss. Protein kinetic measurements were undertaken twice: immediately before the fast and at the end of the 5% weight loss. The other five subjects (VLED group) received 2.5 MJ/day for ~9 days, to again lose ~5% of initial body weight, and this diet was continued until *day 21* to achieve a weight loss of not more than 10% of initial body weight. These subjects, therefore, had protein kinetics measured on three occasions (at baseline, at *day 9* with 5% body weight loss, and at *day 21* with ~7–8% loss of body weight).

Dietary intervention. The maintenance diet for each subject (as energy, 40% fat, 45% carbohydrate, and 15% protein) was freshly prepared and provided as three isoenergetic meals daily, with a 3-day rotating menu. The individual maintenance energy intakes for each subject were based on 1.4× the predicted basal metabolic rate (61). The dietary intake was occasionally altered (increased or decreased by 0.5 MJ/day), with the aim of reversing small, but consistent trends in body weight. The VLED (2.5 MJ/day) was also given daily as three isoenergetic meals and was provided in a 5-day rotating menu. The macronutrient composition of the diet was similar to the standard maintenance diet (40% fat, 45% carbohydrate, and 15% protein). The compositions of both the maintenance and the VLED diets were based on the McCance and Widdowson Food Composition Tables (26). The VLED supplied ~25% of maintenance energy intake and met the following percentage of Reference Nutrient Intake for the UK (17): thiamin, 41%; riboflavin, 35%; niacin, 48%; vitamin B_6, 33%; vitamin B_{12}, 53.3%; vitamin C, 82%; vitamin A, 41.5%; folate, 54%; calcium, 39.8%; sodium, 42.7%; potassium, 14.5%; chloride, 44.2%; phosphorus, 59.3%; magnesium, 20.1%; iron, 3.98%; zinc, 21.1%; copper, 17.4%; selenium, 24.8%; and iodine, 23.7%.

Table 1. *Baseline physical characteristics of subjects in the starvation and VLED groups*

	Starvation Group	VLED Group
Age, yr	39.83±11.63	47.20±7.19
Height, m	1.79±0.05	1.77±0.05
Weight, kg	71.05±9.15	67.04±8.15
Body mass index, kg/m^2	22.25±2.59	21.39±2.49
Fat, %	13.47±4.31	13.68±6.46

Values are means ± SD. Percent fat was measured using the 4-component model of Fuller et al. (27). VLED, very low energy diet. There were no significant differences between the two groups.

Isotope infusion protocols. Two isotope infusions were undertaken successively. After an overnight (12 h) fast, whole body leucine irreversible loss rate, protein synthesis, breakdown, and oxidation were monitored first with a primed, 4-h continuous intravenous infusion of [1-^{13}C]leucine, a preferred method (59, 73). This was followed immediately by a primed, 7-h continuous infusion of [$^{13}C_2$]glycine to determine the rates of synthesis of five hepatic secreted proteins (RBP, TTR, HDL-apoA1, TR, and α_1-AT), based on plateau isotopic enrichment of apolipoprotein B100 (apoB100) [a constituent of very low density lipoprotein (VLDL)-apoB100] as representative of the hepatic precursor pool used to synthesize secreted proteins (34, 36).

For both infusions, an intravenous catheter was inserted into an antecubital vein of one arm for the continuous infusion of the tracer solution, and another in a superficial dorsal hand vein on the contralateral side to obtain arterialized blood, using a procedure previously validated for repeated blood sampling (46). A sample of blood (10 ml) was drawn before the start of the infusion for baseline metabolic measurements and to establish natural isotopic abundance ratios.

A sterile, pyrogen-free 50 mM solution of [1-^{13}C]leucine (Cambridge Isotope Laboratories, Andover, MA) was prepared in 9 g/l of NaCl and after prime doses of this (7.5 μmol/kg) and sodium [^{13}C]bicarbonate (1.75 μmol/kg) was infused (5 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) for 4 h. Blood samples were taken at 120 min and at 20-min intervals thereafter until 240 min. Arterialized blood samples (4.5 ml) were collected into lithium heparin tubes (Sarstedt Monovette, LiHep vacutainer, 5 ml) and stored on ice until centrifuged at 4°C, 1,000 *g*, for 10 min. The plasma was then stored at −20°C until analysis. Breath samples were collected at the same times as the blood samples. Subjects inspired through the nose, held their breath for 5–10 s, and then expired through the mouth via an attached 19-G needle into a polyethylene bag (Simpla Plastics, Cardiff, Wales, UK). Samples of expired air were transferred in triplicate into 15-ml plain (red cap) sterile Vacutainers (Becton Dickinson Vacutainer Systems, Plymouth, UK) and preevacuated to 10^{-2} mbar (20) for analysis of $^{13}CO_2$ enrichment within 48 h. Total carbon dioxide production was measured at rest using an open-circuit ventilated hood system (Deltatrac II, MBM-200, Datex, Instrumentarium, Helsinki, Finland) for 40 min before and after each infusion.

Immediately following the [1-^{13}C]leucine infusion, the [$^{13}C_2$]glycine (Cambridge Isotope Laboratories, Woburn, MA) infusion was started. A sterile pyrogen-free 170 mM solution of [$^{13}C_2$]glycine prepared in 9 g/l of NaCl was infused (15 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) for 7 h, after a prime dose (20 μmol/kg). Blood samples (5 ml) were taken hourly between 3 and 7 h of infusion into Na_2-EDTA tubes containing 5 μl of a cocktail of 20 mg/ml sodium azide, 10 mg/ml merthiolate, and 20 mg/ml soybean trypsin inhibitor. The blood was immediately centrifuged at 4°C, and the plasma was removed and stored at −80°C for later analysis.

Sample analyses. Plasma concentrations of triacylglycerol (TAG), total cholesterol, glucose, and iron were measured on a Cobas Fara analyzer (Roche Diagnostic Systems, Welwyn Garden City, UK) using spectrophotometric assay kits (Unimate 5 Trig, Unimate 5 Chol, Unimate 5 Gluc HK, and Unimate 5 Iron, respectively) supplied by the manufacturer. The same analyzer was used to analyze plasma concentrations of nonesterified fatty acids using an enzymatic spec-

trophotometric assay (Wako NEFA-C, Alpha Laboratories) and plasma β-hydroxybutyrate concentrations using the methodology of Williamson et al. (74).

Plasma retinol was assayed using high performance liquid chromatography with the use of the method of Thurnman et al. (68).

Plasma concentrations of RBP, TTR, HDL-apoA1, α_1-AT, and TR were measured by radial immunodiffusion kits (NLRID; The Binding Site, San Diego, CA). VLDL was separated by ultra-centrifugation, and apoB100 was precipitated with isopropanol, as previously described (34). The HDL fraction was isolated in a 1.21 g/ml NaBr-EDTA gradient by ultracentrifugation at 450,000 *g* and 22°C for 16 h (34).

