British Journal of Nutrition (2010), **104**, 1395–1405
doi:10.1017/S0007114510002060

Presence or absence of carbohydrates and the proportion of fat in a high-protein diet affect appetite suppression but not energy expenditure in normal-weight human subjects fed in energy balance

Margriet A. B. Veldhorst[1,2]*, Klaas R. Westerterp[1,2], Anneke J. A. H. van Vught[1,2] and Margriet S. Westerterp-Plantenga[1,2]

[1]*Department of Human Biology, NUTRIM School for Nutrition, Toxicology and Metabolism, Maastricht University Medical Centre, PO Box 616, 6200 MD Maastricht, The Netherlands*
[2]*Top Institute Food and Nutrition, Wageningen, The Netherlands*

(Received 18 September 2009 – Revised 7 April 2010 – Accepted 21 April 2010 – First published online 22 June 2010)

Two types of relatively high-protein diets, with a normal or low proportion of carbohydrates, have been shown effective for weight loss. The objective was to assess the significance of the presence or absence of carbohydrates and the proportion of fat in high-protein diets for affecting appetite suppression, energy expenditure, and fat oxidation in normal-weight subjects in energy balance. Subjects (aged 23 (SD 3) years and BMI 22·0 (SD 1·9) kg/m^2) were stratified in two groups. Each was offered two diets in a randomised cross-over design: group 1 (*n* 22) – normal protein (NP; 10, 60 and 30 % energy (En%) from protein, carbohydrate and fat), high protein (HP; 30, 40 and 30 En%); group 2 (*n* 23) – normal protein (NP-g; 10, 60 and 30 En%), high protein, carbohydrate-free (HP-0C; 30, 0 and 70 En%) for 2 d; NP-g and HP-0C were preceded by glycogen-lowering exercise (day 1). Appetite was measured throughout day 2 using visual analogue scales (VAS). Energy expenditure (EE) and substrate oxidation (respiratory quotient; RQ) were measured in a respiration chamber (08.00 hours on day 2 until 07.30 hours on day 3). Fasting plasma β-hydroxybutyrate (BHB) concentration was measured (day 3). NP-g and NP did not differ in hunger, EE, RQ and BHB. HP-0C and HP *v.* NP-g and NP, respectively, were lower in hunger ($P<0·05$; $P<0·001$) and RQ ($P<0·01$; $P<0·001$) and higher in EE ($P<0·05$; $P=0·07$) and BHB ($P<0·05$; $P<0·001$). Hunger and RQ were lower with HP-0C than HP (693 (SD 208) *v.* 905 (SD 209) mm VAS × 24 h, $P<0·01$; 0·76 (SD 0·01) *v.* 0·81 (SD 0·02), $P<0·01$); BHB was higher (1349 (SD 653) *v.* 332 (SD 102) μmol/l; $P<0·001$). ΔHunger, ΔRQ, and ΔBHB were larger between HP-0C–NP-g than between HP–NP (−346 (SD 84) *v.* −107 (SD 52) mm VAS × 24 h, $P<0·01$; −0·09 (SD 0·00) *v.* −0·05 (SD 0·00), $P<0·001$; 1115 (SD 627) *v.* 104 (SD 42) μmol/l, $P<0·001$). In conclusion, appetite suppression and fat oxidation were higher on a high-protein diet without than with carbohydrates exchanged for fat. Energy expenditure was not affected by the carbohydrate content of a high-protein diet.

Appetite: Carbohydrates: High-protein diets: Energy expenditure

Two types of relatively high-protein diets, i.e. with a relatively normal[1–6] or a relatively low[7–18] proportion of carbohydrates, have been shown to be successful for body-weight loss and weight maintenance. The proportion of protein in these diets ranged from 20 to 40 % of energy (En%), whereas the proportion of carbohydrates ranged from 4 to 50 En%[1–18]. Diets with less than 38 En% from carbohydrates have been claimed to be low-carbohydrate diets and are indicated to be ketogenic[7–18]. However, in order to be effectively ketogenic, a diet should contain less than 20 g or, depending on energy intake, only 2–6 En% from carbohydrates[19,20]. Because the maximal protein content in a high-protein diet in energy balance is limited to about 30 % of energy[21], the proportion of fat in a high-protein, low-carbohydrate diet always will be relatively high.

Many favourable results have been published with respect to body-weight loss after high-protein, low-carbohydrate, high-fat diets, i.e. weight losses of 4·5 to 12·0 kg compared with 2·5 to 6·5 kg after control diets in 2 to 6 months have been reported[7,9,14,16,17]. After diets relatively high in protein but with normal carbohydrate content, body-weight loss after 2 to 6 months ranged from 4·9 to 8·9 kg compared with 3·4 to 6·9 kg after control diets[1,2,5,6]. Moreover, Johnstone *et al.* showed that weight loss was larger after a high-protein, low-carbohydrate, high-fat diet than after a high-protein, normal-carbohydrate diet for 4 weeks (6·34 *v.* 4·35 kg)[18]. In addition, a large meta-analysis also showed that high-protein, low-carbohydrate, high-fat diets increased body-weight loss compared with control diets[22]. Thus, high-protein, low-carbohydrate, high-fat diets may be more

Abbreviations: EN%, percentage of energy; group 1, diets HP and NP; group 2, diets HO-0C and NP-g; HP, high-protein; HP-0C, high-protein, carbohydrate-free; NP, normal-protein group 1; NP-g, normal-protein group 2 (with glycogen-lowering); RQ, respiratory quotient; VAS, visual analogue scale; W_{max}, maximal power output.

