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Short-term, increasing dietary protein and fat moderately affect energy expenditure, substrate oxidation and uncoupling protein gene expression in rats

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Abstract

Macronutrient composition of diets can influence body-weight development and energy balance. We studied the short-term effects of high-protein (HP) and/or high-fat (HF) diets on energy expenditure (EE) and uncoupling protein (UCP1–3) gene expression. Adult male rats were fed ad libitum with diets containing different protein–fat ratios: adequate protein–normal fat (AP–NF): 20% casein, 5% fat; adequate protein–high fat (AP–HF): 20% casein, 17% fat; high protein–normal fat (HP–NF): 60% casein, 5% fat; high protein–high fat (HP–HF): 60% casein, 17% fat. Wheat starch was used for adjustment of energy content. After 4 days, overnight EE and oxygen consumption, as measured by indirect calorimetry, were higher and body-weight gain was lower in rats fed with HP diets as compared with rats fed diets with adequate protein content ($P<.05$). Exchanging carbohydrates by protein increased fat oxidation in HF diet fed groups. The UCP1 mRNA expression in brown adipose tissue was not significantly different in HP diet fed groups as compared with AP diet fed groups. Expression of different homologues of UCPs positively correlated with nighttime oxygen consumption and EE. Moreover, dietary protein and fat distinctly influenced liver UCP2 and skeletal muscle UCP3 mRNA expressions. These findings demonstrated that a 4-day ad libitum high dietary protein exposure influences energy balance in rats. A function of UCPs in energy balance and dissipating food energy was suggested. Future experiments are focused on the regulation of UCP gene expression by dietary protein, which could be important for body-weight management.

Keywords: High-protein diets; High-fat diets; Substrate oxidation; Uncoupling protein gene expression; Energy expenditure; Rats

1. Introduction

Diet composition is known to influence energy intake and body-weight changes in rats and humans. However, the results are contradictory, and it remains unclear what the relative importance of protein, carbohydrate and fat is. Moreover, the influence of different physiological (genotype, gender, insulin resistance) and feeding conditions (degree of energy restriction, duration of feeding a specific diet) is not yet established [1–3].

In order to achieve a reduction in body fat, weight-reducing diets must attain a negative energy balance. An increase of the protein content of diets at the expense of carbohydrates has brought such an advantage in some feeding studies [4–6]. Further, there is increasing evidence of higher thermic response following protein ingestion as compared with carbohydrates or fat [2,7,8]. The mechanisms for the influence of dietary protein on thermic effects are not clear and are controversially discussed [3,5,9]. Uncoupling protein homologues (UCP1–3) have been shown to lower mitochondrial membrane potential in several mammalian cell expression systems, suggesting uncoupling activity of the respiratory chain from ATP synthesis by generation of a proton leak. In consequence, respiration rate relative to a certain ATP production can increase and energy is wasted as heat [10–12]. Further, expression of UCPs is known to be influenced by factors such as long-term high-fat (HF) or high-protein (HP) intake [13,14]. We could show an organ-specific inverse association of UCP gene expression with feed energy efficiency

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and a positive association with nighttime energy expenditure (EE) and oxygen consumption [13]. Increasing the protein content in an HF diet delayed the development of obesity and improved glucose homeostasis in a mouse model of diet-induced obesity [15].

Here, we examined the short-term effects of different diets with high and low protein and fat content, respectively, on energy metabolism and substrate oxidation rates. We hypothesize that an exposure to high dietary protein in relation to other macronutrients rapidly leads to activation of pathways such as UCP gene expression, which, in turn, enhanced EE and fat oxidation (FO).

2. Methods

2.1. Animals, diets and experimental setup

The experimental protocol was approved by the Ethical Committee on the Use of Animals as Experimental Subjects of the Ministry of Agriculture, Nutrition and Forestry (State Brandenburg, Germany, Permission No. 32-44456). Housing of rats (Shoe-Wist-Han; Charles River, Sulzfeld, Germany), composition of diets, blood and tissue sampling and conservation were essentially as described [16]. In brief, adult male Wistar rats (initial body weight, ~190 g) were housed individually in a climate-controlled room with a 12-h light–dark cycle. Before the feeding experiment, all rats had consumed ad libitum a nonpurified pelleted stock diet (Altromin GmbH, Lage, Germany; crude protein, 190 g/kg; crude fat, 40 g/kg; metabolizable energy, 11.9 MJ/kg). Rats were randomly assigned to experimental diets ($n=10$ per group), which were provided ad libitum for 4 days. The experimental diets (Table 1) have either adequate (AP, about 20% of calories) or high (HP, about 60% of calories) protein content and have either normal (NF, about 13% of calories) or high (HF, about 40% of calories) dietary fat content. A crude protein content of 10–15% (w/w) is considered to be adequate for growing rats fed low-fiber diets containing a balanced amino acid pattern and 5% fat [17]. Protein and fat were exchanged energetically for wheat starch. The metabolizable energy density of the diets was calculated according to the following macronutrient energy contents: casein, 15.7 kJ/g; carbohydrate, 16 kJ/g; and fat, 38 kJ/g. The two HF diets, AP and HP did not differ in fat content and energy density. Water was provided ad libitum. Food intake and body weight was monitored daily. On Day 4 of feeding, EE was measured as described below. Finally, rats were sedated by ether inhalation and killed by decapitation in the postabsorptive state (1–2 h after withdrawal of food) between 0900 and 1030 h. Blood samples were collected from trunk, and plasma was obtained by centrifugation. Samples of liver, skeletal muscle (musculus biceps femoris), interscapular brown adipose tissue (BAT), epididymal fat pad, kidney, pancreas, intestinal mucosa and spleen were collected and stored in liquid nitrogen until analysis.

