Effects of chronic administration of ephedrine during very-low-calorie diets on energy expenditure, protein metabolism and hormone levels in obese subjects

Renato PASQUALI*, Francesco CASIMIRRI*, Nazario MELCHIONDA*, Gabriele GROSSI†, Lucia BORTOLUZZI‡, Antonio Maria MORSELLI LABATE*, Chiara STEFANINI§ and Antonino RAITANO‡

*Istituto di Clinica Medica 1, †Laboratorio Centralizzato, and ‡Farmacia Interna, Ospedale S. Orsola, University Alma Mater of Bologna, Bologna, Italy

(Received 12 April/9 August 1991; accepted 19 August 1991)

1. We investigated the effects of the chronic administration of a sympathomimetic agent on energy expenditure, protein metabolism and levels of thyroid hormones and catecholamines in 10 obese subjects after a 6-week very-low-calorie-diet programme (1965 kJ, 60 g of protein, 45 g of carbohydrates). L-(-)-Ephedrine hydrochloride (50 mg three times a day by mouth) or placebo were administered during 2-week periods (weeks 2–5 of the VLCD programme) in a randomized, double-blind, crossover design. Five subjects began with ephedrine and five with placebo.

2. The results were analysed separately in the two groups. No difference was found between them as regards weight loss during the very-low-calorie diet and drug treatments. Conversely, ephedrine therapy induced a significantly lower daily urinary excretion of nitrogen (and, consequently, a better nitrogen balance) with respect to placebo, independently of the drug sequence. Daily urinary levels of 3-methylhistidine during ephedrine and placebo treatments were similar. The fasting resting metabolic rate (oxygen consumption, ml STP/min) fell significantly during the very-low-calorie diet in both groups, but this effect was partially and significantly prevented by administration of ephedrine. Diet therapy significantly reduced 24 h urine levels of vanillylmandelic acid and homovanillic acid, which, however, increased to pretreatment values during ephedrine treatment. No significant effects were shown on 24 h urinary concentrations of adrenaline, noradrenaline and dopamine during the very-low-calorie diet and/or ephedrine treatment. There were also no effects on the serum levels of thyrotropin, thyroxine, free-tri-iodothyronine and free-thyroxine, but ephedrine significantly prevented a further fall in the serum tri-iodothyronine level and the serum tri-iodothyronine/thyroxine ratio during the very-low-calorie diet.

3. These findings demonstrate that in obese subjects following a very-low-calorie-diet programme, administration of chronic adrenoceptor agonists, such as ephedrine, partially prevented the fall in resting metabolic rate and significantly improved the nitrogen balance. These effects may be of importance in the treatment of patients in whom a reduced capacity for energy expenditure may be involved in their obese state.

INTRODUCTION

It is well established that there are obese subjects who may be characterized by a reduced capacity for energy expenditure [1, 2]. This condition may be related to a genetic trait and/or to environmental and dietary factors [3–5], and can partially explain their propensity to become obese and the frequent relapses of body weight gain that occur in these subjects after dietary-induced weight loss despite the maintenance of a persistently low energy intake [6–8].

Thyroid hormones and the sympathetic nervous system represent the most important regulatory factors of energy balance in humans [9]. Several previous studies performed in laboratory animals have suggested that some models of obesity are characterized by impaired activity of the sympathetic nervous system, and preliminary data in humans appear to be consistent with these findings [10–12]. Ephedrine is a sympathomimetic drug which stimulates thermogenesis in animals [13, 14] as well as in man [11, 15–17]. Animal studies have clearly shown that it may reduce fat content and therefore body weight by mechanisms that involve increased energy expenditure and, to a lesser extent, reduced food intake [13]. In a preliminary clinical trial performed in ten selected obese women with stable weight, who had adapted to a chronically low energy intake, that were treated with a low-calorie diet, we observed that 150 mg of ephedrine/day induced a greater weight loss over 2 months than placebo, thus suggesting that such a thermogenic agent may have a

Key words: catecholamines, energy expenditure, ephedrine, obesity, protein metabolism, thyroid hormones, very-low-calorie diets.

