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Diet induced thermogenesis measured over 24h in a respiration chamber: effect of diet composition

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OBJECTIVE: To study the effect of diet composition on diet-induced thermogenesis (DIT) over 24 h in a respiration chamber.
SUBJECTS: Eight healthy female volunteers (age 27 ± 3 y; body mass index, BMI 23 ± 3 kg/m^2).
DIETS: A high protein and carbohydrate (HP/C) (60:10:30; percentage energy (E%)carbohydrate, fat and protein, respectively) and high fat (HF) (30:60:10 respectively) diet, both isoenergetic, isovolumetric, composed of normal food items and matched for organoleptic properties (taste, smell, appearance).
DESIGN: Subjects spent two 36 h periods each in a respiration chamber consuming both test diets in random order. Components of 24 h energy expenditure (24 h EE): sleeping metabolic rate, DIT and activity induced energy expenditure were measured.
RESULTS: DIT was higher in all subjects while on the HP/C diet (1295 kJ/d *vs* 931 kJ/d; 14.6% *vs* 10.5% of energy intake; $P < 0.02$). There was no significant difference in other components or total 24 h EE, although there was a trend towards higher EE on the HP/C diet.
CONCLUSION: A high protein and carbohydrate diet induces a greater thermic response in healthy individuals when compared to a high fat diet.

Keywords: energy metabolism; physical activity; macronutrients; carbohydrate; protein; fat

Introduction

Dietary factors involved in diet induced thermogenesis (DIT) have been widely studied. Despite this, there is still a discrepancy as to which factors are significant and to what degree. Factors such as energy content,[1–3] palatability,[4–6] frequency[7,8] and timing[9] of meals, have all been studied with varying results. Macronutrient composition of diets is particularly interesting as this may have a direct influence on the development and maintenance of obesity.[10,11]

The measured thermic effect of separate nutrients is highest and most prolonged for protein (20–30% energy content), followed by carbohydrate (5–15%) and fat (0–3%).[12] Bobbioni-Harsch *et al*[13] measured, over 8 h after oral loads of separate macronutrients, an equivalent thermic effect of 8–9% energy content for carbohydrate and fat. It follows that, in theory, experimental diets where the protein to fat ratio is manipulated, would be expected to yield the most significant results. Indeed, studies using diets with varying protein compositions, especially as liquid and/or single nutrient[14–16] preparations have, on the whole, been more successful in establishing a difference in DIT between diets. In studies where food was given as mixed meals, it is most often,[7,17,18] although not always[1,19], the carbohydrate/fat ratio that has been manipulated and results have been inconclusive. Also the majority of studies has been of short duration (60–360 min) and this may not be enough time to fully measure DIT.[20,21] In these studies, subjects have remained stationary, most often in a supine position for the duration of the measurements, which might not be representative of the real life situation. Furthermore, energy expended in fidgeting may erroneously be attributed to DIT. This leaves the debate open as to the significance of diet composition on DIT under free living conditions.

The diets used in the present study were chosen to represent extremes of macronutrient composition, while still enabling us to compile diets comparable with respect to other dietary factors such as palatability, appearance and volume. The diets were compiled using normal food items served in the way they are normally eaten and consumed throughout the day following a normal eating pattern. The aim of this study was to investigate the effect of diets of extreme macronutrient composition on DIT under near physiological conditions in a respiration chamber over the duration of a full day.

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Subjects and methods

Subjects

Eight healthy female subjects, aged 23–33 y, participated in the study. Characteristics of the subjects are described in Table 1.

Experimental design

Subjects each spent two 36 h periods in a respiration chamber, consuming either a high protein and carbohydrate (HP/C) or high fat (HF) diet in random order. Subjects had all been in a respiration chamber on at least one previous occasion and so were familiar with the study environment. Subjects were fed to energy balance as estimated from their 24 h sleeping metabolic rate (SMR), measured during the first night, multiplied by an activity index of 1.6.[22] Before entering the chamber, at 19.00 h, subjects were given a dinner of similar composition to the experimental diet of the following day. In the morning following the first stay in the respiration chamber, anthropometric measurements were conducted (see below).