* **Corresponding author:** Dr Margriet Veldhorst, fax +31 43 3670976, email m.veldhorst@hb.unimaas.nl

effective in reducing body weight up to 6 months than high-protein diets with normal carbohydrate content. Several studies with subjects in energy balance under well-controlled conditions have shown that high-protein diets in general affect the metabolic targets appetite suppression, energy expenditure and fat oxidation[23–25]. The metabolic targets that are suggested to be affected by relatively high-protein, low-carbohydrate, high-fat diets and are hypothesised to contribute to a reduction in body weight are suppression of appetite, increase in energy expenditure and/or increase of fat oxidation[1–18]. However, effects of these diets on these metabolic targets have not been compared under controlled conditions. Therefore, it is not known which of these metabolic targets are especially affected by the absence of carbohydrates and a high fat content in a high-protein diet.

Appetite, energy expenditure, fat oxidation and body composition are affected by obesity, negative energy balance and/or weight loss, therefore, effects of diet composition on these metabolic targets may be confounded by obesity or negative energy balance. In order to actually study effects of the macronutrient composition of the diets on the metabolic targets under highly controlled conditions, we studied normal-weight subjects in energy balance while staying in a respiration chamber. The objective of the present study was to assess the significance of the presence or absence of a normal proportion of carbohydrates and differences in the proportion of fat in a relatively high-protein diet for affecting appetite suppression, energy expenditure, and fat oxidation, measured in a controlled setting in a respiration chamber with normal-weight subjects in energy balance.

Subjects and methods

Subjects

Forty-five healthy volunteers (twenty men and twenty-five women, BMI 18·5–25 kg/m^2, aged 18–40 years) were recruited by advertisements placed on notice boards at the university. All subjects underwent a medical screening procedure and were in good health, non-smokers, not using medication and at most moderate alcohol users (ten or less alcoholic consumptions per week). After medical screening subjects were randomly stratified in two groups based on sex, age and BMI; there were no statistically significant differences between the groups (Table 1). The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethics Committee of the Maastricht University Medical Centre. Written informed consent was obtained from all participants. Subjects received financial compensation for participation in the study. The study was conducted from January 2006 until December 2008.

Experimental sessions

Protocol. Subjects were stratified in two groups. In both groups, subjects were offered two different diets while staying in a respiration chamber for 36 h (20.00 hours on day 1 until 08.00 hours on day 3). The first 12 h of the 36 h period were considered as baseline measurements to familiarise the subjects with the respiration chamber. The data of the last 24 h were included for analyses. The sessions were conducted 4 weeks apart to preclude influences of the menstrual cycle. Subjects were instructed to refrain from heavy exercise for 2 d before the study and not to consume more than two glasses of alcohol 2 d before the study. The day before the study the subjects were provided with meals and fed according to the study diet. Group 1 received a high-protein diet and a normal-protein diet (HP and NP; see Diets and energy intake) in a single-blind, randomised, cross-over design. Group 2 received a high-protein, carbohydrate-free diet and a normal-protein diet (HP-0C and NP-g; see Diets and energy intake) in a single-blind, randomised, cross-over design, both preceded by a glycogen-lowering exercise test that took place at 17.00 hours on day 1, in order to mimic the long term effects of a low-carbohydrate diet that depletes glycogen stores to a great extent[26]. This glycogen-lowering exercise test has been shown before not to affect energy expenditure or respiratory quotient (RQ)[27]; however, an effect on appetite has been shown by Melanson *et al.*[28]. Therefore, in the present study potential effects of the exercise test on appetite ratings and β-hydroxybutyrate were controlled for by including the NP-g diet condition. In order to limit the burden of the subjects, a study with two similar groups each receiving two diets was chosen.

A fasting blood sample was taken at the end of each session to measure the concentration of β-hydroxybutyrate in plasma (08.00 hours on day 3). Ketone bodies, of which β-hydroxybutyrate is the most important in the blood, are produced when whole-body metabolism shifts towards obtaining a greater percentage of energy from lipid sources by a larger fat oxidation when carbohydrate availability is low and fat is available[19].

At the first experimental session body composition was determined.