Table 1
Composition and percentage of macronutrients of purified test diets containing different protein and fat concentrations (AP–NF, adequate protein–normal fat; AP–HF, adequate protein–high fat; HP–NF, high protein–normal fat; HP–HF, high protein–high fat)

	AP–NF	AP–HF	HP–NF	HP–HF
Diet (g/100 g)				
Casein[a]	20	20	60	60
Wheat starch[b]	58	40	18	3
Saccharose[c]	5	5	5	5
Palm kernel fat[d]	3	15	3	15
Sunflower seed oil[e]	2	2	2	2
Cellulose[f]	5	11	5	8
Mineral mixture[g]	5	5	5	5
Vitamin mixture[h]	2	2	2	2
Macronutrient [metabolizable energy (%)]				
Protein	21.6	19.1	63.6	55.0
Carbohydrates	65.3	41.5	23.6	7.3
Fats	13.0	39.5	12.8	37.7
Macronutrient ratios				
Protein–fat ratio	1.66	0.48	4.97	1.46
Carbohydrate–fat ratio	5.02	1.05	1.84	0.19
Protein–carbohydrate ratio	0.33	0.46	2.69	7.50
Metabolizable energy (kJ/100 g)	1512	1680	1500	1716

[a] Dauermilchwerk Peiting GmbH, Landshut, Germany, contained 86% crude protein (% N×6.38).
[b] Heller u. Strauß, Berlin, Germany.
[c] Nordzucker GmbH, Uelzen, Germany.
[d] Union Deutsche Lebensmittelwerke, Hamburg, Germany.
[e] Kunella-Feinkost GmbH, Cottbus, Germany.
[f] Rettenmeier, Ellwangen, Germany.
[g] Mineral mixture per 100 g diet: Ca, 930 mg; P, 730 mg; Mg, 80 mg; Na, 440 mg; K, 710 mg; S, 170 mg; Cl, 360 mg; Fe, 20 mg; Mn, 10 mg; Zn, 3 mg; Cu, 800 mg; J, 40 mg; F, 400 mg; Se, 20 mg; Co, 10 mg, (Altromin GmbH).
[h] Vitamin mixture containing 17.5 g/100 g DL-methionine; vitamin content in 100 g diet: A, 0.45 mg; D_3, 1.3 mg; K_3, 1 mg; B_1, 2 mg; B_2, 2 mg; B_6, 1.5 mg; B_{12}, 3 mg; niacin, 5 mg; pantothenate, 5 mg; folic acid, 1 mg; biotin, 20 mg; choline chloride, 100 mg; *p*-aminobenzoic acid, 10 mg; inositol, 10 mg; E, 16.4 mg (Altromin GmbH).

2.2. Energy expenditure

EE was measured by indirect calorimetry in individual rats as described [13,18]. Rats were housed in metabolism cages for 23 h (0900 to 0800 h of the following day), allowing the separate collection of urine and feces. Food was available between 1900 and 0800 h. Oxygen consumption and CO_2 production were determined every 6 min in an open respirometric system (O_2 and CO_2 analyzers: Magnos 16 and U14, Hartmann & Braun, Frankfurt/Main, Germany). EE was calculated according to Weir [19] as

$$\mathrm{EE\ (kJ)} = 16.17 \times \mathrm{VO_2} + 5.03 \times \mathrm{VCO_2} - 5.98 \times N_{\mathrm{ex}},$$

where VO_2 is the oxygen consumption (liters per day), VCO_2 is the CO_2 production (liters per day) and N_{ex} is the urinary nitrogen excretion (grams per day). Respiratory quotient (RQ) is defined as VCO_2 divided by VO_2. Total EE (TEE) was calculated as a daily mean. In addition, during the dark phase, mean EE (EE_{dark}) was calculated between 1900 and 0600 h. Resting metabolic rate (RMR) was defined as the mean of the 10 lowest values during the

Table 2
Body weight, fat weights and plasma metabolites of rats fed diets with different protein and fat concentrations for 4 days (Control, nonpurified pelleted stock diet; AP–NF, adequate protein–normal fat; AP–HF, adequate protein–high fat; HP–NF, high protein–normal fat; HP–HF, high protein–high fat)

Diet	Control	AP–NF	AP–HF	HP–NF	HP–HF
Body weight					
Final body weight (g)	260±6	260±7	262±6	254±8	248±7
Body-weight increase (g/4 days)	17.4±2.5^{b}	17.2±1.7^{b}	20.0±1.6^{b}	8.0±1.7^{a}	5.7±1.6^{a}
Total epididymal fat pad weight (g)	2.66±0.28	3.09±0.24	3.63±0.32	2.90±0.28	3.38±0.24
Interscapular brown fat (g)	0.24±0.01^{a}	0.28±0.02^{ab}	0.32±0.02^{b}	0.25±0.01^{a}	0.25±0.02^{a}
Plasma metabolites, postabsorptive					
fT_3 (pg/ml)	3.90±0.14	4.24±0.12	4.07±0.13	4.03±0.12	3.94±0.12
FFA (mmol/L)	0.46±0.06^{b}	0.26±0.04^{a}	0.56±0.07^{bc}	0.69±0.05^{c}	0.74±0.07^{c}
TG (mmol/L)	1.04±0.06^{a}	1.26±0.12^{a}	1.82±0.24^{b}	1.18±0.14^{a}	1.23±0.18^{a}
Glucose (mmol/L)	8.37±0.36	7.61±0.28	8.59±0.26	8.05±0.36	8.40±0.17
Urea (μmol/ml)	6.36±0.89^{a}	7.01±0.65^{a}	7.16±0.74^{a}	13.25±2.03^{c}	11.75±1.33^{b}
Leucine–threonine ratio	0.70±0.02^{b}	0.54±0.04^{a}	0.63±0.04^{ab}	1.37±0.06^{d}	1.07±0.05^{c}