RBP, TTR, α_1-AT, and TR were isolated from plasma by sequential immunoprecipitation with anti-human RBP, TTR, α_1-AT, and TR (Behring, Somerville, NJ), as previously described (63). The immunoprecipitates, ApoB100 precipitate and isolated HDL fraction, were subjected to SDS gel electrophoresis to separate protein from antibody or to separate apoprotein from other lipoprotein components. A pure standard of each protein (Sigma, St. Louis, MO) and low molecular weight marker standards (Bio-Rad Laboratories, Richmond, CA) were also included in the gel (63). After staining with Coomassie brilliant blue dye, the bands corresponding to the protein standards were excised and washed several times. The gel bands were then hydrolyzed in 6 mol/l HCl at 110°C for 12 h. The amino acids released from hydrolysis of the proteins were purified by ion-exchange chromatography using AG50W-X8 resin, 100–200 mesh hydrogen form (Bio-Rad Laboratories, Hercules, CA).

RBP, TTR, α_1-AT, TR, apoA1, and apoB100 derived amino acids were converted to the *N*-propyl ester heptafluorobutyramide derivatives, and the isotopic enrichment of glycine was determined by negative chemical ionization gas chromatography-mass spectrometry on a HP6890 gas chromatograph coupled to an HP5973 quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA). The glycine isotope ratio was determined by monitoring ions at mass-to-charge ratios of 293 to 295.

The preparation of the plasma samples for the measurement of isotopic enrichment of α-ketoisocaproate (KIC) is described elsewhere (8). The enrichment of α-KIC in plasma was measured by gas chromatography-mass spectrometry as the tertiary butyl-dimethylsilyl derivative under electron impact ionization with selective ion monitoring (8), with a VG-12–250 quadrupole mass spectrometer (VG MassLab, Manchester, UK). Measurement of breath CO_2 enrichment was as described by Milne and McGaw (50a). Urine nitrogen (N) was measured by the Kjeldahl method (Kjeltec system, Tecator AB, Hoganas, Sweden).

Calculation and statistics. N balance during weight loss was calculated as the difference between N intake and N loss in urine plus an estimate of nonurinary N losses, which for simplicity was assumed to be 0.5 g N/day in the starvation group (assuming little or no fecal loss) (25) and 1.0 g N/day in the VLED group (assuming loss of 0.5 g N/day in feces).

Whole body protein turnover was calculated in two ways (47, 49) that differ in the assumption concerning the partitioning of the infusate between oxidation and synthesis. The results and trends within groups were similar and for simplicity; only data by one approach (24) are presented, which assumes that the tracer is in excess and that an equivalent amount of leucine is oxidized.

In the postabsorptive state, leucine irreversible loss rate (ILR) provides an estimate of protein breakdown ($\mu mol \cdot kg^{-1} \cdot h^{-1}$) based on a one-pool steady-state model (49) from tracer dilution:

$$\text{breakdown} = i[(E_i/E_p) - 1]$$

where i is the tracer infusion rate ($\mu mol \cdot kg^{-1} \cdot h^{-1}$) and E_i and E_p are the tracer-to-(tracer plus tracee) ratios of the infusate and plasma α-KIC (corrected for the natural abundance ratio) at plateau, respectively. Steady isotopic state of plasma α-KIC enrichment was maintained in both the starvation and VLED groups (see Table 3).

Leucine oxidation (Leu_{oxid}; $\mu mol \cdot kg^{-1} \cdot h^{-1}$) from $^{13}CO_2$ excretion in expired breath was calculated as follows:

$$Leu_{oxid} = (\dot{V}CO_2 \times E_{co_2} \times 44.6 \times 60)/(Bwt \times R_f \times E_{KIC}) - i$$

where $\dot{V}CO_2$ is the volume of CO_2 produced, E_{co_2} is the isotopic enrichment of carbon dioxide (atom percent excess), Bwt is body weight (kg), R_f is the fraction of carbon dioxide recovered in the breath during the fasted state (0.81), and E_{KIC} is the enrichment of KIC (16). The product of 44.6 and 60 converts milliliters per minute to micromoles per hour of CO_2. The rate of leucine incorporated into protein (protein synthesis) was estimated from the difference between total leucine irreversible loss rate and endogenous leucine oxidation.

For the hepatic secreted proteins, the fractional synthetic rate (FSR) of each protein was calculated, assuming a precursor-product relationship, as follows:

$$\text{FSR (\%/day)} = [(IE_{t_7} - IE_{t_5})/IE_{pl}] \times 2{,}400/(t_7 - t_5)$$

where $IE_{t_7} - IE_{t_5}$ is the increase in the isotopic enrichment of glycine bound in RBP, TTR, HDL-apoA1, α_1-AT, and TR between 5 and 7 h ($t_7 - t_5$) of the infusion, and IE_{pl} is the isotopic enrichment of glycine bound in VLDL-apoB100 in plasma at plateau levels of enrichment (3).

The plasma enrichment of VLDL-apoB100 bound glycine in plasma is assumed to represent the isotopic enrichment of the intrahepatic glycine pool from which all hepatic secretory proteins are synthesized (3, 36). In addition, the steady isotopic state of the enrichment of VLDL-apoB100 was also shown to be maintained in both the starvation and the VLED group during the continuous infusion of [^{13}C]glycine (see Table 3).

The intravascular absolute synthesis rate (ASR) for each protein was then calculated as follows:

$$\text{intravascular ASR } (mg \cdot kg^{-1} \cdot day^{-1}) = \text{intravascular protein mass (mg/kg)} \times \text{FSR}/100$$

where the intravascular mass of a protein is the product of the plasma volume and the plasma concentration of the protein. The plasma volume of each subject was estimated based on the ideal body weights of the subjects (1), using the equation:

$$\text{plasma volume (liter)} = (\text{ideal weight} \times 0.045) + (\text{excess weight} \times 0.01)$$

where excess weight is the difference between the actual and the ideal weight of the subject.

Statistical analysis. The data are presented as means ± SD. Differences between baseline and 5% body weight loss (starvation and VLED) or 7% body weight loss (within VLED only) were assessed by repeated-measures ANOVA. The effect of rate of 5% weight loss between groups was assessed by split level repeated-measures ANOVA (with subject as blocking factor). Results were also analyzed using analysis of covariance, with baseline values as the covariates, but, because the effect of the covariate was not significant, the outcomes were similar to those obtained by split level repeated-measures ANOVA and will not be described here. A P value of < 0.05 (two-tailed) was taken to represent statistical significance. Analysis was undertaken using SPSS statistical software package (version 10.0, SPSS, Woking, Surrey, UK).

RESULTS

All subjects were lean with no difference between the starvation and VLED groups in the initial body mass index and percent body fat (Table 1). The starvation group lost a mean weight of 3.6 kg (5.05% of body weight), while the VLED group lost 2.9 kg (4.4% body weight). The loss of body weight was not significantly different between the groups or from the

Table 2. *Weight and nitrogen losses in the starvation and VLED groups*

	Starvation Group			VLED Group			
	Baseline	5% Weight Loss	*P* Value	Baseline	5% Weight Loss	7% Weight Loss	*P* Value
Body weight, kg	71.04±9.15	67.45±9.40	$P < 0.001$	67.04±8.15	64.10±8.16	62.46±7.42	$P < 0.001$
Fat, %body weight	13.47±4.31	11.64±5.04	NS	13.68±6.46	12.67±8.44	11.53±6.33	NS
Urine nitrogen, g/day	12.35±1.18	10.83±1.47	$P < 0.001$	12.23±1.94	10.27±3.02	6.36±1.17	$P < 0.001$

Values are means ± SD. Percent fat was measured using the 4-component model of Fuller et al. (27). NS, not significant.

planned 5% weight loss (Table 2). In addition, percent body fat did not differ significantly between the groups at 5% weight loss (Table 2) nor did the change in protein mass. Thus neither the total N losses during 5% weight loss in the starvation and VLED groups (−57 ± 7 vs. −58 ± 22 g N) nor the N loss per kilogram weight loss (−15.9 ± 2.3 vs. −19.7 ± 8.54 g N/kg) differed significantly. During the 5% weight loss, subjects in the VLED group each received a total protein intake of 155 g protein (24.8 g N), corresponding to 17.9 g protein/day. Over the entire period on the VLED, there was ~7% weight loss and a total negative N balance of −82 g N (−17.9 g N/kg weight loss). Throughout the continuous infusion of [^{13}C]leucine and [^{13}C]glycine in the studies of whole body protein turnover and synthetic rates of hepatic proteins, isotopic steady states were maintained in both the starvation and VLED groups (Table 3). In addition, none of these isotopic enrichments at these time points were significantly different from each other using paired *t*-test in both studies.