Diets and energy intake. Group 1 received a diet with 30, 40 and 30 En% from protein, carbohydrate and fat (HP) and a diet with 10, 60 and 30 En% from protein, carbohydrate and fat (NP). Here, the effects of a relatively high and normal proportion of protein were compared while fat was kept constant and the proportion of carbohydrates was within the normal range of 40–60 En%[29]. Group 2 received a diet

Table 1. Subject characteristics of the subjects stratified in group 1 (normal-protein diet and high-protein diet) and group 2 (normal-protein diet and high-protein, carbohydrate-free diet, both preceded by glycogen lowering)*

(Mean values and standard deviations)

	Group 1 (*n* 22)		Group 2 (*n* 23)	
	Mean	SD	Mean	SD
Male (*n*)	10		10	
Female (*n*)	12		13	
Age (years)	23	3	23	2
Height (m)	1·75	0·08	1·74	0·08
Weight (kg)	66·9	8·3	67·1	10·3
BMI (kg/m^2)	21·7	1·5	22·2	2·3
Body fat (%)	20·3	3·1	22·9	6·7

* Body composition was determined using hydrodensitometry and the ^{2}H dilution technique and was subsequently calculated using the equation of Siri[40]. There were no differences between the two groups.

with 30, 0 and 70 En% from protein, carbohydrate and fat (HP-0C) and a diet with 10, 60 and 30 En% from protein, carbohydrate and fat (NP-g). The proportion of protein was the same as in the diets of group 1; however, the HP-0C diet did not contain any carbohydrates and had a high fat content. Subjects performed a glycogen-lowering exercise test.

Subjects were fed with a study diet designed to provide energy balance. For the meals that subjects consumed before the experimental sessions at home, the energy content was based on BMR which was calculated with the equation of Harris–Benedict and multiplied with an activity index of 1·7, which is the average value reported for the general population in The Netherlands[30,31]. In order to reach the required macronutrient compositions, amounts of the foods to be served were calculated accordingly subject-specifically. The macronutrient composition of the diets and the average macronutrient intake and energy density are presented in Table 2. During the screening visit it was tested whether the subjects liked all food items sufficiently. Dietary fibre intake was 18·6 (SD 3·1), 20·4 (SD 2·9), 6·1 (SD 1·8), 20·4 (SD 2·9) g/d in the HP, NP, HP-0C and the NP-g conditions, respectively.

To determine the appropriate level of energy intake for attaining energy balance in the respiration chamber, the sleeping metabolic rate was measured during the first night of the first experimental session and multiplied by an activity index of 1·4, which represents an average value for subjects in a respiration chamber[32]. Energy intake was divided over the meals as 20 % for breakfast (08.00 hours), 40 % for lunch (13.00 hours) and 40 % for dinner (18.00 hours). Subjects were instructed to finish meals within 20 min; on average they finished their meals within 15 min.

Glycogen-lowering exercise test. Before the HP-0C and the NP-g diet, subjects performed a glycogen-lowering exercise test on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) in the afternoon of day 1. Before, at the screening visit, subjects performed an incremental exhaustive exercise test according to the protocol of Kuipers *et al.* on an electronically braked cycle ergometer to determine maximal power output (W_{max})[33], which was 258 (SD 50) W. After a warming-up at 50 % of their W_{max} for 5 min, subjects cycled for 2 min at 90 % of their W_{max} followed by 2 min at 50 % of their W_{max}; this was repeated until subjects were no longer able to maintain the high-intensity exercise. The maximal intensity was then lowered to 80 % of W_{max}. When this intensity also could no longer be maintained, the maximal intensity was decreased to 70 % of W_{max}. The test was ended after exhaustion[27]. Subjects were allowed to consume water during the exercise test. Heart rate was monitored continuously during the exercise with a Polar Sport tester (Polar, Kempele, Finland).

Appetite profile. Appetite profile was measured using 100 mm visual analogue scales (VAS), with the questions 'How hungry are you?' and 'How full do you feel?' that were anchored with 'not at all' and 'extremely'. Subjects were instructed to rate themselves by marking the scale at the point that was most appropriate to their feeling at that time. Questionnaires were completed during each experimental session at 07.55, 08.30, 09.00, 09.30, 10.00, 11.00, 12.00, 12.55, 13.30, 14.00, 14.30, 15.00, 16.00, 17.00, 17.55, 18.30, 19.00, 19.30, 20.00, 21.00 and 22.00 hours on day 2. For the calculation of the 24 h area under the curve (AUC) the VAS ratings were interpolated from the latest measurement at night until the first measurement in the morning[34].

Energy expenditure and substrate oxidation. O_2 consumption and CO_2 production were measured in the respiration chamber[35]. This is a 14 m^3 room furnished with a bed, chair, computer, television, DVD player, telephone, intercom, sink and toilet. The room was ventilated with fresh air at a rate of 70–80 litres/min. The ventilation rate was measured using electronically modified dry gas meters (G6; Schlumberger, Dordrecht, The Netherlands). The analysis system consisted of dual pairs of IR CO_2 (ABB/Hartman&Braun Uras, Frankfurt am Main, Germany) and paramagnetic O_2 analysers (Servomex 4100; Servomex Group Ltd, Crowborough, East Sussex, UK). Data acquisition was performed using custom-built interfaces (IDEE Maastricht University, Maastricht, The Netherlands), a computer (Apple Macintosh, Cupertino, CA, USA), and graphical programming environment (Labview; National Instruments, Austin, TX, USA).

Energy expenditure and carbohydrate, fat and protein oxidation were calculated from the measurements of O_2 consumption, CO_2 production, and urinary N excretion, using the formula of Brouwer[36]. Energy expenditure and 24 h RQ were measured from 08.00 hours on day 2 until 07.30 hours on day 3. Sleeping metabolic rate was defined as the lowest mean energy expenditure measured over 3 consecutive h between 00.00 and 06.00 hours. From the second voiding on day 2 until the first voiding on day 3, 24 h urine was collected. Samples were collected in containers with 10 ml H_2SO_4 to prevent N loss through evaporation. Volume and N concentration were measured, the latter using a nitrogen analyser (Elemental Analyzer; CHN-O-Rapid, Heraeus, Wellesley, MA, USA).