Diets

The macronutrient compositions of the two experimental diets, HP/C and HF, are given in Table 2. The diets were compiled using normal food items and were consumed as breakfast (08.30 h; 23% energy intake (EI)), lunch (12.30 h; 31% EI), dinner (18.30 h; 32% EI) and two snacks (15.00 h; 8% EI and 20.30 h; 6% EI). The diets were isoenergetic and isovolumetric, and were matched as closely as possible for organoleptic properties (taste, smell, appearance). Macronutrient composition of the diets was calculated using the Dutch food composition table[23] and food quotients (FQ) using the equations of Brouwer.[24]

Table 1 Physical characteristics of the eight female subjects

Age (y)	27 ± 3
Height (m)	1.70 ± 0.08
Body mass (kg)	67 ± 12
Body mass index (kg/m^2)	23.3 ± 2.5
Body fat (%)	29 ± 6
Fat-free mass (kg)	47 ± 4

Values are means ± s.d.

Table 2 Energy content, macronutrient composition and food quotients (FQ) of diets

	HP/C[a]	*HF*[b]
Energy content (kJ/d)	8877 ± 735	8921 ± 767
Carbohydrate (%)[c]	61 ± 0.7	30 ± 0.4
Fat (%)[c]	10 ± 0.3	61 ± 0.3
Protein (%)[c]	29 ± 0.5	9 ± 0.3
FQ	0.909 ± 0.001	0.802 ± 0.001

Values are mean ± s.d.
[a]High protein and carbohydrate diet; [b]High fat diet; [c]Percentage of total energy content

Anthropometry and body composition

Anthropometric measurements were carried out in the fasted state. Body weight was measured using a digital scale accurate to 0.01 kg (Sauter, type E1200, Sauter, Ebingen, Germany) and height was measured to the nearest 0.001 m. Body mass index (BMI, kg/m^2) was calculated as body weight (kg) divided by height (m) squared.

Body composition was estimated by using hydrodensitometry and isotope dilution. Body density was determined by underwater weighing with simultaneous assessment of residual lung volume with the helium dilution technique using a spirometer (Volugraph 2000, Mijnhardt, The Netherlands). Total body water (TBW) was determined by deuterium oxide (D_2O) dilution following the Maastricht protocol.[25] Briefly, in the evening, prior to anthropometric measurements, after obtaining a background urine sample, subjects ingested an oral dose of D_2O. Deuterium enrichment was measured in urine from the second voiding of the following morning. Body composition was calculated using the combined equation of Siri.[26]

Indirect calorimetry and physical activity

Oxygen consumption and carbon dioxide production were measured in a respiration chamber.[27] The chamber is a 14 m^3 room furnished with a bed, chair, computer, television, radio-cassette player, telephone, intercom, sink and toilet, and is ventilated with fresh air at a rate of 70–80 l/min. The ventilation rate was measured with a dry gas meter (Schlumberger, type G6, The Netherlands). The concentration of oxygen and carbon dioxide was measured using paramagnetic O_2 analyzers (Hartmann & Braun, type Magnos G6, Hartmann & Braun, Frankfurt, Germany; Servomex, type OA 184A, UK) and infrared CO2 analyzer (Hartmann & Braun, type Uras 3G, Hartmann & Braun, Frankfurt, Germany). During each 15 min period, six samples of outgoing air for each chamber, one sample each of fresh air, zero gas and calibration gas were measured. The gas samples to be measured were selected by a computer which also stored and processed the data.

During daytime, subjects followed a light exercise protocol consisting of six exercise periods of either walking or low intensity bench stepping (25 cm bench with one step per s), each with a duration of 10 min. This was implemented to extend the range of movement and avoid single extreme values of energy expenditure (EE) that could affect the calculation of DIT. EE over 30 min intervals thus ranged between SMR and 4.3 ± 0.8 times SMR. The exercise intervals were evenly spread throughout the day at 10.15 h, 12.10 h, 14.15 h, 16.15 h, 18.15 h and 20.10 h. Apart from the exercise periods, subjects were not restricted in their activities, only sleeping and strenuous physical activity was not allowed. Physical activity was monitored by means of a radar system working on the Doppler principle.

Components of 24 h EE

Twenty-four-hour values for EE (24 h EE), DIT and activity induced energy expenditure (AEE) were calculated from 07.00–07.00 h. EE was calculated from O_2 consumption and CO_2 production using the Weir formula.[28]

Sleeping metabolic rate (SMR) was measured on both nights of each experiment and was defined as the lowest mean EE measured over three consecutive hours between 00.00 h and 07.00 h. The average SMR of the two nights was used in further calculations.

