

Albumin Synthesis Is Diminished in Men Consuming a Predominantly Vegetarian Diet¹

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ABSTRACT Albumin synthesis was calculated in healthy male volunteers consuming diets differing in the relative contribution of protein from animal or vegetable sources. In one study (Study 1, $n = 4$) two isoenergetic and isonitrogenous diets were consumed for a period of 10 d each. One diet (diet A) was animal protein rich (74%), the other one (diet V) contained 67% of vegetable protein. Albumin synthesis rate was measured from L-[²H₅]phenylalanine incorporation (43 mg/kg) at the end of each dietary period. Both albumin fractional synthesis rate (FSR) (5.7 ± 0.6 vs. $6.7 \pm 0.8\%/d$, $P = 0.04$) and absolute synthesis rate (ASR) (123 ± 6 vs. $143 \pm 8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, $P = 0.05$) were reduced after diet V. In a second study (Study 2, $n = 8$) a third dietary treatment was added (Diet VS). This was similar to diet V but supplemented with soy protein (18g/d). The results of study 2 confirmed that albumin synthesis was reduced after diet V (FSR: 5.9 ± 0.3 vs. $6.7 \pm 0.5\%/d$, $P = 0.015$; ASR: 126 ± 7 vs. $146 \pm 9 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, $P = 0.007$), but it also showed that the drop could be prevented by adding supplemental protein to the predominantly vegetarian diet (Diet VS) (FSR: $6.4 \pm 0.3\%/d$, $P = 0.08$; ASR: $140 \pm 7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, $P = 0.03$). Albumin synthesis appears to be modulated by changes in the proportion of animal vs. vegetable protein occurring in the diet. The mechanism might be related to differences in digestibility and consequently in net amino acid availability between diets. *J. Nutr.* 130: 528–533, 2000.

KEY WORDS: • albumin • dietary protein • protein synthesis • L-[²H₅]phenylalanine • humans

Several epidemiological studies indicate that a greater consumption of fruit, vegetables and cereals in the diet is associated with favorable effects on health. The Mediterranean diet, which has been recommended as an example of a healthy diet, being associated with a reduced risk of several diseases such as coronary heart disease and cancer (Keys et al. 1984, Tavani and La Vecchia 1995), is mainly plant-based (Ferro-Luzzi and Branca 1995). However, in many Western societies most of the protein in the diet is supplied by animal-derived foods (Young and Pellett 1994), and a greater contribution of plant food in the diet has been recommended (Committee on Diet and Health et al. 1989, World Health Organization 1990). It is therefore important to consider the role of diets richer in vegetable protein and to study their nutritional and metabolic effects.

The measurement of albumin synthesis rates with stable isotopically labeled amino acids is an important and sensitive tool for the study of the visceral protein response to nutrition and other physiological and/or pathological stimuli. A number of studies, both in animals and humans, have shown that nutrient intake can modulate albumin synthesis and that the protein component of the diet has an important role in regu-

lating albumin (Gersovitz et al. 1980, Hoffenberg et al. 1966, James and Hay 1968, Kelman et al. 1972a, Kirsch et al. 1968, Morgan and Peters 1971, Pain et al. 1978). However, since previous studies have mainly investigated the effects of the level of protein in the diet, it is not known whether protein from different sources might have a differential effect on albumin synthesis.

The aim of this study was to investigate whether the origin of dietary protein from animal vs. vegetable sources can affect visceral protein metabolism, and in particular albumin synthesis, in healthy volunteers. Albumin synthesis was measured using the flooding method with [²H₅]phenylalanine, as originally described by Ballmer et al. (1990) and then modified by Hunter et al. (1995).

METHODS

Experimental design

Healthy free-living male volunteers, nonsmokers and with no known metabolic diseases, took part in two separate studies. Both protocols were approved by the Ethical Committee of the University of Naples (Italy), and each volunteer gave informed written consent before participating in the study.

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TABLE 1

Diet composition

	Diet A	Diet V	Diet VS
Energy, MJ	10.42	10.46	10.51
Total protein, g	78 (12.5) ¹	78 (12)	96 (15)
Animal protein	58	26	26
Vegetable protein	20	52	70
Carbohydrates, g	382 (58)	401 (60)	386 (58)
Fat, g	82 (29.5)	78 (28)	75 (27)
Cholesterol, mg	244	162	162
Fiber, g	23	37	37

¹ Numbers in parentheses indicate % of energy. Diet A, predominantly animal diet; Diet V, predominantly vegetarian diet; Diet VS, predominantly vegetarian diet supplemented with soy protein.

