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Effects of a supra-sustained gelatin–milk protein diet compared with (supra-)sustained milk protein diets on body-weight loss

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Abstract

Diets higher in protein content result in increased satiety and energy expenditure. In the short term, gelatin showed stronger hunger suppression and less subsequent energy intake compared with other proteins. The present study investigated whether a supra-sustained gelatin–milk protein (GMP) diet promotes weight loss compared with a sustained milk protein (SMP) diet and a supra-sustained milk protein (SSMP) diet during an 8-week diet period. A total of seventy-two healthy subjects (31·2 (SD 4·8) kg/m^2; 43 (SD 10) years) followed one of the three diets in a subject-specific amount: SMP, SSMP or GMP diet. During weeks 1–4, energy intake was 100 % of individual energy requirement: 10, 40 and 50 % of energy (En %) as protein, fat and carbohydrate, respectively (SMP diet), and 20, 30 and 50 En % as protein, fat and carbohydrate, respectively (SSMP diet or GMP diet). During weeks 5–8, energy intake was 33 % of individual energy requirement: 30, 35 and 35 En % as protein, fat and carbohydrate, respectively (SMP diet), and 60, 5 and 35 En % as protein, fat and carbohydrate, respectively (SSMP diet or GMP diet). Thus, absolute protein intake was kept constant throughout per subject. Significant decreases in BMI ($P<0·0001$) were similar between the GMP (−1·7 (SD 0·5) kg/m^2) and the SMP (−2·1 (SD 0·8) kg/m^2) and SSMP (−1·6 (SD 0·5) kg/m^2) diets. Decreases in fat-free mass (FFM), fat mass (FM) and FM %, and increases in FFM % were similar between the GMP and both control diets. Changes in RQ differed ($P<0·05$) between the GMP (−0·01 (SD 0·06)) and SSMP (−0·04 (SD 0·04)) diets. Changes in HDL concentrations differed ($P<0·05$) between the GMP (−0·21 (SD 0·18) mmol/l) and the SMP and SSMP diets (−0·08 (SD 0·18) mmol/l and −0·09 (SD 0·26) mmol/l, respectively). In conclusion, a gelatin–milk protein diet does not induce more beneficial effects during an 8-week weight-loss period compared with a SMP or SSMP diet.

Key words: Protein: Weight loss: Body composition: Appetite

Obesity is associated with disorders such as hypertension, hypercholesterolaemia, diabetes and liver disease[1]. Since obesity is a major health concern and the number of people with obesity is still increasing, strategies for weight loss and weight maintenance thereafter are important. Therefore, short-term as well as long-term mechanisms should be targeted. Recent findings suggest that an increased protein intake may serve this goal by (1) an increased satiety, despite similar or lower energy intake, (2) an increased thermogenesis, (3) contribution to storage of fat-free mass (FFM) and (4) lower energy efficiency during overfeeding[2–5].

In previous studies, short-term effects of different protein types, represented in normal and high single-protein breakfasts/diets, on satiety, energy intake and energy expenditure have been investigated[6–14]. First, it has been shown that, under 10 % of energy (En %) as well as under 25 En % protein conditions, energy intake after a single-protein breakfast was less with gelatin compared with casein, soya or whey without glycomacropeptide[8]. Under 10 En % protein conditions, gelatin decreased hunger more than casein after a single-protein breakfast[8] as well as after a single-protein diet for 1 d[13]. Second, over 24 h, it has been shown that gelatin compared with casein, under 10 En % as well as under 25 En % single-protein conditions, resulted in similar effects on total energy expenditure[13]. For both protein types, total energy expenditure was increased with an increased protein content of the diet[12,14]. At the moment, it is not clear whether the beneficial short-term effects of gelatin on hunger and energy

Abbreviations: DPI, daily protein intake; En %, percentage of energy; FFM, fat-free mass; FM, fat mass; GLP, glucagon-like peptide; GMP, supra-sustained gelatin–milk protein; PYY, peptide–tyrosine–tyrosine; REE, resting energy expenditure; RQ, respiratory quotient; SMP, sustained milk protein; SSMP, supra-sustained milk protein.

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expenditure may play a role in the long term during weight loss. Since gelatin is an incomplete protein, because it is deficient in certain essential amino acids, i.e. devoid of tryptophan and imbalanced in methionine, it cannot be used as a single-protein source in a long-term diet. To create a relatively high-protein diet without lacking the essential amino acids, gelatin should be complemented with a complete protein source. In that case, the mechanism of hunger suppression due to gelatin being an incomplete protein[15–18] may not play a role anymore. However, when in our previous experiment, gelatin was added to the diet, over 36 h appetite homeostasis appeared to be stronger in comparison with casein[13], possibly through increased gluconeogenesis[13]. The minimum level of imbalance required to alter protein metabolism in the hypothesised direction and degree that we observed is 10 En% of protein in a diet in a neutral energy balance. This is the same absolute amount of protein that we added to the diet during the weight-loss experiment executed in the present study. The short-term effects on hunger suppression induced by gelatin may relate to a mechanism observed in metazoans, where it has been discovered that the transfer RNA/general control non-derepressible 2/phosphorylation of the α-subunit of eukaryotic initiation factor 2 system in the brain can detect a deficiency of essential amino acids in the diet from a decline in serum amino acid levels, leading to a behavioural response that rejects consumption of imbalanced diets[15,16,18–22]. This shows that the lack of a specific essential amino acid precursor for protein synthesis is a limiting factor of an incomplete protein diet[18]. The inability of an incomplete protein diet to support human life was known already in the early 1800s, when Napoleon's injured soldiers failed to recover on a diet with gelatin as the protein source[17]. A general amino acid control system, which is activated by deprivation via deacylated transfer RNA, has shown conservation of amino acid sensory mechanisms across eukaryotic species[18].

