Brazilian Journal of Medical and Biological Research (2001) 34: 1277-1283
ISSN 0100-879X

Obese women on a low energy rice and bean diet: effects of leucine, arginine or glycine supplementation on protein turnover

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Abstract

This study examined if leucine, arginine or glycine supplementation in adult obese patients (body mass index of 33 ± 4 kg/m^2) consuming a Brazilian low energy and protein diet (4.2 MJ/day and 0.6 g protein/kg) affects protein and amino acid metabolism. After four weeks adaptation to this diet, each subject received supplements of these amino acids (equivalent to 0.2 g protein kg^{-1} day^{-1}) in random order. On the seventh day of each amino acid supplementation, a single-dose ^{15}N-glycine study was carried out. There were no significant differences in protein flux, synthesis or breakdown. The protein flux (grams of nitrogen, gN/9 h) was 55 ± 24 during the nonsupplemented diet intake and 39 ± 10, 44 ± 22 and 58 ± 35 during the leucine-, glycine- and arginine-supplemented diet intake, respectively; protein synthesis (gN/9 h) was 57 ± 24, 36 ± 10, 41 ± 22 and 56 ± 36, respectively; protein breakdown (gN/9 h) was 51 ± 24, 34 ± 10, 32 ± 28 and 53 ± 35, respectively; kinetic balance (gN/9 h) was 3.2 ± 1.8, 4.1 ± 1.7, 3.4 ± 2.9 and 3.9 ± 1.6. There was no difference in amino acid profiles due to leucine, arginine or glycine supplementation. The present results suggest that 0.6 g/kg of dietary protein is enough to maintain protein turnover in obese women consuming a reduced energy diet and that leucine, arginine or glycine supplementation does not change kinetic balance or protein synthesis.

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Research supported by FAPESP (No. 97/09577-4).

Received August 10, 2000
Accepted June 28, 2001

Key words
• Obese women
• Protein
• Glycine
• Arginine
• Leucine
• Stable isotope

Introduction

Obese subjects have increased protein turnover, whole-body protein synthesis and oxidation compared with same age lean adults (1). It has been suggested that protein balance in obese subjects is not maintained with a protein intake lower than 70 g/day when energy is restricted to 1100 kcal/day (4.5 MJ) (2). Garlick et al. (3) have also shown that when energy is restricted, the nitrogen balance decreases and is improved by high protein intake.

Supplementation with amino acids themselves has not been tested as a protein-sparing method during low energy diets in the

treatment of obesity, but as a source of nitrogen one can expect it to be effective. Moreover, some amino acids have known pharmacological properties which could be of potential benefit for protein metabolism (4), and the effect of supplementation with these amino acids during low energy diets on obese persons has not been reported.

Two amino acids, arginine and leucine, are particularly recognized to have pharmacological in addition to nutritional properties. Arginine (L-2-amino-5-guanidinovaleric acid) is an aliphatic amino acid which has an important role in immune function and stimulates the release of growth hormone by the pituitary gland (5). Arginine is also a physiological precursor of nitric oxide (endothelium-derived relaxing factor) and this has been suggested as an explanation for its hypotensive effect on healthy subjects (6). Leucine (L-2-amino-4-methyl valeric acid) is a branched chain essential amino acid that has important roles in protein and glucose metabolism, neurotransmitter synthesis and lymphoid tissue metabolism (7).

This study was intended to test if supplementation with arginine and leucine improves protein and amino acid metabolism in obese patients consuming a low energy diet. To determine if any eventual changes in protein metabolism would be due to their pharmacological properties, we also studied a period of supplementation with glycine, an amino acid with no such effects. All supplementation periods were compared with nonsupplemented (control) ones.

Material and Methods

Subjects

Seven obese women aged 36 ± 8 years were studied. Each subject underwent detailed clinical evaluation and blood tests (complete hematologic cell count, plasma glucose, creatinine and urea levels) and, apart from obesity, no other acute or chronic illnesses were detected. No subjects were pregnant or breastfeeding. After the initial evaluation, a dietary history was obtained and each subject was instructed to eat a local rice and bean diet (see below) and to maintain their usual level of activity.

This study was approved by the local Ethics Committee. Written informed consent was obtained from each subject after detailed information about the purposes and risks of this research.