Study 1. Four volunteers (age = 30 ± 2 y, weight = 76.7 ± 0.7 kg, height = 1.76 ± 0.01 m, body mass index (BMI)³ = 24.7 ± 0.5 kg/m²) participated in this study, which consisted of two experimental periods of 10 d each, in which two different diets (A and V) were provided in random order. The two diets were designed to be isocaloric and isonitrogenous and similar in macronutrient composition, which was representative of a typical Mediterranean diet (Table 1). The main difference between the diets was the source of dietary protein: diet A contained 74% animal and 26% vegetable protein, whereas diet V contained 33% animal and 67% vegetable protein. In diet A, protein was mainly derived from meat and fish (55%) and dairy products (20%), with a smaller contribution from cereals (7.3%) and legumes (3.6%). In diet V, cereals represented the main protein source (35%), followed by meat and fish (27%), legumes (22%) and dairy products (7.8%). Due to the higher amount of fiber in vegetables, the fiber content of the two diets was not equal (23 g/d in diet A vs. 37 g/d in diet V) (Table 1).

Study 2. A different group of eight volunteers (age = 31 ± 2 y, weight = 77.5 ± 2.9 kg, height 1.74 ± 0.02 m, BMI = 25.7 ± 1.2 kg/m²) took part in this study. The design and diets were identical to that described in Study 1 except that a third diet treatment was included (Diet VS). The composition of diet VS was the same as diet V with the addition of a supplement of 18 g of soy protein (Table 1) to make the utilizable protein content the same as diet A. The supplement, provided in the form of a soy drink (Nutricia, Milan, Italy), increased the total daily protein intake by 23% and the contribution of protein derived from vegetable sources to 73%. The three dietary treatments (A, V, VS) were provided in random order.

For both studies, meals were prepared in a single batch and were stored frozen until use. The daily intake was divided into three main meals (breakfast, lunch and dinner) and one snack, and for each dietary period a daily menu was provided in the same order for all subjects. Meals were adjusted for individual estimated energy requirements. The daily energy requirement was calculated by multiplying the resting energy expenditure, measured by indirect calorimetry, by a factor of 1.4 to account for physical activity. To meet individual energy requirements, the basic diets shown in Table 1 were adjusted with small variations in carbohydrate and fat, without modifying the protein intake. Volunteers were asked not to take any medication and to avoid any strenuous exercise or physical activity during the experiments.

On d 11 of each study period, albumin synthesis rates were measured with the flooding method using L-[²H₅]phenylalanine (Hunter et al. 1995). Measurements were made in the postabsorptive state, beginning at 0900 h. A baseline blood sample was taken for measurement of plasma albumin (Study 1) or of albumin, other plasma proteins, amino acids, lipid and hormone concentrations (Study 2) from a sampling line inserted into a forearm vein. A tracer

solution containing L-[²H₅]phenylalanine (MassTrace, Woburn, MA) and unlabeled L-phenylalanine (Ajinomoto, Tokyo, Japan) at 43 mg/kg was then infused at a constant rate over 10 min through a line inserted into a contralateral forearm vein. The enrichment of the solutions was 5 and 10 mole percent excess (MPE), respectively, for the first and second tests in Studies 1 and 2 and 15 MPE for the third test in Study 2. All solutions were sterilized by filtration through a 0.22- μ m pore sterilizing filter (Millipore, Molsheim, France). Blood samples were taken at 5, 10, 15, 30, 50, 70, 90 min for the determination of the L-[²H₅]phenylalanine enrichment in the albumin and in the plasma free amino acid pool. Blood was centrifuged and serum and plasma stored at -70°C until further analysis.

Analytical methods

Serum albumin isotopic enrichment was measured as previously described by McNurlan et al. (1994) and Hunter et al. (1995). Albumin was separated by differential solubility in acid ethanol (Korner and Debro 1956), then solubilized in 0.3 mol/L NaOH at 37°C for 1 h. The protein was extensively washed with 20 g/L of perchloric acid and then hydrolyzed for 24 h in 6 mol/L of HCl. This procedure has been shown to yield a pure albumin preparation in healthy volunteers (Hunter et al. 1995). The determination of the [²H₅]phenylalanine in the hydrolysate was carried out after enzymatic conversion of phenylalanine to β -phenylethylamine, solvent extraction and derivatization as described by Calder et al. (1992). The heptafluorobutyl derivative was injected into an MD800 quadrupole mass spectrometer coupled to a GC8000 series gas chromatograph (GCMS) with computerized data analysis system (Fisons, Wythenshawe, Manchester, United Kingdom). The GCMS was operated under electron impact conditions in splitless mode, and the ions m/z 106 ($m + 2$) and m/z 109 ($m + 5$) were monitored under selective ion recording conditions.