For calculation of DIT, EE was plotted against radar output, both averaged over 30 min periods. The intercept of the regression line at lowest radar output, represents EE in the inactive state (resting metabolic rate, RMR), consisting of SMR and DIT (Figure 1). DIT was determined by subtracting SMR from RMR. AEE was calculated by subtracting RMR from 24 h EE.

Substrate utilization

During both stays in the respiration chamber, 24 h urine was collected from the second voiding on the day of the experiment until the first voiding the following day. Samples were collected in containers that contained 10 ml H_2SO_4 to prevent nitrogen loss through evaporation. Volume and nitrogen concentration were measured, the latter using a nitrogen analyser (Carlo Erba EA1108CHN, Rodano, Italy).

Substrate oxidation was calculated using O_2 consumption, CO_2 production and urinary nitrogen excretion using the formula of Brouwer.[24] Theoretical estimates of DIT were calculated from measured substrate utilization using median values for the thermogenic effect of separate nutrients[12] (carbohydrate 10% EI, fat 1.5% EI, protein 25% EI).

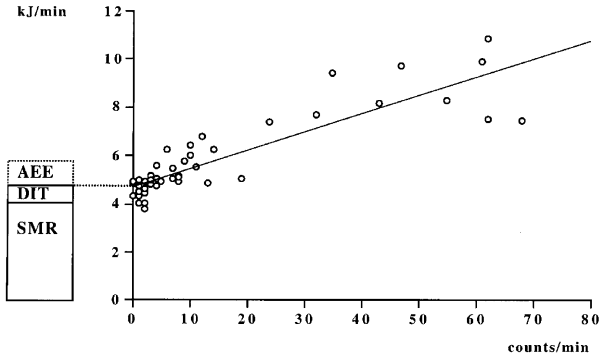

Figure 1 Energy expenditure (EE) plotted against radar output in one subject, each point representing a 30 min average from 07.00–07.00 h, with the calculated linear regression line. Resting metabolic rate (RMR) can be calculated as the y-intercept and includes diet induced thermogenesis (DIT) and sleeping metabolic rate (SMR). AEE-activity induced EE.

Table 3 Energy balance and components of 24 h energy expenditure (24 h EE)

	HP/C	*HF*	P
EI (kJ/d)	8877 ± 735	8921 ± 767	0.41
24 h EE (kJ/d)	9228 ± 760	8894 ± 897	0.08
EI–EE (kJ/d)	− 351 ± 593	28 ± 601	0.05
SMR (kJ/d)	5803 ± 580	5738 ± 691	0.71
DIT (kJ/d)	1295 ± 240	931 ± 315	0.02
DIT (%EI)	14.6 ± 2.9	10.5 ± 3.8	0.02
AEE (kJ/d)	2129 ± 631	2225 ± 694	0.91

Values are mean ± s.d.; *P* values were determined by Students paired *t*-test.
HP/C = high protein and carbohydrate diet; HF = high fat diet; EI = energy intake; SMR = sleeping metabolic rate; DIT = diet induced thermogenesis; AEE = activity induced energy expenditure.

Statistical analysis

All results are expressed as mean ± s.d. Student's paired *t*-test was used to test for significant differences between variables. *P* values were considered significant when < 0.05.

Results

Values for the different components of 24 h EE as well as energy balance are given in Table 3. All values are expressed as kJ/d. In addition, DIT is also given as a percentage of energy intake (%EI). On average, subjects were in energy balance while on the HF diet ($+28 \pm 601$ kJ/d, $P = 0.79$) and were in negative energy balance on the HP/C diet (-351 ± 593 kJ/d, $P = 0.19$). The difference in energy balance between diets was not significant ($P = 0.05$). There was a trend towards higher 24 h EE during the HP/C diet, but the difference was not significant ($P = 0.08$). SMR and AEE did not differ significantly between the diets. Total DIT varied greatly between subjects, whether expressed in absolute terms (HP/C 789–1580 kJ/d; HF 439–1365 kJ/d) or as %EI (HP/C 9.3–17.7; HF 5.2–15.6), but was consistently higher in all subjects while on the HP/C diet ($P < 0.02$). On a group level, the difference in energy balance (334 kJ/d) was approximately equivalent to the difference in DIT (364 kJ/d) between diets. Theoretically calculated DIT, 1338 ± 122 kJ/d for the HP/C and 987 ± 158 kJ/d for the HF diet, was very close to measured DIT, which was 1295 ± 240 kJ/d and 931 ± 315 kJ/d for the HP/C and HF diets, respectively.