Abbreviations: DA, dopamine; HVA, homovanillic acid; 3-MH, 3-methylhistidine; NA, noradrenaline; RMR, resting metabolic rate; T3, tri-iodothyronine; T4, thyroxine; TSH, thyrotropin; VCO2, CO2 production; VLCD, very-low-calorie diet; VMA, vanillylmandelic acid; VO2, O2 consumption.

Correspondence: Professor Renato Pasquali, Istituto di Clinica Medica 1, Ospedale S. Orsola, Via Massarenti 9, 40138 Bologna, Italy.
role in the therapeutic approach to selected obese subjects [18].

This study was planned to investigate the effects of administration of ephedrine on the fasting resting metabolic rate (RMR) and on the thyroid hormone and catecholamine concentrations in a group of obese subjects when following a very-low-calorie-diet (VLCD) programme. Moreover, since recent observations have reported that administration of β-adrenoceptor agonists may have anabolic effects on protein metabolism both in animals [19] and in man [20, 21], we also examined the effects of chronic administration of ephedrine on nitrogen balance and muscle protein breakdown. In fact, the development of a negative nitrogen balance, only partially limited by adequate amounts of high-quality protein intake, represents a crucial tool in the adaptive phenomena that occur during severe and prolonged underfeeding in obese individuals that has not yet been completely investigated.

MATERIALS AND METHODS

Patients

This study was carried out on ten obese in-patients of both sexes admitted to the Istituto di Clinica Medica 1 of the University of Bologna, Italy. The study was performed according to the Helsinki II declaration: all subjects volunteered for the study and gave their informed written consent after a full explanation of the purpose and risks of the study itself had been given. The approval of the Ethic Committee for Pharmacological Therapies of the S. Orsola Hospital, Bologna, was also obtained. The patients’ general characteristics are summarized in Table 1. None of them had diabetes, hypertension or other endocrine and metabolic diseases or any relevant cardiovascular, renal or liver abnormalities. None was taking any drugs before the study, was dieting nor had undergone X-ray examination with iodine compounds in the previous 12 months.

Table 1. General data of the obese subjects participating in the study. Subjects 1-5 formed group 1 and subjects 6-10 formed group 2 (see the Statistics section for details).

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>Height (m)</th>
<th>Body mass index (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>34</td>
<td>120</td>
<td>1.73</td>
<td>40.1</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>43</td>
<td>169.5</td>
<td>1.70</td>
<td>52.6</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>45</td>
<td>104</td>
<td>1.71</td>
<td>35.6</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>42</td>
<td>149.5</td>
<td>1.60</td>
<td>58.4</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>42</td>
<td>112</td>
<td>1.68</td>
<td>39.5</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>33</td>
<td>100.3</td>
<td>1.69</td>
<td>35.1</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>45</td>
<td>88.1</td>
<td>1.55</td>
<td>36.7</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>32</td>
<td>103.4</td>
<td>1.56</td>
<td>42.5</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>37</td>
<td>127.2</td>
<td>1.70</td>
<td>44.0</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>28</td>
<td>115.7</td>
<td>1.76</td>
<td>37.4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>38.1 ± 6.1</td>
<td>119.0 ± 24.5</td>
<td>1.67 ± 0.7</td>
<td>42.2 ± 7.7</td>
</tr>
</tbody>
</table>