Mean whole body protein synthesis decreased in both groups at 5% weight loss ($P = 0.036$ effect of weight loss, Table 4), with no difference between groups. In contrast, responses in protein breakdown differed ($P = 0.012$ interaction term), with an increase in the starvation group and a decrease in the VLED group. The total oxidation rate increased significantly more in the starvation group than in the VLED group ($P = 0.015$ interaction term), which showed little change from baseline.

In the VLED group, the changes in protein synthesis and breakdown became more marked after a further 7% weight loss ($P = 0.011$ for the extra weight loss).

Changes in circulating concentrations of nutrients and metabolites at 5% weight loss in both groups and at 7% weight loss in the VLED group are shown in Table 5. In the starvation group, there were significant decreases in the plasma concentrations of glucose, and significant increases in TAG, cholesterol, and β-hydroxybutyrate compared with baseline values. However, in the VLED group, there was a significant decrease in the plasma concentration of iron and a significant increase in β-hydroxybutyrate, which was less than in the starvation group, and a nonsignificant decrease in cholesterol at 5 and 7% weight loss (Table 5). The changes in all of the above metabolites were significantly different in the starvation group compared with the VLED group (interaction effect, Table 5). The concentration of retinol decreased significantly in the starvation group, but not the VLED group. In contrast, the plasma concentration of iron decreased significantly in the VLED group and not the starvation group (significant interaction with both of these nutrients).

At 5% weight loss, the plasma concentration of TTR decreased by 0.07 g/l in both groups ($P < 0.001$) and RBP by 0.0135 g/l ($P = 0.001$; Table 6). In contrast, α_1-AT markedly increased during starvation, but was unchanged in the VLED group ($P = 0.025$, interaction term; Table 6).

The FSR of the various hepatic export proteins did not differ between the two groups at baseline and increased with 5% weight loss for RBP, TTR, and α_1-AT in both groups, although the changes were significant only for RBP (both groups) and α_1-AT (starvation group only) (Table 7). HDL-apoA1 also increased for the starvation group, but not in the VLED group. The increase in FSR between baseline and 5% weight loss was greater in the starvation group for HDL-apoA1 and RBP (Table 7) and also exceeded the change associated with further weight loss in the VLED group ($P = 0.008$ for HDL-apoA1, $P < 0.001$ for RBP and $P = 0.01$ for α_1-AT).

As with concentration and FSR, the ASR for all of the secreted proteins was not different between groups at baseline (Table 8). At 5% weight loss, the ASR of α_1-AT increased in the starvation group ($P = 0.008$) and significantly more ($P = 0.020$) than the VLED group. Similarly, the ASR for HDL-apoA1 increased in the starvation group ($P = 0.012$) and significantly more than the VLED group ($P = 0.003$). There were no effects of weight loss on ASR for TR, RBP, or TTR (although this decreased at 5% weight loss in the VLED group but returned to baseline after further weight loss). The changes in ASR of HDL-apoA1 ($P = 0.012$) and α_1-AT ($P = 0.02$) in

Table 3. *Isotopic enrichment values during isotopic steady state in the studies of whole body protein turnover and synthetic rates of hepatic protein using [^{13}C]leucine and [^{13}C]glycine, respectively*

Whole Body Protein Turnover Study Using [^{13}C]Leucine Infusion	Isotopic Enrichment at 180 min	Isotopic Enrichment at 200 min	Isotopic Enrichment at 220 min	Isotopic Enrichment at 240 min
Starvation group	4.38±0.29	4.33±0.26	4.40±0.36	4.37±0.35
VLED group	4.20±0.51	4.21±0.52	4.29±0.47	4.33±0.56

Synthetic Rates of Hepatic Proteins Study Using [^{13}C]Glycine Infusion	Isotopic Enrichment at 5 h	Isotopic Enrichment at 6 h	Isotopic Enrichment at 7 h
Starvation group	4.04±0.62	4.34±0.78	4.42±0.95
VLED group	3.87±0.18	4.88±0.92	4.79±0.92

Values are means ± SD; $n = 6$ for starvation and $n = 5$ for VLED groups.

Table 4. *Whole body leucine kinetics before and after a nominal weight loss of 5% body weight in the starvation and VLED groups and after further weight loss in the VLED group*

Leucine Kinetic, $\mu mol \cdot kg^{-1} \cdot h^{-1}$	Starvation Group (Rapid Weight Loss)		VLED Group (Slower Weight Loss)			SPANOVA (≤5% Weight Loss)	
	Baseline	5% Weight Loss	Baseline	5% Weight Loss	7% Weight Loss	*P* Value (effect of weight loss)	*P* Value (interaction)
Synthesis	93.96±6.95	91.36±8.96	97.54±12.69	88.66±13.47	85.67±10.72*	0.036	0.205
Breakdown	104.48±7.20	108.58±10.89	108.61±13.52	100.37±15.64	94.67±12.16*	0.313	0.012
Oxidation	10.52±2.11	17.22±3.01*	11.07±2.41	11.71±5.63	8.99±2.07	0.006	0.015

Values are means ± SD; $n = 6$ for starvation and $n = 5$ for VLED groups. SPANOVA, split level repeated-measures ANOVA. *$P < 0.05$ vs. baseline.

the starvation group at 5% weight loss were greater than those in the VLED group observed at both 5 and 7% weight loss.

The changes in plasma protein concentrations did not always mirror the changes in FSR or ASR (Fig. 1). Figure 1 shows that the concentrations of different proteins do not relate to FSR, and the changes in concentrations of individual proteins often do not parallel changes in FSR. For example, during starvation, the concentration of HDL-apoA1 did not change significantly, despite a major increase in both FSR and ASR, while, in contrast, the concentration declined during the VLED study, but without alteration in FSR or ASR. These same general responses were also seen with RBP. In the case of α_1-AT, the changes in concentration, FSR, and ASR were in parallel, as all increased during starvation but not with VLED (Fig. 1).

