Protein metabolism in obese subjects during a very-low-energy diet1-3

Réjeanne Gougeon, L John Hoffer, Paul B Pencharz, and Errol B Marli$$

ABSTRACT We postulated that the return to nitrogen equilibrium after 3 wk of a negative balance during a very-low-calorie diet (VLCD) providing low-quality protein in obese subjects was due to availability of endogenously originating amino acids from a "pool" that, when depleted, would result in worsening balance. This should be reflected in altered kinetics of protein metabolism with the requirement for increased breakdown to maintain metabolism constant. Seven female obese subjects [body mass index (BMI) = 34.4 ± 1.8 kg/m2] were given a 1.7-MJ/d all-protein diet (16.8 g N) derived from hydrolyzed gelatin (supplemented with tryptophan and methionine) that provides 18% of its amino acids as essential, a multivitamin-mineral supplement, and 16 mmol KCl for 42 d. At baseline (7-d isocaloric diet), and weeks 4 and 6 of VLCD, amino nitrogen flux rate was calculated from the 15N abundance in urinary urea using the oral 15N-glycine method and rates of synthesis (S) and breakdown (B) inferred from N flux. Whole-body N flux did not change from baseline to weeks 4 and 6 (39.5 ± 2.0 vs 37.4 ± 2.0 vs 39.2 ± 1.9 g N/d). By contrast, S and B decreased at weeks 4 and 6 with S decreasing more so that net protein synthesis (S-B) was less positive at week 4 than at baseline (2.2 ± 0.2 and 0.9 ± 0.3 g N/d; P < 0.05) and became negative at week 6 (−0.9 ± 0.2 g N/d; P < 0.05). Concurrently, N equilibrium was achieved by week 4 but returned to negative balance by week 6. A decrease in net protein synthesis may reflect insufficient exogenous amino acid when such diets are used alone for a prolonged period of time and explain the return to negative N balance with net depletion of available endogenous sources. Am J Clin Nutr 1992;56:249S–54S.

KEY WORDS 15N-glycine, very-low-calorie diet, VLCD, obesity, protein turnover, net protein synthesis

Introduction

Very-low-energy diets (VLCD) have continued to be used clinically, apparently with fewer complications (1-4) than had been reported earlier (5-7). Presumably, the use of these protein-supplemented fasts has been rendered safer through rigorous and specialized patient selection and monitoring, adequate protein sources and with vitamin, mineral, and potassium supplementation (7). Bicarbonate supplementation has also been shown to increase N sparing (8). Furthermore, even with such severe energy restriction, nitrogen equilibrium has been achieved at protein intakes of 1.5 g/kg body wt (9-14) although their prolonged use has been shown to result in large losses of lean body mass in some persons (1). However, we have observed (R Gougeon, and EB Marli$$, unpublished observations, 1992) in healthy obese subjects, that nitrogen equilibrium was achieved within 3 wk of VLCD when the protein source was hydrolyzed collagen supplemented with its limiting amino acids, a protein considered to be of low quality. We questioned whether when an individual is given a protein of 18% essential amino acids versus a high-quality protein of 45% essential amino acids the body's own proteins are mobilized to provide some of the missing essential amino acids and to minimize net protein losses in the short term. Were this the case, if the diet is given for a longer period, the organism might catabolize greater amounts of endogenous protein to yield the required essential amino acids, ultimately inducing negative nitrogen balance.

The nitrogen balance technique does not identify the adaptive mechanisms by which protein is conserved or lost during energy or protein restriction. Tracer techniques that estimate rates of whole body protein flux (Q), synthesis (S), and breakdown (B) have been used to study nitrogen sparing in fasting supplemented with protein and carbohydrate (15) or after acute changes in protein intake (16). In protocols of at most 3 wk, others have found that N equilibrium, Q, S, and B were maintained with a 1.5-g high-quality egg protein/kg ideal body wt (IBW) as the sole source of energy (15, 17, 18) whereas with a mixed diet providing 0.8 g protein/kg IBW and 0.7 g carbohydrate/kg IBW, all values fell significantly (15).

Using the oral stable isotope 15N-glycine and the Picou Taylor-Roberts model to obtain data that reflect the integrated fed and fasting cycle, we investigated the kinetics of protein metabolism in response to 6 wk of VLCD with hydrolyzed collagen supplemented with L-tryptophan and DL-methionine as the protein source.