Study design

Each volunteer received a low energy rice and bean diet (4.2 MJ) with a total protein intake of 0.6 g kg^{-1} day^{-1} throughout the 8-week study period. This diet, which has been used in previous similar research protocols, does not have any amino acid deficiency and reflects a typical Brazilian diet (8-10).

After an adaptation period of 4 weeks, the rice and bean diet was supplemented with leucine, arginine and glycine in random order (equivalent to 0.2 g protein kg^{-1} day^{-1}) for 7 days each, so that each subject participated in four study periods. Between 7-day supplementation periods, the subjects received a nonsupplemented rice and bean diet for 2 days (washout period). A period of 7 days on a nonsupplemented diet was also studied (control).

On the 7th day of each period, a 9-h whole-body ^{15}N-glycine single-dose study was carried out (11) after a 12-h fast. Each subject was given six isoenergetic, isonitrogenous meals at 2-h intervals, which provided half the total usual daily energy, protein and supplemented leucine, arginine or glycine intake (i.e., 2.2 MJ, 0.3 g protein kg^{-1} day^{-1} from rice and beans, and an amino acid supplement equivalent to 0.2 g kg^{-1} day^{-1}). Two hours after the first meal, the bladder was emptied for baseline urine enrichment determination and a dose of ^{15}N-glycine (200 mg per subject) was given (0 h).

All urine was collected until the 9th h of study for urea and ammonia ^{15}N enrichment determination. Two blood samples were taken for urea ^{15}N enrichment determination at 0 h and 9 h (12).

Protein turnover

Flux was calculated from the amount of isotope excreted in urine in the end product (urea and ammonia) over the 9-h study period. In addition, the amount of label retained in the urea pool at 9 h was used to adjust the result of flux based on the label excreted in urinary urea (13). Flux was calculated as: Q = d x Ex/ex, where Q is flux, d is the amount of isotope administered, Ex is the amount of end product excreted, and ex is the amount of isotope excreted as the end product over the study period. The end product average flux was taken as the harmonic mean of the estimates of flux based on the excretion of urea-N and ammonia-N, and was used in the calculation of protein synthesis and breakdown rates (12). Protein synthesis and degradation were estimated from: Q = Nint + D = Ex + S, where Nint is nitrogen intake, D is the equivalent of protein degradation, and S is the equivalent of protein synthesis.

Sample analysis

Urine was collected into containers with 5 ml concentrated HCl. Samples were stored at -20°C until triplicate analysis. Aliquots were taken for duplicate determination of total nitrogen, ammonia-N, urea-N, ammonia-^{15}N, urea-^{15}N (14) and free amino acids (15). Total nitrogen was measured by the Kjeldahl method. Urea and ammonia were extracted sequentially by Conway diffusion and titration for mass spectrometry analysis by alkaline aeration. Isotope enrichment was measured with a mass spectrometer (Anca 20-20, Europa Scientific, Cheshire, England). Plasma amino acid was analyzed by high-performance liquid chromatography (Shimadzu Corporation, Tokyo, Japan) after precolumn fluorescence derivatization with ortho-phthaldialdehyde (15).

Statistical analysis

The repeated measures design was used for statistical analysis, with one-way within-subjects ANOVA (for repeated measures or amino acid supplement). Thus, the repeated measure factor had four levels: no supplement, leucine supplement, glycine supplement and arginine supplement, in random order (16). *Post hoc* comparisons were made using the Tukey test. Fasted state was compared to fed state by the paired *t*-test. P values of 0.05 or less were considered significant.

Results

Anthropometric and biochemical data are shown in Table 1. Body mass index and arm fat index decreased significantly from day one to day 35 (adaptation period) and then remained constant up to the end of the study (Table 1). Blood biochemical data did not change significantly. Urinary urea excretion was higher on the first day of the study as compared to any other study period and urinary urea excretion remained constant from week 2 to 8.

Subjects 2 and 3 were excluded from the repeated statistical analysis because samples from one study period were lost for both subjects for technical reasons. There were no significant differences in whole-body protein turnover between the period of non-supplemented rice and bean diet intake and any period of leucine, arginine or glycine supplementation (Table 2). There were also no significant differences in plasma or urinary amino acid profiles between fasted or fed state or between the nonsupplemented and different supplementation periods (Table 3).

Table 1. Clinical, anthropometric and biochemical data of the volunteers throughout the experimental protocol.