β-Hydroxybutyrate. A blood sample was obtained after an overnight fast at 08.00 hours on day 3 and mixed in an EDTA tube, centrifuged at 4°C for 10 min at 3000 rpm. Plasma was stored at −80°C until analysis. The β-hydroxybutyrate concentration was measured with the method of Moore *et al.* using a semi-automated centrifugal spectrophotometer (Cobas Fara; Roche Diagnostics, Basel, Switzerland)[37].

Body composition. Body composition was determined by the three-compartment model, using the hydrodensitometry and ^2H dilution (^2H_2O) technique[38,39] and was calculated using the combined equation of Siri[40].

Statistical analysis

Data are presented as mean values and standard deviations. A repeated-measures ANOVA was used to determine possible differences in appetite, energy expenditure, RQ and β-hydroxybutyrate concentration between the HP and the NP diet and between the HP-0C and the NP-g diet, respectively. A Mann–Whitney *U* test was used to determine differences in appetite, energy expenditure, RQ and β-hydroxybutyrate concentration between the NP-g and the NP diet and between the HP-0C and the HP diet, respectively. In addition, a Mann–Whitney *U* test was used to determine differences in the difference between the HP and the NP diet and the difference between the HP-0C and the NP-g diet in appetite, energy expenditure, RQ and β-hydroxybutyrate concentration.

Table 2. Composition of the meals, average intake per food item (g and kJ), macronutrient intake and energy density of the normal-protein (NP), high-protein (HP), normal-protein (NP-g) and the high-protein, carbohydrate-free (HP-0C) diet*

Diet and meal		Wt of item (g)	Energy content of item (kJ)	Protein content (g)	CHO content (g)	Fat content (g)	Diet and meal		Wt of item (g)	Energy content of item (kJ)	Protein content (g)	CHO content (g)	Fat content (g)
NP							HP						
Breakfast	Whole-wheat bread	94	900	8	40	1	Breakfast	Whole-wheat bread	65	712	6	29	2
	Low-fat margarine	24	333	0	0	8		Margarine	9	296	0	0	7
	Chocolate spread	18	446	1	10	6		Chicken filet	46	331	14	0	2
	Confiture	24	251	0	14	0		Milk	280	579	10	13	4
	Coffee (decaffeinated) or tea	250	0	0	0	0							
	Energy density of meal (kJ/g)			4·7				Energy density of meal (kJ/g)			4·8		
	% of energy from protein, CHO and fat in meal			9/58/31				% of energy from protein, CHO and fat in meal			29/39/31		
Lunch	Soup (bouillon)	200	20	0	1	0	Lunch	Soup (bouillon)	200	20	0	1	0
	Whole-wheat bread	147	1403	12	62	2		Whole-wheat bread	104	1138	9	47	3
	Low-fat margarine	25	353	0	0	9		Soya milk	178	326	6	3	4
	Chocolate spread	30	756	1	17	11		Tomato	20	18	0	0	0
	Cheese	25	276	9	0	3		Cucumber	27	10	0	0	0
	Lettuce	118	22	1	0	0		Feta cheese	63	896	12	4	15
	Cucumber	41	15	0	1	0		Salad dressing	25	250	0	3	5
	Olive oil	5	181	0	0	5		Tuna in water	111	593	29	0	2
	Grape juice	295	840	1	47	0		Fruit yoghurt	198	631	8	27	0
	Energy density of meal (kJ/g)			4·4				Energy density of meal (kJ/g)			4·2		
	% of energy from protein, CHO and fat in meal			11/58/31				% of energy from protein, CHO and fat in meal			31/39/30		
Dinner	Soup (bouillon)	200	20	0	1	0	Dinner	Soup (bouillon)	200	20	0	1	0
	Chinese noodle dish	332	2594	18	80	22		Rice dish with ham	286	2966	55	74	16
	Lettuce	85	16	1	0	0		Soya milk	295	540	11	6	6
	Cucumber	35	13	0	1	0		Muesli bar	18	340	1	10	4
	Olive oil	7	253	0	0	6		Sugar-free syrup	175	0	0	0	0
	Fruit cocktail	170	516	1	28	0							
	Grape juice	165	464	0	26	0							
	Energy density of meal (kJ/g)			3·9				Energy density of meal (kJ/g)			4·0		
	% of energy from protein, CHO and fat in meal			10/62/28				% of energy from protein, CHO and fat in meal			32/41/27		
	Energy density of diet (kJ/g)			4·2				Energy density of diet (kJ/g)			4·2		
	% of energy from protein, CHO and fat in diet			10/60/30				% of energy from protein, CHO and fat in diet			31/40/29		
NP-g							HP-0C						
Breakfast	Whole-wheat bread	89	853	8	38	1	Breakfast	Boiled eggs	117	745	15	0	12
	Low-fat margarine	22	315	0	0	8		Bacon	66	1111	12	0	22
	Chocolate spread	17	423	1	10	6		Coffee (decaffeinated) or tea	250	0	0	0	0