Plasma free [²H₅]phenylalanine enrichment was measured as described by Calder and Smith (1988) with separation of amino acid by ion exchange chromatography followed by derivatization to the tertiarybutyldimethylsilyl derivative and measurement of the isotopic enrichment by GCMS. The MS was operated under electron impact conditions and the ions of mass m/z 336 and m/z 341 were monitored.

Plasma insulin and cortisol concentrations were measured by radioimmunoassay, using commercial kits (Ares Sero Diagnostici, Milan, Italy). Albumin concentration was assessed with the bromocresol green method (Doumas et al. 1971), using an automated analyzer, whereas prealbumin and transferrin concentrations were measured by immunonephelometry (Behring Diagnostics, L'Aquila, Italy). Triacylglycerols and cholesterol concentrations in serum and isolated lipoproteins were measured by enzymatic colorimetric methods on a COBAS-MIRA autoanalyzer (Roche, Basel, Switzerland). Plasma amino acid concentration was assessed by HPLC (Gilson Italia, Milan, Italy).

Calculation of albumin synthesis rate

Albumin fractional synthesis rate (FSR), which represents the percentage of the intravascular albumin pool synthesized per day, was calculated from the [²H₅]phenylalanine enrichment of albumin and the area under the curve of the plasma free [²H₅]phenylalanine (precursor) enrichment, by using the formula (Ballmer et al. 1990):

$$\text{FSR (\%/d)} = \frac{P_2 - P_1}{A} \cdot 100$$

where P_1 and P_2 represent the albumin enrichment between the times T_1 and T_2 (usually 50 and 90 min), corresponding to the portion of the curve when the incorporation of the isotope into protein is almost linear. A is the area of the precursor enrichment between the times adjusted for the delay between albumin synthesis and secretion, as described in detail by Ballmer et al. (1990).

The absolute synthesis rate (ASR), which is the amount of albumin synthesized per day expressed as $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, was calculated by multiplying the FSR by the albumin intravascular mass. The intravascular albumin mass was estimated from plasma volume and plasma albumin concentration. Since a direct measurement of plasma

³ Abbreviations used: ASR, absolute synthesis rate; BMI, body mass index; FSR, fractional synthesis rate; GCMS, gas chromatography mass spectrometer; MPE, mole percent excess.

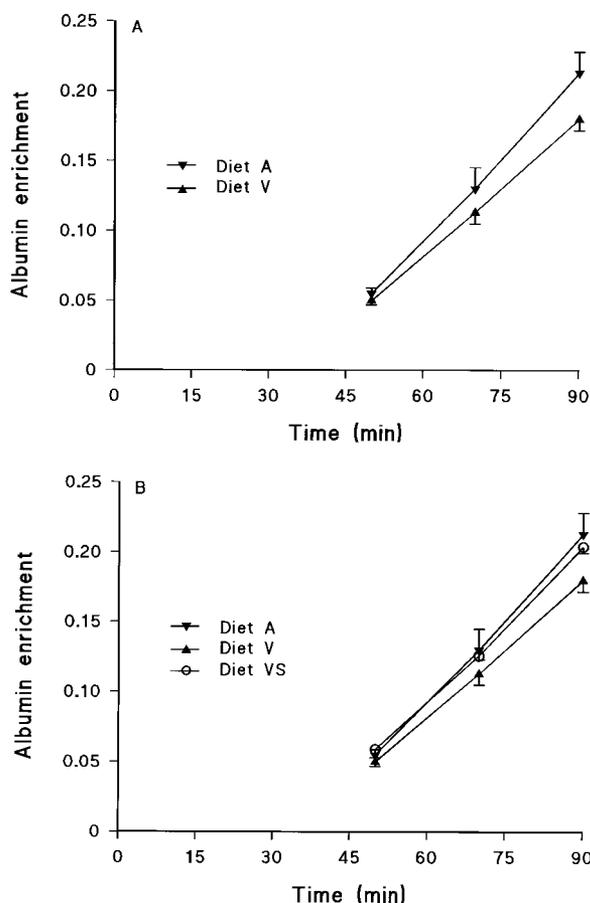


FIGURE 1 Enrichment of phenylalanine in albumin following the injection of L-[$^2\text{H}_5$]phenylalanine in men fed a predominantly animal (Diet A) or vegetarian (Diet V) diet or of a predominantly vegetarian diet supplemented with protein (Diet VS) in study 1 (panel A) and 2 (panel B). Data are expressed as percentage of the enrichment of the injection solution. Values are means \pm SEM ($n = 4$ in A and $n = 8$ in B).

volume with radiolabeled albumin was not permissible in these healthy volunteers, the plasma volume values were predicted from sex, age and weight by using a nomogram (Dagher et al. 1965). The albumin secretion time (T_s), which represents the time interval between the synthesis of albumin molecules and their secretion into the bloodstream, was extrapolated from the curve of the albumin enrichment vs. time by plotting the regression line for the linear part of the curve (between 50 and 90 min) and determining its intercept on the time axis.