Evidence on the role of gluconeogenesis is given by animal model research. *De novo* synthesis of glucose from gluconeogenic precursors is increased by a high-protein diet[23]. The main gluconeogenic organ is the liver. The activity of hepatic phosphoenolpyruvate carboxykinase, an enzyme involved in gluconeogenesis, is increased in rats fed a high-protein diet. The satiating effect of high-protein feeding could be related to the improvement of glucose homeostasis through the modulation of hepatic gluconeogenesis and subsequent glucose metabolism, glucose homeostasis and glucose signalling to the brain.

Therefore, the aim of the present study was to investigate whether the addition of gelatin to a milk protein diet would promote weight loss during a weight-loss period. To investigate this, one intervention diet, a supra-sustained protein diet with gelatin and milk protein as the two protein sources in equal amounts, was compared with two control diets, a sustained and a supra-sustained protein diet with milk protein as the only protein source. The effects of the three diets on body weight, body composition, RQ, resting energy expenditure (REE), eating behaviour, physical activity, post-absorptive appetite profile and relevant blood parameters were determined before and after an 8-week diet period.

Methods and procedures

Subjects

A total of eighty-one subjects aged 18–65 years with a BMI of $\geq 25\,kg/m^2$ were recruited by advertisements on notice boards of Maastricht University and in local newspapers. Subjects underwent a medical screening and were in good health, non-smokers, did not use medication (except for contraceptives), did not have a cow milk allergy and were at most moderate alcohol users. Written informed consent was obtained from all subjects. 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 Ethical Committee of the Maastricht University Medical Center, Maastricht, The Netherlands. During the first week, nine subjects dropped out for personal reasons. The remaining seventy-two subjects all completed the diet period.

Experimental design

The study had a single-blind parallel design. Subjects were randomly assigned to one of three treatment groups: (1) sustained milk protein (SMP) diet (control group 1); (2) supra-sustained milk protein (SSMP) diet (control group 2); (3) supra-sustained gelatin–milk protein (GMP) diet (intervention group). All groups followed an 8-week diet period. At the start and end of this period of time, subjects visited the university for measurements.

Energy intake

During the first 4 weeks of the diet period (weeks 1–4), subjects from all three diet groups consumed a diet that was 100% of their individual estimated energy requirements for energy balance, while during the last 4 weeks (weeks 5–8), they consumed a diet that was 33% of their individual energy requirements. The energy content of the diet was based on estimated subject-specific average daily energy requirements and calculated as the BMR multiplied with a physical activity level of 1·5. BMR was calculated by the Harris–Benedict formula[24].

Diets

During the complete 8-week diet period, subjects from all three diet groups consumed a fixed amount of protein each day, referred to as (supra-)sustained protein diets. This implied that the absolute protein content of each of the three protein diets remained the same during the whole period, while each diet differed in En% from protein between weeks 1–4 and 5–8 of the diet period due to a reduction in energy intake in weeks 5–8. Macronutrient compositions of the three diets are shown in Table 1. The protein content of the two supra-sustained protein diets was twice the amount of the sustained protein diet. Carbohydrate content was kept

constant between the three diet groups in order to prevent a possible effect from carbohydrate, as ingestion of this nutrient results in insulin secretion, and insulin is involved in protein metabolism[25]. All three diets were provided as meal replacements and contained all necessary vitamins, minerals, fatty acids and carbohydrates. The protein content of the SMP and SSMP diets consisted of 100 % milk protein, while the protein content of the GMP diet consisted of 50 % milk protein and 50 % gelatin. The physical form of the meal replacements was a powder, to be dissolved in water, resulting in a semi-solid dish, to be consumed with a spoon. In addition, subjects were instructed to eat four portions of fruit and vegetables each day and drink at least 1·5 litres of water. Energy density of their total diet was thus estimated to be 4 kJ/g.

Measurements

At the start (week 0) and at the end (week 8) of the diet period, subjects visited the university for the following measurements. Subjects came to the university in the morning, after an overnight fast, and were not allowed to eat and drink until all measurements were finished.

Body weight and height. Body weight was measured on a digital scale (BOD POD; Life Measurement, Inc., Concord, CA, USA), with subjects in their underwear, in a fasted state and after voiding their bladder. Height was measured using a wall-mounted stadiometer (Seca Model 225; Seca GmbH, Hamburg, Germany). BMI was calculated as body weight divided by height squared (kg/m^2).

Waist and hip circumference. Waist circumference was measured at the site of the smallest circumference between the rib cage and the ileac crest, with subjects in standing position. Hip circumference was measured at the site of the largest circumference between the waist and the thighs. Both waist and hip circumferences were measured with an accuracy of 1·0 mm. The waist:hip ratio was calculated by dividing the waist circumference by the hip circumference.

Body composition. Body composition was determined according to the three-compartment model based on body weight, body volume as measured with the air displacement plethysmograph[26] and total body water as measured with the ^2H dilution (^2H_2O) technique[27,28], and was calculated using the combined equation of Siri[29].

Resting energy expenditure and respiratory quotient. REE was measured by means of an open-circuit ventilated hood system while subjects were lying supine for 40 min. Gas analyses were performed by a paramagnetic O_2 analyser (Servomex type 500A; Servomex Controls Limited, Crowborough, Sussex, UK) and an infrared CO_2 analyser (Servomex type 500A; Servomex Controls Limited). Calculation of REE was based upon Weir's formula[30]. Respiratory quotient (RQ) was calculated as CO_2 produced/O_2 consumed.

