Response of hepatic proteins to the lowering of habitual dietary protein to the recommended safe level of intake

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Afolabi, Paul R., Farook Jahoor, Neil R. Gibson, and Alan A. Jackson. 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; 10.1152/ajpendo.00036.2004.—The plasma concentrations of albumin, HDL apolipoprotein A1 (apoA1), retinol-binding protein (RBP), transthyretin (TTR), haptoglobin, and fibrinogen were measured, and a stable isotope infusion protocol was used to determine the fractional and absolute synthesis rates of RBP, TTR, and fibrinogen in 12 young adults on three occasions during a reduction of their habitual protein intake from 1.13 to 0.75 g·kg−1·day−1 for 10 days. This study was performed to determine whether healthy adults could maintain the rates of synthesis of selected nutrient transport and positive acute-phase proteins when consuming a protein intake of 0.75 g·kg−1·day−1. During the lower protein intake, the plasma concentration of all the proteins, other than HDL-apoA1, remained unchanged. HDL-apoA1 concentration was significantly reduced (P < 0.05) after 3 days of the lower protein intake, but not at 10 days. The rates of synthesis of RBP and TTR declined significantly (P < 0.05), whereas the rate of synthesis of fibrinogen remained unchanged. The results indicate that, when normal adults consume the recommended safe level of protein, 0.75 g·kg−1·day−1, there is a slower rate of turnover of nutrient transport proteins than on their habitual diet. Hence, healthy individuals consuming this amount of protein may be less able to mount an adequate metabolic response to a stressful stimulus.

THE AVERAGE MINIMUM REQUIREMENT for dietary protein in normal adults is estimated as 0.6 g·kg−1·day−1, which will maintain body weight and nitrogen balance. The recommended safe level of intake was set at 0.75 g·kg−1·day−1 by the Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) to take into account the individual variability within a population (3). However, there is considerable ongoing debate about the adequacy of this recommended protein intake (11, 18). Because habitual protein intake is usually higher, it has been suggested that, although nitrogen balance may be achieved on these lower levels of protein intake, it may be at the expense of metabolic functional capacity (1, 16). We provided such evidence in a previous study of healthy adults consuming a protein intake of 0.6 g·kg−1·day−1 for 7 days (6). There were reductions in whole body protein turnover and the synthesis rates of several nutrient transport proteins, whereas there were simultaneous increases in the synthetic rates of some positive acute-phase proteins, indicative of a low-grade stress response (6). This finding suggested that, although a protein intake of 0.6 g·kg−1·day−1 may be sufficient to maintain nitrogen balance in a healthy adult, it is not sufficient to maintain the synthesis rates of proteins involved in the transportation of nutrients around the body.

More recently, we have shown that the recommended protein intake of 0.75 g·kg−1·day−1 can maintain nitrogen balance in normal adults. This was achieved, however, by a downregulation of whole body protein turnover, suggesting that even this so-called “safe level of protein intake” may not be adequate to maintain the synthesis of rapid-turnover proteins such as the hepatic secretory proteins (4). Thus we hypothesized that a protein intake of 0.75 g·kg−1·day−1 will not be sufficient to maintain adequate rates of synthesis of rapid-turnover nutrient transport proteins as part of the adaptive metabolic response from a habitual intake of ~1 g·kg−1·day−1. This hypothesis was tested by measuring the response of six hepatic secretory proteins: four proteins that are directly involved in nutrient transport and that respond negatively during an acute-phase response, and two proteins that respond positively during an acute-phase response to stress. A stable isotope tracer method was used to measure the synthesis rates of three of these proteins in 12 young adults whose dietary protein intake was reduced from their habitual intake of ~1.0 g·kg−1·day−1 to an intake of 0.75 g·kg−1·day−1. This study is part of a larger study designed to assess the responses of whole body protein kinetics, positive and negative acute-phase proteins, and cellular antioxidant capacity (5).

METHODS

The study protocol was approved by the joint ethics committees of Southampton University Hospital National Health Service Trust and South West Hampshire Health Authority. Signed informed consent was obtained from each subject after the nature of the study had been explained. The studies were carried out in the metabolic ward of the Institute of Human Nutrition at Southampton General Hospital.