Statistics

Data are expressed as mean \pm SEM. In study 1 the differences between the dietary treatments were analyzed by using a two-tailed t test for paired data. In study 2 the differences between the three diets were analyzed by using repeated measures ANOVA for planned comparisons of paired means (diet V vs. diet A or diet VS). A probability of $P < 0.05$ was considered statistically significant.

RESULTS

Study 1. The body weight was not affected by dietary treatments (76.8 ± 1 after diet V vs. 76.7 ± 1 kg after diet A). Plasma albumin concentration did not differ after the two diets (45.0 ± 0.6 vs. 45.3 ± 0.7 g/L).

As expected, the enrichment of phenylalanine in plasma albumin increased almost linearly between 50 and 90 min

(Fig. 1A). The changes in albumin enrichment during this period were used for calculation of synthesis rates. To normalize for differences in the enrichment of injected solution, the data in Figure 1 are expressed as percentage of the injection solution. The albumin secretion times did not differ after the two diets (35.5 ± 1 vs. 35.9 ± 2 min). Albumin FSR after diet V were significantly lower than values measured after diet A, with a mean decrease of 15% (Table 2, $P = 0.039$). Albumin ASR were also decreased after diet V by 15% (Table 2, $P = 0.05$).

Study 2. Study 2 was designed to further confirm the findings of study 1 in a larger number of subjects and to test whether the diminished albumin synthesis observed after diet V could be the result of a difference in net amino acid availability between the two diets. A third dietary treatment similar to diet V but supplemented with vegetable (soy) protein was therefore included (Diet VS).

As in study 1, no difference in body weight was detected after the three diets (77.7 ± 3.0 after diet V, 77.6 ± 2.9 after diet A, 77.8 ± 2.9 kg after diet VS).

Following diet V, the postabsorptive plasma insulin concentration tended to be higher than after diet A ($P = 0.07$) whereas cortisol concentration tended to decrease ($P = 0.11$) (Table 3), resulting in a higher insulin/cortisol ratio (0.124 ± 0.026 vs. 0.180 ± 0.028 , $P = 0.03$). However, the hormone concentrations after diet VS were not different from diet A (insulin/cortisol ratio 0.132 ± 0.023) (Table 3).

No effects of different diets were observed on plasma triacylglycerols (0.58 ± 0.05 vs. 0.58 ± 0.05 and 0.65 ± 0.06 mmol/L), total cholesterol (3.98 ± 0.13 vs. 4.09 ± 0.10 and 4.06 ± 0.16 mmol/L) and HDL-cholesterol serum concentrations (1.22 ± 0.09 vs. 1.23 ± 0.11 and 1.22 ± 0.10 mmol/L) (V vs. A and VS).

Plasma albumin concentration was lower after diet V compared to both diet A ($P = 0.02$) and diet VS ($P = 0.03$) (Table 3). No differences in plasma prealbumin and transferrin concentration were observed between diets V and A (Table 3). Both plasma prealbumin ($P = 0.006$) and transferrin concentrations ($P = 0.008$) were greater after diet VS than after diet V (Table 3).

Postabsorptive plasma amino acid levels did not differ after the consumption of the three diets (data not shown).

The curves of the changes in plasma free phenylalanine enrichment were comparable after each dietary treatment. The plasma free phenylalanine rose to an enrichment close to

TABLE 2

Fractional (FSR) and absolute (ASR) albumin synthesis rates in men after the consumption of a predominantly animal (Diet A) or vegetarian (Diet V) diet (Study 1)

Subject	FSR		ASR	
	Diet A	Diet V	Diet A	Diet V
	%/d		$\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$	
1	9.1	7.5	192	151
2	5.8	4.9	125	105
3	6.1	5.8	118	112
4	5.9	4.5	120	97
Mean	6.7*	5.7	139*	116
SEM	0.8	0.6	18	12

* Significantly different from diet V, $P \leq 0.05$.

TABLE 3

Plasma protein and hormone concentrations in men after the consumption of a predominantly animal (Diet A) or vegetarian (Diet V) diet or of a predominantly vegetarian diet supplemented with soy protein (Diet VS)¹

	Diet A	Diet V	Diet VS
Albumin, g/L	48.9 ± 0.6 ^a	47.7 ± 0.7 ^b	48.9 ± 0.7 ^a
Prealbumin, mg/L	301 ± 15 ^{ab}	292 ± 15 ^b	314 ± 17 ^a
Transferrin, mg/L	2.73 ± 0.14 ^{ab}	2.67 ± 0.09 ^b	2.85 ± 0.13 ^a
Insulin, pmol/L	47.1 ± 10.3	60.5 ± 9.5	50.3 ± 7.7
Cortisol, nmol/L	392 ± 32	341 ± 22	397 ± 30

¹ Values are means ± SEM, *n* = 8. Means in a row without common superscripts differ, *P* < 0.05.