Blood pressure and heart rate. Diastolic and systolic blood pressure, and heart rate were measured with an upper-arm digital blood pressure monitor (OMRON M6; Omron Healthcare Europe BV, Hoofddorp, The Netherlands) while subjects were sitting quietly in a chair.

Eating behaviour. The Dutch translation of a three-factor eating questionnaire[31] was used to determine whether attitude towards food intake changed during the diet period. The first factor of the three-factor eating questionnaire (F1) measures cognitive restrained eating: control of food intake by thought and will power. The second factor (F2) represents disinhibition: an incidental inability to resist eating cues, or inhibition of dietary restraint, and emotional eating. The third factor (F3) examines the subjective feeling of general hunger.

Physical activity. To determine whether physical activity was kept constant during the weight-loss period, subjects filled in the Baecke questionnaire[32] before and after the diet period. From this questionnaire, (1) physical activity at work, (2) sport during leisure time and (3) physical activity during leisure time excluding sport were determined.

Post-absorptive appetite profile. In the morning, after an overnight fast, appetite was scored by 100 mm anchored visual analogue scales. Four questions were asked, anchored with 'not at all' to 'extremely', namely 'How satiated do you feel?', 'How full do you feel?', 'How hungry are you?' and 'How is your desire to eat?'.

Blood parameters. Fasting blood samples were taken for measurements of plasma glucagon-like peptide-1 (GLP-1), peptide–tyrosine–tyrosine (PYY), insulin, glucose, creatinine (serum), HDL, LDL, TAG and NEFA concentrations. For GLP-1, blood was collected into EDTA-containing tubes to which a dipeptidyl peptidase IV inhibitor (10 µl/ml blood) was added. For PYY analysis, blood was collected into EDTA-containing tubes in which a dipeptidyl peptidase IV inhibitor (10 µl/ml blood) and aprotinin (500 kIU/ml blood) were added. For insulin, glucose, HDL, LDL, TAG and NEFA, blood was collected into EDTA-containing tubes. For creatinine, blood was collected in serum separator tubes. After the collection of blood into the tubes, blood samples were

Table 1. Macronutrient compositions of the sustained milk protein (SMP), supra-sustained milk protein (SSMP) and supra-sustained gelatin–milk protein (GMP) diets during the diet period

	Weeks 1–4 (100 % of energy requirements)			Weeks 5–8 (33 % of energy requirements)		
	SMP	SSMP	GMP	SMP	SSMP	GMP
Milk protein (En %)	10	20	10	30	60	30
Gelatin (En %)			10			30
Fat (En %)	40	30	30	35	5	5
Carbohydrate (En %)	50	50	50	35	35	35

immediately centrifuged for 10 min (3000 rpm at 4°C), except for the creatinine tube, which was centrifuged after staying for 60 min at room temperature. Plasma and serum samples were immediately frozen in liquid N_2 and stored at −80°C until further analysis. Plasma active GLP-1 concentrations were analysed by ELISA (EGLP-35K; Linco Research, Inc., St Charles, MO, USA). Plasma concentrations of PYY and insulin were measured by RIA (Linco Research, Inc.). Plasma glucose concentrations were determined using the hexokinase method (Glucose HK CP kit; ABX diagnostics, Montpellier, France). The homeostatic model assessment index was calculated as (fasting glucose (mmol/l) × fasting insulin (mU/l))/22·5. Serum creatinine concentrations were analysed by means of the Jaffe rate method on the Synchron LX20 Pro (Beckman Coulter, Nyon, Switzerland). Plasma total cholesterol and HDL concentrations were analysed using cholesterol oxidase-*p*-aminophenazone reagent (Roche Diagnostics GmbH, Mannheim, Germany). LDL was calculated using the Friedewald formula[33]. Plasma TAG concentrations were analysed with the GPO-Trinder kit (Sigma, St Louis, MO, USA). Plasma NEFA concentrations were analysed using the acylcoenzyme A synthetase-acyclcoenzyme oxidase-MEHA (ACS-ACOD-MEHA) method in the Wako-NEFA-C kit (Wako Chemicals GmbH, Neuss, Germany).

Protein intake. At the start and end of the diet period, subjects collected their urine for 24 h, which was analysed for N to check the compliance with protein intake. Subjects were instructed in detail on how to collect their 24 h urine before each time of collection. Furthermore, subjects completed a questionnaire on frequency and completeness of collection at home, which was discussed with a researcher upon return of the bottles. If the 24 h collection was not complete, subjects collected a second time. Protein intake was calculated from the 24 h N output as follows:

$$\text{Protein intake (g/d)} = \text{N output in 24 h urine (g/d)} \times 6{\cdot}25.$$

Statistical analysis

Data are presented as means and standard deviations, unless otherwise indicated. For each diet group, a repeated-measures ANOVA was carried out for the determination of possible differences between the start (week 0) and the end (week 8) of the diet period in all measured parameters. To determine possible differences between the GMP diet group and the two control diet groups, a factorial ANOVA was carried out. *Post hoc* analyses were made with Fisher's protected least significant difference. To determine the relationships between variables, simple linear regression analyses were performed. The level of statistical significance was set at $P<0{\cdot}05$. Statistical analyses were performed using StatView 5.0 (SAS Institute, Inc., Cary, NC, USA).

Results

Baseline characteristics of the subjects for the three diet groups are presented in Table 2.