Rats were in the postabsorptive state 1–2 h after removal of food offered overnight.
Values are means±S.E.M., $n=10$. Within a row, values without a common superscript differ, $P<.05$. For diet compositions and more details, see Section 2.

measurement period according to a procedure previously described in mice [18]. Net rates of FO, carbohydrate oxidation (CO), and protein oxidation (PO) (in grams per day) were calculated [13] as

$$FO = 1.72 \times (VO_2 - VCO_2) - 1.96 \times N_{ex}$$
$$CO = 4.17 \times VCO_2 - 2.97 \times VO_2 - 2.44 \times N_{ex}$$
$$PO = 6.25 \times N_{ex}.$$

2.3. Assays

Plasma free amino acid and urea concentrations were determined essentially as described [16] Circulating free fatty acids (FFAs) were determined using NEFA C kit (Wako Chemicals GmbH, Neuss, Germany). Concentrations of free triiodothyronine (fT_3) in plasma were measured by chemiluminescence (Advia Centaur, Bayer Vital GmbH, Fernwald, Germany). The other circulating metabolites were determined using colorimetric and enzymatic standard methods (Cobas Mira, Hoffmann La Roche AG, Grenzach-Whylen, Germany). Nitrogen content of diets and urine was determined by Kjeldahl method (Kjeldatherm–Turbosog–Vapodest 45, C. Gerhardt GmbH & Co. KG, Bonn, Germany), and gross energy content of diets was measured for control purposes by means of an adiabatic bomb calorimeter (IKA-Calorimeter C 5000, IKA-Werke GmbH & Co. KG, Staufen, Germany).

2.4. Gene expression

Total RNA from individual tissues (interscapular BAT, liver, hind limb skeletal muscle) was extracted using a single-step acid phenol–guanidine protocol [20], and gene expression was analyzed by Northern blot as described [21]. In brief, 10 μg of total RNA was separated by electrophoresis in a 1% agarose gel containing formaldehyde and blotted by capillary transfer to a nylon membrane (Hybond N, Amersham Biosciences, Freiburg, Germany).

Table 3
Nutrient intake, EE and net oxidation of macronutrients of rats fed diets with different protein and fat concentrations for 4 days (Control, nonpurified pelleted stock diet; AP–NF, adequate protein–normal fat; AP–HF, adequate protein–high fat; HP–NF, high protein–normal fat; HP–HF, high protein–high fat)

Diet	Control	AP–NF	AP–HF	HP–NF	HP–HF
Nutrient intake					
Total food intake (g/4 days)	91.5±1.7^{c}	83.0±2.2^{b}	84.9±2.1^{b}	71.5±1.8^{a}	66.5±2.1^{a}
Metabolizable energy intake (MJ/4 days)	1.56±0.03^{a}	1.49±0.04^{a}	1.74±0.04^{b}	1.45±0.04^{a}	1.50±0.05^{a}
Total N intake (g/4 days)	2.9±0.1^{b}	2.4±0.1^{a}	2.5±0.1^{a}	5.9±0.2 ^{d}	5.3±0.2^{c}
N balance (gN/4 days)	1.73±0.09^{bc}	1.32±0.06^{ab}	1.32±0.04^{a}	1.80±0.18^{c}	2.09±0.17^{c}
Overall daily EE (on Day 4)					
TEE (kJ/h)	8.56±0.3	8.99±0.2	8.63±0.3	8.77±0.2	8.82±0.2
TEE [kJ/(h×kg)]	34.2±0.5	35.3±1.1	33.9±1.1	35.5±0.8	36.3±1.1
RMR (kJ/h)	6.02±0.3	6.41±0.2	6.16±0.3	5.75±0.2	6.03±0.2
RMR [kJ/(h×kg)]	24.0±0.8	25.2±1.0	24.2±1.2	23.2±0.9	24.8±1.0
VO_2 (LO_2/h)	0.41±0.01	0.43±0.01	0.42±0.02	0.43±0.01	0.45±0.01
VO_2 [LO_2/(h×kg)]	1.64±0.02^{a}	1.69±0.05^{ab}	1.66±0.03^{a}	1.78±0.02^{ab}	1.83±0.05^{b}
RQ	0.95±0.00^{d}	0.97±0.01^{d}	0.90±0.01^{c}	0.88±0.01^{b}	0.82±0.00^{a}
Dark-phase EE (Day 4, 1600–0400 h)					
EE_{dark} (kJ/h)	9.5±0.3	10.0±0.2	9.5±0.4	10.2±0.2	10.0±0.3
EE_{dark} [kJ/(h×kg)]	38.0±0.6^{a}	39.0±1.1^{ab}	38.0±0.9^{a}	41.9±0.8^{b}	40.9±1.0^{ab}
$VO_{2,\ dark}$ (LO_2/h)	0.45±0.01	0.47±0.01	0.46±0.02	0.49±0.02	0.50±0.01
$VO_{2,\ dark}$ [LO_2/(h×kg)]	1.80±0.03^{a}	1.85±0.05^{a}	1.83±0.04^{a}	2.03±0.02^{b}	2.05±0.05^{b}
RQ	1.00±0.00^{d}	1.01±0.01^{e}	0.94±0.00^{c}	0.87±0.00^{b}	0.82±0.00^{a}

Values are means±S.E.M., $n=10$. Within a row, values without a common superscript differ, $P<.05$. For diet compositions and more details see Section 2.

