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Combined Effects of Caloric Restriction and Branched-Chain Amino Acid Supplementation on Body Composition and Exercise Performance in Elite Wrestlers

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Twenty-five competitive wrestlers restricted their caloric intake ($28 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) for 19 days, using a hypocaloric control (hC, $n = 6$), hypocaloric high-protein (hHP, $n = 7$), hypocaloric high-branched-chain amino acid (hBCAA, $n = 6$), hypocaloric low-protein (hLP, $n = 6$) diet to determine the effects of caloric restriction on body composition and performances versus control diet (C, $n = 6$). Anthropometric parameters (weight, percent body fat) and adipose tissue (AT) distribution measured by magnetic resonance imaging (MRI) obtained before and after diet, were compared. A significant highest body weight loss (-4 kg , $p < 0.05$) and decrease in the percent of body fat (-17.3% , $p < 0.05$) were observed for subjects of the hBCAA group. Subjects of the hBCAA group exhibited a significant reduction (-34.4% , $p < 0.05$) in abdominal visceral adipose tissue (VAT). There was no change in aerobic (VO_2max) ($p > 0.75$) and anaerobic capacities (Wingate test) ($p > 0.81$), and in muscular strength ($p > 0.82$). We conclude that under our experimental conditions, the combination of moderate energy restriction and BCAA supplementation induced significant and preferential losses of VAT, and allowed maintenance of a high level of performance.

Key words: Caloric restriction, branched-chain amino acids, magnetic resonance imaging, body composition, aerobic and anaerobic parameters, muscular strength

Introduction

Prior to competing, all wrestlers are required to attain a specific body weight (weight class). The majority of wrestlers attempt to lose body weight, maximise the amount of lean tissue and minimise the amount of body fat.

Numerous methods are employed to reduce body weight for wrestling competition. Wrestlers can reduce body weight

either rapidly or gradually. Reduction of body weight in less than 1 week is defined by Fogelholm (9) as rapid body weight reduction. Reduction over a longer period (> 7 days) is defined as gradual body weight reduction. The main difference is that gradual body weight reduction is accomplished by negative energy balance, whereas active or passive dehydration (fluid loss) is a necessary part of rapid body weight loss. The primary goal of restriction of energy intake is the restriction in body fat. But previous studies in moderately active men have shown that substantial reductions in body weight include loss of both lean and fat tissues (22). Therefore, wrestlers cutting weight through the restriction of energy intake can expect to lose muscle mass. Results of the effects of gradual body weight reduction on aerobic performance in athletes are variable. It has been shown that during gradual body weight reduction, maximal oxygen intake (VO_2max) might deteriorate (16,38), remain unchanged (26) or might improve (24). When reducing body weight gradually with a normal carbohydrate diet (50% of energy intake, $2.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$), anaerobic performance was impaired (28). Conversely, anaerobic performance was not affected after body weight loss with a higher intake of carbohydrates ($4.1 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) (28). After gradual body weight reduction, the isometric endurance capacity of the skeletal muscle was reduced (13,36) while the endurance capacity during isokinetic exercise was unaffected (36).

Steen and McKinney (34) found that college wrestlers from several universities consumed diets composed of approximately 15% protein, 33 to 37% fat and 43 to 47% carbohydrate with the composition being somewhat dependent on the time of the season (pre-season, mid-season, etc.). During periods of weight reduction, the authors found that 37% of the wrestlers studied consumed less than two-thirds of the Recommended Dietary Allowances (RDA) for energy. At the reduced level of energy intake, all macronutrients were significantly lower, although the average protein intake remained at $0.9 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (14), which was almost the RDA for these subjects (Committee on Dietary Allowances 1989). Based on nitrogen balance, Walberg et al. (36) suggested that a protein intake of $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ was insufficient to maintain nitrogen balance during body weight reduction, whereas $1.6 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ was enough. It has been shown that in catabolic states such as starvation, the plasma concentrations of branched-chain amino acids (BCAA), i.e. leucine and valine, were selectively increased (33). It has become widely accepted that BCAA and leucine in particular can directly stimulate protein synthesis.

Table 1 Descriptive diets composition¹.

Variable	C group (n = 6)	hC group (n = 6)	hHP group (n = 7)	hBCAA group (n = 6)	hLP group (n = 6)
Carbohydrates (%) ²	55	55	60	60	60
Proteins (%)	12	12	25	20	15
Fats (%)	33	33	15	20	25
Energy intake (kcal · kg ⁻¹ · day ⁻¹)	40	28	28	28	28

¹ C = normocaloric control diet; hC = hypocaloric diet; hHP = high-protein diet; hBCAA = high-branched-chain amino acids diet; hLP = low-protein diet

² percentage of calorie intake

Louard et al. (25) observed that in normal man, BCAA infusion suppresses skeletal muscle proteolysis independently of any rise of plasma insulin. Furthermore, Schena et al. (31) concluded that the administration of a suitable amount of BCAA can prevent the ubiquitous muscle loss observed during high altitude exposure. BCAA administration potentiated the release of some anabolic hormones, mainly human growth hormone (GH) (17), known to accelerate amino acid uptake and protein synthesis in skeletal muscle (32). It may thus be suggested that under conditions of increased protein catabolism (hypercaloric diet), BCAA will help minimise muscle wasting.

The purpose of this study was to investigate the effects of 19-day qualitatively and quantitatively varied energy intakes on body composition and exercise performance in elite male wrestlers.