90% of that of the injected solution after 10 min and declined almost linearly thereafter (results not shown). Similarly to study 1, L-[²H₅]phenylalanine enrichment in plasma albumin increased almost linearly between 50 and 90 min after the injection of the isotope solution (Fig. 1B).

Albumin secretion time was not affected by the three dietary treatments. However, as already shown in study 1, albumin synthesis rates were diminished following diet V. The FSR was 10% lower than after diet A (*P* = 0.015) (Table 4). The addition of soy protein to the predominantly vegetarian diet tended to raise the mean FSR by 7% (*P* = 0.08). As shown in Table 4, diet V was followed by a reduction in ASR in all subjects, with an average decrease of 12% (*P* = 0.007). This drop was reversed by diet VS (*P* = 0.03) (Table 4).

DISCUSSION

Albumin synthesis can be influenced by varying the relative contribution of protein from animal and vegetable sources in the diet. Healthy men showed lower albumin synthesis rates when consuming a diet containing 63% of vegetable protein (diet V) compared to an isonitrogenous and isocaloric diet in which only 26% of protein was of vegetable origin (Diet A). If data from both study 1 and 2 are combined (*n* = 12), the predominantly vegetarian diet resulted in an average decrease

in albumin synthesis rates of 12% when expressed as FSR (5.8 ± 0.3 vs. 6.7 ± 0.4%/d, *P* = 0.004) and 13% when expressed as ASR (123 ± vs. 143 ± 8 mg · kg⁻¹ · d⁻¹, *P* = 0.003). These measurements were carried out in the postabsorptive state. The differences found are therefore not representative of an acute effect of absorbing a meal of different composition, but rather, they reflect long-term adaptive changes in the basal rate of albumin synthesis in response to dietary changes.

The two diets, although equivalent in macronutrient composition, including the amount of crude protein, differed in the quality of the protein. This was due to a lower content of essential amino acids (amino acid score) and lower digestibility of the predominantly vegetarian diet. The approximate digestibilities estimated from the FAO/WHO/UNU values for individual food items were 92% for diet A and 87% for diet V (FAO/WHO/UNU 1985). The amino acid score for the diets calculated from the amino acid composition of individual item was 1 for diet A and 0.86 for diet V (Carnovale and Marletta 1997). Therefore the approximate adjusted daily protein intake for diet V was about 19% lower than for diet A and the average daily intake of utilizable protein was 0.93 g · kg⁻¹ · d⁻¹ for diet A and 0.75 g · kg⁻¹ · d⁻¹ for diet V.

Albumin synthesis has been shown to be reduced in conditions of protein energy malnutrition, in particular when protein intake is restricted (James and Hay 1968, Kirsch et al. 1968, Morgan and Peters 1971, Pain et al. 1978, Weidel et al. 1994). A 25% reduction in albumin synthesis has also been shown in young adults by reducing the protein content of an isocaloric liquid-formula diet from 1.5 to 0.4 g · kg⁻¹ · d⁻¹ for 2 wk (Gersovitz et al. 1980) and a similar (Hoffenberg et al. 1966) or a much greater effect (Kelman et al. 1972a) has been reported in volunteers when the protein intake is reduced from 70 to 10 g/d for 4–6 wk. Therefore, albumin synthesis is responsive to large reductions in dietary protein intake. However, in the present study, albumin synthesis was altered by relatively small variations in dietary protein content within the recommended safe range (FAO/WHO/UNU 1985). The reduced albumin synthesis is unlikely to be the result of altered energy balance between the two dietary treatments since the body weight of the volunteers did not change.

The reduction in albumin synthesis observed after diet V might reflect a fine physiological regulation of this major liver

TABLE 4

Fractional (FSR) and absolute (ASR) albumin synthesis rates in men after the consumption of a predominantly animal (Diet A) or vegetarian (Diet V) diet or of a predominantly vegetarian diet supplemented with soy protein (Diet VS) (Study 2)¹

Subject	FSR			ASR		
	Diet A	Diet V	Diet VS	Diet A	Diet V	Diet VS
	%/d			mg · kg ⁻¹ · d ⁻¹		
1	7.2	5.1	7.1	161	107	156
2	8.3	6.7	7.6	174	131	154
3	7.3	6.7	6.4	152	144	141
4	7.0	5.2	6.5	155	113	146
5	3.9	4.0	4.3	92	90	100
6	6.2	6.3	5.9	128	127	119
7	6.9	7.0	6.9	154	151	151
8	6.6	6.3	6.6	149	141	155
Mean	6.7 ^a	5.9 ^b	6.4 ^{ab}	146 ^a	126 ^b	140 ^a
SEM	0.5	0.3	0.3	9	7	7