There was a significant difference between the FQ and RQ of each diet. The figures for the HP/C diet were: FQ 0.909 ± 0.001; RQ 0.921 ± 0.012 ($P < 0.05$) and for the HF diet: FQ 0.802 ± 0.001; RQ 0.864 ± 0.024 ($P < 0.001$). On the HP/C diet 70:12:18% and on the HF diet 51:38:11% of energy expended was derived from carbohydrate (CHO), fat and protein, respectively. Utilization was significantly different from intake in all cases, with the exception of fat oxidation on the HP/C diet, and being most significant ($P < 0.0001$) for protein on the HP/C and fat on the HF diet.

Discussion

The results of the present study are consistent with the findings of Le Blanc *et al*[29], amongst others,[30] that protein induces a greater thermic response than other nutrients. Also diminished thermic response to diets with a high fat content has been reported.[10,13] Comparison of studies is, however hampered by methodological differences. The majority of studies have measured the thermic effect of single test meals using a ventilated hood with the duration of measurement ranging between 60 and 360 min. DIT has been found to continue well beyond this period,[20,21] indicated by EE not returning to baseline at cessation of measurements, especially in the case of protein, and therefore some of the DIT will be missed. Subjects have been required to remain stationary for the duration of the experiment, usually in a supine position, this does not represent physiological conditions under which DIT normally occurs. Also as movement is not taken into account, energy expended by fidgeting, especially towards the end of the measurement period may be erroneously attributed to DIT and affect the results.[31] Extrapolation of measurements of short duration over days or even years may be inaccurate.[32] Studies conducted in a respiration chamber over extended periods have been scarce[17,18] and have, with one exception,[30] not been able to show a difference in relation to diet composition. This may be due to varying methods of calculating DIT and less extreme diets than used in the present study.

The diets were chosen to represent extremes of macronutrient composition. Although they do not represent any diets habitually consumed on a national level, the composition of the diets in this study falls approximately within the range of normal proportional consumption of macronutrients (protein 10.6–42.7% EI, CHO 3.1–81.7% EI, fat 6.3–54.2% EI) in different populations.[33] Subgroups of populations (for example, body builders[34] and the obese) may, however habitually consume diets of extreme composition. In compiling the diets, the relative amounts of protein and fat (as the most and least thermogenic nutrients) were maximized and minimized in order to achieve diets that would potentially produce differing results in DIT. The moderating factor in determining diet composition was the need to take into account other dietary factors that may contribute to DIT. Sensory stimulation, including palatability, produced by food has been found by some,[4,6,35,36] to affect the magnitude of the cephalic or predigestive phase of DIT. The increase in the activity of the sympathetic nervous system may account for as much as 25–40% of total DIT.[37] For this reason we adjusted the diets to be as similar as possible with regard to all sensory factors. Familiarity of food has also been found to affect DIT[38] and for this reason, both diets were composed of food items familiar to the subjects with the exception of Quorn, which is a vegetarian meat substitute that has a similar taste, consistency and appearance to chicken when served, as in the present diets. Naturally, the diets fulfilled the basic criterion of being isoenergetic and isovolumetric.

The method of calculating DIT from data obtained in the respiration chamber is crucial. In the present study, DIT was calculated over 24 h, using the intercept of the linear regression between EE and physical activity to determine RMR and by subtracting SMR to determine DIT. Schutz *et al*[39] first calculated DIT using the individual relationship between EE and activity in each subject to determine mean RMR, from which (BMR, the basal metabolic rate measured separately) was subtracted to determine DIT. SMR as opposed to BMR was used as baseline EE in this study as it is the more reproducible of the two.[31] To further ensure stable values, we used the mean SMR of both nights of each experiment, as this value correlated marginally better with fat free mass (FFM) than either single night or the lowest measured SMR of the nights. Schutz *et al*[39] proposed DIT only be measured during waking hours, since most of the thermogenic response to feeding occurs during the time subjects are awake and sleep is known to depress EE. DIT may however continue well beyond this period, especially if an evening snack is consumed. Tataranni *et al*[40] found that 15 h calculations underestimated DIT, when compared to measurements conducted in subjects in the fed or fasted state. However, calculations where DIT was calculated as 24 h RMR above SMR, were similar to the difference in 24 h EE when subjects were fed or fasted.