Table 2. *Continued*

Diet and meal		Wt of item (g)	Energy content of item (kJ)	Protein content (g)	CHO content (g)	Fat content (g)	Diet and meal		Wt of item (g)	Energy content of item (kJ)	Protein content (g)	CHO content (g)	Fat content (g)
	Confiture	22	238	0	13	0							
	Coffee (decaffeinated) or tea	250	0	0	0	0							
	Energy density of meal (kJ/g)			4·6				Energy density of meal (kJ/g)			4·3		
	% of energy from protein, CHO and fat in meal			9/59/32				% of energy from protein, CHO and fat in meal			27/0/73		
Lunch	Soup (bouillon)	200	20	0	1	0	Lunch	Soup (bouillon)	250	26	0	1	0
	Whole-wheat bread	142	1355	12	60	2		Salami	55	924	11	0	18
	Low-fat margarine	20	282	0	0	7		Tuna in oil	108	924	29	0	10
	Chocolate spread	26	655	1	15	10		Garden cress	11	4	0	0	0
	Cheese	22	243	7	0	3		French cheese	59	494	13	0	6
	Lettuce	115	21	1	0	0		Lettuce	103	19	1	0	0
	Cucumber	39	14	0	1	0		Mushrooms	27	25	1	0	0
	Olive oil	7	242	0	0	6		Olive oil	35	1266	0	0	32
	Grape juice	287	818	1	46	0		Sugar-free syrup	200	0	0	0	0
	Energy density of meal (kJ/g)			4·3				Energy density of meal (kJ/g)			4·3		
	% of energy from protein, CHO and fat in meal			11/59/30				% of energy from protein, CHO and fat in meal			28/0/71		
Dinner	Soup (bouillon)	200	20	0	1	0	Dinner	Soup (bouillon)	250	26	0	1	0
	Chinese noodle dish	303	2368	17	73	20		Chicken meat	65	474	20	0	3
	Lettuce	83	19	1	0	0		Tuna in oil	108	924	29	0	10
	Cucumber	34	12	0	1	0		Garden cress	11	4	0	0	0
	Olive oil	7	253	0	0	6		Cheese	54	915	14	0	17
	Fruit cocktail	165	502	1	27	0		Lettuce	125	23	1	0	0
	Grape juice	175	498	0	28	0		Mushrooms	32	30	2	0	0
								Olive oil	34	1230	0	0	31
								Sugar-free syrup	250	0	0	0	0
	Energy density of meal (kJ/g)			3·8				Energy density of meal (kJ/g)			3·9		
	% of energy from protein, CHO and fat in meal			10/62/28				% of energy from protein, CHO and fat in meal			33/0/67		
	Energy density of diet (kJ/g)			4·1				Energy density of diet (kJ/g)			4·1		
	% of energy from protein, CHO and fat in diet			10/60/30				% of energy from protein, CHO and fat in diet			30/0/70		

CHO, carbohydrate.

* Macronutrient composition of the food items was based on food tables. The amounts of food items were adjusted to subject-specific energy requirements; the data shown here are based on average energy intake of 9·65 (SD 0·7) MJ/d in group 1 (NP and HP) and 9·14 (SD 1·3) MJ/d in group 2 (NP-g and HP-0C).

A repeated-measures ANOVA was used to test whether macronutrient balances were significantly different from zero and whether macronutrient balances were different between the HP diet and the NP diet and between the HP-0C diet and the NP-g diet. A Mann–Whitney *U* test was used to test whether macronutrient balances were different between the NP-g diet and the NP diet and between the HP-0C diet and the HP diet. Possible relationships between macronutrient oxidation and appetite profile were studied using regression analyses. $P<0{\cdot}05$ was regarded as statistically significant. Statistical procedures were performed using StatView 5.0 (1998; SAS Institute, Inc., Cary, NC, USA).

Results

There were no differences between the NP diet and the NP-g diet in appetite profile, energy expenditure, RQ or β-hydroxybutyrate concentration (Table 3). Thus, the glycogen-lowering exercise test did not affect these parameters. Moreover, differences between these variables did not differ between men and women; therefore the data were analysed together.

Appetite profile

Hunger was 10 % lower after the HP diet than after the NP diet ($P<0{\cdot}05$; Table 3) and was 33 % lower after the HP-0C diet than after the NP-g diet ($P<0{\cdot}001$). There was no difference in hunger between the NP-g and the NP diet, whereas hunger was 27 % lower after the HP-0C diet than after the HP diet (*z*-score: 4·1; $P<0{\cdot}01$). The Δhunger between the HP-0C and the NP-g diet was larger than the Δhunger between the HP and the NP diet (*z*-score: 5·1; $P<0{\cdot}01$).

Fullness was 21 % higher after the HP diet than after the NP diet ($P<0{\cdot}05$) and was 28 % higher after the HP-0C diet than after the NP-g diet ($P<0{\cdot}001$). There was no difference in fullness between the NP-g and the NP diet or between the HP-0C and the HP diet. The Δfullness between the HP-0C and the NP-g diet was higher than the Δfullness between the HP and the NP diet (*z*-score: 5·1; $P<0{\cdot}01$).

