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ORIGINAL CONTRIBUTION

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Long-term dietary high protein intake up-regulates tissue specific gene expression of uncoupling proteins 1 and 2 in rats

Summary *Background* The consequences of chronic high protein (HP) diets are discussed controversially and are not well understood. Rats adapted to HP exposure show an increased amino acid and fat oxidation and lower feed energy efficiency. We hypothesized that the dietary protein level can affect gene expression of uncoupling protein (UCP) homologues which is suggested to be involved in thermogenesis, substrate oxidation, and energy expenditure. *Aim of the study* To assess the mRNA expression of UCP homologues in various tissues of rats fed HP diets and to relate UCP gene expression to various parameters of substrate and energy metabolism. To obtain further indications for the possible involvement of UCP in reducing feed energy efficiency under conditions of HP exposure. *Methods* Adult rats were adapted to casein based diets containing either 13.8 % (adequate, AP), 25.7 % (medium, MP), or 51.3 % (high, HP) crude protein. Rats were fed for 8 wk and killed in the postabsorptive state. Energy expenditure and mRNA expression were measured using indirect calorimetry and Northern blot analysis, respectively. Pearson correlation coefficients were calculated to determine relationships between UCP mRNA expression and metabolic parameters. *Results* Hepatic UCP2 mRNA expression was increased by MP and HP diets compared to AP diet. In skeletal muscle UCP2 mRNA expression was lowest under MP conditions. UCP1 mRNA expression in brown adipose tissue (BAT) was significantly increased by HP exposure. The values were inversely associated with feed energy efficiency and positively with energy expenditure and oxygen consumption in the dark period. Skeletal muscle UCP2 and –3 mRNA expression strongly correlated with the plasma free fatty acid concentration, whereas BAT UCP1 and hepatic UCP2 gene expression did not. *Conclusions* Our results indicate that hepatic UCP2 and BAT UCP1 mRNA expression is related to the level of dietary protein intake. This suggests a role of UCPs in substrate oxidation and in thermogenesis under conditions of HP exposure.

Key words high protein diets – uncoupling protein – gene expression – energy expenditure – rats

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Introduction

The tolerable upper level of chronic protein or amino acid intake under various conditions not leading to health risks is still a matter of discussion and remains to be clearly defined [1–4]. Therefore, it is important to clarify whether the chronic intake of high protein diets has adverse or beneficial effects. Undesirable metabolic effects have been reported in man such as increased insulin secretion or renal net acid excretion with ~1.6 times the recommended intake of dietary protein [2].

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In animal studies beneficial effects of a long-term high protein (HP) exposure (about 50 % dry matter intake) have been shown such as reduced lipid deposition [5]. An increase in lean body mass and a reduction in body fat were noted when the level of protein in the diet of rats was enhanced from 10 to 20–25 % [6]. However, HP exposure during gestation (twice the gestation requirements) in rats resulted in greater total and relative fat mass and decreased total energy expenditure in the offspring [7]. Finally, HP diets are increasingly used in weight reducing therapies because they seem to reduce energy intake and to favor the loss of body fat [5, 8].

Metabolic adaptation to a dietary high protein (HP) supply involves an increase in amino acid transport and oxidation [9, 10]. In addition, long-term HP exposure results in decreased feed energy efficiency (body weight gain divided by energy intake) and considerably less adipose tissue mass in rats compared to adequate protein (AP) diets [5, 10]. This could be due to an increased fat oxidation and a less efficient ATP production per liter of oxygen consumed or could result from an increase in metabolic rate or a diminished energy absorption in the gut.