Subjects. Twelve young adults (6 men and 6 women) were recruited for the study. They were in good health on the basis of a complete medical history and physical examination. Before the dietary intervention, the height, weight, and body composition (by bioelectrical impedance) of each subject were measured. The physical characteristics of the subjects are shown in Table 1.

Study design. Subjects underwent a 7-h stable isotope infusion while in the fasted state on three occasions: at baseline while consuming their habitual diet and on days 3 and 10 while consuming a diet which provided 0.75 g·kg−1·day−1 of protein. The habitual...
Table 1. Physical characteristics and intakes of energy and protein of all subjects

<table>
<thead>
<tr>
<th>Subjects (n = 12)</th>
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<tbody>
<tr>
<td>Sex</td>
<td>6M, 6F</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>25.6 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Height, m</td>
<td>1.72 ± 0.02</td>
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</tr>
<tr>
<td>Weight, kg</td>
<td>70.3 ± 3.6</td>
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</tr>
<tr>
<td>Lean body mass, kg</td>
<td>56.0 ± 5.7</td>
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<tr>
<td>Fat mass, kg</td>
<td>14.2 ± 1.6</td>
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### Habitual diet

<table>
<thead>
<tr>
<th>Energy intake, kJ·kg⁻¹·day⁻¹</th>
<th>154 ± 4.7</th>
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<tbody>
<tr>
<td>Protein intake, g·kg⁻¹·day⁻¹</td>
<td>1.13 ± 0.04</td>
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</table>

### Experimental diet

<table>
<thead>
<tr>
<th>Energy intake, kJ·kg⁻¹·day⁻¹</th>
<th>154.7 ± 4.7</th>
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</thead>
<tbody>
<tr>
<td>Protein intake, g·kg⁻¹·day⁻¹</td>
<td>0.75 ± 0.00*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from habitual protein intake, P < 0.05.

Protein intake of each subject was determined from 5-day weighed-diet records. For 2 days before the first (baseline) isotope infusion, the subjects consumed a diet based on their habitual intakes of protein and energy. This was followed by 10 days of a diet that provided the same amount of energy but a lower protein intake of 0.75 g·kg⁻¹·day⁻¹. Throughout the intervention, diets were supplied as ready-made meals and food portions (Marks and Spencer, Southampton, UK). The energy contents of both diets were similar, and the amount of food offered to the subjects was assessed on the basis of their resting energy expenditure measured by indirect calorimetry (Gas Exchange Measurement System; Europa Scientific, Crewe, UK) multiplied by a factor of 1.5 to account for physical activity. The dietary energy and protein intakes of the subjects are summarized in Table 1.

Isotope infusion protocol. The rates of synthesis of three hepatic secretory proteins, retinol-binding protein (RBP), transthyretin (TTR), and fibrinogen, were measured from the rates of incorporation of [13C₂]glycine into the proteins by use of plasma very low density lipoprotein apolipoprotein B-100 (VLDL-apoB-100) isotopic enrichment at plateau to represent that of the glycine precursor pool from which the liver synthesized the other plasma proteins.

A sterile solution of [13C₂]glycine (Cambridge Isotope Laboratories, Woburn, MA) was prepared in 9 g/l NaCl by the pharmacy at Southampton General Hospital and confirmed to be sterile and pyrogen free before use. After a 12-h overnight fast, the subjects arrived at the metabolic unit of the Southampton General Hospital and underwent a 7-h isotope infusion protocol. Intravenous catheters were inserted into superficial veins of both arms, one for continuous infusion of the tracer solution and the other for repeated blood sampling. A sample of blood (10 ml) was drawn before the start of the infusion. A priming dose of [13C₂]glycine (20 μmol/kg) was then injected and was immediately followed by a continuous infusion of [13C₂]glycine (15 μmol·kg⁻¹·h⁻¹) for 7 h. Additional 5-ml blood samples were taken hourly from 3 to 7 h.

Sample analyses. Blood was drawn into prechilled tubes containing Na₂EDTA and a cocktail of sodium azide, merthiolate, and soybean trypsin inhibitor. The blood was immediately centrifuged at 4°C, and the plasma was removed and stored at −70°C for later analysis.