Compliance to the diets

Baseline daily protein intake (DPI) was 0·8 (SD 0·3), 0·9 (SD 0·3) and 1·0 (SD 0·6) g/kg per d for the SMP, SSMP and GMP diet groups, respectively. At the end of the diet period, the SSMP and GMP diet groups had a DPI of 1·1 (SD 0·3) and 1·2 (SD 0·5) g/kg per d, respectively, which was significantly higher for both supra-sustained protein diet groups ($P<0{\cdot}01$) compared with the DPI of 0·8 (SD 0·3) g/kg per d for the SMP diet group. DPI was not significantly different between the two supra-sustained protein diet groups.

Body weight and BMI

In all groups, weighing of the subjects after 4 weeks showed that not all subjects remained weight stable during the first 4 weeks as was intended. They started to lose body weight immediately, so weight loss and its effects have been reported over the full 8 weeks.

In all three diet groups, body weight and BMI were significantly decreased after the diet period ($P<0{\cdot}0001$; Fig. 1). The decreases in body weight and BMI were similar between the GMP diet group compared with each of the control groups (not significant).

Waist:hip ratio

The waist:hip ratio was significantly decreased after the diet period in the SSMP and GMP diet groups ($P<0{\cdot}01$; Table 2), but did not change in the SMP diet group (NS). The changes in the waist:hip ratio were similar between the GMP diet group compared with each of the control groups (NS).

Body composition

FFM (kg), fat mass (FM, kg) and FM expressed as a percentage of body weight were decreased after the diet period in all three diet groups (Fig. 2(A), (B) and (D)), while FFM expressed as a percentage of body weight was increased in all three diet groups (Fig. 2(C)). The changes in FFM (kg or %) and FM (kg or %) were similar between the GMP diet group compared with each of the control groups (NS).

Respiratory quotient

After the diet period, RQ significantly decreased in the SMP and SSMP diet groups ($P<0{\cdot}01$; Table 2), but did not change in the GMP diet group (NS). The changes over time in RQ were significantly different between the SSMP and GMP diet groups ($P<0{\cdot}05$), with a stronger decrease in RQ for the SSMP diet group. The changes over time in RQ were similar between the SMP and GMP diet groups (NS).

Resting energy expenditure as a function of fat-free mass

REE is plotted as a function of FFM at baseline (week 0) and after the diet period (week 8) for the SMP, SSMP and GMP diet groups (Fig. 3(A), (B) and (C), respectively). In all three

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Table 2. Subject characteristics and measured variables of the three diet groups before and after the diets
(Mean values and standard deviations)

	Baseline (week 0)						End weight loss (week 8)					
	SMP		SSMP		GMP		SMP		SSMP		GMP	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
n	29		22		21		–		–		–	
Male	4		7		5		–		–		–	
Female	25		15		16		–		–		–	
Age (years)	43	10	43	10	44	10	–		–		–	
Height (m)	1·67	0·07	1·73	0·08	1·68	0·12	–		–		–	
Waist:hip ratio	0·91	0·05	0·92	0·06	0·93	0·06	0·90	0·05	0·90**	0·07	0·91**	0·05
Respiratory quotient†	0·83	0·04	0·84	0·04	0·83	0·06	0·81**	0·03	0·81**	0·04	0·82	0·03
Diastole (mmHg)	81	10	81	8	82	12	76**	9	72***	8	75**	11
Systole (mmHg)	130	17	127	10	129	16	118***	10	117***	11	117**	14
Heart rate (beats/min)	67	10	71	10	69	12	64*	9	64***	8	66	10
TFEQ1‡ (dietary restraint)	9	4	8	5	10	5	11**	5	10*	4	11*	5
TFEQ2‡ (disinhibition)	6	3	6	2	7	3	5**	3	5	3	5**	3
TFEQ3‡ (hunger)	5	3	5	3	6	4	3**	2	4	3	4**	3
Baecke (work)	2·73	0·57	2·52	0·60	2·87	0·51	2·75	0·56	2·56	0·59	2·85	0·52
Baecke (sport)	2·58	1·03	2·56	0·63	2·61	1·09	2·64	0·88	2·64	0·77	2·63	0·92
Baecke (leisure)	3·03	0·62	2·91	0·49	3·00	0·69	3·20*	0·61	3·03	0·62	3·11	0·58
Baecke (total)	8·34	1·51	7·99	1·23	8·48	1·72	8·58	1·32	8·23	1·38	8·59	1·59
VAS (satiety)	47	16	41	18	36	25	45	16	41	22	39	18
VAS (fullness)	40	16	34	18	43	26	44	16	42	22	36	15
VAS (hunger)	35	17	31	16	38	24	33	17	27	18	35	20
VAS (desire to eat)	35	20	32	19	47	21	37	18	27	17	38	19

SMP, sustained milk protein; SSMP, supra-sustained milk protein; GMP, supra-sustained gelatin–milk protein; TFEQ, three-factor eating questionnaire; VAS, visual analogue scale.
Mean values were significantly different over time within one diet group: * $P<0·05$, ** $P<0·01$, *** $P<0·0001$.
† Mean values were significantly different over time between the SSMP and GMP diets ($P<0·05$).
‡ Factors 1, 2 and 3, respectively, of the TFEQ.