Uncoupling protein (UCP) 1 and its homologues UCP2 and UCP3 have been shown to lower mitochondrial membrane potential in several mammalian cell expression systems suggesting uncoupling activity of oxidative phosphorylation by generation of a proton leak. This provides an alternative route for protons to reenter the mitochondrial matrix. As a consequence, respiration rate relative to a certain ATP production can increase. Therefore, the efficiency of energy metabolism can decrease and energy will be wasted as heat [for review: 11–15]. We hypothesized that feeding HP diets leads to an activation of UCP gene expression which in turn contributes to lower metabolic efficiency and an increase in fat oxidation. Therefore, we studied whether mRNA expression of different UCP homologues in various tissues is altered in rats when exposed to HP diets. In addition, we related various parameters of substrate and energy metabolism to changes in UCP mRNA expression in order to obtain further indications for their physiological functions.

Materials and methods

Experimental design

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. 32/48-3560-0/3). Housing of rats (Shoe-Wist-Han; Charles River, Sulzfeld, Germany), composition of diets, blood and tissue sampling and conservation was essentially as described [10].

In brief, male adult Wistar rats (body weight ~ 230 g) were housed individually in a climate controlled room with a 12-h light:dark cycle. Rats were switched from a commercial rat diet (190 g/kg crude protein, 40 g/kg fat; Altromin GmbH, Lage, Germany) and randomly assigned to experimental diets (n = 10 per group) which were consumed ad libitum for 8 wk. The experimental diets (Table 1) contained either 13.8 % (adequate protein; AP), 25.7 % (medium protein; MP), or 51.3 % (high protein; HP) crude protein (casein). A crude protein content of 10–15 % is considered to be adequate for growing rats fed low-fiber diets containing a balanced amino acid pattern and 5 % fat [16]. Protein was exchanged isoenergetically for wheat starch, and supplemented with DL-methionine (0.35 g/100 g diet). Water was provided ad libitum. Food intake was monitored daily and body weight was monitored weekly. After 8 wk of feeding energy expenditure 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 h and 1030 h. Blood samples were collected from trunk and plasma was obtained by centrifugation. Samples of liver, skeletal muscle (m. biceps femoris), interscapular brown adipose tissue (BAT), epididymal fat pad, intestinal mucosa, and spleen were collected and stored in liquid nitrogen until analysis.

Table 1 Composition of purified test diets containing different protein concentrations (*AP* adequate protein; *MP* medium protein; *HP* high protein)

Diet	AP	MP g/100 g	HP
Casein[1]	15	30	60
Wheat starch[2]	58	43	13
Saccharose[3]	10	10	10
Palm kernel fat[4]	3	3	3
Soy bean oil[5]	2	2	2
Cellulose[6]	5	5	5
Mineral mixture[7]	5	5	5
Vitamin mixture[8]	2[9]	2[9]	2[9]
Crude protein	13.8	25.7	51.3
Gross energy, kJ/g	16.8	17.9	19.9

[1] Dauermilchwerk Peiting GmbH, Landshut, Germany, contained 86 % crude protein (% N × 6.38); [2] Heller u. Strauß, Berlin, Germany; [3] Nordzucker GmbH, Uelzen, Germany; [4] Union Deutsche Lebensmittelwerke, Hamburg, Germany; [5] Kunella-Feinkost GmbH, Cottbus, Germany; [6] Rettenmeier, Ellwangen, Germany; [7] 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, Lage, Germany); [8] 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, Lage, Germany)

Energy expenditure

Energy expenditure (EE) of individual rats was measured using indirect calorimetry as described [17]. Rats were housed in metabolism cages for 23 h of a day allowing collection of urine and feces separately. Food was available between 1900 h 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 [18] as

$$EE\ [kJ] = 16.17 \times VO_2 + 5.03 \times VCO_2 - 5.98 \times N_{ex}$$

where VO_2 is the oxygen consumption (l/d), VCO_2 is the CO_2 production (l/d), and N_{ex} is the urinary nitrogen excretion (g/d). 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 h and 0600 h. Resting metabolic rate (RMR) was defined as the mean of the 10 lowest values during the measurement period according to a procedure previously described in mice [17]. Net oxidation rates of fat (FO), carbohydrate (CO), and protein (PO) were calculated in g/d [19] as

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

Assays

Plasma free amino acid and urea concentrations were determined essentially as described [10]. Circulating free fatty acids (FFA) 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-Wyhlen, 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 by means of an adiabatic bomb calorimeter (IKA-Calorimeter C 5000, IKA-Werke GmbH & Co. KG, Staufen, Germany).