Plasma concentrations of albumin, fibrinogen, haptoglobin, HDL-apoA1, RBP, and TTR were measured by radial immunodiffusion using NL RID kits (The Binding Site, San Diego, CA). VLDL was separated by ultracentrifugation and apoB-100 precipitated with isopropyl alcohol, as previously described (6, 7). Fibrinogen was extracted as fibrin clots by thrombin precipitation (7).

RBP and TTR were isolated from plasma by sequential immunoprecipitation with anti-human RBP and TTR (Behring, Somerville, NJ), as previously described (14). The immunoprecipitates were separated by SDS-gel electrophoresis. After staining with Coomassie brilliant blue dye, the bands corresponding to the protein standards were cut out and washed several times. The gel bands, apoB-100 precipitates, and fibrin clots 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 (AG50W-X8 resin, 100–200 mesh, hydrogen form; Bio-Rad Laboratories, Hercules, CA).

RBP, TTR, and apoB-100-derived amino acids were converted to the N-propyl ester heptfluorobutyramide derivatives, and the isotopic enrichment of glycine was determined by negative chemical ionization gas chromatography-mass spectrometry on an HP 6890 gas chromatograph coupled to an HP 5973 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 amino acids released from the fibrin clot were converted to the N-acetyl, N-propyl ester derivatives, and the isotopic enrichment of glycine was determined by gas chromatography-combustion isotope ratio mass spectrometry (ThermoQuest Finnigan DELTAplus XL) by monitoring ions at mass-to-charge ratios of 44 and 45.

Calculations and statistics. The fractional synthesis rates (FSR) of RBP, TTR and fibrinogen were calculated, assuming a precursor-product relationship, using the equation:

$$\text{FSR} (\%\text{day}^{-1}) = \left[\frac{(E_t - E_5)}{E_5}\right] \times 2,400/t_5$$

where \(E_t - E_5\) is the increase in the isotopic enrichment of glycine bound in RBP, TTR, and fibrinogen from 5 to 7 h (\(t_7 - t_5\)) of the infusion, and \(E_5\) is the isotopic enrichment of glycine bound in VLDL-apoB-100 in plasma at plateau levels of enrichment. In this case, the plasma enrichment of VLDL-apoB-100-bound glycine in plasma is assumed to represent the isotopic enrichment of the intraplatelet glycine pool from which all hepatic secretory proteins are synthesized.

The intravascular absolute synthesis rate (ASR) for each protein was estimated as the product of the FSR and the intravascular mass of the protein: intravascular ASR (mg·kg⁻¹·day⁻¹) = intravascular protein mass × FSR, 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 calculated on the basis of the ideal body weights of the subjects (10) by use of the equation: plasma volume (liters) = (ideal weight × 0.045) + (excess weight × 0.01), where excess weight is the difference between the actual weight and the ideal weight of the subject.

The data are expressed as means ± SE for each group. Differences between baseline and days 3 and 10 of the low protein intake were assessed by ANOVA. A probability of 5% (P < 0.05) was taken to represent statistical significance.

RESULTS

All the subjects were within the normal range of ideal body weight and body mass index (Table 1). The subjects had a mean habitual consumption for energy of 155 ± 5 kJ·kg⁻¹·day⁻¹ and for protein of 1.13 g·kg⁻¹·day⁻¹.

The plasma concentrations of three of the four negative acute-phase proteins, albumin, RBP, and TTR, did not change from baseline after the lower-protein diet had been consumed for 3 or 10 days (Table 2 and Fig. 1). The concentration of HDL-apoA1, however, was significantly lower (P < 0.05) on day 3 but by day 10 had recovered to a value that was not different from baseline. Compared with rates at baseline, both the FSR and ASR of TTR were significantly slower after 3 and 10 days of the dietary intervention (Fig. 1). Similarly, both the FSR and ASR of RBP were significantly slower after 10 days of the dietary intervention.

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of synthesis and degradation of RBP and TTR are similar to the responses in whole body protein kinetics for these same subjects while they consumed 0.75 g protein·kg⁻¹·day⁻¹ (4).