	Experimental day				
	1	35	42	49	56
Heart rate (bpm)	72 ± 8 (7)	-	-	-	85 ± 9 (6)
Systolic blood pressure (mmHg)	120 ± 6 (7)	-	-	-	125 ± 7 (6)
Diastolic blood pressure (mmHg)	79 ± 4 (7)	-	-	-	82 ± 10 (6)
Height (cm)	156 ± 7 (7)	-	-	-	-
Arm length (cm)	35 ± 3 (7)	-	-	-	-
Triceps skinfold (mm)	31 ± 4 (7)*	30 ± 4 (7)	-	-	28 ± 3 (6)
Fat arm index (mm/dm²)	2.6 ± 0.5 (7)*	2.5 ± 0.5 (7)	-	-	2.3 ± 0.3 (6)
Arm circumference (cm)	36 ± 4 (7)	35 ± 3 (7)	-	-	34 ± 4 (6)*
Muscular circumference (cm)	26 ± 3 (7)	26 ± 3 (7)	-	-	25 ± 3 (6)
Weight (kg)	82 ± 18 (7)*	78 ± 18 (7)	78 ± 18 (7)	77 ± 18 (7)	78 ± 19 (6)
Body mass index (kg/m²)	33 ± 4 (7)*	32 ± 5 (7)	31 ± 5 (7)	31 ± 5 (7)	31 ± 5 (6)
Blood data					
Hemoglobin (g/dl)	14 ± 1 (7)	13 ± 1 (7)	-	-	13 ± 1 (6)
Triglycerides (mg/dl)	140 ± 39 (5)	-	-	-	113 ± 28 (5)
Cholesterol (mg/dl)	155 ± 52 (5)	-	-	-	142 ± 19 (5)
HDL cholesterol (mg/dl)	30 ± 2 (5)	-	-	-	35 ± 5 (4)
Albumin (g/l)	49 ± 4 (7)	53 ± 4 (7)	-	-	50 ± 5 (6)
Iron (µg/dl)	72 ± 12 (7)	84 ± 15 (7)	-	-	78 ± 10 (6)
Total iron-binding capacity (µg/dl)	303 ± 39 (7)	288 ± 29 (7)	-	-	298 ± 41 (6)
ß-Carotene (mg/dl)	118 ± 41 (7)	147 ± 29 (7)	-	-	141 ± 58 (6)
Vitamin A (mg/dl)	45 ± 20 (7)	36 ± 7 (7)	-	-	35 ± 18 (6)
Vitamin C (mg/dl)	0.26 ± 0.09 (7)	0.31 ± 0.13 (7)	-	-	0.25 ± 0.09 (6)
Glucose (mg/dl)	95 ± 6 (7)	89 ± 9 (6)	-	-	80 ± 8 (7)
Folic acid (ng/ml)	8 ± 2 (6)	7 ± 1 (6)	-	-	13 ± 1 (3)
Urine data					
Creatinine (g/day)	1.4 ± 0.4 (6)	1.2 ± 0.4 (7)	1.3 ± 0.5 (7)	1.2 ± 0.3 (7)	1.1 ± 0.2 (5)
Urea (g/day)	25 ± 5 (5)*	17 ± 4 (7)	-	14 ± 4 (7)	17 ± 4 (5)

Data are reported as means ± SD, with the number of subjects given in parentheses. *P<0.01 compared to day 1 (ANOVA/Tukey test). Fat arm index = triceps skinfold/arm length; muscular circumference = arm circumference - π triceps skinfold; body mass index = weight/height.

Table 2. Kinetic data after a single dose of ^{15}N-glycine during the periods of no supplementation and supplementation with leucine, glycine or arginine (in random order).