Methods

Subjects and diet

Seven healthy obese female subjects were admitted to the Clinical Investigation Unit of the Royal Victoria Hospital. Their

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clinical characteristics are shown in Table 1. Each was informed of the nature, purpose, and possible risks of the study and consent was obtained according to a hospital Human Ethics Committee, which had approved the protocol. Their age range was from 24 to 44 y. Clinical and laboratory evaluations showed no evidence of diabetes mellitus, hepatic, cardiovascular, renal or pulmonary dysfunction, or guilt. The subjects did not smoke during the study and were confined to the sedentary life of the hospital ward with no other exercise performed.

Upon admission, all subjects were given a weight-maintaining liquid-formula diet (Ensure) supplemented in some with Poly
cose (both from Ross Laboratories, Montreal) for 7 d of baseline to provide the energy requirement calculated from the resting metabolic rate according to the Harris-Benedict equation, multiplied by 1.5. It provided 10.4–13.3 MJ/d with constant 80 g of protein (12.8 g N). Then each subject received a formula diet with 1.7 MJ (400 kcal) and 16.8 g N/d (Bariatric International Inc, Lachine, Quebec) for 42 d. The protein was partially hydrolyzed collagen, palatably flavored and fortified with L-tryptophan and DL-methionine. The total daily amino acid intake was 93 g/d of which 18.3% were essential amino acids (by analysis by automated ion-exchange chromatography); its amino acid content has been reported previously (8). It met the FAO/WHO/ UNU estimates of amino acid requirements as indicated for adults on weight-maintaining diets (19). This formula was supplemented daily with one tablet of a multivitamin, multimineral preparation (Centrume Forte, Cyanamid Canada Inc, Montreal) and 16 mmol potassium as KCl (Slow K, Ciba Pharmaceuticals, Dorval, Quebec). It was given in three equally divided doses except on the days of $^{15}$N-glycine studies (see below). Water intake was $\geq$ 1.5 L/d. No other beverages were allowed. At day 43, the subjects were refed, as previously described (8, 20), a 3.35 MJ/d (800 kcal/d) diet composed of regular foods with a low carbohydrate content to minimize water retention. Most subjects were discharged with a 4.6 MJ/d (1100-kcal/d) diet prescription.

While in the hospital, vital signs were monitored lying and standing twice daily. Twenty-four-hour urine collections were performed daily in containers with 10 mL concentrated hydrochloric acid as preservative. Weight, water intake, and urine ketones were recorded daily (Chemstrip µg 5000, Boehringer Mannheim, Montreal). Overnight fasted serum electrolytes, calcium, phosphorus, uric acid, liver and kidney function tests, venous blood gases, complete blood counts, and an electrocardiogram were performed weekly.

Procedures and isotope studies

The protein turnover studies were done three times on days 4, 5, and 6 of baseline and days 24, 25, 26 and 39, 40, 41 of VLCD starting at 0800 of the first day and ending at 2000 of the third day. Ninety-nine atom-percent excess $^{15}$N-glycine (MSD isotopes, Merck Frosst Canada, Dorval, Quebec) was given at a rate of 0.5 mg $^{15}$N/kg body wt $^{-1}$ 24 h $^{-1}$ in repeated doses prepared in 5 mL distilled water, every 3 h for 60 h to achieve steady-state isotopic enrichment in urine area. The day’s formula was divided into six equal doses given every 3 h at the same time as the oral $^{15}$N-glycine intake. Urine collections were made at precise 3-h intervals in containers with hydrochloric acid as preservative. They were analyzed for total nitrogen, urea, and ammonia nitrogen. $^{15}$N enrichment of urea nitrogen was measured with a dual-inlet, double-collector isotope-ratio mass spectrometer (Vacuum Generators, Micromass 602D, Winsford, Cheshire, UK). Enrichment was corrected for background values determined on a urine sample taken before the test.

The method requires that within 60 h the abundances of $^{15}$N in urinary urea have reached a plateau. The mean plateau value was calculated from measurements of $^{15}$N abundance in samples (usually 4–6) determined to be at isotopic steady state and before $^{15}$N recycling. The rate of entry of nitrogen (Q) into the metabolic nitrogen pool can be calculated from the mean plateau value, assuming that the fraction of the administered isotope that is excrated as urinary $^{15}$N-urea is the same as the fraction of total amino-nitrogen entering the metabolic pool excrated as urinary nitrogen (21–24). Nitrogen intake (I) is known and total rootary nitrogen (E) was measured. Thus protein synthesis (S) and breakdown (B) were calculated from the Picou-Taylor-Roberts equation: Q = I + B = S + E (25).