The blots were probed with ^{32}P-labeled probes in a hybridization solution containing sodium phosphate (0.5 mol/L), EDTA (1 mmol/L), sodium dodecyl sulfate (SDS; 7%) and bovine serum albumin (1%) at 63°C overnight and washed twice with saline sodium citrate (SSC) 2×SSC–0.1% SDS for 20 min at room temperature, twice with 0.1×SSC–0.1% SDS for 20 min at 42°C and twice with 0.1×SSC–0.1% SDS for 20 min at 63°C. An Instant Imager (A202401, Canberra Packard GmbH, Dreieich, Germany) was used for analysis and quantification of radiolabeled signals. For hybridization, complete cDNA probes for rat UCP1 and UCP2 were kindly provided by Prof. Daniel Ricquier (CNRS, Paris, France) as reported before [22], and a UCP3 cDNA probe was kindly provided by Dr. Martin Klingenspor (University of Marburg, Germany).

2.5. Statistical analysis

Data are reported as means±S.E.M. Differences between mean values were determined by ANOVA, followed by comparisons using the Newman–Keuls multiple range test (WinSTAT, version 1999.2, R. Fitch software, Staufen, Germany). Pearson correlation coefficients were calculated to determine the relationship between selected parameters. Differences with $P<.05$ were considered statistically significant if not stated otherwise.

3. Results

Rats exposed to HP diets gained less weight during 4 days than the other experimental groups, with lowest weight gain in the high protein–high fat (HP–HF) group (Table 2). Bodyweight changes were two to three times larger ($P<.05$) in controls and in the AP groups as compared with the HP groups. On the other hand, total energy intake of HP diet fed rats was not different ($P>.05$) from controls and adequate protein–normal fat (AP–NF) rats (Table 3). However, adequate protein–high fat (AP–HF) rats consumed more energy compared with all other groups, which was due to an active hyperphagia, that is, an increased food intake. Interestingly, the HP–HF group consumed less food compared with all other groups, indicating that the hyperphagia in the AP–HF group is not due to the increased dietary fat alone. Nitrogen balance was positive in all groups but lower in the AP groups as compared with the HP groups. As expected, total nitrogen intake was lower in the AP groups and higher in the HP groups as compared with controls. The level was twice as high in HP compared with AP diet fed groups (Table 3). Total epididymal fat pad weight was not different between controls and feeding groups at the end of the short-term feeding period. The absolute and relative weights of the liver, spleen and pancreas were not affected by experimental diets (data not shown). However, kidney weights were significantly lower in controls and AP diet fed groups compared with HP diet fed groups. Interscapular BAT weight was largest in the AP–HF group (Table 2).

Postabsorptive concentrations of plasma glucose and levels of fT_3 did not differ among the groups (Table 2). However, plasma FFA levels were significantly lower in AP–NF diet fed rats as compared with all other experimental groups. Plasma triglyceride (TG) concentrations were found to be highest in the AP–HF group. Both the plasma urea concentrations and the leucine–threonine ratios were increased dose dependently with increasing dietary nitrogen or protein intake, which corresponded to observations made earlier in a long-term feeding study [13]. The corresponding plasma leucine concentrations were 156±8, 213±14, 201±12, 356±15 and 273±21 μmol/L for control, AP–NF, AP–HF, high protein–normal fat (HP–NF) and HP–HF