	No supplement	Leucine	Glycine	Arginine
Total urine nitrogen, urea and ammonia after 9 h of ^{15}N-glycine infusion (gN/9 h)				
Ammonia	0.22 ± 0.23	0.26 ± 0.23	0.15 ± 0.15	0.18 ± 0.12
Urea	1.04 ± 1.32	1.33 ± 0.78	1.32 ± 0.69	1.22 ± 0.93
Total nitrogen	2.34 ± 1.80	2.56 ± 0.93	2.36 ± 0.73	1.94 ± 1.14
Urine ammonia ^{15}N enrichment at baseline and after 9 h of ^{15}N-glycine infusion (atom%excess)				
Baseline	0.38 ± 0.01	0.38 ± 0.02	0.38 ± 0.01	0.38 ± 0.01
After 9 h	0.47 ± 0.04	0.48 ± 0.05	0.54 ± 0.15	0.47 ± 0.07
Urine urea ^{15}N enrichment at baseline and after 9 h of ^{15}N-glycine infusion (atom%excess)				
Baseline	0.38 ± 0.01	0.38 ± 0.02	0.38 ± 0.01	0.38 ± 0.01
After 9 h	0.45 ± 0.04a	0.49 ± 0.02b	0.48 ± 0.05c	0.46 ± 0.03d
Blood urea ^{15}N enrichment at baseline and after 9 h of ^{15}N-glycine infusion (atom%excess)				
Baseline	0.38 ± 0.01	0.38 ± 0.01	0.37 ± 0.05	0.38 ± 0.01
After 9 h	0.41 ± 0.02	0.40 ± 0.02	0.40 ± 0.02	0.40 ± 0.01
Protein kinetic data (gN/9 h)				
Whole-body flux	55 ± 23	39 ± 10	44 ± 21	58 ± 33
Synthesis	54 ± 24	36 ± 10	41 ± 22	56 ± 36
Breakdown	51 ± 24	34 ± 10	32 ± 28	53 ± 35
Balance (synthesis-breakdown)	3.2 ± 1.8	4.1 ± 1.7	3.4 ± 2.9	3.9 ± 1.6

Data are reported as means ± SD. Repeated ANOVA: a<b, a<c, a = d, b = c = d. gN = grams of nitrogen.

Table 3. Plasma and urinary amino acids before (fast) and after (fed) the kinetic study in obese subjects receiving a low energy rice and bean diet.

Supplement	Urine data (µmol/l)								Plasma data (µmol/l)							
	Leucine		Glycine		Arginine		Without supplement		Leucine		Glycine		Arginine		Without supplement	
	Fast	Fed	Fast	Fed	Fast	Fed	Fast	Fed	Fast	Fed	Fast	Fed	Fast	Fed	Fast	Fed
Alanine	145	139	214	143	147	165	128	83	172	260	195	206	147	240	268	171
	161	172	116	129	74	197	99	48	93	171	155	118	101	226	191	150
Arginine	69	145	249	107	148	111	310	114	409	390	348	292	279	431	400	337
	61	161	233	96	139	98	452	77	247	186	197	73	100	270	140	98
Aspartic acid	26	39	52	33	27	33	52	39	80	89	55	51	49	69	68	59
	21	25	40	27	11	28	43	32	50	36	29	21	14	28	23	10
Glutamic acid	53	81	171	60	64	108	86	124	353	412	385	352	313	340	416	365
	29	66	142	70	59	143	48	125	162	153	299	65	53	134	132	84
Glycine	833	1753	2559	2319	796	1912	3706	763	386	307	318	187	140	379	294	188
	867	1980	2640	3507	468	2794	3796	413	419	209	295	82	79	546	177	112
Isoleucine	23	62	23	23	11	25	20	11	58	101	67	80	58	72	76	70
	20	89	13	22	13	25	15	5	26	109	32	42	10	31	21	19
Leucine	28	36	32	35	30	31	45	18	334	371	209	202	160	238	188	186
	32	34	17	32	19	34	54	13	206	192	111	68	51	112	94	50
Methionine	15	9	18	10	19	12	14	9	134	152	158	74	59	136	171	136
	15	7	8	13	12	12	16	6	131	187	196	86	79	198	169	153
Phenylalanine	48	52	69	50	48	42	69	36	107	131	85	83	64	110	94	81
	37	46	59	65	37	70	62	20	75	58	39	28	23	52	38	26
Serine	115	109	263	205	84	139	204	115	305	264	181	150	123	343	217	160
	136	130	216	339	46	128	210	113	354	111	111	54	65	439	130	73
Threonine	149	159	292	231	134	181	221	149	211	210	185	149	135	201	216	170
	124	137	116	273	118	200	183	79	144	76	138	51	34	155	76	36
Tyrosine	63	68	87	71	62	63	102	61	87	95	64	63	53	81	68	64
	43	37	52	68	35	58	77	22	65	46	26	24	19	42	28	12
Valine	20	20	30	19	20	17	35	17	229	215	262	229	197	279	295	245
	19	8	17	10	12	18	28	10	131	89	159	58	28	137	128	60

Data are reported as means ± SD.