Protocol

The protocol consisted of a 6-week treatment with a VLCD programme. On admission, all patients followed a 3-day standardized diet providing 8368 kJ and 250 g of carbohydrates. During this period, baseline data were collected and clinical, laboratory and diagnostic procedures were performed. All patients then received a VLCD providing 1965 kJ in the form of 60 g of proteins (20% as egg albumin and 80% as milk casein), 5 g of lipids (3.2 g as lecithin and 1.8 g as esterified fatty acids) and 45 g of carbohydrates (as maltodextrin). The diet was administered as liquid formula (Daily Dose M; Gazzoni SpA, Bologna, Italy) three times a day for 6 weeks. It contained all essential vitamins, micronutrients and trace elements to produce recommended daily allowances for the normal population and has been previously published in detailed form [22]. Water was allowed ad libitum (minimum 2 litres/day); allopurinol (100–300 mg/day) and ursooxycholic acid (300 mg/day in the evening) were also administered for the entire period of the study, in order to prevent the serum level of uric acid increasing and to reduce cholesterol concentrations in the bile, thus preventing gallstone formation [23]. Compliance with the nutritional programme was checked throughout the study period by determining the daily urinary excretion of sodium and potassium and urinary levels of acetoacetate (Ketur test; Boehringer-Biochimia-Robin, Milan, Italy).

Since during the first week on a VLCD, large water losses can mask fat loss and induce a high degree of variability between subjects [22], we started pharmacological treatment in the second week of the VLCD. All patients were assigned to a cross-over double-blind treatment with oral L-(−)-ephedrine hydrochloride (50 mg three times a day before each meal; Carlo Erba, Milan, Italy) or placebo for 2 weeks each. The sequence of each treatment was randomly assigned, so that five patients began with ephedrine and five began with placebo without any washout period between the treatments. Drugs were not administered during the sixth week of the VLCD programme.

Measurements

Fasting RMR. Energy expenditure was measured by indirect calorimetry with a Metabolic Measurement Cart (Beckman Instruments Inc., Schiller Park, IL, U.S.A.). RMR was determined in the morning (08.00–08.30 hours) after an overnight fast. Patients had been lying down for approximately 45–60 min before testing. Briefly, the Metabolic Measurements Cart is an instrument using a polarographic O₂ sensor (OM 11) and CO₂ sensor (LB2) calibrated to gases of known composition (16% O₂ and 4% CO₂) every 2–3 h and contains a barometer, a temperature sensor and a volume transducer, which are calibrated to independent instruments. The air exhaled by the subject through the mask is collected and passes through tubing to a gas collection drum from which a continuous aliquot (500 ml/min) is
Effects of ephedrine during very-low-calorie diets in obese subjects

withdrawn and passed through the gas analyser, volume transducer and temperature sensor. A programmable computer integrates data, performs calculations and prints the integrated values measured and calculated at predetermined time intervals (1 min). Measurements were performed for 30 min and the average of 30 consecutive integrated 1 min \( V_O_2 \) and \( V_CO_2 \) production values was used. Only equilibrated measurements were averaged (coefficients of variation for both \( V_O_2 \) and \( V_CO_2 \) less than 5%).

RMR measurements were obtained in basal conditions and at the end of each 2-week pharmacological treatment with either ephedrine or placebo.

Biochemistry. To obtain urinary 3-methylhistidinid (3-MH) concentrations during the baseline period, an adequate 24 h urine collection was made by each subject after 3 days of a meat-free diet, following all the procedures previously described by Lukaski & Mendez [24]. Daily urinary 3-MH excretion was then measured weekly. Assays were performed on urine samples stored at \(-20^\circ C\) until analysis. 3-MH was measured with an Aminonoranalyzer (Kontron, Liquimat III) using the ionic exchange resin technique after the urine samples had been deproteinized with trichloroacetic acid (pH 2) and filtered on Acrodisc (0.45 \( \mu m \); Gelman, Milano, Italy). Urinary excretion of nitrogen was measured during the VLCD in urine samples collected every day, frozen at \(-20^\circ C\) at the end of the collection, and then analysed as individual samples, each of which represented a pool of 7 days. The pool was made by adding together a quantity of urine (1 ml/l) from each day's sample. Urinary nitrogen excretion was determined by the standard Kjeldahl technique [25]. Assuming that faecal, integumental and miscellaneous nitrogen loss were constant (approximately 1.25 g/day) [26], nitrogen balance was calculated according to the formula:

\[
\text{Nitrogen input-nitrogen output (urinary nitrogen)} + 1.25 \text{ g of nitrogen}
\]

Determinations of the serum creatinine level and of the daily urinary excretion of creatinine in the same urine sample were made by using the enzymic colorimetric method (Creatinine PAP; Boehringer, Mannheim, Germany).