DISCUSSION

The extent of weight loss during starvation and during the consumption of VLED (18, 20, 43) is known to alter physical, psychological, and metabolic function. This study indicates that whole body protein kinetics, circulating concentrations of various nutrients and metabolites, and synthetic rates of specific hepatic secretory proteins are independent of the magnitude of weight loss but are affected by different degrees of dietary restriction that cause different rates of weight loss. The reasons for undertaking severe dietary restriction (VLED, 75% food restricted) are because *1*) diets as low as 330 kcal were used for weight reduction (14), and diets as low as 600 kcal/day are still used for this purpose (66); *2*) such restriction is common in hospitalized patients; and *3*) it allows comparisons with similar degrees of restriction reported in animal studies (38).

At a nominal 5% weight loss, whole body protein oxidation and the synthetic rates of specific hepatic export proteins were increased in the starvation group (rapid weight loss) compared with subjects receiving VLED (slower rate of weight loss), where most of these parameters were either unchanged or reduced. The increase in whole body protein oxidation during starvation supports previous studies with lean individuals (40, 53, 69, 70). This contrasts with the VLED treatment, where protein synthesis, breakdown, and oxidation were reduced during weight loss (significant at 7% weight loss relative to the baseline values for synthesis and breakdown only). This agrees with earlier observations that long-term nutrient restriction, either with low-protein or protein-free diets, leads to an adaptation with reduced protein turnover and lowered amino acid catabolism (9, 11). The technique of constant infusion of a low concentration of leucine as a tracer, which was used in this study, is the preferred method for measuring protein kinetics (59, 73). However, like in other tracer methods, it has not been fully validated in all nutritional and inflammatory states.

The apparent discrepancy at 5% weight loss between the higher rate of leucine oxidation in starvation than VLED (17.2 vs. 11.7 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, $P = 0.012$), yet with similar rates of urine N excretion (10.83 vs. 10.27 g N/day; not significant), reflects the fact that the isotope kinetics, measured after an overnight fast, represent oxidation of endogenous protein, whereas the urinary losses over 24 h will also include catabolism of the dietary protein supplied in the VLED. Diurnal protein metabolism, including production of urea, is known to follow fed or fasted conditions, with the magnitude influenced by absolute N intake (56, 58). Although urinary N loss did not differ between the two dietary treatments, the loss of endogenous protein was greater (both absolute and relative to body weight) for the starvation group.

Provision of even a limited supply of carbohydrate and amino acids by VLED may reduce the dependency on endog-

Table 5. *Circulating concentrations of nutrients and metabolites in 11 lean men before and after a nominal weight loss of 5% body weight in the starvation and VLED groups and after further weight loss in the VLED group*

Plasma Concentration	Starvation Group (Rapid Weight Loss)		VLED Group (Slower Weight Loss)			SPANOVA (≤5% Weight Loss)	
	Baseline	5% Weight Loss	Baseline	5% Weight Loss	7% Weight Loss	*P* Value (effect of weight loss)	*P* Value (interaction)
Glucose, mmol/l	5.07±0.27	3.14±0.39*	5.06±1.00	4.33±0.46	4.7±0.46	0.013	0.001
NEFA, mmol/l	0.66±0.83	1.11±0.10*	0.44±0.09	0.51±0.23	0.58±0.23	0.001	0.001
β-Hydroxybutyrate, μmol/l	75±54	5062±744*	128±95	636±536*	834±106*	0.001	0.001
TAG, mmol/l	0.79±0.12	1.14±0.31*	0.86±0.17	0.84±0.16	0.83±0.24	0.018	0.023
Cholesterol, mmol/l	4.19±0.19	4.70±0.54*	4.94±1.23	4.24±0.84	4.42±0.19	0.015	0.766
Retinol, μmol/l	1.68±0.18	1.00±0.19*	1.61±0.11	1.43±0.44	1.20±0.19	0.01	0.001
Iron, μmol/l	23.38±9.64	29.41±7.95	24.42±7.75	13.15±5.99*	10.94±4.29*	0.131	0.004

Values are means ± SD; $n = 6$ for starvation and $n = 5$ for VLED groups. NEFA, nonesterified fatty acid; TAG, triacylglycerol. *$P < 0.05$ vs. baseline.

Table 6. *The concentration of plasma proteins in 11 lean men before (0% weight loss) and after a nominal weight loss of 5% body weight in the starvation and VLED groups and after further weight loss in the VLED group*

Plasma Protein Concentration, g/l	Starvation Group (Rapid Weight Loss)		VLED Group (Slower Weight Loss)			SPANOVA (≤5% Weight Loss)	
	Baseline	5% Weight Loss	Baseline	5% Weight Loss	7% Weight Loss	*P* Value (effect of weight loss)	*P* Value (interaction)
HDL-apoA1	1.24±0.19	1.26±0.21	1.48±0.25	1.23±0.27*	1.06±0.14*	0.107	0.063
RBP	0.031±0.005	0.018±0.007*	0.039±0.005	0.025±0.008*	0.026±0.004*	0.001	1.000
TTR	0.20±0.03	0.13±0.02*	0.22±0.02	0.15±0.01*	0.21±0.09	0.0005	0.464
α_1-AT	0.78±0.11	1.04±0.18*	0.87±0.13	0.86±0.09	0.89±0.22	0.038	0.025
TR	1.64±0.33	1.55±0.20	1.65±0.42	1.55±0.23	1.50±0.30	0.400	0.992

Values are means ± SD; $n = 6$ for starvation and $n = 5$ for VLED groups. HDL-apoA1, high-density lipoprotein apolipoprotein A1; RBP, retinol binding protein; TTR, transthyretin; α_1-AT, α_1-antitrypsin; TR, transferrin. *$P < 0.05$ vs. baseline.

enous carbon from amino acids to support gluconeogenesis and maintain glycemia. Indeed, infusion of glucose to fasted cattle decreases urinary N losses by ~50% (55). In the undernourished state, utilization of amino acids for gluconeogenesis will result in elevated net protein catabolism and increase excretion of N and oxidation of ketogenic amino acids, such as leucine, released from protein degradation.

The changes in circulating metabolites observed during starvation and VLED are those expected, with the starvation group showing greater reduction in glucose and retinol concentrations along with greater increments in nonesterified fatty acid and β-hydroxybutyrate concentrations, reflecting increased lipolysis and ketogenesis. These changes also provide the biochemical validation of starvation and VLED. The reason for the decrease in circulating iron concentration only in the VLED group is unclear, but interpretation would be aided by measurements of circulating iron carrier proteins. Starvation and the VLED also differed in their impact on synthesis of liver export proteins. The changes in FSR and ASR of the measured proteins also tended to be greater during starvation than in VLED, particularly for RBP (FSR), α_1-AT (ASR), and HDL-apoA1 (both FSR and ASR). Indeed, these remained greater for the fasted group even compared with greater weight loss during VLED. However, an initial decrease in ASR of TTR observed in the VLED group at 5% body weight loss returned back to baseline values upon further weight loss. Recent dietary intake has been suggested to affect physical and psychological function independently of the magnitude of weight loss (29, 37, 39, 41), and the present study suggests the same also applies to aspects of metabolic function. Understanding the underlying mechanisms that control protein synthesis also requires appreciation of the subtle specificities involved. This was clearly illustrated by the starvation-induced increases in FSR (and ASR) of HDL-apoA1 and α_1-AT, but not for TR or TTR. A number of potential regulators are probably involved. For example, previous studies have shown that HDL-apoA1 can act both as an anti-inflammatory protein by suppressing the effects of cytokines on their induction of adhesion molecules by endothelial cells and as an antioxidant by reducing oxidized low-density lipoprotein, which has been implicated in the inflammation process involved in cardiovascular disease (42). Results from this study support previous reports of increased concentrations of HDL apoA1 and HDL cholesterol during anoxeria nervosa (54), along with the modulation of the HDL apoA1 gene by dietary restriction (2, 5, 31). Although the precise mechanism(s) involved in the regulation of HDL-apoA1 gene or changes in plasma concentration of HDL-apoA1 during starvation is still not known, the increased change in HDL-apoA1 synthesis may be indicative of a change in lipid (TAG) and cholesterol transport accompanied by a possible suppression of cytokines that might be released during starvation.