It is important to note that the changes in the plasma concentration of HDL-apoA1 appeared transient, with a reduced concentration at 3 days, which was not different from the baseline concentration after 10 days of consumption of the lower-protein diet. There are two points of interest. First, there is a clear indication of a time course in the response. This was identified because the study at day 3 coincided with a lower plasma concentration. Without daily measurements of the concentration of other plasma proteins, we cannot know whether similar patterns of change occurred but with a different time course so that they were not identified in the present study.

Second, the observation that the plasma concentration of HDL-apoA1 returned to the baseline value after 10 days on the diet implies that this was the preferred concentration and that the value on day 3 was lowered as a part of the adaptive response. The lower plasma concentration of HDL-apoA1 during the early phase of the dietary intervention may indicate that lipid and cholesterol transport was modified during adaptation to the lower protein intake. For RBP and TTR, a similar plasma concentration was maintained throughout the study period, suggesting that this is the preferred state. It needs to be determined whether a change in synthesis and turnover of these two proteins exerts any effect on the delivery of nutrients to tissues and thereby carries functional consequence for cells or tissues.

There were no changes in the plasma concentrations of fibrinogen or haptoglobin in response to the dietary intervention (Table 1) nor in the FSR or ASR of fibrinogen (Fig. 1).

### DISCUSSION

This study was performed to determine the changes in amino acid and protein metabolism when normal adult humans consume the safe level of dietary protein, 0.75 g·kg⁻¹·day⁻¹, recommended by FAO/WHO/UNU (3). Previously, we have reported that nitrogen balance is reestablished after this lower protein diet is consumed for 3–4 days (4). Here, we were interested to determine the extent to which it was possible to maintain the plasma concentrations and the rate of synthesis of hepatic secretory proteins. The data show that the consumption of 0.75 g protein·kg⁻¹·day⁻¹ led to changes in both the plasma concentration and rates of synthesis of some proteins. These effects were, however, selective and different for different secretory proteins. For three of the four nutrient transport proteins, albumin, TTR, and RBP, the plasma concentrations were not different after 3 or 10 days of consumption of the lower-protein diet. However, there was a significant fall in the rate of synthesis, by day 3 for TTR and by day 10 for RBP. The plasma concentration of HDL-apoA1 was lower by day 3. However, by day 10 of the dietary intervention, the plasma concentration of HDL-apoA1 was not different from the baseline value. There were no identifiable changes in the plasma concentrations of haptoglobin and fibrinogen, and the synthesis rate of fibrinogen appeared unchanged. These results suggest that, despite the maintenance of the plasma concentration or pool size of most hepatic secretory proteins during adaptation to this lower intake of dietary protein, there may be important, but less obvious, changes in their rates of synthesis.

The pool size of a protein is a reflection of the balance between its rate of synthesis and catabolism and its partitioning between the intravascular and extravascular compartments. It has long been known that, on lower-protein diets, there is a tendency for the intravascular albumin pool to be maintained at the cost of the extravascular pool. Less clear is the extent to which this may apply to other plasma proteins. To maintain the plasma pools of RBP and TTR during consumption of 0.75 g·kg⁻¹·day⁻¹ of protein over a period of 10 days, any reduction in their rates of synthesis would have to be matched by equal reductions in their rates of catabolism. Our data suggest that the plasma pool sizes of RBP and TTR were maintained due to the downregulation of the rates of both synthesis and catabolism as an intrinsic part of the adaptative response to the lower protein intake. The reduction in the rates of synthesis and degradation of RBP and TTR are similar to the responses in whole body protein kinetics for these same subjects while they consumed 0.75 g protein·kg⁻¹·day⁻¹ (4).