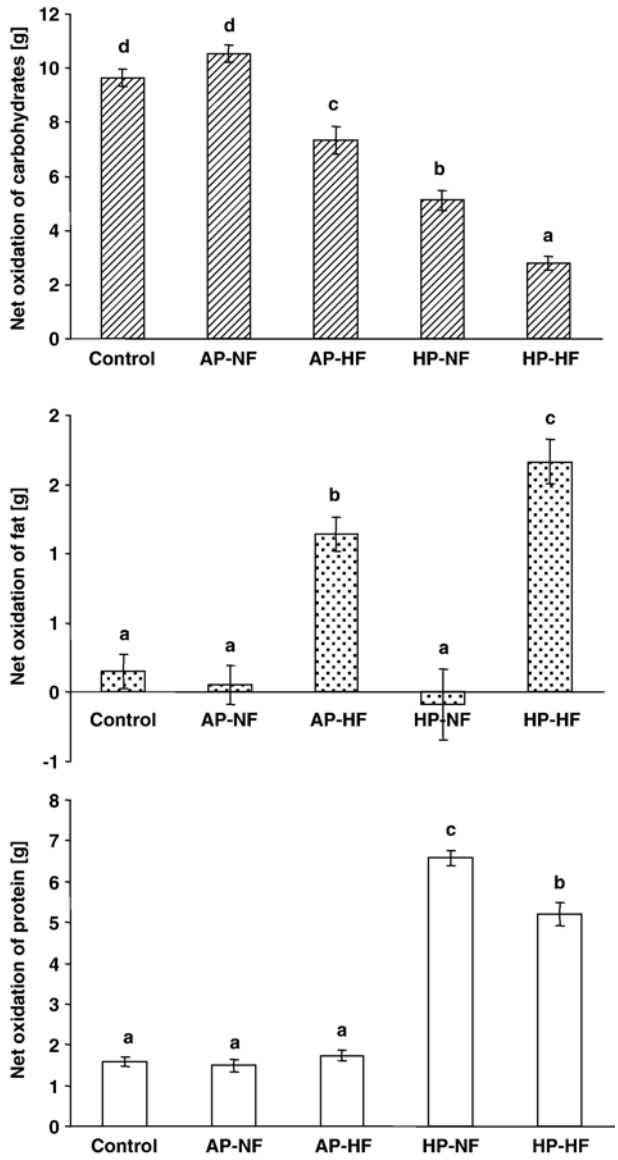

Fig. 1. Net oxidation of macronutrients of rats fed diets with different protein and fat concentrations for 4 days (Control, nonpurified pelleted stock diet; AP–NF, adequate protein–normal fat; AP–HF, adequate protein–high fat; HP–NF, high protein–normal fat; HP–HF, high protein–high fat). Data are means±S.E.M., $n=10$. Columns without a common superscript differ, $P<.05$. For diet compositions and more details, see Section 2.

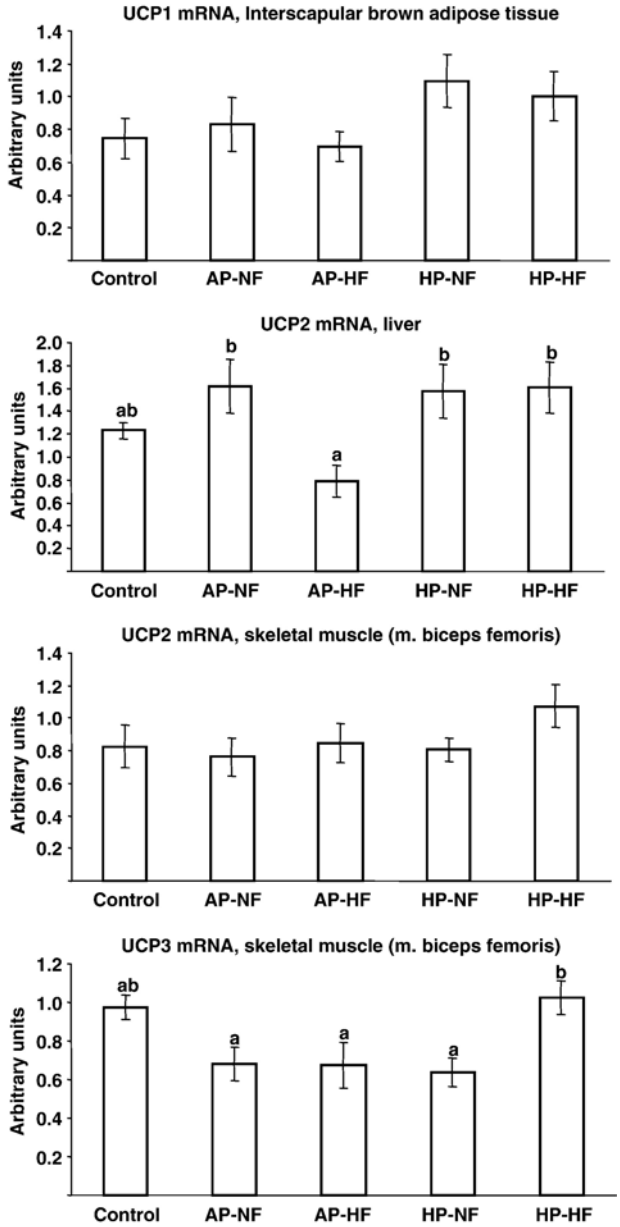

Fig. 2. Gene expression of UCP homologues in various tissues of rats fed diets with different protein and fat concentrations for 4 days (Control, nonpurified pelleted stock diet; AP–NF, adequate protein–normal fat; AP–HF, adequate protein–high fat; HP–NF, high protein–normal fat; HP–HF, high protein–high fat). Rats were in the postabsorptive state 1–2 h after removal of food offered overnight. Data are means±S.E.M., $n=10$. Columns without a common superscript differ, $P<.05$. For diet compositions and more details, see Section 2.

diet fed rats, respectively. The plasma threonine concentrations were 230±9, 395±12, 323±17, 264±17 and 254±15 μmol/L for control, AP–NF, AP–HF, HP–NF and HP–HF diet fed rats, respectively.

Overall daily TEE and RMR did not differ ($P>.05$) between groups fed diets with different macronutrient combinations. However, EE was higher during nighttime when related to body weight in the groups exposed to HP diets as compared with controls. The effect was significant for the HP–NF group. Body-weight-related oxygen consumption during nighttime was also significantly higher in the HP groups as compared with controls and the AP groups. The fat content of the experimental diets did not have an additional influence on oxygen consumption.

Generally, RQ is indicative for the substrate oxidation. FO causes values close to 0.7, whereas CO results in an RQ of 1.0 and PO has values in between [23]. We found lower RQ values with a decrease in the carbohydrate moiety in experimental diets (Table 3). This was indicative for a higher proportion of PO and FO and a relative decrease in CO in the HP groups as compared with the AP groups. Comparing RQ values of both groups fed an HF diet revealed significantly lower values in the case of an exposure to HP. This is indicative for a higher FO in the HP–HF group as compared with the AP–HF group (Fig. 1). The net oxidation of fat was 1.14±0.13 and 1.67±0.16 for rats fed the AP–HF and HP–HF diets, respectively. As expected, the values for net oxidation of carbohydrates and protein were dependent on the content of these macronutrients in the diets.