Northern blot analysis

Total RNA from individual tissues was extracted using a single-step acid phenol-guanidine protocol [20] 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). The blots were probed with ^{32}P-labeled probes in a hybridization solution containing sodium phosphate (0.5 M), EDTA (1 mmol/l), sodium dodecyl sulfate (SDS) (7%) and bovine serum albumin (1%) at 63 °C over night, and washed twice with saline sodium citrate (SSC) 2xSSC-0.1% SDS for 20 min at room temperature, twice with 0.1xSSC-0.1% SDS for 20 min at 42 °C and twice with 0.1xSSC-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 previously [22], and a UCP3 cDNA probe was kindly provided by Dr. Martin Klingenspor (University of Marburg, Germany). For leptin a rat specific 244bp cDNA fragment was obtained by reverse transcription and PCR amplification of rat mRNA isolated from white fat using following leptin specific primers: CCTGTGGCTTTGGTCCTATCTG (forward) and AGGCAAGCTGGTGAGGATCTG (reverse).

Statistical analysis

Data are reported as means ± SEM. Differences between mean values were determined by ANOVA followed by comparisons using the Newman-Keuls multiple range test (WinSTAT®, vers. 1999.2, R. Fitch software, Staufen, Germany). Pearson correlation coefficients were calculated to determine the relationship between selected parameters. Differences with $P < 0.05$ were considered statistically significant if not stated otherwise.

Results

Rats fed the MP and HP diets had a significantly lower final body weight and a lower epididymal fat pad weight than AP diet fed rats (Table 2). Correspondingly, the body weight gain throughout the study was decreased with increasing dietary protein content. However, there was no significant difference in these parameters between MP and HP fed rats, although the protein content of HP diet was twice that of the MP diet.

Postabsorptive concentrations of FFA were significantly higher in rats fed the HP diet but not in rats fed the MP diet compared to those fed the AP diet (Table 2). Thyroid hormones are related to the rate of energy expenditure; however, plasma fT3 levels were not significantly affected by dietary protein exposure. However, plasma urea concentrations were enhanced dose-dependently with increasing dietary protein concentration

Table 2 Body weight, fat weights and plasma metabolites of rats fed for 8 wk diets with different protein concentrations (*AP* adequate, 13.8 %; *MP* medium, 25.7 %; *HP* high, 51.3 %). Rats were in the postabsorptive state, 1–2 h after removal of food offered overnight[1–3]

Diet	AP	MP	HP
Body weight			
Final body weight (g)	445 ± 12[b]	415 ± 10[a]	409 ± 8[a]
Body weight increase (g/8 wk)	209 ± 11[b]	182 ± 9[a, b]	177 ± 9[a]
Total epididymal fat pad weight (g)	14.7 ± 1.7[b]	11.0 ± 1.0[a]	10.0 ± 0.9[a]
Plasma metabolites			
FFA (mmol/l)	0.95 ± 0.08[a]	0.95 ± 0.10[a, b]	1.27 ± 0.13[b]
fT_3 (pg/ml)	3.63 ± 0.11	3.61 ± 0.11	3.46 ± 0.25
Urea (µmol/ml)	5.60 ± 0.23[a]	8.96 ± 0.47[b]	11.25 ± 0.57[c]

[1] Values are means ± SEM, $n = 10$. Within a row, values without a common superscript differ, $P < 0.05$; [2] For diet compositions and more details see Material and Methods. [3] *FFA* free fatty acids; *fT_3* free triiodothyronine

(Table 2). Plasma leucine concentrations were 182 ± 15, 253 ± 57, and 315 ± 73 µmol/l for AP, MP, and HP diet fed rats, respectively. In contrast, plasma threonine concentrations were 487 ± 101, 343 ± 53, and 242 ± 61 µmol/l for AP, MP, and HP diet fed rats, respectively. The resulting ratios of the corresponding leucine to threonine concentrations were 0.37, 0.74 and 1.30 for AP, MP, and HP diets fed rats, and appear to be directly related to the dietary protein intake.