Material and Methods

Subjects

Thirty-one male wrestlers from the French National Institute of Sports volunteered to serve as subjects in this study. Testing was conducted mid-season. The study was approved by Cochin-Port Royal Hospital Ethics Committee. All subjects were informed of risks and signed consent forms. All were highly trained in prolonged exercise and had been wrestling for many years. None reported ever using anabolic steroids. The subjects were randomly assigned to one of five groups and consumed diets which varied in their energy and macronutrient composition (four levels of dietary protein intake, detailed in Table 1):

- Normocaloric control (n = 6): C
- Hypocaloric control (n = 6): hC
- Hypocaloric high-protein (n = 7): hHP
- Hypocaloric high-branched-chain amino acid (n = 6): hBCAA
- Hypocaloric low-protein (n = 6): hLP

Anthropometric measurements

Height, weight and BMI

Height of the barefoot subject was measured to the nearest 0.1 cm. Before and immediately after the dietary period, body weight (BW) of subjects wearing same shorts was measured on the same standard medical balance with an accuracy of ± 100 g. Body Mass Index (kg · m⁻²) was then calculated (5).

Skinfold thickness

The anthropometric assessment consisted of three skinfold sites: chest, quadriceps and abdomen, which were measured with a Harpenden skinfold calliper (18). A minimum of two measurements were made at each skinfold site by the same experienced investigator at each time of measurement. The values were averaged and used to estimate body fat percentage using an equation developed by Jackson and Pollock (18).

MRI

Magnetic resonance images were taken at 0.5 Tesla (General Electric, Milwaukee, WIS, U.S.A.). Before and after the dietary period, subjects were positioned in the magnet in a supine feet-first-position. A multislice spin-echo sequence (TR = 500 ms, TE = 25 ms) was used to obtain T1-weighted images with a reasonable contrast between adipose tissue (AT) and lean tissue.

The magnetic resonance examination consisted of two series of 7 mm thick (3 mm gap) axial images. One series was taken at midhigh, and two other series were taken in the abdomen at the level of the umbilicus.

As discussed by Ross et al. (30), magnetic resonance imaging (MRI) pixel intensity value for a given tissue may not be consistent from slice to slice or between individuals. To set a common dynamic range for all images, a 1.5-cm diameter reference tube was filled to capacity with a solution of Gadolinium-DOTA (0.5 mmol · l⁻¹) and placed on either of the sides of the subjects during data acquisition. The end points of the signal range were 0 for air and 4095 (maximum value for the 12 bit integers returned by the MRI system) for the reference tube. All the images were transferred onto personal Iris Computer (Silicon graphics) for further analysis.

Segmentation of Adipose Tissue (AT)

Two large regions of interest (ROI) were drawn manually. One ROI included the largest cross-sectional area of subcutaneous adipose tissue (SAT) and the other ROI included the largest cross-sectional area of visceral adipose tissue (VAT). The next step consisted of highlighting and counting the pixels which correspond to AT in each ROI. The reference tube allows determination of the threshold of AT. The threshold selected for AT was based on the analysis of a sample of typical images and their respective gray level histograms. The optimal threshold for AT was 180 (on a scale of 256); above this value, pixels were considered as representing AT. The next step involved

highlighting the pixels that were counted as AT in response to the selected threshold. Each slice was reviewed using an interactive slice editor program that allowed verification and, when necessary, correction of the segmentation result. The operation was facilitated by superimposing the original gray level image on the binary segmented image using a transparency mode. Visceral and subcutaneous adipose tissue regions on each abdominal slice were then assigned different colour codes. Similar operations were performed on each midthigh slice to identify adipose tissue and muscle tissue (MT).

Calculation of areas

The areas of the respective AT regions in each slice were computed automatically by summing AT pixels and multiplying by the pixel surface area. A previous investigation (30) verified that the error in the spatial dimensions of MR images using this system ranged from 0.1 to 1.5%. Thus the AT area on the MR images is fairly accurate.

Diet analysis

Wrestlers recorded their daily weighed-meals intake over seven successive days in notebooks supplied by the investigators. One training session was held to show the subjects how to keep records. During the weeks of the recording, the investigators met with the subjects and reminded them to record the amount and type of all foods consumed. The complete records of food intake of all subjects were analysed using Dietetique I software (copyright S.D. Grun for SDG ©1986-1987, Database by Mouton-Pastre-Peres for SPEFS ©1985-1986-1987) before the beginning of the study. Total daily intake of energy, protein, carbohydrate and fat were estimated daily and averaged for one week.

Dietary protocol

During the study, all the meals of the wrestlers were eaten in the same refectory. Estimates of the nutrient compositions of food ingested were made by weighing foods and recording the energy content of each portion. All wrestlers generally ate from a common menu. A dietician gave instructions on what to choose to obtain the desired diet, according to the specifications of the diet group.

The 19-day diet period began immediately following the period of nutrient composition food intake analysis. In all groups, diets exceeded RDA for protein intake ($0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). Subjects on hypocaloric diets consumed the same food and received a commercial supplement depending on the group they belonged to. The experimental diets were isocaloric and the daily energy intake of each subject (except C subjects) was estimated to be $28 \text{ kcal} \cdot \text{kg}^{-1}$. The commercial dietary supplements used in the present study represented $3.6 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$.

Subjects of the C group consumed *ad libitum* (normocaloric diet), while subjects of the hC group consumed a $28 \text{ kcal} \cdot \text{kg}^{-1}$ body weight $\cdot \text{day}^{-1}$ hypocaloric diet without any supplementation.

The subjects of the hHP group consumed a hypocaloric diet ($24.4 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and a dietary supplement which consisted of a mixture of soja protein ($0.9 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) so that

protein intake was increased to $2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$; 25% of the total energy intake ($6.1 \text{ g BCAA}/100 \text{ g protein}$: 46% leucine, 22% isoleucine and 32% valine) with 60% carbohydrate and 15% fat.

The hBCAA diet consisted of a hypocaloric diet ($24.4 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and a protein dietary supplement enriched with $0.9 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ branched-chain amino acids ($51.9 \text{ g BCAA}/100 \text{ g protein}$: 76% leucine, 19% isoleucine and 5% valine), approximately 20% protein, 60% carbohydrate and 20% fat.