Analytical methods

Venous blood samples were drawn with minimal stasis in the overnight-fasted state at the end of the baseline diet and of each week of the VLCD and on days 1 and 3 of each $^{15}$N-glycine study. The samples were divided into tubes containing cold 10% (wt/vol) perchloric acid or containing heparin and one-tenth of the volume of blood as aprotinin (Trasylol, 10 000 Kallikrein inhibitor units/mL; FBA Pharmaceuticals, Pointe Claire, Quebec). These samples were cooled and were centrifuged at 4 $^\circ$C, and aliquots of plasma were stored at $-20$ $^\circ$C. Perchloric acid supernatants were assayed for 3-hydroxybutyrate and acetocetate by enzymic microfluorometric methods (26); heparin and aprotinin-containing plasma was assayed for insulin by single-antibody, charcoal precipitation radioimmunoassay with use of human standards and labeled hormone from Novo Research Laboratory (Copenhagen) by a method previously described (27). Plasma values were corrected for dilution by aprotinin, based on concurrently measured hematocrit. Blood samples were added to oxalate-fluoride tubes for glucose and urea-nitrogen measurements and to tubes without antioxidant for creatinine and electrolyte determinations by standard automated techniques in the hospital clinical laboratory. After thorough mixing, aliquots of the 24-h urine collections were analyzed daily for urea, creatinine, and electrolytes (sodium, potassium, chloride) and frozen at $-20$ $^\circ$C until assayed for nitrogen components.

Daily urinary urea nitrogen and creatinine were obtained by the autoanalyzer method and daily ammonium nitrogen was obtained by using a specific ion electrode (Orion Research Inc, Cambridge, MA). Total urinary nitrogen was measured with an automated microKjeldahl technique by using a single-channel
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TABLE 2
Electrolyte, metabolic, and acid-base responses to 6 wk of VLCD, by week*

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum sodium (mmol/L)</td>
<td>140.9 ± 0.8</td>
<td>140.0 ± 0.8</td>
<td>139.9 ± 0.7</td>
<td>141.7 ± 1.2</td>
<td>140.8 ± 0.8</td>
<td>142.3 ± 0.9</td>
<td>141.6 ± 0.9</td>
</tr>
<tr>
<td>Serum chloride (mmol/L)</td>
<td>104.4 ± 0.48</td>
<td>105.4 ± 1.0</td>
<td>105.4 ± 1.0</td>
<td>106.9 ± 2.0</td>
<td>105.7 ± 1.0</td>
<td>107.2 ± 1.5</td>
<td>106.0 ± 1.1</td>
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<tr>
<td>Serum potassium (mmol/L)</td>
<td>4.13 ± 0.11</td>
<td>3.9 ± 0.05</td>
<td>3.90 ± 0.04</td>
<td>3.79 ± 0.06</td>
<td>3.72 ± 0.05</td>
<td>3.88 ± 0.04</td>
<td>3.80 ± 0.05</td>
</tr>
<tr>
<td>Serum urea (mmol/L)</td>
<td>4.33 ± 0.28</td>
<td>5.92 ± 0.42</td>
<td>5.48 ± 0.24</td>
<td>5.27 ± 0.24</td>
<td>5.90 ± 0.20</td>
<td>5.65 ± 0.26</td>
<td>5.60 ± 0.43</td>
</tr>
<tr>
<td>Serum uric acid (mmol/L)</td>
<td>289 ± 16</td>
<td>352 ± 39</td>
<td>357 ± 27</td>
<td>307 ± 29</td>
<td>284 ± 26</td>
<td>293 ± 28</td>
<td>248 ± 28</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)‡</td>
<td>4.75 ± 0.14</td>
<td>4.48 ± 0.27</td>
<td>3.96 ± 0.31</td>
<td>3.68 ± 0.33</td>
<td>3.72 ± 0.30</td>
<td>3.84 ± 0.34</td>
<td>4.00 ± 0.35</td>
</tr>
<tr>
<td>Plasma triglyceride (mmol/L)</td>
<td>1.43 ± 0.30</td>
<td>0.89 ± 0.11</td>
<td>0.91 ± 0.12</td>
<td>0.86 ± 0.13</td>
<td>0.92 ± 0.11</td>
<td>0.81 ± 0.09</td>
<td>0.99 ± 0.12</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>5.9 ± 0.5</td>
<td>4.8 ± 0.3</td>
<td>4.4 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>4.4 ± 0.2</td>
<td>4.6 ± 0.3</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>Plasma insulin (pmol/L)</td>
<td>171 ± 29</td>
<td>81 ± 11</td>
<td>78 ± 12</td>
<td>61 ± 2</td>
<td>78 ± 11</td>
<td>76 ± 7</td>
<td>100 ± 13</td>
</tr>
<tr>
<td>Blood 3-OHB (μmol/L)§</td>
<td>13 ± 7</td>
<td>657 ± 102</td>
<td>943 ± 228</td>
<td>980 ± 78</td>
<td>968 ± 174</td>
<td>1115 ± 321</td>
<td>856 ± 86</td>
</tr>
<tr>
<td>Venous blood pH</td>
<td>7.35 ± 0.01</td>
<td>7.35 ± 0.005</td>
<td>7.34 ± 0.01</td>
<td>7.34 ± 0.01</td>
<td>7.37 ± 0.01</td>
<td>7.34 ± 0.07</td>
<td>7.35 ± 0.01</td>
</tr>
<tr>
<td>Venous blood HCO₃⁻ (mmol/L)</td>
<td>27.4 ± 0.6</td>
<td>25.5 ± 0.4</td>
<td>25.3 ± 0.9</td>
<td>25.3 ± 0.6</td>
<td>25.5 ± 0.7</td>
<td>24.8 ± 0.7</td>
<td>24.6 ± 1.2</td>
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*SEM; n = 7.
† Significantly different from baseline, P < 0.05.
‡ Serum total cholesterol is presented.
§ 3-hydroxybutyrate.