Patterns of hunger and fullness ratings over time were similar between all conditions (data not shown). Hunger and fullness ratings were continuously lower and higher, respectively, after HP *v.* NP, HP-0C *v.* NP-g and HP-0C *v.* HP with significant differences at various time points.

Energy intake, expenditure and balance

Energy intake was not different between group 1 and 2; 9·65 (SD 0·70) MJ/d in group 1 and 9·14 (SD 1·30) MJ/d in group 2, respectively (Table 3; NS).

Energy expenditure was 4 % higher after the HP diet than after the NP diet ($P<0{\cdot}05$; Table 3) and tended to be higher after the HP-0C diet than after the NP-g diet ($P=0{\cdot}07$). There was no difference in energy expenditure between the NP-g and the NP diet or between the HP-0C and the HP diet. The Δenergy expenditure between the HP-0C and the NP-g diet was not different from the Δenergy expenditure between the HP and the NP diet.

Energy balance was not different from zero after the HP diet, whereas the subjects were slightly in positive energy balance after the NP diet ($P<0{\cdot}05$; Table 3). Energy balance was not different from zero both after the HP-0C diet and the NP-g diet. The energy balance was not different between the NP-g diet and the NP diet or between the HP-0C diet and the HP diet.

Respiratory quotient

The RQ was lower after the HP diet than after the NP diet ($P<0{\cdot}01$; Table 3) and was lower after the HP-0C diet than after the NP-g diet ($P<0{\cdot}001$). There was no difference in RQ between the NP-g and the NP diet whereas the RQ was lower after the HP-0C diet than after the HP diet (*z*-score: 4·9; $P<0{\cdot}01$). The ΔRQ between the HP-0C and the NP-g diet was larger than the ΔRQ between the HP and the NP diet (*z*-score: 5·3; $P<0{\cdot}001$).

β-Hydroxybutyrate

The β-hydroxybutyrate concentration was higher after the HP diet than after the NP diet ($P<0{\cdot}05$; Table 3) and was higher after the HP-0C diet than after the NP-g diet ($P<0{\cdot}001$). There was no difference in β-hydroxybutyrate concentration between the NP-g and the NP diet, whereas the β-hydroxybutyrate concentration was higher after the HP-0C diet than after the HP diet (*z*-score: 5·8; $P<0{\cdot}001$). The Δβ-hydroxybutyrate concentration between the HP-0C and the NP-g diet was larger than the Δβ-hydroxybutyrate concentration between the HP and the NP diet (*z*-score: 5·3; $P<0{\cdot}001$).

Macronutrient balances

Protein, carbohydrate and fat intake, oxidation and balance are shown in Table 4.

After the HP diet, protein balance was positive and fat balance was negative ($P<0{\cdot}01$). After the NP-g diet, protein and fat balance were negative ($P<0{\cdot}05$ and $P<0{\cdot}01$) and carbohydrate balance was positive ($P<0{\cdot}01$). After the HP-0C diet, protein balance was positive ($P<0{\cdot}01$) and carbohydrate balance was negative ($P<0{\cdot}01$). Protein and fat balance was different between HP and NP ($P<0{\cdot}01$ and $P<0{\cdot}001$), whereas protein, carbohydrate and fat balance was different between HP-0C and NP-g ($P<0{\cdot}001$ for all). Protein, carbohydrate and fat balance was different between NP-g and NP ($P<0{\cdot}001$ for all) and carbohydrate and fat balance was different between HP-0C and HP ($P<0{\cdot}001$ for all). There was no relationship between macronutrient oxidation and appetite profile.

Discussion

The presence or absence of a normal proportion of carbohydrates, and consequently differences in the proportion of fat, in a relatively high-protein diet significantly affected the metabolic targets appetite suppression and fat oxidation in healthy normal-weight subjects who were in energy balance and were studied under highly controlled conditions. Both males and females were included to conduct a study that is relevant for a larger part of the population. Moreover, differences between the variables were not different between men and women. Yet, this mixed-sex population may have increased variance in outcomes, since sex-specific effects of similar diets have been shown before in that appetite-related

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Table 3. Appetite profile, energy intake (MJ/d), energy expenditure (MJ/d), energy balance (MJ/d), 24 h respiratory quotient (RQ) and β-hydroxybutyrate concentration (μmol/l) after a high protein (HP), normal-protein (NP), high-protein, carbohydrate-free (HP-0C), or a normal-protein (NP-g) diet for 2 d in twenty-two (NP and HP) and twenty-three (NP-g and HP-0C) healthy subjects (males and females)†

(Mean values and standard deviations)