The hLP diet consisted of a hypocaloric diet ($24.4 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and a dietary supplement of $0.9 \text{ g glucose} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ($3.6 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$), approximately 15% protein, 60% carbohydrate and 25% fat.

Exercise testing

During the two days prior to the dietary program, the maximal oxygen uptake (VO_2max) was determined using an open system to analyse the expired gases (Sensor Medics 2900, Sensor Medics corporation, Yorba Linda, CA, USA). On the morning of the first day, the subjects exercised on a treadmill according to an incremental protocol for VO_2max determination ($\text{VO}_2\text{max T}$). The exercise session of $\text{VO}_2\text{max T}$ consisted of a five min warm-up at $12 \text{ km} \cdot \text{h}^{-1}$ followed by an incremental test during which successive speeds of $1 \text{ km} \cdot \text{h}^{-1}$ were reached every two minutes. On the second morning, they exercised on an arm ergometer (881 E, Monark-Crescent A.B., Valberg, Sweden) for VO_2max determination ($\text{VO}_2\text{max U}$). The exercise session of $\text{VO}_2\text{max U}$ consisted of a two min warm-up at 36.8 W followed by incremental work during which successive loads of 18.4 W were reached every two minutes. A pedalling frequency of 75 rpm was selected until 110 W was obtained and then upwards to 85 rpm. The test protocol was adjusted to ensure attainment of maximal effort within 15 to 20 min. Oxygen intake, minute ventilation, respiratory exchange ratio (R) and heart rate (HR) were continuously monitored. VO_2max was the value obtained over 60 s when two of the following criteria were retained: 1) an increase in exercise intensity produced no further increase in VO_2 , 2) a respiratory exchange ratio > 1.1 , and 3) visual signs of tiredness and distress on the treadmill or with the crankarm ergometer.

The first afternoon, right isometric knee extensions were performed using an isokinetic dynamometer (Cybex II, Lumex Inc., N.Y., U.S.A.). The subjects were in a sitting position, securely strapped into the muscle-test chair. The seated posture met the following specifications: hip angle 100° , knee angle 80° . The experimental procedure comprised the following steps: 1) subjects were asked to perform maximal isometric contractions of short duration (2-2 s) of the knee extensor muscles. The maximal force was measured, and the best performance after 4 trials was selected as maximal voluntary isometric contraction (MVC). Approximately 2-3 minutes elapsed between each of the 4 trials. 2) following a sufficient recovery period lasting between 15 and 17 minutes, subjects maintained a prolonged isometric contraction of the knee extensor muscles at 50% MV as long as possible. The required force of contraction was shown by an oscilloscope placed in front of the subject. The endurance time for this submaximal muscle contraction was measured until the subject was unable to maintain the required tension.

The second afternoon, the anaerobic capacity was determined using the Wingate Anaerobic Capacity Test (2). Subjects completed a 30-sec Wingate Anaerobic Capacity Test on a Monark cycle ergometer (Monark-Crescent A.B., Valberg, Sweden) with two clips and adapted with an electronic reed switch to measure speed (revolutions per minute, rpm). The resistance for each subject ($0.098 \cdot \text{kg}^{-1}$ body weight) was determined according to Evans and Quinney (7). Five sec revolutions were counted for 30 sec using an electronic rpm counter. From these measurements, peak power, total power and mean power were calculated (2).

All physical performance tests were conducted two days prior to the initiation of the study and the two last days of the dietary period.

Blood sample analyses

Blood samples of approximately 10 ml were collected from an antecubital vein at rest, and at the end of the incremental treadmill exercise. Blood samples were collected before and after the dietary program, and plasma was immediately obtained by centrifugation and frozen until assays were performed within 30 days. At rest, glucose, lactate and glycerol were assayed in neutralised blood samples using an enzymatic method (3). Free fatty acids (FFA) were assayed according to the method of Ho (12). Because low caloric diets are known to affect the regulation of peripheral thyroid hormones (27) and because protein and amino acid intakes promote a rise in growth hormone serum levels (19,27), the endocrine responses to diets were tested. Hormone determinations were made in duplicate using commercially available radioimmunoassay kits (CIS-bio International, Gif-sur-Yvette, France); insulin (SB-INSI-5), growth hormone (GH) (SB-GH) and triiodothyronine (RIA-gnost T3).

Statistical analysis

All data are given as means \pm standard error of the mean (SEM). Significant time (pre- and post-diet measurements) and diet effects (C, hC, hHP, hBCAA, hLP) were determined by a two-way repeated measures analysis of variance (ANOVA). If the F-value indicated significance between group variance, comparisons between pre- and post-values in each experimental group (C, hC, hHP, hBCAA and hLP) were determined using a paired Student's t-test, $p < 0.05$ after adjustment using the Bonferroni correction was considered to indicate a significant difference between groups. The percentage differences between measurements obtained pre- and post-dietary period in each group were compared with an unpaired Student's t-test. A level of $p < 0.05$ was selected to indicate significant differences between mean values.