Data for nutrient intake and energy expenditure are presented in Table 3. Total gross energy intake was significantly higher in HP diet fed rats as compared to AP diet fed rats. Total nitrogen intake was increased with increasing dietary protein content as expected and was more than 4 times as high in HP as compared to AP rats. Feed energy efficiency decreased with increasing dietary protein.

Mean RMR and TEE were not affected by dietary protein exposure (Table 3). However, body weight related 24 h oxygen consumption (VO_2) showed a tendency to be increased in MP and HP rats ($P < 0.06$), and VO_2 during the dark phase relative to the body weight was significantly increased in MP and HP groups. RQ values were significantly lower with MP and HP diet as compared to the AP diet. This reflects a significant increase in protein and fat oxidation and a decrease in carbohy-

Table 3 Nutrient intake, energy expenditure, and net oxidation of macronutrients of rats fed for 8 wk diets with different protein concentrations (*AP* adequate, 13.8 %; *MP* medium, 25.7 %; *HP* high, 51.3 %)[1–3]

Diet	AP	MP	HP
Nutrient intake			
Total gross energy intake (MJ)	20.7 ± 0.5[a]	20.7 ± 0.5[a]	23.0 ± 0.4[b]
Total N intake (g)	22.5 ± 0.5[a]	52.0 ± 1.3[b]	100.0 ± 1.9[c]
Mean feed energy efficiency (g/MJ)[4]	9.62 ± 0.25[c]	8.90 ± 0.21[b]	7.73 ± 0.32[a]
Overall daily energy expenditure (d 56)			
TEE (kJ/h)	10.6 ± 0.2	10.8 ± 0.4	10.4 ± 0.4
TEE (kJ/(h × kg))	23.8 ± 0.6	25.9 ± 0.7	24.9 ± 0.7
RMR (kJ/h)	7.58 ± 0.19	7.15 ± 0.22	7.13 ± 0.33
RMR (kJ/(h × kg))	17.0 ± 0.6	17.2 ± 0.4	17.1 ± 0.7
VO_2 (lO_2/h)	0.51 ± 0.01	0.52 ± 0.02	0.52 ± 0.02
VO_2 (lO_2/(h × kg))	1.14 ± 0.03	1.26 ± 0.03	1.24 ± 0.04
RQ	0.97 ± 0.01[c]	0.92 ± 0.01[b]	0.86 ± 0.01[a]
Dark phase energy expenditure (d 56, 1900 h – 0600 h)			
EE_{dark} (kJ/h)	11.3 ± 0.2	11.6 ± 0.4	11.1 ± 0.5
EE_{dark} (kJ/(h × kg))	25.4 ± 0.7	28.0 ± 0.8	26.7 ± 0.9
$VO_{2,\,dark}$ (lO_2/h)	0.54 ± 0.01	0.57 ± 0.02	0.56 ± 0.02
$VO_{2,\,dark}$ (lO_2/(h × kg))	1.21 ± 0.03[a]	1.37 ± 0.04[b]	1.33 ± 0.05[b]
Net oxidation of macronutrients			
FO (g/d)	0.35 ± 0.12[a]	0.77 ± 0.15[a, b]	1.16 ± 0.23[b]
CO (g/d)	12.14 ± 0.26[c]	9.06 ± 0.68[b]	4.98 ± 0.50[a]
PO (g/d)	1.46 ± 0.08[a]	3.64 ± 0.30[b]	6.19 ± 0.57[c]