¹ Means in a row without common superscripts differ, *P* < 0.05.

export protein by the level of amino acids in the diet. Although no differences were detected in postabsorptive plasma amino acid concentrations, differences in the absorption kinetics and/or net release of amino acids in the portal vein after each meal might affect albumin synthesis. Liver perfusion studies (Flaim et al. 1982, John and Miller 1969, Kelman et al. 1972b, Kirsch et al. 1969) indicate that the amino acid supply to the liver is an important factor in regulating albumin synthesis. Studies in humans also show that albumin synthesis acutely responds to oral nutrient intake (Hunter et al. 1995) or to the addition of protein to the diet (Cayol et al. 1997). Thus, the small difference in net amino acid availability between diets might explain the decrease in albumin synthesis observed with diet V. This hypothesis is further supported by the results of Study 2, in which a diet similar to V but supplemented with soy protein (diet VS) was also tested. The daily amount of crude protein provided by diet VS was higher than the other two diets (96 vs. 78 g/d), but the utilizable protein was similar to that of diet A (72 vs. 74 g/d) when the values were corrected for estimated digestibility and amino acid score (88 and 0.88%, respectively, for diet VS). Because diet VS increased the mean albumin synthesis rates to values close to those obtained after diet A, small changes in net protein absorption may play a primary role in explaining the lower albumin synthesis rates after consumption of the predominantly vegetarian diet.

A specific effect of vegetable protein and/or an indirect effect of fiber metabolism might also be implicated in the regulation of albumin synthesis, either through a direct effect on the liver or indirectly through differences in hormonal responses. Insulin has been shown to have a stimulatory role in albumin synthesis (De Feo et al. 1993, Flaim et al. 1985, Peavy et al. 1985) and it might represent one of the regulatory factors involved in the response of albumin synthesis to dietary nutrients. However, in this study the plasma insulin was slightly higher ($P = 0.07$) rather than lower after diet V, so insulin alone is unlikely to explain the observed lower rate of albumin synthesis after diet V.

Despite a reduction in albumin synthesis rates, increasing the percentage of protein derived from vegetable sources in the diet did not consistently affect plasma albumin concentration. When all the data from studies 1 and 2 are combined ($n = 12$) no significant difference in plasma albumin concentration between diets A and V was apparent ($P = 0.09$). This is in agreement with the findings of Gersovitz et al. (1980), who observed a significant decrease in albumin synthesis rates in young adult volunteers following a 2-wk protein-restricted diet, without showing any changes in plasma albumin concentration. Similarly, Scalfi et al. (1990) did not show any difference in plasma albumin concentration over twenty days in five groups of obese patients given four types of very-low-calorie diets providing different amounts of protein. However, when protein-restricted diets are provided for 4 to 6 wk a significant drop in both albumin synthesis rate and plasma albumin concentration has been documented (Hoffenberg et al. 1966, Kelman et al. 1972a), confirming that plasma albumin concentration does not reflect short-term changes in liver synthesis, partially due to the large body albumin mass, with a relatively slow turnover rate. There is evidence that in conditions of reduced albumin synthesis, such as following protein restriction, albumin degradation rate is also reduced, and the net transfer of extravascular albumin into the intravascular pool is enhanced (Hoffenberg et al. 1966, James and Hay 1968, Kelman et al. 1972a, Kirsch et al. 1968, Weidel et al. 1994). Although the kidney contributes little to albumin loss in healthy subjects, it has also been shown that diets contain-

ing only vegetable protein reduce the fractional clearance of albumin by the kidney compared to diets containing meat (Kontessis et al. 1990 and 1995). These regulatory mechanisms might counteract the drop in albumin synthesis and prevent and/or minimize any changes in plasma albumin concentration.

The rates of albumin synthesis measured in both studies are in the range previously reported using the same or different methods (Ballmer et al. 1990, Cayol et al. 1997, Hunter et al. 1995, Olufemi et al. 1990). The plasma volume for the calculation of the absolute synthesis rates was estimated using a nomogram and was not directly assessed after each dietary treatment, on the assumption that the two diets did not have a specific effect on the intravascular fluid redistribution. This assumption, although not directly proven, is supported by the findings of no detectable changes in hematocrit before each test (data not shown).