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### Table 2. Mean plasma concentration of plasma proteins in 12 normal adults at baseline and on days 3 and 10 of a protein intake of 0.75 g·kg⁻¹·day⁻¹

<table>
<thead>
<tr>
<th>Plasma Protein</th>
<th>Baseline (g/l)</th>
<th>Day 3 (g/l)</th>
<th>Day 10 (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>35.4±3.6</td>
<td>32.5±2.9</td>
<td>32.0±2.7</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2.29±0.152</td>
<td>2.23±0.132</td>
<td>2.34±0.239</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>0.79±0.12</td>
<td>0.8±0.122</td>
<td>0.76±0.11</td>
</tr>
<tr>
<td>HDL-apoA1</td>
<td>1.56±0.13</td>
<td>1.32±0.096*</td>
<td>1.46±0.053</td>
</tr>
<tr>
<td>Retinol-binding protein</td>
<td>0.02±0.003</td>
<td>0.02±0.003</td>
<td>0.02±0.002</td>
</tr>
<tr>
<td>Transthyretin</td>
<td>0.22±0.01</td>
<td>0.21±0.01</td>
<td>0.23±0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE in g/l. apoA1, apolipoprotein A1. *P < 0.05 vs. baseline.

Fig. 1. Twelve normal adults received a primed continuous intravenous infusion of [13C2]glycine for 7 h at baseline (open bars) and after day 3 (hatched bars) and day 10 (filled bars) of consuming a diet that provided a low but adequate level of protein to determine the fractional (FSR) and absolute synthesis rates (ASR) and concentrations of plasma transthyretin (A), retinol-binding protein (B), and fibrinogen (C). Values are means ± SE. Significant differences were assessed by ANOVA. P < 0.05 was taken to represent statistical significance.
The combination of the present results with those of earlier reports shows that, when healthy individuals decrease their protein consumption from habitual, \( -1.1 \text{ g kg}^{-1} \text{day}^{-1} \) down to 0.75 \( \text{g kg}^{-1} \text{day}^{-1} \), there is a series of metabolic responses. These responses include the downregulation of whole body protein turnover (4), a decrease in the rate of glutathione synthesis and cellular antioxidant protection (5), and a downregulation in the synthesis of individual nutrient transport proteins. These changes indicate a selective sacrifice of function and imply a limitation on the ability of healthy individuals to maintain important metabolic functions. The question that remains unanswered is whether this state is less than optimal and whether, for example, the reduction in the rate of synthesis of specific nutrient transport proteins in any way compromises tissue function. Although this loss of capacity may not always express itself as a change in metabolic behavior, the concern would be that there has been a loss of reserve capacity. The cost of this loss of reserve capacity would be expected to manifest itself in situations where the individual is challenged by a stressful situation or stimulus. These findings would, therefore, support the arguments put forward by Young et al. (19) that a diet that provides 0.75 \( \text{g protein} \text{kg}^{-1} \text{day}^{-1} \) in normal adults may not be adequate to mount and maintain an acceptable level in otherwise healthy individuals.

In a previous study, Jackson et al. (6) reported faster rates of synthesis of two positive acute-phase proteins in healthy subjects who had consumed a diet that provided 0.6 \( \text{g protein} \text{kg}^{-1} \text{day}^{-1} \) for 7 days. This response was associated with higher plasma concentrations of IL-6, suggesting that consumption of the average minimum requirement for protein intake had elicited an “acute-phase response” in these otherwise healthy subjects. In the present study, there was no change in the concentration of haptoglobin and fibrinogen or in the synthesis of fibrinogen. This suggested that the response of haptoglobin and fibrinogen had not been elicited at a higher protein intake that provided an increase of 0.15 \( \text{g protein} \text{kg}^{-1} \text{day}^{-1} \). These findings suggest that, although a dietary protein intake of 0.75 \( \text{g kg}^{-1} \text{day}^{-1} \) may not be sufficient to maintain synthesis of all nutrient transport proteins in healthy adults, it is sufficient to prevent a stress-like acute-phase response.

In conclusion, our present study shows that, for normal adults consuming the safe level of protein intake, 0.75 \( \text{g kg}^{-1} \text{day}^{-1} \), as recommended by FAO/WHO/UNU (3), adaptive metabolic responses are brought into play. It was not possible to maintain the rate of synthesis of important nutrient transport proteins. As a result, the metabolic capacity to cope with stress may be impaired in healthy adults consuming this amount of protein. There is a need for additional studies to be conducted to determine cellular and tissue effects of these changes in nutrient transport proteins and the minimum level of protein intake that will maintain the metabolic capacity at an acceptable level in otherwise healthy individuals.

ACKNOWLEDGMENTS

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GRANTS

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REFERENCES