The increase in α_1-AT synthesis observed for the starvation but not the VLED group would suggest involvement of stress-related events, because synthesis of acute phase proteins is responsive to cytokines and stress hormones, including the corticosteroids, catecholamines, and glucagon. In addition, α_1-AT is known to be an anti-proteolytic enzyme, which is involved in the inhibition of neutrophil elastase, thereby protecting extracellular structures from proteolysis by neutrophil elastase released by activated or disintegrating neutrophils (13). Insulin may also be involved, because this is known to decrease to a greater extent during total fasting than when small amounts of carbohydrate are consumed (32) and has differential effects on the synthesis of hepatic export proteins in humans [e.g., a decrease in the synthesis of fibrinogen and an increase in albumin (15, 16)] and also reduces fibrinogen synthesis in swine without an overall effect in metabolism of

Table 7. *The FSR (%/day) of plasma proteins in 11 lean men before (0% weight loss) and after a nominal weight loss of 5% body weight in the starvation and VLED groups and after further weight loss in the VLED group*

FSR of Plasma Protein, %/day	Starvation Group (Rapid Weight Loss)		VLED Group (Slower Weight Loss)			SPANOVA (≤5% Weight Loss)	
	Baseline	5% Weight Loss	Baseline	5% Weight Loss	~7% Weight Loss	*P* Value (effect of weight loss)	*P* Value (interaction)
HDL-apoA1	31.33±7.45	56.64±11.39*	30.03±4.39	28.51±3.38	31.52±11.14	0.008	0.003
RBP	37.98±8.74	68.62±18.17*	33.65±5.84	40.77±9.77*	36.29±17.32*	0.0005	0.007
TTR	41.06±5.76	52.69±17.2	37.41±2.9	40.61±9.3	41.76±1.54	0.146	0.376
α_1-AT	26.12±4.53	54.48±21.45*	22.52±8.05	29.63±9.06	34.78±19.35	0.010	0.088
TR	21.07±7.42	29.1±9.40	16.02±2.98	20.64±3.19	22.05±9.79	0.049	0.584

Values are means ± SD; $n = 6$ for starvation and $n = 5$ for VLED groups. FSR, fractional synthetic rate. *$P < 0.05$ vs. baseline.

Table 8. *The ASR (mg·kg^{-1}·day^{-1}) of plasma proteins in 11 lean men before (0% weight loss) and after a nominal weight loss of 5% body weight in the starvation and VLED groups and after further weight loss in the VLED group*

ASR of Plasma Protein, mg·kg^{-1}·day^{-1}	Starvation Group (Rapid Weight Loss)		VLED Group (Slower Weight Loss)			SPANOVA (≤5% Weight Loss)	
	Baseline	5% Weight Loss	Baseline	5% Weight Loss	7% Weight Loss	*P* Value (effect of weight loss)	*P* Value (interaction)
HDL-apoA1	18.25±6.07	35.35±13.39*	21.53±5.73	17.66±5.91	16.79±5.73	0.032	0.003
RBP	0.56±0.21	0.64±0.36	0.61±0.07	0.50±0.13	0.49±0.30	0.753	0.079
TTR	3.75±0.918	3.28±1.23	4.08±0.93	2.95±0.842*	4.18±1.525	0.069	0.413
α_1-AT	9.38±1.38	27.22±11.20*	9.44±3.57	13.02±5.03	16.92±10.70	0.002	0.02
TR	16.83±10.39	22.23±9.58	13.13±6.03	16.19±4.50	17.44±9.98	0.638	0.138

Values are means ± SD; $n = 6$ for starvation and $n = 5$ for VLED groups. ASR, absolute synthesis rate. *$P < 0.05$ vs. baseline.

constitutive liver proteins (4). Specific nutrients may also play a role, e.g., iron status regulates synthesis of the carrier protein, TR, in the isolated, perfused rat liver (52) and a human hepatoma cell line (6). Furthermore, plasma concentrations and patterns of amino acids differ between subjects receiving no food (67) or very low calorie diets (28, 71).

A low protein diet in humans (0.6 k·kg^{-1}·day^{-1}) for 7 days increased acute protein synthesis (haptoglobin and fibrinogen), whereas the synthesis of nutrient transport proteins (albumin, VLDL-apoB100, and HDL-apoA1) decreased (40). This was associated with an increase in the plasma concentration of interleukin-6 and suggested that a low-grade inflammatory process was responsible (33). Cytokines were not measured in the present study, but the known responsiveness of α_1-AT to proinflammatory cytokines would suggest these may have increased in the starvation group (10). Although the liver is considered the dominant source of the export proteins measured in this study, other tissues may also contribute to plasma concentrations. For example, in the rat, RBP mRNA is produced in extra hepatic tissues (e.g., kidney mRNA levels of 5–10% of that of the liver and lungs, spleen, brain, stomach, heart, and skeletal muscle, 1–3% of the level found in the liver) (64). Interestingly, this extra-hepatic RBP does not appear to perform all the physiological functions of hepatic RBP (57).

A reduction in the plasma concentration of a secreted protein has often been interpreted as being due to a reduction in synthesis, such as cited for RBP and TTR, which are widely used as markers of visceral protein status (35, 51, 60). It has also been assumed that the changes are due to reduced availability of amino acids or dietary protein (3). While some support for this assumption comes from animal studies (7, 12), the present study shows this cannot be applied generally. For example, the synthesis rates of RBP and TTR were unchanged, despite significant decreases in plasma concentrations. Other proteins, such as HDL-apoA1, showed no significant concentration change during starvation but a major increase in FSR and ASR, whereas α_1-AT showed simultaneous increases in concentration, FSR, and ASR. For some, but not all proteins, therefore, a clear dissociation exists between plasma concentration and synthesis rates. Factors that contribute to these