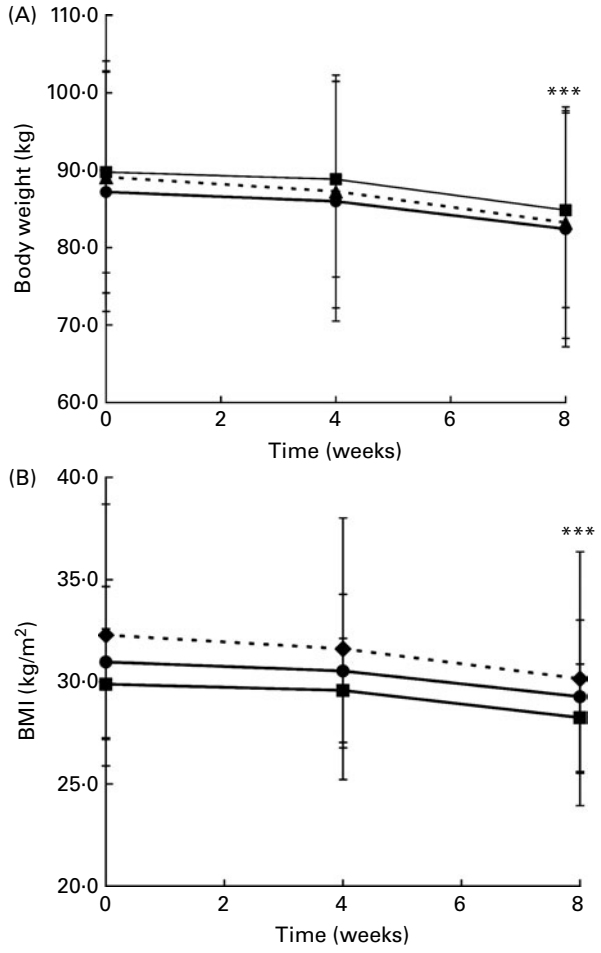

Fig. 1. (A) Body weight and (B) BMI for the sustained milk protein (SMP), supra-sustained milk protein (SSMP) and supra-sustained gelatin–milk protein (GMP) diet groups before, during and after the 8-week diet period. Values are means, with standard deviations represented by vertical bars. Mean values were significantly different over time between weeks 8 and 0 for each diet group (*** $P<0·0001$). No significant difference in change over time from week 0 to 8 between the GMP diet and the two control diets. (A) --▲--, SMP; —■—, SSMP; —●—, GMP. (B) --◆--, SMP; —■—, SSMP; —●—, GMP.

diet groups, a significant linear relationship was present between REE (MJ/d) and FFM (kg) at baseline as well as after the weight-loss period ($P<0·0001$). For each diet group, to determine whether the REE as a function of FFM had changed significantly over time, as shown by the regression lines at week 0 and week 8, the FFM (kg) values from week 8 were filled in by the slope equation of week 0 to result in a calculated REE of week 8. The calculated and measured REE of week 8 were analysed with repeated-measures ANOVA to assess any changes in REE as a function of FFM. In the SMP and GMP diet groups, REE as a function of FFM decreased significantly ($P<0·0001$ and $P<0·01$, respectively), while in the SSMP diet group, REE as a function of FFM did not change significantly. However, the changes over time in REE as a function of FFM were not significantly different between the GMP diet group compared with each of the control groups.

Blood pressure and heart rate

Diastole and systole significantly decreased after the diet period in all three diet groups ($P<0·01$ or $P<0·0001$; Table 2). Heart rate significantly decreased in the SMP and SSMP diet groups ($P<0·05$ and $P<0·0001$, respectively, Table 2) after weight loss, but not in the GMP diet group (NS). The changes over time in diastole, systole and heart rate were similar between the GMP diet group compared with each of the control groups (NS).

Eating behaviour

Dietary restraint (factor 1 of the three-factor eating questionnaire; Table 2) increased significantly in all three diet groups ($P<0·05$ or $P<0·01$). Disinhibition (factor 2) and general hunger (factor 3) significantly decreased in the SMP and GMP diet groups ($P<0·01$), but the decrease did not reach significance in the SSMP diet group (NS). The changes over time in dietary restraint, disinhibition and general hunger were similar between the GMP diet group compared with each of the control groups (NS).

Physical activity

Physical activity (Baecke work, sport, leisure or total; Table 2) did not significantly change over time in all three diet groups. Changes over time in physical activity were similar between the GMP diet group compared with each of the control groups (NS).

Post-absorptive appetite profile

Post-absorptive scores for satiety, fullness, hunger and desire to eat did not significantly change over time in all three diet groups (Table 2), and changes over time were similar between the GMP diet group compared with each of the control groups (NS).

Blood parameters

Fasting plasma concentrations at baseline and after the diet period are presented in Table 3. Plasma GLP-1 and glucose concentrations significantly decreased and increased, respectively, in the GMP diet group, while no significant changes over time occurred in the SMP and SSMP diet groups. Plasma PYY, insulin and LDL concentrations, and homeostatic model assessment index significantly decreased in all three diet groups. Plasma HDL concentrations significantly decreased in the SMP and GMP diet groups, but did not significantly change in the SSMP diet group. Plasma TAG concentrations did not significantly change over time in all three diet groups. Plasma NEFA and serum creatinine concentrations significantly increased in the SMP diet group, but no significant changes occurred in the SSMP and GMP diet groups. Changes over time in all blood variables were similar between the GMP diet group and the two control groups (NS), except for the plasma HDL concentrations, which decreased more over time in the GMP diet group compared with both control groups ($P<0·05$).