Gene expression of UCPs was only marginally affected by short-term dietary exposure (Fig. 2). UCP1 mRNA expression in BAT was not significantly different in the HP groups as compared with controls and the AP groups. The expression of liver UCP2 was significantly lower in AP–HF diet fed rats as compared with the other groups. In skeletal muscle, higher mRNA expression values were found for UCP2 and UCP3 in HP–HF diet fed rats as compared with the other groups, but the values were significantly different only for UCP3.

Positive correlations were obtained between nighttime EE or oxygen consumption and mRNA expression of UCP1 in BAT, UCP2 in liver and UCP2 and UCP3 in skeletal muscle

Table 4
Pearson correlation coefficients between UCP1, UCP2 and UCP3 mRNA expression in BAT, liver and skeletal muscle (musculus biceps femoris) and EE, N intake, macronutrient oxidation based on RQ and postabsorptive circulating metabolites in rats fed diets with different protein and fat concentrations for 4 days

Parameter	UCP1, BAT	UCP2, liver	UCP2, skeletal muscle	UCP3, skeletal muscle
EE	ns	ns	.319	.372
EE_{dark}	.274	.284	.366	.322
VO_2 [LO_2/(h×kg)]	.295	.222[a]	.332	ns
$VO_{2,\ dark}$ [LO_2/(h×kg)]	.305	.264	.360	.324
N intake	.361	.388	ns	ns
CO	−.243	ns	−.257	ns
FO	ns	ns	.226[a]	ns
PO	.274	.247	ns	ns
FFA concentration, plasma	.265	ns	ns	ns
TG concentration, plasma	ns	−.334	ns	ns
Glucose concentration, plasma	ns	−.215[a]	−.254	ns
fT_3 concentration, plasma	ns	ns	.237[a]	ns

$n=40$; correlation coefficients were significant ($P<.05$) if not otherwise stated; correlation coefficients not significant ($P>.1$) are labeled ns. For diet compositions and more details, see Section 2. EI, energy intake; $VO_{2,\ dark}$, dark-phase oxygen consumption.

[a] Correlation coefficient of borderline significance ($.1>P>.05$).

(Table 4). Further, a positive correlation was found between N intake or PO and gene expression of BAT UCP1 and liver UCP2. Interestingly, there were negative correlations between net oxidation of carbohydrates and UCP1 gene expression in BAT, between plasma TG levels and liver UCP2 gene expression and between plasma glucose concentrations and skeletal muscle UCP2 mRNA expression.

4. Discussion

We examined a short-term exposure of rats to diets with different macronutrient ratios regarding energy metabolism. This was performed to test our hypothesis that a high content of dietary protein in relation to carbohydrates and fat increases EE and FO without a need for long-term adaptation. Our results suggest that dietary protein is the main macronutrient responsible for variations in EE under ad libitum feeding conditions, that is, without restriction of the energy intake. Body-weight development was significantly lower and nighttime oxygen consumption was higher in HP diet fed groups, and this effect was independent of the fat or carbohydrate content of the experimental diets. Rats are nocturnal animals and feed preferentially during nighttime. Therefore, it is reasonable to conclude that higher nighttime values for oxygen consumption of HP diet fed rats compared with AP diet fed rats indicate a higher thermic effect of food with high protein content. It has been shown repeatedly in animal and human studies [2,4,5,7,8] that high dietary protein increases the thermic effect of food. However, other studies did not find changes in energy efficiency ratio or thermic effect of food as a function of the dietary protein content [3,24]. For example, a short-term replacement of dietary carbohydrate with protein under energy-restrictive conditions was not able to blunt a diet-induced fall in resting EE or to increase the thermic effect of food to a level large enough to facilitate weight loss in patients with type 2 diabetes [24]. Because these studies were carried out under conditions of energy restriction, we suggest that the thermic effects of dietary proteins are prevented under conditions of energy deficiency. On the other hand, it could mean that conversions of amino acids to glucose via gluconeogenesis and the degradation of amino acids induced by HP diets, which are considered to be costly processes, are indeed not responsible for thermic effects of proteins [3]. Thus, it remains to be elucidated which mechanisms are causing thermic effects of HP meals and how they are regulated.

HF diets have been shown to lead to hyperphagia in various animal and human studies [15,25,26]. However, this hyperphagia was abolished when the protein–carbohydrate ratio was increased. HP–HF diet fed rats even showed a reduced food intake. It thus seems that high fat causes hyperphagia only in combination with high carbohydrate levels. This is in line with human studies that showed that the weight-reducing effect of low-carbohydrate, HF diets was mainly due to a decreased energy intake [27].