[1] Values are means ± SEM, $n = 10$. Within a row, values without a common superscript differ, $P < 0.05$. [2] For diet compositions and more details see Material and Methods. [3] *CO* carbohydrate oxidation; *EE* energy expenditure; *FO* fat oxidation; *PO* protein oxidation; *RMR* resting metabolic rate; *RQ* respiratory quotient; *TEE* total energy expenditure; *VO_2* oxygen consumption. [4] Body weight gain (g) divided by energy intake (MJ)

drate oxidation with MP and HP exposure (Table 3). The urinary nitrogen excretions were 0.23 ± 0.01, 0.58 ± 0.05, and 0.99 ± 0.09 g/d for AP, MP, and HP diet fed rats, respectively.

UCP2 mRNA expression in liver was influenced by MP and HP diet exposure (Table 4). The expression of UCP2 in skeletal muscle was not increased by HP exposure and actually decreased in MP diet fed rats compared to AP diet fed rats. There was no effect of dietary protein on UCP2 mRNA levels in spleen, intestinal mucosa, and WAT, on UCP3 mRNA levels in skeletal muscle, and on leptin mRNA levels in BAT and in WAT. As expected, WAT leptin mRNA expression was correlated with the epididymal fat pad weight ($r = 0.620$, $P < 0.001$) which reflects the association of leptin gene expression with body fat mass. UCP1 mRNA expression in BAT was significantly higher with HP diet but not with MP diet as compared to AP diet (Table 4).

The data were used to compute correlations with UCP mRNA expression levels (Table 4). Positive correlations were obtained between UCP1 mRNA expression in BAT and VO_2 in the dark phase ($P < 0.01$) and TEE ($P < 0.05$), whereas correlations were negative for BAT UCP1 mRNA expression and body weight ($P < 0.1$), epididymal fat pad weight ($P < 0.05$), and feed energy efficiency ($P < 0.05$). There was no correlation between plasma FFA concentrations and BAT UCP1 gene expression. A negative correlation was computed between hepatic UCP2 mRNA expression and body weight. Further, expression levels of skeletal muscle UCP2 and UCP3 positively correlated with plasma FFA concentrations.

Discussion

This study shows that the intake level of dietary protein is associated with UCP2 mRNA expression in liver and skeletal muscle. Furthermore, there is a clear relationship between dietary protein level, increased UCP1 gene expression in BAT, reduced feed energy efficiency, and a simultaneous raise in VO_2 and EE during the dark phase. This might be causally involved in the observed decreased body and epididymal fat pad weight and supports the suggested role of UCP1 in nonshivering thermogenesis [14] under conditions of HP exposure. A comparable increase in UCP1 gene expression was reported in mice fed high-fat diet as compared to low-fat controls [23]. However, the authors did not consider that the high-fat diet contained 31 % casein (wt/wt), whereas the casein concentration in the low-fat diet was only 18 % casein and that food (protein) intake was significantly higher in high-fat diet fed mice as compared to low-fat controls [23]. Therefore, based on our results an additional influence of dietary protein on UCP1 gene expression in the high-fat diet study [22] cannot be excluded. Generally, the function of UCP1-mediated thermogenesis has been established convincingly in rodents [24] and a defect or non functional BAT thermogenesis (e. g. in thermoneutrality) is considered to be causative for the development of obesity in mice [23]. On the other hand, there are results showing that UCP1-deficient mice do not develop obesity following high-fat adipogenic diets [25]. The alternative thermogenic mechanisms in UCP1-deficient mice leading to resistance to diet-induced obesity and a greater proportion of fat oxidation remain to be identified [25]. Although the physiologic functions of UCP2 have not been fully elucidated we suggest a role of hepatic UCP2 in thermogenesis under conditions of HP feeding: There is a negative correlation of liver UCP2 expression with body weight and epididymal fat weight and a numerically positive correlation with oxygen consumption in the dark period. Furthermore, UCP2 expression in liver shows the strongest correlation with postabsorptive plasma urea concentrations (Table 5) and with postabsorptive ratios of plasma leucine to threonine concentrations (not shown), the lat-