Hormones. Baseline blood samples for thyroid hormone analysis were collected on the same day that the subjects began the VLCD programme, then at weekly intervals and at the end of each pharmacological treatment. Samples were drawn in the morning (08.00–08.30 hours) after a 12 h overnight fast from an antecubital vein kept patent with saline (150 mmol/l NaCl), and were subsequently stored frozen at \(-20^\circ C\) until assayed. All assays were performed in duplicate. Thyrotropin (TSH), thyroxine (\( T_4 \)) and tri-iodothyronine (\( T_3 \)) were measured by an r.i.a. method with reagents obtained from Diagnostic Product Co. (Los Angeles, CA, U.S.A.); free \( T_4 \) and free \( T_3 \) fractions were determined with reagents obtained from Schavo (Cinisello Balsamo, Milano, Italy). The inter- and intra-assay coefficients of variation were respectively 13% and 9% for TSH, 6% and 5% for \( T_3 \), 8% and 6% for \( T_4 \), 11% and 8% for free \( T_4 \), 12% and 9% for free \( T_3 \). A 24 h urine collection was also obtained from each subject at weekly intervals and after each 2-week pharmacological period. Urine samples were acidified with HCl (0.1 mol/l of urine) during collection; the volume of each 24 h sample was immediately measured, and aliquots were taken and frozen at \(-70^\circ C\) until assayed for dopamine (DA), adrenaline, noradrenaline (NA), vanillylmandelic acid (3-methoxy-4-hydroxymandelic acid, VMA) and homovanillic acid (3-methoxy-4-hydroxy-phenilacetic acid, HVA), as previously described [27]. Urinary concentrations were expressed as \( \mu g \) of adrenaline, NA and DA/g of creatinine and as mg of metabolites (VMA and HVA)/g of creatinine.

Statistics

To examine the effects of ephedrine and placebo, the subjects were divided into two groups according to the sequence of pharmacological treatment: group 1 (\( n = 5 \)) started with ephedrine and then continued with placebo, and group 2 (\( n = 5 \)) received the drugs in the reverse order. No significant differences (Student's t test after log transformation) were seen in the subjects' general data between the two groups (see Table 1). The distribution of all variables showed no significant deviation from normality (\( P > 0.20 \)) with the Kolmogorov–Smirnov test. Because of the cross-over experimental protocol, analysis of variance and analysis of co-variance were used to take account of all the factors present in the statistical design [28, 29]. In the evaluation of weight loss and nitrogen balance, the factors taken into account by the analysis of variance test were the pharmacological treatment (ephedrine versus placebo) and the duration of each pharmacological treatment (week 1 and week 2 were examined separately). RMR was expressed as the percentage difference with respect to the previous measurement (week 3 versus basal and week 5 versus week 3) and the significant difference between ephedrine and placebo treatment was examined by means of analysis of variance. For the evaluation of thyroid hormones, catecholamines and 3-MH the analysis of co-variance test was applied with the basal values as co-variates. When variations of the data during the VLCD (after week 3, ephedrine or placebo treatment, and week 6) were compared with basal values, analysis of variance was used. Results are expressed as means ± SD.

RESULTS

Subjects

Tolerance of the diet regimen and the pharmacological therapy was good in all subjects and no side effects were observed throughout the study.

Body weight

Body weight loss was 17.0 ± 3.5 kg in the whole group of subjects, 17.9 ± 3.4 kg in group 1 and 16.0 ± 3.7 kg (not
significant) in group 2. Variations in body weight during the VLCD in subjects grouped according to the sequence of the pharmacological treatment is reported in Fig. 1. No significant difference (analysis of variance) in weight loss during placebo and ephedrine treatments was found.