Results

Body weight

Analysis of variance (ANOVA) showed that there is a global effect of both time and diet on BW changes ($p < 0.05$, Table 2). BW losses recorded in all hypoenergy groups were significantly higher than those observed in control subjects (Fig. 1, $p < 0.05$). Moreover, the highest BW loss was observed for subjects of

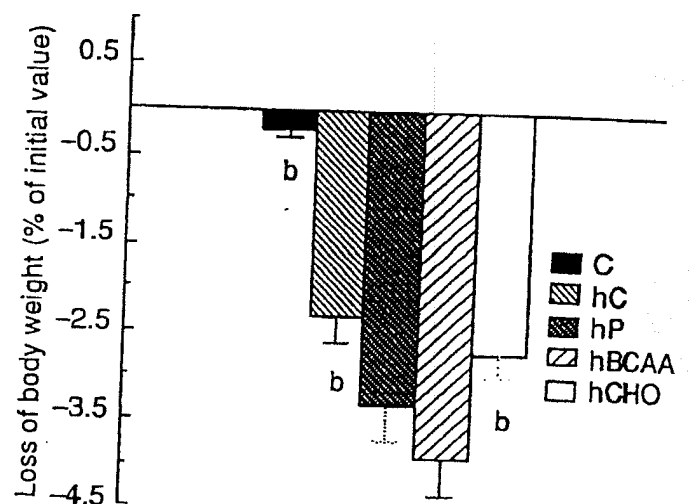


Fig. 1 Comparison between the relative losses of weight of different diets: normocaloric-control (C, $n = 6$), hypocaloric-control (hC, $n = 6$), hypocaloric high-protein (hP, $n = 7$), hypocaloric high-branched-chain amino acid (hBCAA, $n = 6$) and hypocaloric low-protein (hCHO, $n = 6$). $X \pm SD$. ^b significant vs hBCAA group, $p < 0.05$ (paired t-test).

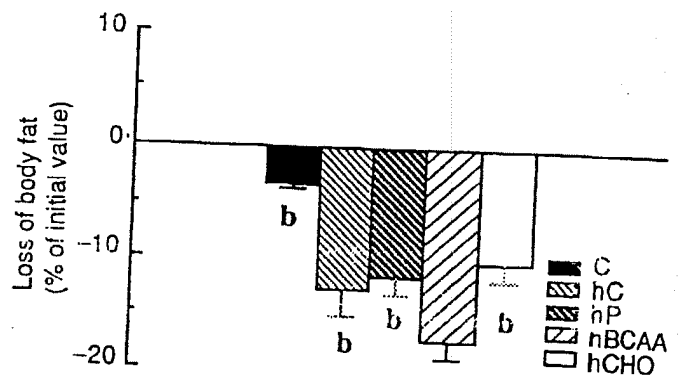


Fig. 2 Comparison between the relative losses of body fat of different diets: normocaloric-control (C, $n = 6$), hypocaloric-control (hC, $n = 6$), hypocaloric high-protein (hP, $n = 7$), hypocaloric high-branched, chain amino acid (hBCAA, $n = 6$) and hypocaloric low-protein (hCHO, $n = 6$). $X \pm SD$. ^b significant vs hBCAA group, $p < 0.05$ (paired t-test).

the hBCAA group, who reduced significantly their BW (4 kg, -5.4% , Fig. 1).

Body composition

Body fat estimated by skinfold thickness

There was a global effect of both time and diet on body fat changes (Table 2, $p < 0.05$). The decrease in the percentage of body fat (BF) of hBCAA subjects was significantly higher than that recorded in the other hypoenergy groups ($p < 0.05$). Subjects receiving BCAA supplementation lost an average of 17.3% of their estimated % BF using skinfold measures (Fig. 2).

Body fat estimated by MRI

Adipose tissue and lean tissue were clearly identified due to large differences in signal on magnetic resonance images (MRI).

Table 2 Pre-diet descriptive characteristics of subjects and post-diet changes in selected anthropometric and magnetic resonance imaging (MRI) variables¹.

Variable	C group (n = 6)		hC group (n = 6)		hHP group (n = 7)		hBCAA group (n = 6)		hLP group (n = 6)	
	Pre-diet	Post-diet	Pre-diet	Post-diet	Pre-diet	Post-diet	Pre-diet	Post-diet	Pre-diet	Post-diet
Anthropometry										
Age (y)	22 ± 4	22 ± 4	23 ± 4	23 ± 4	24 ± 4	24 ± 4	23 ± 4	23 ± 4	23 ± 4	23 ± 4
BW (kg) ²	65.2 ± 10.9	65.1 ± 10.8 ^b	82.7 ± 9.9	80.8 ± 10.1 ^b	75.9 ± 10.1	73.5 ± 10.2 ^b	74.4 ± 11.1	70.4 ± 10.4 ^c	72.8 ± 7.0	70.9 ± 7.0 ^b
BMI (kg·m ⁻²) ³	22.6 ± 0.6	22.5 ± 0.6	26.1 ± 1.0	25.5 ± 0.7	24.8 ± 0.5	24.0 ± 0.4	25.1 ± 1.0	24.2 ± 0.8	24.0 ± 0.8	23.4 ± 0.6
BF4 (%)	5.8 ± 2.0	5.6 ± 1.8	7.8 ± 3.4	6.8 ± 2.6 ^{b,c}	7.8 ± 1.9	6.9 ± 1.6 ^{b,c}	8.1 ± 3.1	6.6 ± 2.1 ^c	6.9 ± 1.4	6.2 ± 1.4 ^{b,c}
MRI⁵ (abdomen)										
SAT ⁶ (cm ²)	20.4 ± 3.5	21.1 ± 5.0	53.7 ± 8.1	44.6 ± 6.9 ^c	54.0 ± 3.0	42.8 ± 4.5 ^c	52.2 ± 2.0	37.8 ± 1.7 ^c	51.9 ± 9.8	42.6 ± 9.9 ^c
VAT ⁷ (cm ²)	55.5 ± 0.5	55.9 ± 1.0	60.2 ± 1.5	51.8 ± 1.5 ^{b,c}	59.4 ± 2.4	45.9 ± 2.1 ^{b,c}	56.3 ± 1.6	36.9 ± 1.6 ^c	56.5 ± 6.9	44.9 ± 1.9 ^{b,c}
MRI⁸ (thigh)										
AT (cm ²) ⁹	17.3 ± 3.8	16.9 ± 3.8	32.2 ± 3.6	28.3 ± 3.0 ^c	30.5 ± 3.8	25.8 ± 2.7 ^c	28.5 ± 2.2	21.8 ± 2.1 ^c	27.5 ± 4.9	24.6 ± 4.5 ^c
MT (cm ²) ¹⁰	178.0 ± 9.2	177.5 ± 9.4	226.4 ± 12.5	215.1 ± 10.8	208.2 ± 12.9	193.3 ± 8.9	208.7 ± 10.5	200.9 ± 10.3	207.4 ± 10.5	193.9 ± 8.9