Discussion

In this study, a protein intake of 0.6 g kg^{-1} day^{-1} was sufficient to maintain protein turnover in obese women on a reduced energy diet. During the experimental protocol, as expected, there was a drop in anthropometric measurements, but from around the fourth week on, after the adaptation period, there was anthropometric and biochemical stabilization. This may reflect the occurrence of energy expenditure reduction in response to the reduced energy intake, a phenomenon demonstrated by other authors (17). For this research, this stabilization represented, in fact, an advantage, as it eliminated the possibility of misinterpretation of results due to changes in body weight.

The rates of whole-body protein flux were measured using a single oral dose of ^{15}N-

glycine (3,13). There is evidence that the method of ^{15}N-glycine infusion employed in whole-body protein turnover studies (i.e., constant infusion, repeated or single dose) does not affect the results (3,13). Besides, the use of a single dose permits the repetition of the study for many times, allowing the follow-up of time course changes in protein synthesis and breakdown (3). For whole-body protein turnover studies it is assumed that the nitrogen pool is homogenous and that the nitrogen exchange among different pools is constant under the experimental conditions used. However, the hypothesis of homogeneity and stability of the metabolic pool of body nitrogen is valid only under exceptional circumstances (12,18). For this reason, the fact that in the present investigation the same subject was studied under different conditions seems to be advantageous. Also, since this permitted the comparison of results with baseline and with every other period, any possible methodological problem would have been diluted in the overall experiment (19). Although this method implies an oversimplification of the complex reactions of protein kinetics *in vivo*, when it is applied under controlled experimental conditions it affords useful information concerning the dynamics of human protein metabolism (20).

Previous studies have shown that 0.6 g protein kg^{-1} day^{-1} is sufficient to keep the nitrogen equilibrium in healthy Brazilian persons on a rice and bean diet (9,10). It is possible that obese subjects have the same protein requirements, which remain unchanged during the low energy diet, as also proposed by others. Solini et al. (21) found no differences in leucine flux, oxidation or non-oxidative disposal between non-diabetic obese women and normal women. Other studies conducted on eutrophic subjects receiving 0.5 g protein kg^{-1} day^{-1} (10,22) found a kinetic balance close to that of the present study. Vazquez et al. (23) showed that an increase of the protein composition of isoenergetic weight reduction diets (2.5 KJ/day) from 50 to 70 g/day leads to no changes in nitrogen balance.

In the present study, whole-body nitrogen flux (around 39-58 mg nitrogen kg^{-1} h^{-1}) was higher than that obtained for control subjects (around 17-46 mg nitrogen kg^{-1} h^{-1}) (10,13) with a mean body mass index of 25 kg/m^2. Protein synthesis and breakdown were also higher than values reported in the literature for normal body weight individuals (10,11,22) but similar to those reported for obese adolescents (24). One of these studies (10) was done using the same protocol/environmental conditions as the present study. It should also be pointed out that the variation in the data obtained here for obese subjects was similar to that observed in other studies of protein metabolism (1-3,20,21,24).

Leucine, arginine or glycine supplementation (0.2 g kg^{-1} day^{-1}) did not improve kinetic balance or protein synthesis compared with the baseline diet period. It is possible that, as the subjects had no changes in protein metabolism induced by the low energy diet, any eventual effects of supplementation remained undetectable.

Urine and plasma amino acid profile did not show any remarkable differences, with the exception of high mean plasma values for phenylalanine, leucine and methionine when compared to literature values for eutrophic women (25). Plasma amino acid levels were not modified by supplementation. Kihlberg et al. (19) have described high plasma levels of phenylalanine and leucine and also of isoleucine, valine, lysine, tyrosine, proline and glutamic acid in obese women. These results suggest that obese women may have a particular amino acid profile, but this should be confirmed by further studies.

In conclusion, the present study does not support the use of leucine, arginine or glycine supplementation during low energy diets for obese subjects with the objective of decreasing protein breakdown rate and/or

increasing protein synthesis rate. Obese subjects are able to keep a stable protein metabolism on a diet containing 0.6 g protein kg^{-1} day^{-1} and providing an energy intake of 4.2 MJ/day. Further trials with longer follow-up periods are needed to determine if nitrogen kinetic balance is maintained during very low energy treatment regimens (or even drug treatments). In addition, we confirm that single-dose protein turnover studies are reliable, fast, inexpensive and easy to carry out in hospitalized patients. They can be used in short whole-body protein turnover studies in patients for whom long, continuous isotope infusion with gas and blood collection would be difficult or even impossible.

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