Nitrogen balance

In the whole sample of subjects, the cumulative nitrogen balance during the VLCD (0-42 days) was 

\[-70.90 \pm 90.02 \text{ g, equal to 1.68 \pm 2.14 g/day.}\]

The results of the nitrogen balance studies in the two groups are reported in Table 2. No significant difference was observed in the data obtained during weeks 1 and 6, whereas nitrogen balance values during ephedrine treatment were significantly \((F=8.22; P=0.008)\) less negative than with placebo, regardless of the drug sequence. The duration of treatment (i.e. values observed in the second week of each treatment versus values observed after the first week) did not show any significant effect \((F=0.43; P=\text{not significant})\).

3-MH

Baseline values of urinary excretion of 3-MH in group 1 were \(243.3 \pm 148.5 \mu\text{mol/day}\) in group 1 and \(195.6 \pm 33.1 \mu\text{mol/day}\) in group 2. After the first week of the VLCD no significant change was found \((231 \pm 36.3 \mu\text{mol/day}\) in group 1 and \(181.2 \pm 35.1 \mu\text{mol/day}\) in group 2\). Values after ephedrine and placebo treatment in group 1 \((186.2 \pm 26.2 \mu\text{mol/day}\) and \(158.8 \pm 56.7 \mu\text{mol/day}, \text{respectively}\) as well as in group 2 \((159.8 \pm 34.5 \mu\text{mol/day}, \text{and 153.8} \pm 60.7 \mu\text{mol/day})\) were not significantly different. Similarly, no difference was found between the two groups (nor versus basal values) in values observed at the sixth week of VLCD (196.8 \pm 64.4 \mu\text{mol/day} and 161.0 \pm 34.7 \mu\text{mol/day}, respectively).

Fasting RMR

Compared with baseline values, RMR decreased significantly during placebo, whereas no significant fall occurred after administration of ephedrine. Values of RMR \((V_{O_{2}}, \text{ml STP/min})\) in group 1 were 274.6 \pm 49.6 at baseline, 261.0 \pm 44.9 after ephedrine (week 3) and 237.2 \pm 36.5 after placebo (week 5). Group 2, however, had values of 254.6 \pm 47.8 at baseline, 226.6 \pm 43.7 after placebo (week 3) and 224.4 \pm 40.2 after ephedrine (week 5). Percentage changes in RMR during ephedrine and placebo treatments were significantly lower during ephedrine than during placebo \((F=6.54; P=0.021).\) In particular, in group 2 with ephedrine treatment the RMR was stabilized and did not fall further (Table 3).

Thyroid hormones

With respect to basal concentrations, no significant variations were observed in serum levels of TSH, T$_3$, free T$_4$ and free T$_3$. In contrast, the serum T$_3$ level, the serum T$_3$/T$_4$ ratio and the serum free T$_3$/free T$_4$ ratio fell significantly during the VLCD (weeks 1–6) (analysis of variance versus basal values). Administration of ephedrine, however, did not affect the serum levels of TSH, T$_4$, free T$_4$ and free T$_3$, whereas it induced a higher serum level of T$_3$ \((F=3.92; P=0.060)\) and a higher serum T$_3$/T$_4$ ratio \((F=5.79; P=0.029)\) with respect to placebo (this effect was particularly evident in group 2) (Table 4).