¹ x ± SEM. C = normocaloric control group; hC = hypocaloric diet; hHP = high-protein diet; hBCAA = high-branched-chain amino acids diet; hLP = low-protein diet
² BW = Body weight (kg)
³ BMI (kg · m⁻²) = body mass index
⁴ BF = Body fat (using skinfold thickness)
⁵ MRI = magnetic resonance images obtained at umbilicus level
⁶ SAT = subcutaneous adipose tissue
⁷ VAT = visceral adipose tissue
⁸ MRI = magnetic resonance images obtained at midhigh level
⁹ AT = Adipose tissue
¹⁰ MT = Muscle tissue
^a Significant vs C group, p < 0.05 (paired t-test)
^b Significant vs hBCAA group, p < 0.05 (paired t-test)
^c Significant difference compared with pre-diet values vs post-diet values, p < 0.05 (paired t-test)

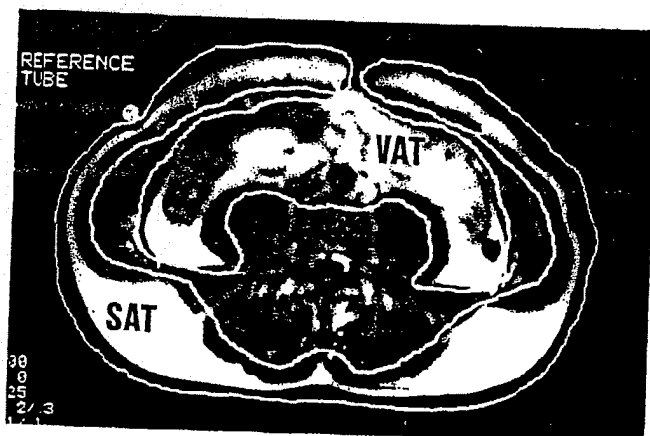


Fig. 3 Magnetic resonance imaging: Axial T1-weighted image obtained at the level of umbilicus. Two large regions of interest (ROI A and ROI B) are drawn manually. The ROI A includes the largest cross-sectional area of subcutaneous adipose tissue (AT) and the ROI B includes the largest cross-sectional area of visceral adipose tissue. The next step consists in highlighting and counting the pixels which correspond to AT in each ROI. The reference tube allows to determine the threshold of AT.

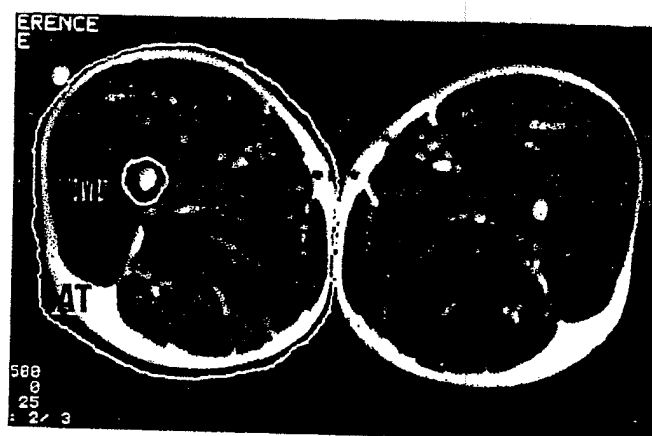


Fig. 4 Magnetic resonance imaging: Axial T1-weighted image obtained at midhigh.

Fig. 3 shows an abdominal cross-sectional image obtained at the level of the umbilicus and Fig. 4 a cross-sectional image obtained at midhigh. The areas of AT at these two different levels are shown in Table 2. As shown by Fig. 3, the SAT and VAT compartments are clearly distinguished from lean tissue and, thus, were segmented in a straight forward manner. ANOVA indi-

cated a significant effect of diets on SAT area changes (p < 0.05). The percent changes in SAT were not significantly different between hypoenergy groups (p = 0.12, Fig. 5). Analysis of variance showed a global effect of both time and diet on VAT area changes (p < 0.05). The VAT losses (-34.4%) observed in the hBCAA group were significantly higher than those recorded in other groups (p < 0.05, Fig. 5).

As shown in Fig. 4, AT at midhigh were clearly distinguished from muscle tissue. Analysis of variance showed a significant effect of both time and diet on thigh muscle AT (p < 0.05). After

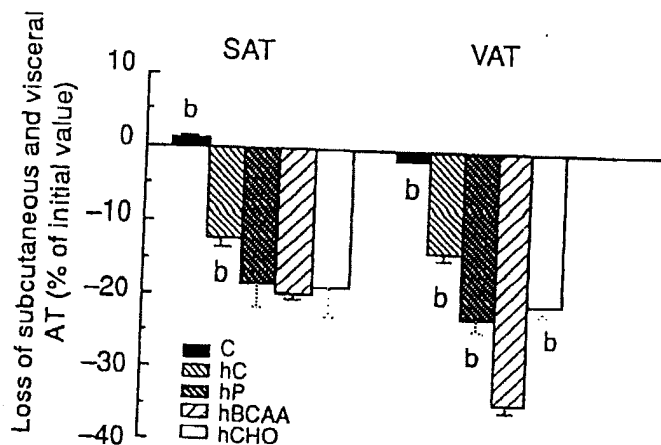


Fig. 5 Comparison between the relative losses of subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) of different diets: normocaloric-control (C, $n = 6$), hypocaloric-control (hC, $n = 6$), hypocaloric high-protein (hP, $n = 7$), hypocaloric high-branched-chain amino acid (hBCAA, $n = 6$) and hypocaloric low-protein (hCHO, $n = 6$). $X \pm SD$. ^b significant vs hBCAA group, $p < 0.05$ (paired t-test). Transverse abdominal images acquired at the level of the abdomen (umbilic) by resonance magnetic imaging (MRI).