	HP		NP		Δ HP − NP			HP-0C		NP-g		Δ HP-0C−NP-g					
	Mean	SD	Mean	SD	Mean	SD	HP *v.* NP: *P*‡	Mean	SD	Mean	SD	Mean	SD	Δ HP-0C *v.* NP-g: *P*‡	NP-g *v.* NP, *z*-score: *P*§	HP-0C *v.* HP, *z*-score and *P*§	Δ HP-0C −NP-g *v.* Δ HP − NP, *z*-score and *P*§
Hunger (mm VAS×24 h)	950	209	1057	223	−107	52	<0·05	693	208	1039	247	−346	84	<0·001	NS	4·1 (<0·01)	5·1 (<0·01)
Fullness (mm VAS × 24 h)	989	203	815	198	174	43	<0·05	998	258	782	196	216	54	<0·001	NS	NS	4·8 (<0·01)
Energy intake (MJ/d)	9·65	0·70	9·65	0·70			NS	9·14	1·30	9·14	1·30			NS	NS	NS	
Energy expenditure (MJ/d)	9·75	0·70	9·35	0·70	0·40	0·46	<0·05	9·23	1·10	9·04	1·20	0·18	0·44	NS (*P*=0·07)	NS	NS	NS
Energy balance (MJ/d)‖	−0·10	0·36	0·30*	0·40	0·40	0·38	<0·05	−0·09	0·47	0·10	0·38	0·19	0·45	NS	NS	NS	NS
24 h RQ	0·81	0·02	0·86	0·02	−0·05	0·00	<0·01	0·76	0·01	0·85	0·02	−0·09	0·00	<0·001	NS	4·9 (<0·01)	5·3 (<0·001)
β-Hydroxybutyrate (μmol/l)	332	102	228	88	104	42	<0·05	1349	653	234	226	1115	627	<0·001	NS	5·8 (<0·001)	5·3 (<0·001)

VAS, visual analogue scale.

* Energy balance (MJ/d) was significantly different from zero ($P<0·05$; repeated-measures ANOVA).

† The macronutrient composition of NP was 10, 60 and 30 % of energy (En%) from protein, carbohydrate and fat; for HP it was 30, 40 and 30 En%; for NP-g it was 10, 60 and 30 En%; for HP-0C it was 30, 0 and 70 En%.

‡ Repeated-measures ANOVA for comparison of HP *v.* NP and HP-0C *v.* NP-g.

§ Mann–Whitney *U* test for comparison of NP-g *v.* NP, HP-0C *v.* HP, and Δ HP-0C − NP-g *v.* Δ HP − NP.

‖ Energy balance (MJ/d) = energy intake (MJ/d) − energy expenditure (MJ/d).

Table 4. Macronutrient intake, oxidation and balance (all MJ/d) after a normal-protein (NP), high-protein (HP), normal-protein (NP-g) or a high-protein, carbohydrate-free (HP-0C) diet for 2 d in twenty-two (NP and HP) and twenty-three (NP-g and HP-0C) healthy normal-weight subjects (males and females)†

(Mean values and standard deviations)

	Intake		Expenditure/oxidation		Balance‡				
	Mean	SD	Mean	SD	Mean	SD	ANOVA§	HP *v.* NP and HP-0C *v.* NP-g‖	NP-g *v.* NP and HP-0C *v.* HP¶
NP									
Protein (MJ/d)	0·98	0·14	0·97	0·24	0·01	0·23			
Carbohydrates (MJ/d)	5·76	0·73	5·43	0·85	0·33	0·58			
Fat (MJ/d)	2·90	0·39	2·84	0·43	0·06	0·21			
HP									
Protein (MJ/d)	2·96	0·35	2·45	0·35	0·51	0·22	**	**	
Carbohydrates (MJ/d)	3·87	0·59	3·65	0·71	0·22	0·24			
Fat (MJ/d)	2·82	0·41	3·62	0·68	−0·80	0·45	**	***	
NP-g									
Protein (MJ/d)	0·92	0·13	1·12	0·28	−0·20	0·20	*		***
Carbohydrates (MJ/d)	5·52	0·70	4·41	0·89	1·11	0·69	**		***
Fat (MJ/d)	2·74	0·39	3·54	0·69	−0·80	0·62	**		***
HP-0C									
Protein (MJ/d)	2·73	0·37	2·08	0·32	0·65	0·24	**	***	
Carbohydrates (MJ/d)	0·03	0·08	1·01	0·51	−0·97	0·51	**	***	***
Fat (MJ/d)	6·40	0·88	6·16	0·85	0·24	0·60		***	***

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† The macronutrient composition of NP was 10, 60 and 30 % of energy (En%) from protein, carbohydrate and fat; for HP it was 30, 40 and 30 En%; for NP-g it was 10, 60 and 30 En%; for HP-0C it was 30, 0 and 70 En%.

‡ Macronutrient balance (MJ/d) = macronutrient intake (MJ/d) − macronutrient oxidation (MJ/d).

§ Repeated-measures ANOVA for macronutrient balance was different from zero.

‖ Repeated-measures ANOVA for comparison of macronutrient balance HP *v.* NP and HP-0C *v.* NP-g.

¶ Mann–Whitney *U* test for comparison of NP-g *v.* NP and HP-0C *v.* HP.

effects were larger in women than in men, whereas energy metabolism-related effects were larger in men than in women[41]. Here, due to the limited number of subjects from the same sex, these differences may not have appeared. On the other hand, the differences due to the diets were that large, that these may have dominated any possible sex differences in these variables.

It is impossible to determine whether the observed effects of the HP-0C diet are due to a lack of carbohydrates or to elevated fat levels. Because the maximal protein content in a high-protein diet in energy balance is limited[21], the proportion of fat in a high-protein, low-carbohydrate diet always will be relatively high. The observed effects may be due to the low carbohydrate content as well as the high fat content, promoting together with a relatively high protein content a high fat oxidation and elevated β-hydroxybutyrate concentration.