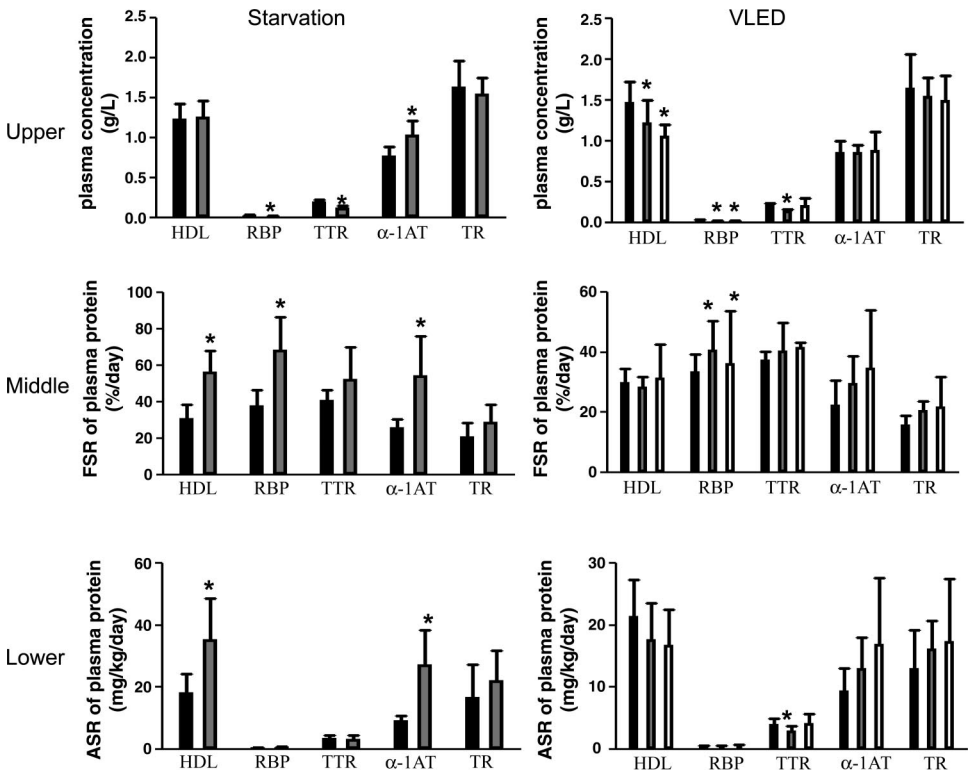

Fig. 1. Plasma concentration (*upper*), fractional synthetic rate (FSR) (*middle*), and absolute synthesis rate (ASR) (*lower*) of high-density lipoprotein apolipoprotein A1 (HDL), retinol binding protein (RBP), transthyretin (TTR), α_1-antitrypsin (α_1-AT), and transferrin (TR) in the starvation group (*left*) and very low energy diet (VLED) group (*right*) at baseline (solid bars), 5% weight loss (shaded bars), and also at 7% weight loss (open bars in VLED group only). Values are means ± SD. Significant differences from baseline values are indicated (*$P < 0.05$; paired *t*-test).

apparent discrepancies include independent effects on the catabolism of individual proteins and changes in the distribution of individual proteins between the intra- and extracellular spaces. The catabolism of plasma proteins occurs in a variety of tissues (50), including the liver (45), and probably under different control mechanisms. In addition, RBP is lost in urine, especially under inflammatory stress conditions (65). The metabolic response to starvation will probably vary according to initial body composition, age, and sex, so the results of this study undertaken in lean male adults should not be extrapolated to other groups.

It is difficult to link our observation on the concentration and kinetics of liver secretory proteins with health implications at high and slow rates of weight loss, without full knowledge of the interorgan transport of the nutrients they carry and the functional consequences of this transport. However, starvation appears to induce a greater inflammatory response than VLED, and this represents an added stress. The α_1-AT concentration and its synthetic rate is a marker of this inflammatory process, and the increase in ASR of HDL-apoA1 could be interpreted as part of a protective anti-inflammatory process. Starvation as a means of weight loss is now no longer recommended as a method for long-term weight loss and maintenance, and the biochemical data from this study are consistent with this view. In addition, there are a number of other important biochemical and functional differences between starvation and VLED. These are shown in Table 5 and include psychological function, such as subjective feelings of fatigue, and physical function, such as physical activity and basal metabolic rate (data not shown in this study), which is often transiently increased during starvation (18) but not VLED.

These findings add to the understanding of the mechanisms involved in the responses to different degrees of dietary restriction in normal weight men that may result from, for example, prolonged restricted dietary intake before surgery, hunger strikes, stunt fasts, or natural disasters. Such understanding may lead to improved management strategies of these or similar situations. However, we should not extrapolate our findings to overweight or obese individuals undergoing weight loss who may respond differently to starvation and VLED than our initially lean subjects. In addition, we do not know how these mechanisms may be modified by coexistent disease or injury, and these remain areas for further investigation.

In summary, this study suggests that the concentration and synthetic rates of several hepatic proteins respond to weight loss, but these changes are probably due more to the degree of dietary restriction (starvation vs. VLED) than the magnitude of weight loss. The study also challenges the concept of tight coupling between synthesis and circulating concentration of specific proteins, including those suggested as markers of visceral protein status (e.g., RBP, TTR).

The differences in whole body protein kinetics, especially the difference between synthesis and breakdown during the rapid weight loss of starvation and the slower weight loss of VLED, have implications for the function of cells and tissues and strategies for the endogenous provision of amino acids.

ACKNOWLEDGMENTS

We are very grateful to Margaret Frazer and Melanie Del Rosario for kind assistance and invaluable technical help with these studies, and to Dr. John Jackson for constructive discussions.

GRANTS

This grant was supported by Slimming World, Alfreton, UK, and the Scottish Executive Environment and Rural Affairs Department.