In conclusion, this study showed that liver protein metabolism can be modulated by varying the contribution of animal and plant derived foods in the diet. In particular albumin synthesis is reduced following consumption of a predominantly vegetarian diet for 10 d. It is unlikely to represent a specific effect of vegetable protein because supplementation with soy protein reversed the effect of the vegetarian diet. Albumin synthesis might be responsive to a small reduction in amino acid availability, a consequence of the lower digestibility and amino acid score and higher fiber content of the vegetable-rich diet, thus showing the exquisite sensitivity of albumin synthesis to small changes in protein composition or intake. It is not known whether this effect is limited to albumin or whether it represents a general response of liver protein synthesis to dietary changes.

LITERATURE CITED

- Ballmer, P. E., McNurlan, M. A., Milne, E., Heys, S. D., Buchan, V., Calder, A. G. & Garlick, P. J. (1990) Measurement of albumin synthesis in humans: a new approach employing stable isotopes. *Am. J. Physiol.* 259: E797-E803.
- Calder, A. G., Anderson, S. E., Grant, I., McNurlan, M. A. & Garlick, P. J. (1992) The determination of low d_5 -phenylalanine enrichment (0.002–0.09 atoms percent excess), after conversion to phenylethylamine, in relation to protein turnover studies by gas chromatography/electron ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 6: 421–424.
- Calder, A. G. & Smith, A. (1988) 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.
- Carnovale, E. & Marletta, L. (1997) *Tabelle di composizione degli Alimenti*. Istituto Nazionale della Nutrizione, Edra, Milano.
- Cayol, M., Boirie, Y., Rambourdin, F., Prugnaud, J., Gachon, P., Beaufrère, B. & Obled, C. (1997) Influence of protein intake on whole body and splanchnic leucine kinetics in humans. *Am. J. Physiol.* 272: E584–E591.
- Committee on Diet and Health, Food and Nutrition Board, Commission on Life Sciences & National Research Council (U.S.) (1989) *Diet and health: implications for reducing chronic diseases*. National Academy Press, Washington D.C.
- Dagher, F. J., Lyons, J. M., Finlayson, D. C., Shamsai, J. & Moore, F. D. (1965) Blood volume measurement: a critical study. Prediction of normal values: controlled measurements of sequential changes: choice of a bedside method. *Adv. Surg.* 1: 69–109.
- De Feo, P., Volpi E., Lucidi, P., Gruciani, G., Reboldi, G., Siepi, D., Mannarino, E., Santeusano, F., Brunetti, P. & Bolli, G. B. (1993) Physiological increments in plasma insulin concentrations have selective and different effects on synthesis of hepatic proteins in normal humans. *Diabetes* 42: 995–1002.
- Doumas, B. T., Watson, W. & Biggs, H. G. (1971) Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chim. Acta* 31: 97–96.
- FAO/WHO/UNU (1985) *Energy and protein requirements*. WHO Technical Report Series 724: Geneva.
- Ferro-Luzzi, A. & Branca, F. (1995) Mediterranean diet, Italian-style: prototype of a healthy diet. *Am. J. Clin. Nutr.* 61(suppl.): 1338S–1345S.
- Flaim, K. E., Hutson, S. M., Lloyd, C. E., Taylor, J. M., Shiman, R. & Jefferson, L. S. (1985) Direct effect of insulin on albumin gene expression in primary rat hepatocytes. *Am. J. Physiol.* 249: E447–E453.
- Flaim, K. E., Peavy, D. E., Everson, W. V. & Jefferson, L. S. (1982) The role of