Suppression of appetite was significantly affected by the absence of a normal proportion of carbohydrates and the proportion of fat in a high-protein diet. Coinciding with the reduced appetite there was an increased dietary fat oxidation after the HP diet compared with the NP diet and an even greater fat oxidation after the HP-0C diet. Inhibition of dietary fat oxidation has been shown to increase food intake[42,43], whereas increased fatty acid oxidation is suggested to reduce appetite[44–46]. This is likely to be due to stimulation of carnitine palmitoyl transferase-1, a catalyst of the rate-limiting step in mitochondrial fatty acid oxidation, which has been shown to inhibit eating[44]. A greater fat oxidation together with a lower appetite was for instance also observed in human subjects that consumed diacylglycerols instead of TAG[47]. Increased fat oxidation when carbohydrate availability is low and fat availability high results in the production of ketone bodies, i.e. β-hydroxybutyrate[19], as was also observed in the present study. The β-hydroxybutyrate concentration after the HP diet was increased but within the normal range[47]. However, after the HP-0C diet the β-hydroxybutyrate concentration was increased dramatically, to a level comparable with that after weight loss[18]. In rats, intracerebroventricular infusion or subcutaneous injection of β-hydroxybutyrate reduced food intake[48,49]. Higher β-hydroxybutyrate concentrations coinciding with reduced appetite in human subjects also have been reported[18,46,50,51]. Taken together, the increased dietary fat oxidation and increase in β-hydroxybutyrate concentration, i.e. a ketogenic state, is likely to contribute to the appetite-suppressive effect of high-protein, low-carbohydrate, high-fat diets. The same mechanism may play a role in a high-protein, normal-carbohydrate diet, but to a much smaller extent.

The present study also clearly shows that the presence or absence of a normal proportion of carbohydrates and the proportion of fat in a relatively high-protein diet do not affect energy expenditure differently. Therefore, the increase in energy expenditure that has been observed with high-protein diets[23–25] is mainly due to the relatively high proportion of protein and not the presence or absence of a normal proportion of carbohydrates or the fat content.

The effects of the two types of high-protein diets were compared using two groups of subjects, without significant differences in subject characteristics. The glycogen-lowering exercise performed by the subjects in group 2 and not by those in group 1 is unlikely to have affected appetite, energy expenditure or β-hydroxybutyrate concentration, since these parameters were not different between the NP and the NP-g diet. Nevertheless, macronutrient oxidation was affected by the exercise: protein and fat balances were lower whereas carbohydrate balance was more positive after the NP-g diet compared with the NP diet. Glycogen depletion has been shown to increase rates of muscle proteolysis and branched-chain amino acid oxidation, resulting in relatively increased protein oxidation, hence a negative protein balance[52]. Regarding the positive carbohydrate balance it is likely that the surplus of carbohydrates was used to restore body glycogen stores[53,54]. Despite differences in macronutrient oxidation there was no difference in RQ between the NP diet and the NP-g diet.

The dietary fibre content was not the same in all four diets, which may have affected metabolic targets. Although there were no differences between the HP, NP and NP-g diet, the fibre content of the HP-0C diet was significantly lower than that of the other diets. Raben *et al.* showed that a high-fibre meal decreased diet-induced thermogenesis and fat oxidation and increased fullness[55]. However, in the study of Raben *et al.* the relative fibre content was much higher than in the present study (4·7 g/MJ *v.* about 2 g/MJ)[55]. Moreover, if the higher fibre content indeed increased fullness, the appetite-suppressive effect of the HP-0C diet was even more pronounced since fibre content was lower in the HP-0C diet. The increased appetite suppression and fat oxidation after the high-protein, carbohydrate-free, high-fat diet thus are not attributable to the lower fibre content of the diet. Weight and volume of the meals as well as the energy density and palatability were similar between diets; they did not explain differences in appetite ratings. Appetite suppression was shown by reduction in hunger and increased fullness, as measured by VAS. As mentioned before, it is important to study effects on metabolic targets when subjects are in energy balance. When fed in energy balance, the outcome only can be appetite profile expressed as VAS ratings and not actual energy intake[56].

In conclusion, the presence or absence of a normal proportion of carbohydrates and the proportion of dietary fat in a relatively high-protein diet significantly affected the metabolic targets of appetite suppression and increased fat oxidation. The absence of a normal proportion of carbohydrates and a high fat content in a relatively high-protein diet induced a large increase in the concentration of β-hydroxybutyrate. An increased dietary fat oxidation and increase in the concentration of β-hydroxybutyrate, i.e. a ketogenic state, may contribute to the increased appetite suppression on a high-protein, low-carbohydrate, high-fat diet. Energy expenditure was not affected differently by the presence or absence of a normal proportion of carbohydrates and the proportion of fat in a high-protein diet.

Acknowledgements

This research was supported by the Top Institute Food and Nutrition, The Netherlands.

M. A. B. V., K. R. W., and M. S. W.-P. designed the study. M. A. B. V. and M. S. W.-P. collected and analysed the data. M. A. B. V. wrote the manuscript and K. R. W., A. J. A. H. v. V. and M. S. W.-P. contributed to the interpretation of the data and reviewed the manuscript.

None of the authors had a personal or financial conflict of interest.

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