Autoanalyzer (Technicon, Tarrytown, NY) (28). All determinations were performed in duplicate or triplicate. The computations of the daily nitrogen balance were based on the measured intake minus the measured urinary total nitrogen and estimated fecal (0.4 g) (29) and skin, hair, and secretion (5 mg/kg body wt) losses (30).

Statistical methods

The data were analyzed by analysis of variance (ANOVA) using a repeated-measures design. Calculations were made with the Primer Biostatistics (McGraw Hill Inc, Montreal) on a Hewlett-Packard Vectra computer (Sunnyvale, CA). Significant differences (P < 0.05) were identified by the Newman-Keuls multiple range test. Linear-regression analysis was used to calculate correlation coefficients. Data in figures and tables are presented as mean ± SE.

Results

Weight was maintained during the baseline diet. The weight loss at the end of day 42 of VLCD was 12.8 ± 0.9 kg. There were no untoward clinical responses during the baseline diet or VLCD. Resting electrocardiograms and liver-function studies showed no alteration. There was no significant change in serum alkaline phosphatase, creatinine, total protein or albumin, hemoglobin, or hematocrit (data not shown). White blood counts, neutrophils, and lymphocytes decreased progressively during the VLCD (31). The metabolic responses are shown in Table 2 and Figures 1 and 2. Serum sodium and chloride showed no significant changes. Serum urea increased at week 1 and remained significantly greater than baseline throughout. There was a significant decline in serum potassium at weeks 3 and 6 but the values remained in the normal range. Mean serum uric acid increased significantly at weeks 2 and 3 then decreased to values lower than baseline at week 6. Serum total cholesterol declined (P < 0.05) by week 2 of the VLCD and remained lower than baseline throughout. Serum triglycerides decreased 40% by week 1, then remained constant. Postabsorptive plasma glucose decreased significantly from baseline from day 7 onward, and
plasma insulin followed the same pattern. Blood 3-hydroxybutyrate increased markedly from baseline to steady-state concentrations from 2 to 6 weeks of VLCD. There were no changes in blood pH, and the small decrease in blood bicarbonate with the diet was not statistically significant.

**Nitrogen balance**

During the VLCD, mean daily nitrogen balance became significantly negative with lowest values on days 3–6. It returned progressively to values not different from 0 by day 21 (Fig 1) and remaining in equilibrium until day 36, after which it became negative again. There was a corresponding marked increase in urinary urea nitrogen excretion during the first days of the VLCD to 18.1 ± 0.9 g/d (646 ± 35 mmol/d) followed by a progressive decline to near steady-state values of 14.6 ± 0.5 g/d (522 ± 20 mmol/d) and a trend toward greater excretion by day 42 (16.4 ± 0.5 g/d, 586 ± 20 mmol/d).

Urine creatinine excretion did not vary from baseline with VLCD and the ratio of urea nitrogen to creatinine presented the same pattern as the nitrogen balance (not shown). The mean cumulative nitrogen loss totaled 721 ± 11 g at day 42. Urine ammonium nitrogen increased from low values at baseline [0.21 ± 0.02 g/d (14.7 ± 1.4 mmol/d)] by fourfold to 0.80 ± 0.07 g/d (57.1 ± 4.9 mmol/d) by day 4 of VLCD and remained at that level thereafter.