REFERENCES

1. **[Anon]. Metropolitan height and weight tables.** *Stat Bull Metrop Life Found* 64: 3–9, 1983.
2. **Adlouni A, Ghalim N, Saile R, Hda N, Parra HJ, Benslimane A.** Beneficial effect on serum apo AI, apo B and Lp AI levels of Ramadan fasting. *Clin Chim Acta* 271: 179–189, 1998.
3. **Afolabi PR, Jahoor F, Gibson NR, Jackson AA.** Response of hepatic proteins to the lowering of habitual dietary protein to the recommended safe level of intake. *Am J Physiol Endocrinol Metab* 287: E327–E330, 2004.
4. **Ahlman B, Charlton M, Fu A, Berg C, O'Brien P, Nair KS.** Insulin's effect on synthesis rates of liver proteins. A swine model comparing various precursors of protein synthesis. *Diabetes* 50: 947–954, 2001.
5. **Akanji AO, Mojiminiyi OA, Abdella N.** Beneficial changes in serum apo A-1 and its ratio to apo B and HDL in stable hyperlipidaemic subjects after Ramadan fasting in Kuwait. *Eur J Clin Nutr* 54: 508–513, 2000.
6. **Barnum-Huckins K, Adrian GS.** Iron regulation of transferrin synthesis in the human hepatoma cell line HepG2. *Cell Biol Int* 24: 71–77, 2000.
7. **Bell AW, Burhans WS, Overton TR.** Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. *Proc Nutr Soc* 59: 119–126, 2000.
8. **Calder AG, Smith A.** Stable isotope ratio analysis of leucine and ketoisocaproic acid in blood plasma by gas chromatography/mass spectrometry. Use of tertiary butyldimethylsilyl derivatives. *Rapid Commun Mass Spectrom* 2: 14–16, 1998.
9. **Castaneda C, Dolnikowski GG, Dallal GE, Evans WJ, Crim MC.** Protein turnover and energy metabolism of elderly women fed a low-protein diet. *Am J Clin Nutr* 62: 40–48, 1995.
10. **Castell JV, Gomez-Lechon MJ, David M, Andus T, Geiger T, Trullenque R, Fabra R, Heinrich PC.** Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett* 242: 237–239, 1989.
11. **Clugston GA, Garlick PJ.** The response of whole-body protein turnover to feeding in obese subjects given a protein-free, low-energy diet for three weeks. *Hum Nutr Clin Nutr* 36: 391–397, 1982.
12. **Connell A, Calder AG, Anderson SE, Lobley GE.** Hepatic protein synthesis in the sheep: effect of intake as monitored by use of stable-isotope-labelled glycine, leucine and phenylalanine. *Br J Nutr* 77: 255–271, 1997.
13. **Crystal RG.** Alpha 1-antitrypsin deficiency, emphysema, and liver disease. Genetic basis and strategies for therapy. *J Clin Invest* 85: 1343–1352, 1990.
14. **Davies HJ, Baird IM, Fowler J, Mills IH, Baillie JE, Rattan S, Howard AN.** Metabolic response to low- and very-low-calorie diets. *Am J Clin Nutr* 49: 745–751, 1989.
15. **De Feo P, Gaisano MG, Haymond MW.** Differential effects of insulin deficiency on albumin and fibrinogen synthesis in humans. *J Clin Invest* 88: 833–840, 1991.
16. **De Feo P, Volpi E, Lucidi P, Cruciani G, Reboldi G, Siepi D, Mannarino E, Santeusanio F, Brunetti P, Bolli GB.** Physiological increments in plasma insulin concentrations have selective and different effects on synthesis of hepatic proteins in normal humans. *Diabetes* 42: 995–1002, 1993.
17. **Department of Health.** *Dietary Reference Values for Food, Energy and Nutrients for the United Kingdom*. London: HMSO, 1991.
18. **Elia M.** Effect of starvation and very low calorie diets on protein-energy interrelationships in lean and obese subjects. In: *Protein-Energy Interactions*, edited by Scrimshaw NS and Schurch B. Lausanne, Switzerland: IDECG, 1992, p. 249–284.
19. **Elia M.** Hunger disease. *Clin Nutr* 19: 379–386, 2000.
20. **Elia M.** Metabolic response to starvation, injury and sepsis. *In: Artificial Nutritional Support in Clinical Practice*, edited by Payne-James J, Grimble G, and Silk D. London: Greenwich Medical Media, 2001.
21. **Elia M.** Tissue distribution and energetics in weigh loss and undernutrition. In: *Physiology, Stress and Malnutrition: Functional Correlates and Nutritional Intervention*, edited by Kinney JM and Tucker HN. Philadelphia: Lippincott-Raven, 1997, p. 383–411.

23. **Elia M, Wood S, Khan K, Pullicino E.** Ketone body metabolism in lean male adults during short-term starvation, with particular reference to forearm muscle metabolism. *Clin Sci (Lond)* 78: 579–584, 1990.
24. **Faber P, Johnstone AM, Gibney ER, Elia M, Stubbs RJ, Duthie GG, Calder AG, Lobley GE.** The effect of rate of weight loss on erythrocyte glutathione concentration and synthesis in healthy obese men. *Clin Sci (Lond)* 102: 569–577, 2002.
25. **Food and Agriculture Organization Of The United Nations/World Health Organization/The United Nations University.** *Energy and Protein Requirements.* Geneva: WHO, 1985.
26. **Food Standards Agency.** *McCance and Widdowson's The Composition of Foods*. London: The Royal Society of Chemistry and Foods Standards Agency, 2002.
27. **Fuller NJ, Jebb SA, Laskey MA, Coward WA, Elia M.** Four-component model for the assessment of body composition in humans: comparison with alternative methods and evaluation of the density and hydration of fat-free mass. *Clin Sci (Lond)* 82: 687–693, 1992.
28. **Gatti E, Porrini M, Noe D, Crovetti R, Testolin G.** Plasma amino acids changes in obese patients on very low-calorie diets. *Int J Vitam Nutr Res* 64: 81–85, 1994.
29. **Gibney RG.** *The Physical, Psychological and Metabolic Effects Of Nutritional Depletion and Subsequent Repletion* (PhD thesis). Cambridge, UK: University of Cambridge, 2002.
30. **Grande F, Anderson T, Keys A.** Changes in basal metabolic rate in man in semistarvation and refeeding. *J Appl Physiol* 12: 230–238, 1958.
31. **Haas MJ, Pun K, Reinacher D, Wong NC, Mooradian AD.** Effects of ketoacidosis on rat apolipoprotein A1 gene expression: a link with acidosis but not with ketones. *J Mol Endocrinol* 25: 129–139, 2000.
32. **Hoffer LJ, Forse RA.** Protein metabolic effects of a prolonged fast and hypocaloric refeeding. *Am J Physiol Endocrinol Metab* 258: E832–E840, 1990.
33. **Jackson AA, Phillips G, McClelland I, Jahoor F.** Synthesis of hepatic secretory proteins in normal adults consuming a diet marginally adequate in protein. *Am J Physiol Gastrointest Liver Physiol* 281: G1179–G1187, 2001.
34. **Jackson AA, Phillips G, McClelland I, Jahoor F.** Synthesis of hepatic secretory proteins in normal adults consuming a diet marginally adequate in protein. *Am J Physiol Gastrointest Liver Physiol* 281: G1179–G1187, 2001.
35. **Jahoor F, Gazzard B, Phillips G, Sharpstone D, Delrosario M, Frazer M, Heird W, Smith R, Jackson A.** The acute-phase protein response to human immunodeficiency virus infection in human subjects. *Am J Physiol Endocrinol Metab* 276: E1092–E1098, 1999.
36. **Jahoor F, Sivakumar B, Del Rosario M, Frazer EM.** Isolation of acute-phase proteins from plasma for determination of fractional synthesis rates by a stable isotope tracer technique. *Anal Biochem* 236: 95–100, 1996.
37. **Jennings G, Elia M.** Effect of dietary restriction on the response of a2-macroglobulin during an acute phase response. *JPEN J Parenter Enteral Nutr* 18: 510–515, 1994.
38. **Jennings G, Elia M.** Effect of dietary restriction on the response of alpha 2-macroglobulin during an acute phase response. *JPEN J Parenter Enteral Nutr* 18: 510–515, 1994.
39. **Jennings G, Elia M.** Independent effects of protein and energy deficiency on acute-phase protein response in rats. *Nutrition* 7: 430–434, 1991.
40. **Jensen MD, Miles JM, Gerich JE, Cryer PE, Haymond MW.** Preservation of insulin effects on glucose production and proteolysis during fasting. *Am J Physiol Endocrinol Metab* 254: E700–E707, 1988.
41. **Johnstone AM.** *Weight Loss and Human Obesity* (PhD thesis). Aberdeen, UK: University of Aberdeen, 2001.
42. **Kaysen GA.** Association between inflammation and malnutrition as risk factors of cardiovascular disease. *Blood Purif* 24: 51–55, 2006.
43. **Keys A, Brozek J, Henschel A, Mickelsen O, Taylor HL.** *The Biology of Human Starvation.* Minneapolis, MN: University of Minnesota Press, 1950, Chapt. 17, p. 81–535.
44. **Kleitman N.** Basal metabolism in prolonged fasting in man. *Am J Physiol* 77: 33–44, 1926.
45. **Koj A.** Extrahepatic synthesis of acute phase proteins. In: *The Acute Phase Response*, edited by Gordon AH, Koj A. New York: Elsevier, 1985, p. 221–246.
46. **Kurpad A, Elia M.** The effect of repeated hand warming in metabolic studies. *Med Sci Res* 23: 51–52, 1995.
47. **Lobley GE, Shen X, Le G, Bremner DM, Milne E, Calder AG, Anderson SE, Dennison N.** Oxidation of essential amino acids by the ovine gastrointestinal tract. *Br J Nutr* 89: 617–630, 2003.
48. **Mansell PI, Fellows IW, Macdonald IA.** Enhanced thermogenic response to epinephrine after 48-h starvation in humans. *Am J Physiol Regul Integr Comp Physiol* 258: R87–R93, 1990.
49. **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-^{3}C]leucine. *Am J Physiol Endocrinol Metab* 238: E473–E479, 1980.
50. **Maxwell JL, Terracio L, Borg TK, Baynes JW, Thorpe SR.** A fluorescent residualizing label for studies on protein uptake and catabolism in vivo and in vitro. *Biochem J* 267: 155–162, 1990.