Our previous results suggest that UCPs could be involved in food energy dissipation [13]. UCPs are mitochondrial proteins considered to be involved in lowering membrane potential and increasing oxygen consumption rate relative to a certain ATP production, therefore dissipating energy as heat [12]. UCP1 present in BAT is responsible for BAT thermogenesis stimulated by sympathetic activation, but the exact biological roles of UCP2 and UCP3 are not yet clear. Their proposed functions include regulation of ATP synthesis, control of oxygen species and control of substrate and fatty acid oxidation in addition to possible thermogenic functions in analogy to UCP1 [10–12,28]. Gene expression of UCP2 and UCP3 was shown to be affected when EE and substrate oxidation were changed [10,12]. Here, we found only moderate changes of mRNA expressions of UCPs between groups fed for 4 days with experimental diets. However, significant positive correlations of all UCPs studied with nighttime EE and oxygen consumption support their involvement in postprandial thermogenic processes. The positive correlation of BAT UCP1 and liver UCP2 gene expression with nitrogen intake confirmed our previous observations in long-term HP diet fed rats [13]. No relation was found between fat or carbohydrate intake and the expression level of UCPs, and no correlation was found between skeletal muscle UCP2 and UCP3 gene expression and protein intake. However, plasma glucose concentrations negatively correlated with skeletal muscle UCP2 gene expression, supporting a role of UCP2 in glucose homeostasis. A predominant glucose oxidation was suggested for HSA-mUCP1 transgenic mice with directed expression of UCP1 in skeletal muscle during nighttime activity, which could explain increased insulin sensitivity and a stimulated reliance on glucose for muscle fuel oxidation [29]. Overall, these results indicate an HP-diet-dependent function of BAT UCP1 and liver UCP2 in energy dissipation. In addition, the results confirmed our previous observation that expression of UCPs in liver and skeletal muscle is regulated differently [13,30]. However, UCP gene expression needs to be confirmed by measurement of UCP protein levels since it has been shown, especially for UCP2, that mRNA expression does not always correlate with encoded protein content [13].

Interestingly, the level of FO was found to be significantly higher in rats fed an HF diet low in carbohydrates but high in protein concentration (HP–HF) as compared with a diet high in fat and high in carbohydrates but adequate in protein (AP–HF; Fig. 1). This is consistent with the reports that a low-carbohydrate diet without restriction in energy as fat or protein induced a greater reduction in body weight and fat mass in humans than a low-fat diet [2,31,32]. We have shown higher proportions of FO based on lower RQ values in long-term HP as compared with AP diet fed rats [13]. Although the mechanism for this remains unclear, we assume that UCPs might be involved in this process [10,11,28]. Higher gene expressions of liver UCP2 and skeletal muscle UCP3 in the HP–HF group as compared

with the AP–HF group could be compatible with an increased fatty acid oxidation. In analogy, an increased liver UCP2 expression was found in obesity-prone mice fed diets supplemented with epigallocatechin gallate (green tee bioactive polyphenol), which prevented the development of diet-induced obesity and increased the FO [30]. Further, we have shown in mice that the oxidation of exogenously administered ^{13}C-palmitic acid measured as $^{13}CO_2$ exhalation clearly increased when epigallocatechin gallate was supplemented to diets (M. Friedrich, S. Klaus and K.J. Petzke, unpublished data). It is interesting to note that, in the present study, a negative correlation of liver UCP2 gene expression with plasma TG concentrations was found and that highest TG concentrations were measured in rats fed the AP–HF diet, characterized by lowest UCP2 gene expression levels in liver, as compared with other experimental diet groups.

It was reported recently that diets in which protein is increased at the extent of carbohydrate not only facilitate body-weight reduction but also improve insulin resistance and lipid metabolism [1,5,33,34]. In this short-term study, lower plasma TG concentrations were evident in the HP–HF group as compared with the AP–HF group. In addition, nitrogen balance was found to be more positive in the HP–HF group as compared with the AP–HF group, indicating advantages for lean body mass development. Metabolic consequences of HP diets are still controversially discussed [27,35,36]. One of the consequences is the clear increase in the leucine–threonine ratio in plasma of HP diet fed rats, which was proposed to be an indicator of protein nutritional status [13] and which could regulate hormonal secretion, intracellular signaling and protein balance [37–39].

In summary, this study demonstrated that HP diets or an increase of the protein–carbohydrate ratio in HF diets promoted a greater proportion of FO during short-term exposure in rats. In addition, UCPs could be involved in this process. Therefore, HP diets may have important implications to lower body fat.

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References

[1] Luscombe-Marsh ND, Noakes M, Wittert GA, Keogh JB, Foster P, Clifton PM. Carbohydrate-restricted diets high in either monounsaturated fat or protein are equally effective at promoting fat loss and improving blood lipids. Am J Clin Nutr 2005;81:762–72.

[2] Due A, Toubro S, Skov AR, Astrup A. Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: a randomised 1-year trial. Int J Obes Relat Metab Disord 2004;28:1283–90.

[3] Marsset-Baglieri A, Fromentin G, Tome D, Bensaid A, Makkarios L, Even PC. Increasing the protein content in a carbohydrate-free diet enhances fat loss during 35% but not 75% energy restriction in rats. J Nutr 2004;134:2646–52.

[4] Westerterp-Plantenga MS. The significance of protein in food intake and body weight regulation. Curr Opin Clin Nutr Metab Care 2003;6:635–8.

[5] Halton TL, Hu FB. The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. J Am Coll Nutr 2004;23:373–85.

[6] Layman DK, Evans E, Baum JI, Seyler J, Erickson DJ, Boileau RA. Dietary protein and exercise have additive effects on body composition during weight loss in adult women. J Nutr 2005; 135:1903–10.

[7] Mikkelsen PB, Toubro S, Astrup A. Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate. Am J Clin Nutr 2000;72:1135–41.

[8] Johnston CS, Day CS, Swan PD. Postprandial thermogenesis is increased 100% on a high-protein, low-fat diet versus a high-carbohydrate, low-fat diet in healthy, young women. J Am Coll Nutr 2002;21:55–61.

[9] Crovetti R, Porrini M, Santangelo A, Testolin G. The influence of thermic effect of food on satiety. Eur J Clin Nutr 1998;52:482–8.