Catecholamines

Urinary adrenaline, NA and DA concentrations did not show any significant variation during the VLCD or during pharmacological treatment (Table 5). Conversely, urinary VMA and HVA concentrations were significantly affected by diet and ephedrine therapy. In fact, the VLCD significantly reduced the urinary concentrations of these metabolites (analysis of variance versus basal values: \(P<0.05)\), whereas administration of ephedrine reversed them and produced values that were higher than the pretreatment values (ephedrine versus placebo: HVA, \(F=8.39, P=0.012; \text{VMA, } F=12.67, P=0.032\) (Table 5).
**DISCUSSION**

The results of the present study indicate that administration of ephedrine during a VLCD programme in obese subjects partially prevented the drop in RMR and maintained the serum protein-sparing effect as documented by a significant reduction in urinary nitrogen loss and an improvement in the nitrogen balance. Body weight loss showed no difference between the ephedrine and placebo therapies as would be expected on the basis of the short period of pharmacological treatment. Much more time is in fact needed to obtain significant effects with thermogenic agents in non-selected obese subjects treated with hypocaloric diets [30].

With long-term hypocaloric feeding and during VLCDs in obese individuals there is a significant fall in basal [6] and dietary- [8] and exercise- [8] stimulated energy expenditure and this is usually interpreted as an adaptive phenomenon that is in some way also linked to the decrease in lean body mass and the reduced availability of metabolizable substrates. The reduction in energy expenditure capacity has been suggested as a factor that could favour, in time, the propensity to regain lost weight in post-obese subjects [8]. In line with this hypothesis, recent studies have reported that a sustained decrement in the RMR (expressed per fat-free mass) may follow massive weight loss (e.g. in massively obese subjects during VLCD treatment), which can remain low for several months after weight reduction despite an increase in caloric consumption to a level that allows weight stability [7]. Our study provides evidence that chronic adrenoceptor stimulation may partially prevent a marked decrease in RMR during VLCDs, although it does not completely eliminate it. Previous studies on the effects of chronic administration of thermogenic compounds on basal and stimulated energy expenditure in humans were only performed in normal-weight subjects without any dietary restriction. Acheson et al. [21] and Scheidegger et al. [31] found that chronic administration of terbutaline significantly increased the basal metabolic rate in small groups of normal-weight individuals and these results were partially confirmed by Astrup et al. [15] after administration of ephedrine (20 mg three times daily) for 2 months in five non-obese women who claimed to have a weight problem despite a low food intake. In the same subjects, moreover, these authors found that VO2, after acute administration of ephedrine, was increased after 4 and 12 weeks of treatment, thus suggesting an improved capacity for adrenoceptor agonists to induce thermogenesis even after their prolonged administration [15]. The observations in the present study, therefore, extend the hypothesis one step further in that they demonstrate that chronically induced adrenergic stimulation minimizes the drop in RMR that follows severe caloric restriction in obese subjects. Whether this effect is...
Therefore, the variations in the activity of the noradrenergic pathways were parallel to those observed for RMR values. However, administration of adrenoceptor agonists also seems to interact with either thyroid hormone secretion or metabolism. In previous studies it was found that terbutaline, administered to normal-weight subjects fed on a normal diet, significantly increased the serum T₃ level and the serum T₃/T₄ ratio [14, 32], although this effect has not been confirmed by others [35]. In our study we found that both the serum T₃ level and the serum T₃/T₄ ratio were significantly increased by administration of ephedrine but no significant effect was observed on the serum levels of the free T₃ and free T₄ fractions or on their ratio in serum during the VLCD programme. These observations do not support the hypothesis that the effects of the catecholamines on energy expenditure are mediated by the thyroid hormones. Moreover, they agree with other studies, which have shown that the amount of the decrease in the serum level of T₃ during dieting was not correlated with that of RMR [15, 36] and, therefore, does not explain the reduction in energy expenditure observed in chronically underfed obese subjects. On the

### Table 4. Serum TSH, T₃ (total and free) and T₄ (total and free) concentrations before and after 2 weeks of ephedrine or placebo treatment in obese subjects on a 6-week VLCD programme grouped according to the sequence of drug treatment.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pharmacological treatment (ephedrine versus placebo)</th>
<th>Sequence of pharmacological treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>TSH</td>
<td>1.08</td>
<td>NS</td>
</tr>
<tr>
<td>T₃</td>
<td>3.92</td>
<td>0.069</td>
</tr>
<tr>
<td>T₄</td>
<td>0.42</td>
<td>NS</td>
</tr>
<tr>
<td>Free T₃</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Free T₄</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>T₃/T₄</td>
<td>5.79</td>
<td>0.029</td>
</tr>
<tr>
<td>Free T₃/free T₄</td>
<td>0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