50a. **Milne E, McGraw BA.** The applicability of evacuated serological tubes for the collection of breath for isotopic analysis of CO_2 by isotopic ratio mass spectrometry. *Biomed Environ Mass Spectrom* 15: 467–472, 1988.

51. **Morlese JF, Forrester T, Jahoor F.** Acute-phase protein response to infection in severe malnutrition. *Am J Physiol Endocrinol Metab* 275: E112–E117, 1998.
52. **Morton AG, Tavill AS.** The role of iron in the regulation of hepatic transferrin synthesis. *Br J Haematol* 36: 383–394, 1977.
53. **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* 46: 557–562, 1987.
54. **Ohwada R, Hotta M, Oikawa S, Takano K.** Etiology of hypercholesterolemia in patients with anorexia nervosa. *Int J Eat Disord* 39: 598–601, 2006.
55. **Orskov ER, Meehan DE, MacLeod NA, Kyle DJ.** Effect of glucose supply on fasting nitrogen excretion and effect of level and type of volatile fatty acid infusion on response to protein infusion in cattle. *Br J Nutr* 81: 389–393, 1999.
56. **Price GM, Halliday D, Pacy PJ, Quevedo MR, Millward DJ.** Nitrogen homeostasis in man: influence of protein intake on the amplitude of diurnal cycling of body nitrogen. *Clin Sci (Lond)* 86: 91–102, 1994.
57. **Quadro L, Blaner WS, Hamberger L, Novikoff PM, Vogel S, Piantedosi R, Gottesman ME, Colantuoni V.** The role of extrahepatic retinol binding protein in the mobilization of retinoid stores. *J Lipid Res* 45: 1975–1982, 2004.
58. **Quevedo MR, Price GM, Halliday D, Pacy PJ, Millward DJ.** Nitrogen homoeostasis in man: diurnal changes in nitrogen excretion, leucine oxidation and whole body leucine kinetics during a reduction from a high to a moderate protein intake. *Clin Sci (Lond)* 86: 185–193, 1994.
59. **Reeds PJ, Davis TA.** Of flux and flooding: the advantages and problems of different isotopic methods for quantifying protein turnover in vivo: I. Methods based on the dilution of a tracer. *Curr Opin Clin Nutr Metab Care* 2: 23–28, 1999.
60. **Reid M, Badaloo A, Forrester T, Morlese JF, Heird WC, Jahoor F.** The acute-phase protein response to infection in edematous and non-edematous protein-energy malnutrition. *Am J Clin Nutr* 76: 1409–1415, 2002.
61. **Schofield WN, Schofield C, James WPT.** Basal metabolic rate. *Hum Nutr Clin Nutr* 39: 1–96, 1985.
62. **Shenkin A, Cederblad G, Elia M, Isaksson B.** Laboratory assessment of protein-energy status. *Clin Chim Acta* 252: 1–56, 1996.
63. **Sivakumar B, Jahoor F, Burrin DG, Frazer EM, Reeds PJ.** Fractional synthesis rates of retinol-binding protein, transthyretin, and a new peptide measured by stable isotope techniques in neonatal pigs. *J Biol Chem* 269: 26196–26200, 1994.
64. **Soprano DR, Soprano KJ, Goodman DS.** Retinol-binding protein messenger RNA levels in the liver and in extrahepatic tissues of the rat. *J Lipid Res* 27: 166–171, 1986.
65. **Stephensen CB, Alvarez JO, Kohatsu J, Hardmeier R, Kennedy JI Jr, Gammon RB Jr.** Vitamin A is excreted in the urine during acute infection. *Am J Clin Nutr* 60: 388–392, 1994.
66. **Strychar I.** Diet in the management of weight loss. *CMAJ* 174: 56–63, 2006.
67. **Swendseid ME, Umezawa CY, Drenick E.** Plasma amino acid levels in obese subjects before, during, and after starvation. *Am J Clin Nutr* 22: 740–743, 1969.

68. **Thurnham DI, Smith E, Flora PS.** Concurrent liquid chromatographic assay of retinol, alpha-tocopherol, beta-carotene, alpha-carotene, lycopene and beta-cryptoxanthin in plasma, with tocopherol acetate as internal standard. *Clin Chem* 34: 377–381, 1988.
69. **Tsalikian E, Howard C, Gerich JE, Haymond MW.** Increased leucine flux in short-term fasted human subjects: evidence for increased proteolysis. *Am J Physiol Endocrinol Metab* 247: E323–E327, 1984.
70. **Umpleby AM, Scobie IN, Boroujerdi MA, Sonksen PH.** The effect of starvation in leucine, alanine and glucose metabolism in obese subjects. *Eur J Clin Invest* 25: 619–625, 1995.
71. **Vila R, Granada ML, Gutierrez RM, Fernandez-Lopez JA, Remesar X, Formiguera X, Foz M, Alemany M.** Short-term effects of a hypocaloric diet on nitrogen excretion in morbid obese women. *Eur J Clin Nutr* 55: 186–191, 2001.
72. **Vitkova M, Klimcakova E, Kovacikova M, Valle C, Moro C, Polak J, Hanacek J, Capel F, Viguerie N, Richterova B, Bajzova M, Hejnova J, Stich V, Langin D.** Plasma levels and adipose tissue messenger ribonucleic acid expression of retinol-binding protein 4 are reduced during calorie restriction in obese subjects but are not related to diet-induced changes in insulin sensitivity. *J Clin Endocrinol Metab* 92: 2330–2335, 2007.
73. **Watt PW, Lindsay Y, Scrimgeour CM, Chien PA, Gibson JN, Taylor DJ, Rennie MJ.** Isolation of aminoacyl-tRNA and its labeling with stable-isotope tracers: use in studies of human tissue protein synthesis. *Proc Natl Acad Sci USA* 88: 5892–5896, 1991.
74. **Williamson DH, Mellanby J, Krebs HA.** Enzymatic determination of D(-)-B-hydroxybutyric acid and acetoacetic acid in blood. *Biochem J* 82: 90–96, 1962.
75. **Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB.** Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 436: 356–362, 2005.
76. **Zauner C, Schneeweiss B, Kranz A, Madl C, Ratheiser K, Kramer L, Roth E, Schneider B, Lenz K.** Resting energy expenditure in short-term starvation is increased as a result of an increase in serum norepinephrine. *Am J Clin Nutr* 71: 1511–1515, 2000.