Table 4 Gene expression of UCP homologues and leptin in various tissues of rats fed diets with different protein concentrations (*AP* adequate, 13.8 %; *MP* medium, 25.7 %; *HP* high, 51.3 %). Rats were in the postabsorptive state, 1–2 h after removal of food offered overnight[1–3]

Diet	AP	MP	HP
UCP1, BAT (AU)	0.95 ± 0.08[a]	0.94 ± 0.12[a]	1.44 ± 0.11[b]
UCP2 (AU)			
Liver	0.76 ± 0.12[a]	1.03 ± 0.04[b]	1.09 ± 0.04[b]
Spleen	1.08 ± 0.10	0.92 ± 0.06	1.13 ± 0.07
Mucosa (proximal jejunum, 30 cm)	0.72 ± 0.08	0.65 ± 0.05	0.74 ± 0.04
WAT	0.83 ± 0.06	0.82 ± 0.09	0.74 ± 0.06
Skeletal muscle	0.71 ± 0.10[a, b]	0.45 ± 0.05[a]	0.90 ± 0.12[b]
UCP3, skeletal muscle (AU)	0.77 ± 0.12	0.57 ± 0.11	0.86 ± 0.07
Leptin (AU)			
BAT	1.02 ± 0.11	0.92 ± 0.13	1.04 ± 0.07
WAT	0.99 ± 0.20	0.92 ± 0.13	1.04 ± 0.07

[1] Values are means ± SEM, $n = 10$. Within a row, values without a common superscript differ, $P < 0.05$. [2] For diet compositions and more details see Material and Methods. [3] *AU* arbitrary units; *BAT* interscapular brown adipose tissue; *UCP* uncoupling protein; *WAT* white adipose tissue

Table 5 Pearson correlation coefficients between UCP1, UCP2, and UCP3 mRNA expression in BAT, liver and skeletal muscle (m. biceps femoris) and parameters of postabsorptive circulating metabolites in rats fed diets with different protein concentrations[1–4]

Parameter	UCP2, Liver	UCP2, Skeletal muscle	UCP3, Skeletal muscle	UCP1 BAT
Body weight	–0.346	ns	ns	–0.273[2]
Epididymal fat pad weight	–0.270[2]	ns	ns	–0.358
$VO_{2,\,dark}$, $lO_2/(h \times kg)$	0.274[2]	ns	ns	0.444
EE_{dark}	ns	ns	ns	0.347
FEE, g body weight gain/MJ EI	ns	–0.306	–0.270[2]	–0.365
Urea concentration, plasma	0.524	ns	ns	0.367
FFA concentration, plasma	ns	0.586	0.368	ns
fT_3 concentration, plasma	–0.272[2]	ns	ns	0.355

[1] $n = 30$; correlation coefficients were significant ($P < 0.05$) if not otherwise stated; one-sided test; correlation coefficients not significant ($P > 0.1$) are labeled ns. [2] Correlation coefficient of borderline significance ($0.1 > P > 0.05$). [3] For diet compositions and more details see Material and Methods. [4] *BAT* interscapular brown adipose tissue; EE_{dark} energy expenditure in dark cycle; *EI* energy intake; *FEE* feed energy efficiency; *FFA* free fatty acids; fT_3 free triiodothyronine; $VO_{2,\,dark}$ dark phase oxygen consumption in; *UCP* uncoupling protein

ter two reflecting rates of protein and amino acid oxidation. In addition, there was a positive correlation of hepatic UCP2 gene expression with net protein oxidation ($P = 0.003$). These findings might indicate a possible functional relationship between liver UCP2 and amino acid oxidation which is induced by HP exposure [10].