British Journal of Nutrition

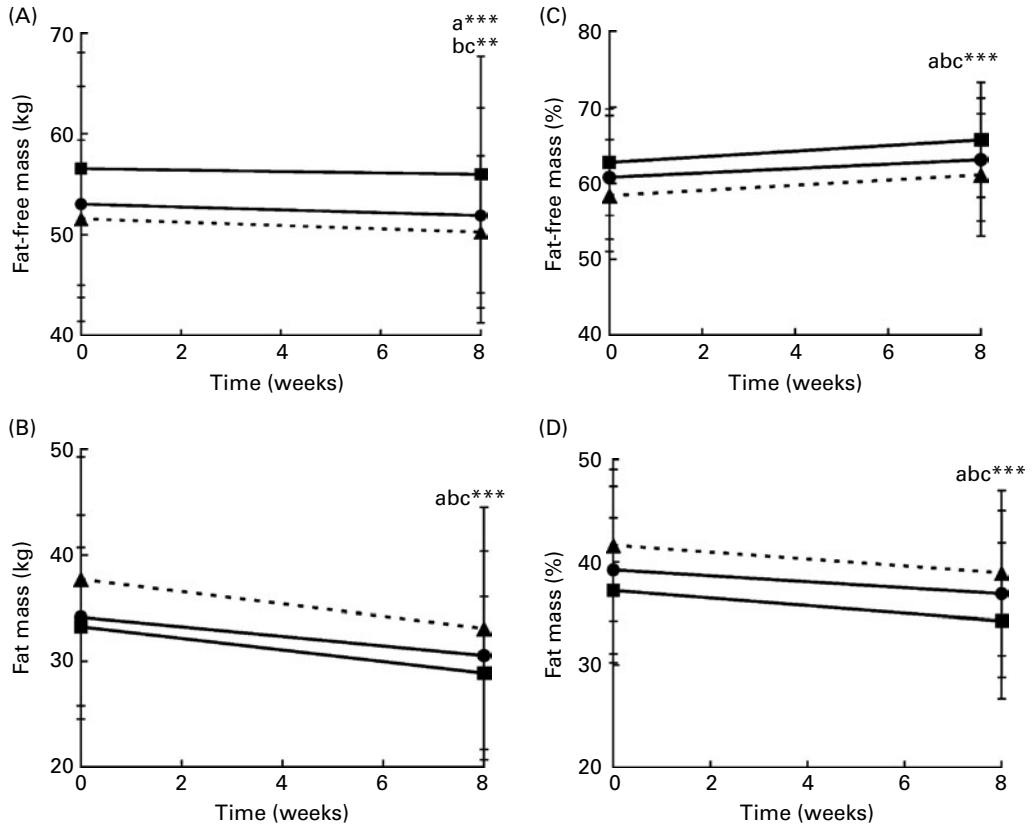

Fig. 2. (A) Fat-free mass (kg), (B) FM (kg), (C) fat-free mass expressed as a percentage of body weight and (D) FM expressed as a percentage of body weight for the sustained milk protein (SMP, --▲--), supra-sustained milk protein (SSMP, —■—) and supra-sustained gelatin–milk protein (GMP, —●—) diet groups before and after the 8-week diet period. Values are means, with standard deviations represented by vertical bars. Mean values were significantly different over time within one diet group: ** $P<0{\cdot}01$, *** $P<0{\cdot}0001$, [a] SMP diet, [b] SSMP diet, [c] GMP diet. There was no significant difference in change over time between the GMP diet and the two control diets.

Discussion

In the present study, we investigated whether the addition of gelatin to a milk protein diet promotes weight loss during a diet period. The results show that this was not observed. Changes over the 8-week diet period in body weight, BMI, waist:hip ratio, body composition, REE as a function of FFM, blood pressure, heart rate, eating behaviour, physical activity, post-absorptive appetite profile, plasma/serum GLP-1, PYY, insulin, glucose, creatinine, LDL, TAG and NEFA concentrations and homeostatic model assessment index were not significantly different between the GMP diet group and the SMP and SSMP diet groups. The GMP diet group differed from the SSMP diet group in showing a smaller decrease in RQ over time, and differed from both the SMP and SSMP diet groups in showing a stronger decrease in plasma HDL concentrations over time. This may indicate that the effect of additional gelatin as a protein does not improve all metabolic parameters as observed usually in relatively high-protein diets[5].

Compliance in the present study was confirmed with the 24 h urinary N results. Although subjects were instructed in detail on how to collect their 24 h urine before each time of collection and completed a questionnaire on frequency and completeness of collection at home, which was discussed with a researcher upon return of the bottles, and although they had to collect a second time if collection had been incomplete, we cannot be completely sure that all 24 h urine was collected, since we did not use a marker. For instance, it could be that the subjects were less accurate towards the end of the weight-loss period, and that some collection was forgotten. In that case, we would have measured a value that was too low. However, we observed for the SMP group a stable DPI after 8 weeks, as was expected, and for the SSMP and GMP diet groups, we observed a significantly increased DPI after 8 weeks, following the protocol. The DPI of the SMP diet group at the end of the weight-loss period was 0·8 g/kg per d, which is the required minimum amount of DPI as recommended by the WHO[34]. The DPI of the supra-sustained protein diet groups, being 1·1 and 1·2 g/kg per d, was significantly higher compared with the sustained protein diet group, while protein intake was similar between the two supra-sustained protein diet groups.

In all three diet groups, body weight and BMI were significantly reduced after the 8-week weight-loss period, while these decreases over time were not different between the GMP diet and the two control diets. The results show that adding gelatin to a SMP diet, while creating a GMP diet, does not result in larger effects on weight loss compared with a sustained and supra-sustained protein diet with milk protein as the only protein source. Thus, the beneficial