A potential source of error is the measurement of physical activity by a radar system. Radar detects only the time the subjects move, but does not provide information on the intensity of the activity. However, variations in movement intensity are restricted by the size of the respiration chamber. We also addressed the possible problem of a few extreme points influencing the slope of the relationship between EE and radar output, causing the intercept to be over- or underestimated and consequently altering measured DIT. To avoid this, we implemented a light exercise protocol to ensure greater range of EE and so eliminate the effect of erratic spontaneous physical activity. Exercise has been cited as one factor possibly affecting DIT,[1,41] although results have not been conclusive. The exercise periods in the present protocol were, however, light enough not to affect DIT. This was confirmed by also calculating DIT excluding the data collected during exercise periods (six times 30 min.) from analysis and extrapolating data over 24 h. This did not affect the significance of the results. Including an exercise protocol is also appropriate, as this further simulates free living conditions.

Energy requirements of subjects were estimated by multiplying measured SMR on the first night by a physical activity index (PAI) of 1.6, as this was found to be the mean PAI in a previous study by Schrauwen *et al.*[22] On a group level, this proved to be an accurate

estimate, although there was great variation with regard to energy balance between subjects, with three subjects being consistently underfed and one subject overfed. It was inevitable that there would be a positive or negative energy balance on one of the diets, resulting from the difference in DIT and the fact that the diets were isoenergetic. For this reason it was, however preferable, to express DIT results as a proportion of EI as opposed to EE.

FQ differed significantly from RQ on each diet. Calculation of substrate utilization using the Brouwer formula[24] confirmed that substrates were oxidized in a different proportion to those ingested. This was anticipated, as previous studies have shown the need for an adaptation period, before substrate utilization is adjusted to substrate ingestion.[22] Thus, the slightly higher RQ value on the HP/C diet, might be explained by a carry-over effect of the unusual (high carbohydrate) dinner on the night before entering the chamber and the higher RQ value on the HF diet by subjects oxidizing some glycogen. For practical reasons, we only provided one meal of similar composition prior to the test diets of the following day. However, had subjects consumed the test diets for a longer period prior to the measurements, and thus had time to adapt RQ to FQ, our results may have been even more convincing. Now, especially on the HF diet, subjects were temporarily storing part of the dietary fat and using glycogen which might have reduced the difference in DIT between the two diets.

Conclusion

The results of the present study, clearly indicate that macronutrient composition of the diet has a direct impact on the magnitude of DIT, irrespective of other dietary factors, and as such may, over long periods of time, contribute to the development and maintenance of obesity.

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Appendix 1 Example of diets containing 8900 kJ

High protein and carbohydrate diet (HP/C)		*High fat diet (HF)*	
	Breakfast		
White bread	120 g	Croissant	80 g
Linera 3%[a]	5 g	Butter	11 g
Jam	30 g	Jam (low calorie)	15 g
Quark based herb cheese	20 g	Cream cheese (Boursin)	15 g
Fat-free fruit yoghurt	200 g		
Drink[b]	150 g	Drink	400 g
	Lunch		
Mushroom soup	250 g	Mushroom soup	200 g
+ fat-free milk powder	35 g	+ double cream	50 g
Mixed salad (iceberg lettuce, tomato, and cucumber)	250 g	Mixed salad (iceberg lettuce, tomato, and cucumber)	250 g
Cottage cheese	60 g		
Tuna fish in brine	80 g	Smoked mackerel	25 g
Salad cream (5% fat)	30 g	Salad cream (40% fat)	30 g
Fresh orange juice	250 g	No calorie orange drink	250 g
+ fantomalt powder[c]	33 g	Drink	150 g
	Snack		
Peaches in syrup	150 g	Peaches in syrup	100 g
Fat-free yoghurt	100 g	Whipped double cream	20 g
Quark	100 g	Drink	250 g
+ artificial sweetener			
	Dinner		
Pasta (cooked weight)	200 g	Pasta (cooked weight)	150 g
Tomato sauce	200 g	Tomato sauce	175 g
+ chicken fillet pieces	225 g	+ quorn[d]	75 g
		+ vegetable oil	35 g
Cucumber (raw)	100 g	Cucumber (raw)	100 g
Drink	150 g	Drink	450 g
	Snack		
Vanilla pudding (fat-free)	150 g	Vanilla pudding	75 g
		+ double cream	20 g
Drink	200 g	Drink	250 g
Total weight	3088 g		3176 g

[a]Low fat butter substitute; [b]Where not otherwise specified, subjects could drink either water, tea or coffee (max. two cups); [c]Maltodextrine - Nutricia, The Netherlands; [d] Vegetarian meat substitute.