Available knowledge seems to indicate that regulation of basal and meal-stimulated energy expenditure in vivo is dependent on the interaction between thyroid hormones and the sympathetic nervous system [32]. In subjects given a VLCD, a significant decrease in the serum T₃ level occurs owing to a reduced activity of the S'-deiodinase enzymes in peripheral tissues [33]. Moreover, severe food restriction also reduces the release of NA from peripheral sympathetic neurons [4] and significantly reduces the concentrations of catecholamines in the blood and of their metabolites in urine [34]. These observations suggest that reduced T₃ formation and NA release may contribute to the fall in RMR during VLCD treatment. We found that a VLCD induced a significant decrease in urinary VMA and HVA levels, which, however, returned to near-pre-treatment levels after the administration of ephedrine. Therefore, the variations in the activity of the noradrenergic pathways were parallel to those observed for RMR transient or persists for the entire duration of hypocaloric treatment obviously requires further study.

Table 4. Serum TSH, T₃ (total and free) and T₄ (total and free) concentrations before and after 2 weeks of ephedrine or placebo treatment in obese subjects on a 6-week VLCD programme grouped according to the sequence of drug treatment. Group 1 began with ephedrine (weeks 2 and 3; 50 mg three times a day) then continued with placebo (weeks 4 and 5), and group 2 received the drugs in the reverse order. Values are means ± s. Abbreviation: NS, not significant. Statistical analysis (analysis of co-variance):
other hand, our results seem to indicate the possibility that ephedrine may have interfered with thyroid-hormone-transport proteins, namely thyroid-binding globulin (to which 75% of T3 is bound), preventing the fall that usually occurs during a VLCD [23] or, possibly, stimulating its own synthesis.

Our study also provides some information on the effects of adrenoceptor agonists on body composition and protein metabolism during underfeeding in man. It is well known that severe hypocaloric feeding produces a significant protein loss even if adequate protein intake is allowed [23, 37]. Administration of 150 mg of ephedrine/day significantly reduced the nitrogen loss and therefore improved the nitrogen balance in all subjects independently of the sequence of the pharmacological treatment. Daily urinary 3-MH levels fell throughout the VLCD programme, but no significant ephedrine-dependent effect was observed during that period. Since urinary excretion of 3-MH appears to be a valid index of skeletal muscle protein breakdown [38], our data indicate that ephedrine may have stimulated protein accumulation without affecting muscle protein breakdown, as previously suggested by studies on laboratory animals and by preliminary observations in humans. In fact, phenethanolamines/β-adrenoceptor agonists have been shown to inhibit atrophy in acutely and chronically denervated rat soleus muscle [39], and clenbuterol, a β2-adrenoceptor agonist, has been shown to significantly increase protein mass and tissue RNA content in food-restricted (not fasting) rats [19]. Moreover, in different types of obese rodents, administration of BRL26830A, a new non-typical adrenoceptor agonist, has increased lipid-free dry weight and skeletal muscle weight [20]. In seven lean healthy young men fed on a normal diet, Acheson et al. [21] observed that 2 weeks of terbutaline sulphate (15 mg daily) treatment significantly reduced urinary and integumental nitrogen loss and increased lean body mass. In obese human subjects fed an energy-restricted diet, administration of BRL26830A caused a significant decrease in body nitrogen loss [20] and this effect was confirmed over 6 weeks of BRL26830A treatment in hospitalized patients fed on a diet providing 8368 kJ (2000 kcal) less than maintenance requirements [20]. In an 8-week study, other authors found that urinary nitrogen loss was reduced by this compound in subjects fed with an 3344 kJ (800 kcal) diet, although this effect was not significant during the last week of treatment (cited in [20]). Although it is still not at all clear whether the effects of adrenoceptor stimulants are transitory or persist over time, they appear to have an important role in whole protein economy, particularly during severely restricted diets when weight loss should be achieved while maintaining the protein balance, thus