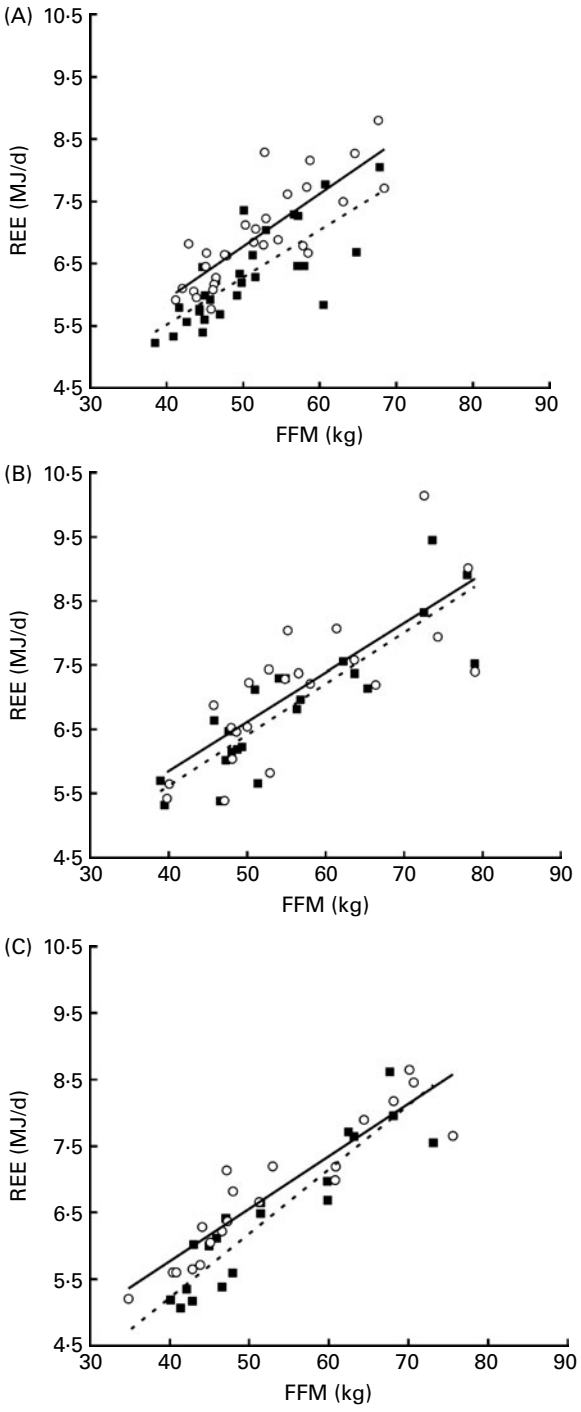

Fig. 3. Resting energy expenditure (REE) as a function of fat-free mass (FFM) plotted for week 0 (baseline, ○, trendline —) and for week 8 (after weight loss, ■, trendline ····) for the sustained milk protein (SMP, A), supra-sustained milk protein (SSMP, B) and supra-sustained gelatin–milk protein (GMP, C) diet groups. Regression equation of SMP diet, week 0: REE = 0·085 FFM + 2·54 (R^2 0·67, P<0·0001); week 8: REE = 0·076 FFM + 2·47 (R^2 0·60, P<0·0001). Regression equation for the SSMP diet, week 0: REE = 0·077 FFM + 2·78 (R^2 0·60, P<0·0001); week 8: REE = 0·079 FFM + 2·44 (R^2 0·75, P<0·0001). Regression equation for the GMP diet, week 0: REE = 0·079 FFM + 2·61 (R^2 0·85, P<0·0001); week 8: REE = 0·097 FFM + 1·36 (R^2 0·87, P<0·0001).

short-term effect of gelatin on hunger suppression[8] was not present anymore, as expected from the now complete protein that was consumed. Moreover, also the appetite homeostasis-promoting effect from gluconeogenesis, as has been hypothesised based upon a previous study[13], did not seem to play a role over the longer term. Although changes in fasting plasma GLP-1 and PYY concentrations over time were observed, these changes in the so-called appetite hormones did not affect the post-absorptive appetite profile in all three diets. The addition of gelatin to the diet did not result in a different effect on the post-absorptive appetite profile compared with both control diets, and therefore did not contribute to promote differences in weight loss. Apart from the possible appetite homeostasis effect through gluconeogenesis that we described in the introduction, gluconeogenesis also could have provided a larger energy expenditure, which also could have promoted a negative energy balance. In a 36 h study, we have shown that in energy balance, the increase in energy expenditure on a relatively high-protein diet was a function of the increase in gluconeogenesis[35]. The contribution of this increase in gluconeogenesis to increase in energy expenditure was 42 %. Yet, this energy expenditure-promoting effect through gluconeogenesis from an incomplete protein diet providing a surplus of amino acids as observed over 36 h obviously did not affect longer-term energy balance and weight loss.

With respect to eating behaviour, changes over time in dietary restraint, disinhibition and general feelings of hunger were also not different between the GMP diet group and the two control groups, and therefore did not contribute to promote differences in weight loss. In most weight-loss studies, relatively high-protein diets are considered during *ad libitum* energy intakes[5,36–38]. In absolute terms, the 'relatively high' intakes in these studies may just meet the required minimum amount of DPI (0·8 g/kg per d) as recommended by the WHO, and are in fact normal-protein diets. In addition, the 'relatively normal' protein intakes in these studies may be lower than the required minimum amount of DPI, and are in fact low-protein diets[2,39]. This may contribute to the observed 'increases' in satiety with the 'relatively high' protein diets, relative to decreases in satiety in their low-protein control groups. As in the present study, the SMP diet group had already a protein intake of 0·8 g/kg per d (which is the required minimum amount of DPI), while the supra-sustained protein diet groups had even higher protein intakes of 1·1 and 1·2 g/kg per d during energy restriction, we compared absolutely normal-(sustained) protein diets with absolutely high-(supra-sustained) protein diets; this is why all three diets were successful in weight loss. Then, although during the GMP diet, protein intake was higher compared with the SMP diet, no differences in appetite profile and weight loss were observed among those diets. This may suggest that the required minimum absolute amount of protein intake is sufficient to accomplish the beneficial effects of protein on satiety and weight loss during energy restriction. The effect of a sustained (absolute) required minimum amount of protein intake on the appetite profile being sufficient, is in line with the 'protein leverage hypothesis', which implies that maintaining