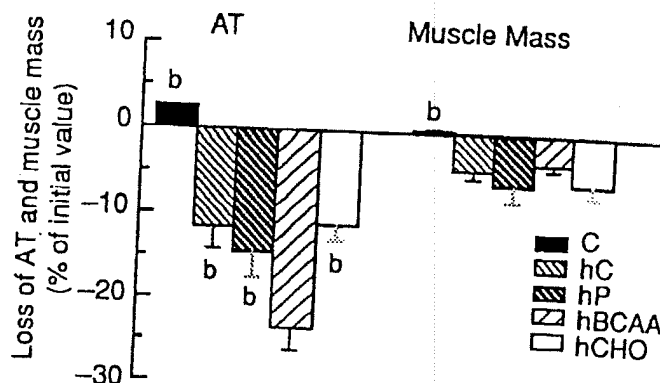


Fig. 6 Comparison between the relative losses of subcutaneous adipose tissue (SAT) and Muscle Mass of different diets: normocaloric-control (C, $n = 6$), hypocaloric-control (hC, $n = 6$), hypocaloric high-protein (hP, $n = 7$), hypocaloric high-branched-chain amino acid (hBCAA, $n = 6$) and hypocaloric low-protein (hCHO, $n = 6$). $X \pm SD$. ^b significant vs hBCAA group, $p < 0.05$ (paired t-test). Cross-sectional images acquired at mid thigh by resonance magnetic imaging (MRI).

Table 3 Pre-diet values and post-diet changes in aerobic capacities, anaerobic capacities and in muscular strength¹.

Variable	C group (n = 6)		hC group (n = 6)		hHP group (n = 7)		hBCAA group (n = 6)		hLP group (n = 6)	
	Pre-diet	Post-diet	Pre-diet	Post-diet	Pre-diet	Post-diet	Pre-diet	Post-diet	Pre-diet	Post-diet
$VO_{2max} T$ ($l \cdot min^{-1}$) ²	3.9 ± 0.4	3.9 ± 0.5	4.7 ± 0.5	4.5 ± 0.4	4.4 ± 0.5	4.2 ± 0.6	4.3 ± 0.4	4.2 ± 0.4	4.1 ± 0.4	4.2 ± 0.4
$VO_{2max} T$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	60.5 ± 2.2	60.9 ± 3.2	55.0 ± 1.2	56.2 ± 1.6	58.6 ± 1.8	59.4 ± 2.1	57.6 ± 2.1	59.5 ± 1.7	56.6 ± 0.7	59.2 ± 1.9
$VO_{2max} U$ ($l \cdot min^{-1}$) ³	2.9 ± 0.5	2.9 ± 0.4	3.6 ± 0.4	3.4 ± 0.3	3.5 ± 0.7	3.5 ± 0.5	3.4 ± 0.6	3.3 ± 0.2	3.0 ± 0.3	3.0 ± 0.2
$VO_{2max} U$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	44.8 ± 3.0	44.4 ± 1.7	43.9 ± 2.8	43.0 ± 1.9	45.7 ± 2.7	47.4 ± 2.2	45.6 ± 2.3	46.0 ± 1.9	40.8 ± 2.4	45.3 ± 1.6
PO (watt) ⁴	571.7 ± 71.4	566.8 ± 65.6	756.5 ± 157.2	767.6 ± 168.7	723.6 ± 57.9	735.5 ± 56.0	760.9 ± 172.8	755.2 ± 206.8	621.3 ± 43.4	715.1 ± 97.3
MVC (N · m) ⁵	255.5 ± 63.3	254.5 ± 37.9	345.5 ± 64.8	358.0 ± 62.1	314.4 ± 73.7	311.7 ± 77.6	302.5 ± 73.1	300.1 ± 75.4	282.0 ± 46.0	285.1 ± 65.0
Endurance time at 50% MVC (s) ⁶	77.8 ± 20.5	75.5 ± 12.5	77.2 ± 17.9	80.2 ± 10.6	78.7 ± 12.9	90.9 ± 20.6	74.5 ± 28.1	83.0 ± 27.4	85.5 ± 17.9	80.5 ± 13.3

¹ $x \pm SEM$, C = normocaloric control group; hC = hypocaloric diet; hHP = high-protein diet; hBCAA = high branched-chain amino acids diet; hLP = low-protein diet
² $VO_{2max} T$ = measurements at maximal oxygen uptake
³ $VO_{2max} U$ = measurements at maximal oxygen uptake
⁴ PO = peak power output (anaerobic Wingate test)
⁵ MVC = maximal isometric voluntary contraction
⁶ Endurance time of the knee extensor muscle at 50% of MVC

the 19-day hBCAA diet, losses of AT were significantly higher (-23.4%) than those observed in other hypoenergy groups ($p < 0.05$, Fig. 6). However, muscle mass loss was not significantly affected by the diets in hypoenergy groups.

Exercise capacity

The 19-day diet program did not significantly affect VO_{2max} measured either on the treadmill or using the crankarm ergometer in any group (Table 3).

Nineteen days of caloric restriction did not reduce peak power output in any group. Dieting did not significantly alter the subject's capacity to perform anaerobic work.

The MVC of the knee extensor muscle was unaffected by the dietary protocol (Table 3) as well as the endurance time of the knee extensor muscle at 50% MVC.