The different patterns of UCP expression in liver and skeletal muscle following dietary HP exposure support the notion that tissue specific UCP expression is differentially regulated as suggested earlier [26] and as concluded from studies in mice [23, 27]. Intestine and liver as splanchnic tissues are the organs which receive dietary amino acids during the first pass and represent the principal site of overall amino acid catabolism, whereas amino acid metabolism in skeletal muscle includes the utilization of amino acids for energy (leucine) and protein synthesis (all proteinogenic amino acids), as well as nitrogen transfer (glutamine), and glucose metabolism (alanine). A tissue specific regulation of UCP expression in response to dietary protein intake thus seems not surprising. However, we found dose-dependent higher UCP2 mRNA levels with increasing dietary protein exposure among splanchnic tissues only in liver and, in contrast, no effects of dietary protein on intestinal mucosa UCP2 gene expression (Table 4).

Interestingly, with increasing dietary protein level the plasma leucine levels rise. This results from the preferential release of branched chain amino acids from the splanchnic bed into the body because the liver does not contain appreciable amounts of branched chain amino acid transaminases [28]. In contrast, plasma threonine decreases due to catabolization by the rate limiting hepatic threonine dehydratase (EC 4.2.1.16), inducible by increasing protein intake [29]. Similar observations can be also made in healthy human subjects [30]. Thus, we propose that the leucine/threonine ratio in the plasma can be used as an indicator of protein nutritional status: the ratio is directly associated to the dietary protein intake.

Plasma levels of FFA, urea and some indispensable amino acids showed significant differences between diet groups. This is not surprising since rats were killed about 1–2 h after food withdrawal, i. e. in the postabsorptive state. For example, in rats long-term-adapted to the same protein levels as used in this study but fasted for 20 h [10], there was no difference in plasma urea concentration of rats fed a HP or an AP diet (unpublished observation by KJ Petzke). Also plasma FFA levels can show different patterns in different nutritional states (postabsorptive, fasted). It has been proposed that UCPs could have a role in fatty acid metabolism, possibly as a fatty acid carrier in cycling of anionic fatty acids across the inner mitochondrial membrane [11, 12, 31]. In contrast to other reports where a positive relation between plasma FFA level and hepatic UCP2 gene expression was observed (e. g. during starvation, cold exposure, overnutrition, fat enriched diet) [12, 31–35], the plasma FFA concentration under our conditions was not related to hepatic UCP2 and BAT UCP1 gene expression. This is somewhat surprising since both plasma FFA levels and hepatic UCP2 expression were increased with the HP feeding (Tables 2 and 4). On the other hand, a positive correlation was evident between skeletal muscle UCP2 and UCP3 gene expression and plasma FFA concentrations (Table 5).

The observed changes in UCP gene expression need to be confirmed by measurement of UCP protein levels since it has been shown that, especially for UCP2, mRNA expression does not always correlate well with UCP2 protein content [36]. However, we suggest that the pattern of UCP expression in different tissues reflects long-term adaptive changes related to dietary protein intake rather than acute nutritional effects on UCP expression. This is supported by findings from a different study where rats were long-term-adapted (18 weeks) to the same protein levels as used in the present study [10]. In contrast to the present study, rats had been fasted for 20 h before sacrifice, but UCP2 and 3 expressions

showed the same dependency on dietary protein levels as shown here [unpublished observations by KJ Petzke].

Conclusions

The results presented here suggest that gene expression of UCP2 and of UCP1 and to a lesser extent of UCP3 in liver and skeletal muscle of rats is dependent on the level of dietary protein intake. Increased BAT UCP1 expression was accompanied by higher EE under HP exposure. We found a higher hepatic UCP2 mRNA expression under HP exposure. These findings might indicate a role of hepatic UCP2 in decreasing the metabolic efficiency, a function known of UCP1 in BAT. Therefore, the study provides additional information about adaptive processes involved in the response to variations in the protein content of the diet which is important for risk-benefit estimation of high protein exposure. Further studies are required to investigate tissue specific and dietary amino acid dependent regulatory mechanisms of UCP gene expression and to elucidate the suggested involvement of UCPs in the control of substrate oxidation and energy expenditure under conditions of dietary HP intake.

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