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pharmacological treatment (ephedrine versus placebo)</th>
<th>Sequence of pharmacological treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Adrenaline (nmol/l of creatinine)</td>
<td>2.71</td>
<td>NS</td>
</tr>
<tr>
<td>NA (nmol/l of creatinine)</td>
<td>0.57</td>
<td>NS</td>
</tr>
<tr>
<td>DA (nmol/l of creatinine)</td>
<td>0.11</td>
<td>NS</td>
</tr>
<tr>
<td>HMA (µmol/l of creatinine)</td>
<td>8.39</td>
<td>0.012</td>
</tr>
<tr>
<td>VMA (µmol/l of creatinine)</td>
<td>12.67</td>
<td>0.032</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Week on VLCD therapy ...</th>
<th>Basal</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>64.9±13.6</td>
<td>78.6±73.7</td>
<td>82.4±52.8</td>
<td>110.8±75.3</td>
<td>80.5±35.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>39.3±16.4</td>
<td>68.2±23.5</td>
<td>45.8±31.1</td>
<td>43.1±24.0</td>
<td>49.2±15.0</td>
<td></td>
</tr>
<tr>
<td>NA (nmol/l of creatinine)</td>
<td>269.8±6.0</td>
<td>344.0±160.2</td>
<td>193.3±255.1</td>
<td>300.4±231.7</td>
<td>295.0±100.3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>168.4±32.5</td>
<td>161.3±42.5</td>
<td>258.9±54.9</td>
<td>185.0±41.4</td>
<td>215.1±39.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1710.2±534.1</td>
<td>1717.1±551.8</td>
<td>1748.7±311.9</td>
<td>1726.1±930.8</td>
<td>1750.2±490.0</td>
<td></td>
</tr>
<tr>
<td>DA (nmol/l of creatinine)</td>
<td>1298.2±340.2</td>
<td>1178.7±233.8</td>
<td>1303.4±312.8</td>
<td>1532.3±391.1</td>
<td>1410.3±290.5</td>
<td></td>
</tr>
<tr>
<td>HMA (µmol/l of creatinine)</td>
<td>14.27±3.40</td>
<td>13.99±1.59</td>
<td>20.75±2.74</td>
<td>9.88±4.99</td>
<td>9.39±5.05</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>14.16±6.59</td>
<td>8.78±2.14</td>
<td>7.52±2.30</td>
<td>11.63±4.39</td>
<td>9.93±4.28</td>
<td></td>
</tr>
<tr>
<td>VMA (µmol/l of creatinine)</td>
<td>13.52±5.15</td>
<td>14.13±11.55</td>
<td>21.49±10.59</td>
<td>7.36±5.09</td>
<td>7.32±5.25</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.49±1.06</td>
<td>7.56±3.08</td>
<td>6.55±2.93</td>
<td>15.64±6.56</td>
<td>10.14±6.10</td>
<td></td>
</tr>
</tbody>
</table>
selectively reducing fat. Finally, it seems possible that this objective can be achieved through the administration of drugs such as the one used in this study.

In conclusion, it was found that administration of ephedrine, a sympathomimetic agent, during VLCD treatment in obese subjects partially prevented the RMR from falling, decreased urinary nitrogen loss and significantly improved the nitrogen balance. These observations may be important in the treatment of obesity, particularly when a defective energy expenditure capacity is considered to be involved.

ACKNOWLEDGMENTS

We thank the nursing staff of our group for their help, Dr Sandro Piazzoli for 3-HIM measurements, and Mrs Susan West and Juliet Macan for their secretarial assistance. This work was supported by a grant from the Consiglio Nazionale delle Ricerche (no. 88.03494.04).

REFERENCES