Blood metabolic changes

Plasma concentrations of glucose, lactate and insulin were unchanged after the 19-day dietary regimen (Table 4). However, the nitrogen-enriched (N-enriched) diets induced lower blood concentrations of FFA (-25% , -35.7% , in the hBCAA and hHP groups, respectively) and higher blood concentrations of glycerol (62.9% , 58.7% , in hBCAA and hHP groups, respectively, $p < 0.05$).

Table 4 Post-diet and post-exercise (treadmill exercise) changes in circulating metabolite and hormone concentrations¹.

Variable	C group (n = 6)		hC group (n = 6)		hHP group (n = 7)		hBCAA group (n = 6)		hLP group (n = 6)	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Before Diet										
Glucose (mmol · l ⁻¹)	4.09 ± 0.46	5.28 ± 0.37	5.28 ± 0.78	6.85 ± 0.32	4.14 ± 0.20	7.37 ± 0.60 ^a	4.43 ± 0.32	7.52 ± 0.36 ^a	4.50 ± 0.21	7.37 ± 0.45 ^a
Insulin (mU · l ⁻¹)	16.02 ± 3.15	22.23 ± 5.66	20.94 ± 4.27	27.36 ± 9.04	16.25 ± 3.79	20.29 ± 4.40	14.62 ± 2.39	19.89 ± 1.64	21.25 ± 5.54	31.59 ± 9.16
Lactate (mmol · l ⁻¹)	1.11 ± 0.17	10.29 ± 1.00 ^a	2.12 ± 0.26	10.75 ± 1.48 ^a	1.83 ± 0.22	10.46 ± 0.58 ^a	2.33 ± 0.24	10.54 ± 1.00 ^a	1.77 ± 0.18	12.54 ± 1.36 ^a
FFA (mmol · l ⁻¹) ²	0.12 ± 0.02	0.30 ± 0.07 ^a	0.13 ± 0.01	0.33 ± 0.07 ^a	0.14 ± 0.03	0.30 ± 0.07	0.16 ± 0.01	0.32 ± 0.05 ^a	0.13 ± 0.06	0.19 ± 0.02
Glycerol (mmol · l ⁻¹)	13.8 ± 1.9	28.3 ± 1.9 ^a	15.4 ± 2.4	38.8 ± 1.2 ^a	15.0 ± 1.0	31.0 ± 3.6 ^a	14.0 ± 2.9	35.0 ± 4.1 ^a	16.7 ± 1.7	29.8 ± 4.3 ^a
T3 (mU · l ⁻¹) ³	1.83 ± 0.06		1.69 ± 1.09		1.81 ± 0.08		1.87 ± 1.08		1.70 ± 0.14	
GH (mU · l ⁻¹) ⁴	0.87 ± 0.11	13.96 ± 7.39 ^a	0.87 ± 0.41	14.17 ± 6.51 ^a	0.89 ± 0.38	12.67 ± 3.40 ^a	0.77 ± 0.57	14.00 ± 3.98 ^a	0.74 ± 0.42	12.65 ± 5.90 ^a
After Diet										
Glucose (mmol · l ⁻¹)	3.26 ± 0.20	5.37 ± 0.24 ^a	3.69 ± 0.41	5.05 ± 0.59	3.91 ± 0.30	6.36 ± 0.24 ^a	3.63 ± 0.23	6.13 ± 0.35 ^a	4.10 ± 0.12	6.05 ± 0.29 ^a
Insulin (mU · l ⁻¹)	21.31 ± 3.31	23.35 ± 3.70	15.30 ± 1.27	16.50 ± 0.81	19.34 ± 3.43	20.43 ± 4.45	17.49 ± 1.48	18.30 ± 2.48	25.17 ± 5.10	26.73 ± 4.78
Lactate (mmol · l ⁻¹)	1.10 ± 0.18	8.32 ± 0.61 ^a	1.18 ± 0.17	8.84 ± 1.42 ^a	1.31 ± 0.17	10.46 ± 1.10 ^a	1.83 ± 0.34	9.73 ± 1.40 ^a	1.48 ± 0.38	11.21 ± 1.58 ^a
FFA (mmol · l ⁻¹)	0.13 ± 0.01	0.30 ± 0.07 ^a	0.10 ± 0.01	0.21 ± 0.03 ^a	0.09 ± 0.01 ^c	0.27 ± 0.06 ^a	0.12 ± 0.01 ^c	0.18 ± 0.02 ^c	0.15 ± 0.05	0.21 ± 0.03 ^a
Glycerol (mmol · l ⁻¹)	11.1 ± 2.4	27.8 ± 2.2 ^a	15.0 ± 2.0	23.4 ± 2.0 ^a	23.8 ± 1.3 ^c	27.7 ± 1.6 ^a	22.8 ± 1.4 ^c	25.3 ± 2.5 ^c	14.8 ± 1.3	31.0 ± 5.7 ^a
T3 (mU · l ⁻¹)	1.83 ± 0.06		1.77 ± 0.09		1.58 ± 0.09 ^c		1.68 ± 0.06 ^c		1.73 ± 0.13	
GH (mU · l ⁻¹)	0.92 ± 0.10	15.26 ± 6.14 ^{ab}	1.22 ± 0.42 ^{bc}	19.80 ± 4.90 ^{ab}	2.71 ± 1.05 ^c	25.81 ± 3.10 ^{ac}	2.85 ± 0.03 ^c	26.30 ± 3.43 ^{ac}	0.90 ± 0.10 ^{bc}	15.87 ± 1.98 ^{ab}

¹ x ± SEM. C = normocaloric control group; hC = hypocaloric diet; hHP = high-protein diet; hBCAA = high-branched-chain amino acids diet; hLP = low-protein diet
² FFA = fat fatty acids
³ T3 = tri-iodothyronine
⁴ GH = human growth hormone
^a Significant difference compared with pre-exercise values vs post-exercise values, p < 0.05 (paired t-test)
^b Significant difference vs BCAA values, p < 0.05 (paired t-test)
^c Significant difference compared with pre-diet values vs post-diet value, p < 0.05 (paired t-test)

Plasma T3 concentration was significantly decreased after 19-day N-enriched diets (p < 0.05). Prior to dietary restriction, resting GH concentrations were similar for all groups. The 19-day diet affected all groups equally, resulting in a significant rise at rest (p < 0.05). In conditions of pre- or post-diet, exercise increased the GH concentrations for all groups. However, the N-enriched diets induced a significant increase in post-exercise plasma GH concentrations (p < 0.05).