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Table 3. Fasting blood variables of the three diet groups before and after the diets
(Means values and standard deviations)

	Baseline (week 0)						End weight loss (week 8)					
	SMP		SSMP		GMP		SMP		SSMP		GMP	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GLP-1 (pmol/l)	1·6	1·6	2·4	2·8	1·5	1·0	1·3	0·9	2·0	2·1	1·3*	0·7
PYY (pg/ml)	80	37	56	49	62	31	20***	13	18**	18	18***	17
Insulin (μU/ml)	15·99	6·12	14·12	4·90	13·35	6·01	12·21**	5·31	10·09**	3·90	10·79**	4·75
Glucose (mmol/l)	5·15	0·40	5·14	0·41	4·97	0·37	5·14	0·55	5·10	0·46	5·22**	0·51
HOMA index	3·70	1·60	3·25	1·23	2·97	1·37	2·87*	1·51	2·31**	1·03	2·53*	1·20
Creatinine (μmol/l)	74	13	78	14	81	23	82*	16	83	15	82	14
HDL (mmol/l)†	1·42	0·38	1·38	0·38	1·56	0·37	1·34*	0·33	1·30	0·023	1·35***	0·31
LDL (mmol/l)	3·68	0·71	3·90	0·92	3·65	0·95	2·97***	0·62	3·09**	1·08	3·04**	0·73
TAG (mmol/l)	1·27	0·71	1·20	0·54	1·03	0·42	1·18	0·34	1·07	0·37	1·10	0·33
NEFA (mmol/l)	480	140	466	165	508	230	582**	193	532	162	523	165

SMP, sustained milk protein; SSMP, supra-sustained milk protein; GMP, supra-sustained gelatin–milk protein; GLP-1, glucagon-like peptide-1; PYY, peptide–tyrosine–tyrosine; HOMA, homeostatic model assessment.
Mean values were significantly different over time within one diet group: * $P<0·05$, ** $P<0·01$, *** $P<0·0001$.
† Mean values were significantly different over time between the GMP diet and the two control diets ($P<0·05$).

absolute DPI is prioritised over fat and carbohydrate intakes, regardless of macronutrient composition of the diets[40].

In all three diet groups, absolute FFM and FM decreased as a result of body weight loss. However, when expressing FFM and FM as a percentage of body weight, in all three diet groups, FFM percentage increased, while FM percentage decreased. As physical activity did not change over time in all three diet groups, physical activity was not involved in the improvement in body composition. This may indicate that in all three diet groups, body composition improved due to sparing of FFM as a result of keeping the DPI at minimum required levels or even higher. Although body composition improved in all three diet groups, the addition of gelatin to a milk protein diet did not result in different effects on body composition compared with the two milk protein diets. Protein intake was 0·4 and 0·1 g/kg per d higher in the GMP diet group compared with the SMP and SSMP diet groups, but this higher intake did not result in a higher preservation of FFM. Thus, the addition of gelatin to a SMP diet, or the exchange of energy from milk protein with energy from gelatin in a supra-sustained protein diet does not affect body composition differentially. In addition, in both milk protein diet groups, RQ decreased, while RQ remained similar in the GMP diet group. The change in RQ over time was different between the SSMP and GMP diet groups, which may indicate that gelatin has less potential to increase post-absorptive fat oxidation during weight loss. However, the difference in RQ change over time did not result in a less favourable effect of the GMP diet on body composition.

REE as a function of FFM decreased significantly over time in the SMP and GMP diet groups, while the change over time was not significant in the SSMP diet group. Previous studies have already observed that the decrease in REE is the result of compensatory changes in energy expenditure to a decrease in body weight[41]. Thus, only in the SSMP diet group, energy expenditure was sustained despite a negative energy balance; this phenomenon was not reached in the SMP and GMP diet groups, and may need a considerably higher protein intake of the quality of a complete protein.

Regarding health benefits, all three diets resulted in beneficial decreases in diastole, systole, heart rate (not significant for the GMP diet group), and fasting plasma insulin and LDL concentrations, while the GMP diet did not show different effects on these parameters over time compared with both milk protein diets. However, the observed higher decrease in fasting plasma HDL concentration with the GMP diet showed a less favourable effect of gelatin compared with the SMP and SSMP diets. The relative increase in plasma glucose concentrations and decrease in HDL concentrations suggest increased degradation of the amino acids in the slightly imbalanced diet via gluconeogenesis, and fewer precursor amino acid substrates for HDL. This may be expected, as gelatin is clearly an incomplete protein.

Milk protein, either at the level of SMP, or at the level of SSMP, has beneficial effects on weight loss during a negative energy balance, with respect to loss of body mass, body composition, fasting plasma insulin and LDL concentrations.

Addition of gelatin to milk protein is not able to improve these factors under negative energy balance conditions.

The present study shows that any appetite reduction seen with short-term gelatin feeding does not carry over in the longer term, when it is added to a complete protein in the diet. However, the very small physiological effects seen with the addition of an incomplete protein to an already sustained protein diet may be reassuring to those using elevated levels of amino acid supplements for muscle building and training for other athletic activities. We conclude that a GMP diet does not induce more beneficial effects during an 8-week weight-loss period compared with a SMP and SSMP diets. The larger decrease in HDL with the GMP diet may indicate a less favourable metabolic effect. This effect supports using better-quality protein diets.

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