**Kinetics of protein metabolism**

With the repeated oral doses of 15N-glycine, enrichment of urinary urea nitrogen reached a plateau in most subjects between 36 and 45 h. The results calculated from the mean plateau value are shown in Figures 2 A, B, and C. Rates of nitrogen flux (Q) did not change from baseline as an effect of VLCD (Fig 2, A). However, the mean rates of protein synthesis (S) and breakdown (B) both decreased from baseline (P < 0.05) (Fig 2, B), with the decrease in S greater than that in B by 4 wk. This resulted in a significantly lower net protein synthesis (S − B) at week 4 than at baseline, which decreased further to become negative at week 6 (Fig 2, C). No significant linear correlations were found between parameters body weight and nitrogen flux, weight B, and plasma glucose and nitrogen flux, at baseline and at weeks 4 or 6 of VLCD.

**Discussion**

This study compared the kinetics of protein metabolism in obese subjects during 6 wk of VLCD with 18% essential amino acid content to those of a prior isocaloric diet with higher quality protein. A VLCD has been shown to achieve nitrogen sparing by weeks 3–4 of treatment when the protein source was collagen hydrolyzate. This might imply that nitrogen conservation in this specific setting is not strongly influenced by protein quality (14). However, interindividual variability in nitrogen loss makes it difficult to assess protein quality and/or prolongation of diet as factors modifying nitrogen balance, a common limitation in the interpretation of nitrogen balance data.

We have also observed no difference in mean nitrogen balance between two groups of obese subjects given VLCDs of different protein quality for 4 wk (data not reported). Nitrogen equilibrium was achieved in both at weeks 3–4. In the current study, with collagen-based protein, though nitrogen equilibrium was also reached in all subjects by week 4 it became negative by week 6. The principal component responsible for this increase was urea nitrogen excretion, excluding a role for the chronic metabolic acidosis causing loss via augmented ammoniagenesis. The 15N-glycine results showed that whole-body nitrogen flux was maintained both at weeks 4 and 6 of diet. Furthermore, the time required to reach a plateau level of urinary 15N-urea enrichment was not different at weeks 4 and 6 than at baseline; it would
have been delayed as has been suggested (16) if the turnover of the urea pool had been reduced as an effect of diet. However, with VLCD, there was a decrease in S and B. The decrease in S was surprising because exogenous protein was given in amounts comparable to those reported to have maintained S when energy was restricted. It may thus be explained by the difference in the quality of the protein given in our study. S has been shown to fall during a brief total fast (18) or a low protein intake (15), indicating that when energy is restricted protein has to be provided for synthesis in amounts that appear to be greater than those recommended when sufficient energy is available (4, 17, 21) or when the essential amino acid content is low and may become insufficient when energy is restricted as is indicated in our study. Sufficient energy with restricted protein has been associated with a slight decrease in nitrogen flux and increases in S and B, suggesting a more efficient use of exogenous protein for anabolic purposes (16) in this setting. Nitrogen flux was shown to decrease when energy and protein were reduced (33). In our study, maintenance of nitrogen flux with prolongation of diet was at the cost of greater B than S and a decrease in net protein synthesis (S − B).

An increase in B at week 6 compared with week 4 sufficient to induce a significant (P < 0.05) decrease in S − B between weeks 4 and 6 probably reflects a need to access endogenous protein sources to counterbalance the missing amino acids of a protein with lower essential amino acid content and allow the same rate of protein synthesis at week 6. The net protein loss at week 6 was reflected in the negative nitrogen balance at that time.

Prolongation of the diet to week 6 thus had measurable deleterious effects on the kinetics of whole-body protein metabolism and indicates that the use of such diets should perhaps be limited to short periods of time, if given under the conditions and using the type of protein source studied. It is worth noting, however, that 1) the total negative nitrogen balance over the 6 wk was small in relation to total body protein stores, 2) the magnitude of the negative nitrogen balance even at 6 wk was only ~2 g/d and appeared relatively constant for the last 4 d, and 3) the net negative protein synthesis (S − B) also represented a rather small 1 g N/d. Furthermore, the loss of such a substantial amount of body weight as that observed would be expected to be associated with an obligate decrease in total body protein by virtue of the loss both from adipose tissue and from muscle and other protein-rich tissues merely from the standpoint of lesser requirements for maintenance at the lower weight (34).

The mechanism(s) of the changes observed in protein metabolism are not explained on the basis of other data obtained. As noted, the acidosis was mild and did not increase after 4 wk to mandate protein loss via renal ammoniagenesis (that also remained constant). No further fall in plasma insulin was observed; indeed, there was even a slight rise from week 5 to 6, and a substantial rise between the nadir at 3 wk to the values at 6 wk. We thus attribute these kinetic changes to the state of endogenous protein available versus that supplied from the diet, and once again postulate a major influence of exogenous protein supply itself upon protein metabolism in vivo (25, 35).

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