Discussion

Dietary restrictions may cause a loss of muscle mass and, if sustained, ultimately reduce work capacity. The changes in percent body fat and lean body mass can be viewed as indicative of the subjects response to the regimen of dietary restriction and training (38). Within the 19-day confines of this study, the results of body composition analysis indicate that during periods of energy restriction, BCAA supplementation was able to maximise loss of VAT and thigh AT.

Results of the effects of gradual body weight reduction on aerobic performance in athletes are variable. VO₂max might deteriorate (16,38), remain unchanged (26) or improve (24). In our study, VO₂ was maintained in all groups. This finding supports the idea that aerobic working power was not affected by diet-induced weight loss. The present results are in agreement with the data of Armstrong et al. (1), Houston et al. (15) who found no significant reduction in maximal oxygen consumption occurring as a result of rapid weight loss.

The results of the present study showed that maximal anaerobic power output was not altered by weight loss (Table 3), similarly, the maximum voluntary force of isometric contraction and the endurance capacity at 50% MVC remained stable for all

groups (Table 3). Dietary carbohydrate intake might be important for preservation of muscle endurance (36). When reporting decreased anaerobic performance after a diet low in carbohydrate intake, Horswill et al. (13) demonstrated the importance of carbohydrates in wrestling-type performance. According to McMurray et al. (28) anaerobic performance was not affected after body weight loss with a reasonable intake of carbohydrates. An hypocaloric diet (24 kcal · kg⁻¹) with a high-carbohydrate content (4.2 g · kg⁻¹, 70% of energy intake) is sufficient to keep athletes in weight-class events and to continue training by maintaining adequate muscle glycogen stores (6). In our study, carbohydrate intake in all hypocaloric diets represents 4.1 or 4.5 g · kg⁻¹. This may partly explain why muscular performance was maintained.

In the present study, all hypocaloric diets showed an effect on body composition: both body weight and body fat diminished. When comparing the diets themselves, those, which tended to be most effective in weight reduction were the ones enriched with nitrogen compounds. Examining the two N-enriched diets, significant results for diminishing visceral abdominal adipose tissue occurred in the hBCAA diet. MRI changes are associated with parallel changes in skinfold thickness measurements. Hence, although the methods differ in absolute terms, they are relatively consistent between each other.

Gradual body weight reduction has been studied in 3 case reports and in a limited number of other studies. In studies of females by Inger and Sundgot-Borgen (16) and Walberg-Ranking et al. (38), the duration of body weight reduction varied between 1 and 12 weeks. Weekly body weight loss varied between 0.3 and 3.8 kg · week⁻¹. Widerman and Hagan (38) concluded that a highly trained wrestler can lose 8% of his original body weight, drop two weight classifications, and maintain or

improve his level of fitness and subsequent performance. In all studies (8, 10, 11, 26), the reduction of dietary energy intake was the primary technique for gradual body weight loss. The daily energy content of weight-reducing diet was typically between 18 and 32 kcal · kg⁻¹ body weight. In general, the dietary fat content was low (12 to 33% of total energy intake) and both protein and carbohydrate contents were high (9).

GH has been reported to have anabolic effects on muscle tissue (35). Previous reports have indicated that hypocaloric diets cause an increase in GH above control levels (21, 28, 29). Our results in hypocaloric groups are consistent with this notion. N-enriched diets altered plasma GH concentrations at rest. This agrees with the effects of dietary protein and amino acids on GH release (4, 23). On the other hand, it has been speculated that the increase in GH release was able to mobilise fat stores for energy production (19). This is consistent with the hypothesis that a high-protein, low-calorie diet maintains protein synthesis while energy needs are primarily met by endogenous and exogenous fat (4).

The results of the present study showed that the total concentration of T3 declined in both hHP and hBCAA groups. It is a well-known fact that plasma T3 concentrations decreased with low-calorie diets (20, 27). Why in the present study the decrease in plasma T3 was only observed in hHP and hBCAA groups remains to be determined.

Our results show that all hypocaloric diets induce a decrease in the muscle mass. Despite this decrease in the muscle mass, the muscle performance was maintained in all hypocaloric groups. The contribution of the different muscle components to the muscle mass changes may be questioned. Under the experimental conditions of the present study, it is difficult to determine whether the muscle mass loss is due to decreased fluids, proteolysis, and/or decreased glycogen and fat stores.

The compliance and tolerance of the subjects following their respective diets were good. Because the study began three months before competition, we did not show the direct effect of dieting on performances in competition. The nutrition strategy for many wrestlers before competitions was based on rapid weight loss. Therefore, this study provided them with a new approach to weight loss techniques.

In conclusion, results of the present study suggest that maintaining a high level of carbohydrate intake, no hypocaloric diets alter performance. Moreover, energy restriction diets induce a reduction in the body mass and favourable modifications in body composition. A high-branched-chain amino acid diet produces the highest losses in body fat. This can partly be explained as a result of specific hormonal adaptations. However, these observations should be confirmed using a greater number of subjects.

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