- amino acids in the regulation of protein synthesis in perfused rat liver. *J. Biol. Chem.* 257: 2932–2938.
- Gersovitz, M., Munro, H. N., Udall, J. & Young, V. R. (1980) Albumin synthesis in young and elderly subjects using a new stable isotope methodology: response to level of protein intake. *Metabolism* 29: 1075–1086.
- Hoffenberg, R., Black, E. & Brock, J. F. (1966) Albumin and γ -globulin tracer studies in protein depletion states. *J. Clin. Invest.* 45: 143.
- Hunter, K. A., Ballmer, P. E., Anderson, S. E., Broom, J. & Garlick, P. J. (1995) Acute stimulation of albumin synthesis rate with oral meal feeding in healthy subjects measured with [ring- $^2\text{H}_6$]phenylalanine. *Clin. Sci.* 88: 235–242.
- James, W. P. T. & Hay, A. M. (1968) Albumin metabolism: effect of the nutritional state and the dietary protein intake. *J. Clin. Invest.* 47: 1958–1972.
- John, D. W. & Miller, L. L. (1969) Regulation of net biosynthesis of serum albumin and acute phase plasma proteins. *J. Biol. Chem.* 244: 6134–6142.
- Kelman, L., Saunders, S. J., Frith, L., Wicht, S. & Corrigan, A. (1972a) Effects of dietary protein restriction on albumin synthesis, albumin catabolism, and the plasma aminogram. *Am. J. Clin. Nutr.* 25: 1174–1178.
- Kelman, L., Saunders, S. J., Wicht, S., Frith, L., Corrigan, A., Kirsch, R. E. & Terblanche, J. (1972b) The effects of amino acids on albumin synthesis by isolated perfused rat liver. *Biochem. J.* 129: 805–809.
- Keys, A., Menotti, A., Aravanis, C., Blackburn, H., Djordjevic, B. S., Buzina, R., Dontas, A. S., Fidanza, F., Karvonen, M. J., Kimura, N., Mohaček, I., Nedeljović, S., Puudu, V., Punsar, S., Taylor, H. L., Conti, S., Kromhout, D. & Toshima, H. (1984) The seven countries study: 2,289 deaths in 15 years. *Prev. Med.* 13: 141–154.
- Kirsch, R. E., Frith, L., Black, E. & Hoffenberg, R. (1968) Regulation of albumin synthesis and catabolism by alteration of dietary protein. *Nature* 217: 578–579.
- Kirsch, R. E., Saunders, S. J., Frith, L., Wicht, S., Kelman, L. & Brock, J. F. (1969) Plasma amino acid concentration and the regulation of albumin synthesis. *Am. J. Clin. Nutr.* 22: 1559–1562.
- Kontessis, P., Bossinakou, I., Sarika, L., Iliopoulou, E., Papantoniou, A., Trevisan, R., Roussi, D., Stipsanelli, K., Grigorakis, S. & Souvatzoglou, A. (1995) Renal, metabolic, and hormonal responses to proteins of different origin in normotensive, nonproteinuric type I diabetic patients. *Diabetes Care* 18: 1233–1240.
- Kontessis, P., Jones, S., Dodds, R., Trevisan, R., Nosadini, R., Fioretto, P., Borsato, M., Sacerdoti, D. & Viberti, G. (1990) Renal, metabolic and hormonal responses to ingestion of animal and vegetable proteins. *Kidney Int.* 38: 136–144.
- Korner, A. & Debro, J. R. (1956) Solubility of albumin in alcohol after precipitation in trichloroacetic acid: a simplified procedure for separation of albumin. *Nature (London)* 178: 1067.
- McNurlan, M. A., Essén, P., Thorell, A., Calder, A. G., Anderson, S. E., Ljungqvist, O., Sandgren, A., Grant, I., Tjäder, I., Ballmer, P. E., Wernerman, J. & Garlick, P. J. (1994) Response of protein synthesis in human skeletal muscle to insulin: an investigation with [$^2\text{H}_6$]phenylalanine. *Am. J. Physiol.* 267: E102–E108.
- Morgan, E. H. & Peters, T. (1971) The biosynthesis of rat serum albumin. Effect of protein depletion and refeeding on albumin and transferrin synthesis. *J. Biol. Chem.* 246: 3500–3507.
- Olufemi, O. S., Humes, P., Whittaker, P. G., Read, M. A., Lind, T. & Halliday, D. (1990) Albumin synthetic rate: a comparison of arginine and alpha-ketoisocaproate precursor methods using stable isotope techniques. *Eur. J. Clin. Nutr.* 44: 351–361.
- Pain, V. M., Clemens, M. J. & Garlick, P. J. (1978) The effect of dietary protein deficiency on albumin synthesis and on the concentration of active albumin messenger ribonucleic acid in rat liver. *Biochem. J.* 172: 129–135.
- Peavy, D. E., Taylor, J. M. & Jefferson, L. S. (1985) Time course of changes in albumin synthesis and mRNA in diabetic and insulin treated rats. *Am. J. Physiol.* 248: E656–E663.
- Scafili, L., Laviano, A., Reed, L. A., Borrelli, R. & Contaldo, F. (1990) Albumin and labile-protein serum concentrations during very-low-calorie diets with different compositions. *Am. J. Clin. Nutr.* 51: 338–342.
- Tavani, A. & La Vecchia, C. (1995) Fruit and vegetable consumption and cancer risk in a mediterranean population. *Am. J. Clin. Nutr.* 61(suppl.): 1374S–1377S.
- Weidel, S. E., Smith, G. & Fleck, A. (1994) The effects of experimental malnutrition on albumin metabolism and distribution in rabbits. *Br. J. Nutr.* 72: 369–384.
- World Health Organization (1990) Diet, nutrition and the prevention of chronic diseases. WHO Technical Report Series 797: Geneva.
- Young, V. R. & Pellett, P. L. (1994) Plant proteins in relation to human protein and amino acid nutrition. *Am. J. Clin. Nutr.* 59(suppl.): 1203S–1212S.