[10] Rousset S, Alves-Guerra MC, Mozo J, Miroux B, Cassard-Doulcier AM, Bouillaud F, et al. The biology of mitochondrial uncoupling proteins. Diabetes 2004;53(Suppl 1):S130–5.

[11] Dulloo AG, Samec S. Uncoupling proteins: their roles in adaptive thermogenesis and substrate metabolism reconsidered. Br J Nutr 2001;86:123–39.

[12] Boss O, Hagen T, Lowell BB. Uncoupling proteins 2 and 3, potential regulators of mitochondrial energy metabolism. Diabetes 2000;49:143–56.

[13] Petzke KJ, Friedrich M, Metges CC, Klaus S. Long-term dietary high protein intake up-regulates tissue specific gene expression of uncoupling proteins 1 and 2 in rats. Eur J Nutr 2005;44:414–21.

[14] Surwit RS, Wang S, Petro AE, Sanchis D, Raimbault S, Ricquier D, et al. Diet-induced changes in uncoupling proteins in obesity-prone and obesity-resistant strains of mice. Proc Natl Acad Sci U S A 1998;95:4061–5.

[15] Klaus S. Increasing the protein/carbohydrate ratio in a high fat diet delays adiposity and improves glucose homeostasis in mice. J Nutr 2005;135:1854–8.

[16] Petzke KJ, Elsner A, Proll J, Thielecke F, Metges CC. Long-term high protein intake does not increase oxidative stress in rats. J Nutr 2000;130:2889–96.

[17] National Research Council, Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council. Nutrient requirement of the laboratory rat. Nutrient requirements of laboratory animals, 4th rev ed. Washington (DC): National Academy Press; 1995. p. 11–79.

[18] Klaus S, Münzberg H, Trüloff C, Heldmaier G. Physiology of transgenic mice with brown fat ablation: obesity is due to lowered body temperature. Am J Physiol 1998;274:R287–93.

[19] Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949;109:1–9.

[20] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–9.

[21] Boeuf S, Klingenspor M, van Hal N, Schneider T, Keijer J, Klaus S. Differential gene expression in white and brown preadipocytes. Physiol Genomics 2001;7:15–25.

[22] Klaus S, Seivert A, Boeuf S. Effect of the beta-3-adrenergic agonist Cl316,243 on functional differentiation of white and brown adipocytes in primary cell culture. Biochim Biophys Acta (Mol Cell Res) 2001;1539:85–92.

[23] Jequier E, Acheson K, Schutz Y. Assessment of energy expenditure and fuel utilization in man. Annu Rev Nutr 1987;7:187–208.

[24] Luscombe ND, Clifton PM, Noakes M, Parker B, Wittert G. Effects of energy-restricted diets containing increased protein on weight loss,

resting energy expenditure, and the thermic effect of feeding in type 2 diabetes. Diabetes Care 2002;25:652–7.
[25] Ramirez I, Tordoff MG, Friedman MI. Dietary hyperphagia and obesity: what causes them? Physiol Behav 1989;45:163–8.
[26] Warwick ZS. Probing the causes of high-fat diet hyperphagia: a mechanistic and behavioral dissection. Neurosci Biobehav Rev 1996;20:155–61.
[27] Astrup A, Larsen TM, Harper A. Atkins and other low-carbohydrate diets: hoax or an effective tool for weight loss? Lancet 2004;364:897–9.
[28] Schrauwen P, Hesselink M. UCP2 and UCP3 in muscle controlling body metabolism. J Exp Biol 2002;205:2275–85.
[29] Klaus S, Rudolph B, Dohrmann C, Wehr R. Expression of uncoupling protein 1 in skeletal muscle decreases muscle energy efficiency and affects thermoregulation and substrate oxidation. Physiol Genomics 2005;21:193–200.
[30] Klaus S, Pültz S, Thöne-Reineke C, Wolfram S. Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. Int J Obes Relat Metab Disord 2005;29:615–23.
[31] Brehm BJ, Seeley RJ, Daniels SR, D'Alessio DA. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. J Clin Endocrinol Metab 2003;88:1617–23.
[32] Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, et al. A randomized trial of a low-carbohydrate diet for obesity. N Engl J Med 2003;348:2082–90.
[33] Hu FB. Protein, body weight, and cardiovascular health. Am J Clin Nutr 2005;82(Suppl):242S–7S.
[34] Lacroix M, Gaudichon C, Martin A, Morens C, Mathe V, Tome D, et al. A long-term high-protein diet markedly reduces adipose tissue without major side effects in Wistar male rats. Am J Physiol Regul Integr Comp Physiol 2004;287:934–42.
[35] Metges CC, Barth CA. Metabolic consequences of a high dietary protein intake in adulthood: assessment of the available evidence. J Nutr 2000;130:886–9.
[36] Eisenstein J, Roberts SB, Dallal G, Saltzman E. High-protein weight-loss diets: are they safe and do they work? A review of the experimental and epidemiologic data. Nutr Rev 2002;60:189–200.
[37] Garlick PJ. The role of leucine in the regulation of protein metabolism. J Nutr 2005;135:1553S–6S.
[38] Nair KS, Short KR. Hormonal and signaling role of branched-chain amino acids. J Nutr 2005;135:1547S–52S.
[39] Matsuzaki K, Kato H, Sakai R, Toue S, Amao M, Kimura T. Transcriptomics and metabolomics of dietary leucine excess. J Nutr 2005;135:1571S–5S.