

# No long-term weight maintenance effects of gelatin in a supra-sustained protein diet.

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# No long-term weight maintenance effects of gelatin in a supra-sustained protein diet

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## ABSTRACT

In the short-term, gelatin showed stronger hunger suppression and less energy intake compared with other proteins. This study investigated if a supra-sustained gelatin–milk protein (GMP) diet improves weight maintenance (WM) compared with a sustained milk protein (SMP) diet and supra-sustained milk protein (SSMP) diet during a 4-months WM period after 8-week weight loss (WL) in sixty-five healthy subjects ( $28.6 \pm 3.4 \text{ kg/m}^2$ ;  $44 \pm 10$  years). Absolute protein intake was kept constant (sustained) throughout per subject. Diets were: protein(P)/fat(F)/carbohydrate(C): 15/40/45% of energy (En%) (SMP) and 30/25/45 En% (SSMP or GMP) for weeks 9–16. Diets on weeks 17–24: P/F/C: 30/35/35 En% (SMP) and 60/5/35 En% (SSMP or GMP). From weeks 8 to 16, and weeks 16 to 24, changes in BMI were similar between the GMP ( $-0.4 \pm 0.6$  and  $0.3 \pm 0.7 \text{ kg/m}^2$  respectively), and the SMP ( $-0.7 \pm 0.9$  and  $0.1 \pm 0.7 \text{ kg/m}^2$  respectively) and SSMP ( $-0.6 \pm 0.6$  and  $0.3 \pm 0.6 \text{ kg/m}^2$  respectively) diets. Sparing of fat free mass (FFM): increases/decreases in FFM%/fat-mass% from weeks 8 to 16 were similar between the GMP and both control diets, and maintained from weeks 16 to 24. In conclusion, all 3 diets resulted in a successful WM period, while a GMP diet does not improve body weight maintenance and related variables after weight loss compared with a SMP and SSMP diet.

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## 1. Introduction

Obesity is associated with disorders such as hypertension, hypercholesterolemia, 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 necessary. Therefore, short-term as well as long-term mechanisms should be affected. Recent findings suggest that an increased protein intake may serve this goal by 1) sustained satiety, despite similar or lower energy intake, 2) sustained thermogenesis, 3) sparing of fat free mass, and 4) lower energy efficiency during the period of weight maintenance [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 were investigated [6–14]. Firstly, it was shown that, under 10 En% as well as under 25 En% protein conditions, energy intake after a single-protein breakfast was less with gelatin compared with casein, soy 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 one day [13]. Since gelatin is an incomplete protein, because it is deficient in certain essential amino

acids, i.e. devoid of tryptophan and imbalanced in methionine, these results may relate to a mechanism observed in metazoans, where it was discovered that the tRNA/GCN2/p-eIF2 $\alpha$  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 behavioral response that rejects consumption of imbalanced diets [15–18], and thus appears as hunger suppression. This phenomenon has been observed since the 1970s as excesses or deficiencies of amino acids in the diet depressing food intake in rats [19]. Later, Fromentin et al. showed that the parabrachial nuclei are involved in the learned aversion to an amino acid devoid diet [20]. In a review Gietzen and Rogers explain that given a choice, herbivores and omnivores select a diet containing higher levels of protein or amino acid mixtures, even if the choices contain balanced indispensable amino acid profiles; this shows that nitrogen per se can become limiting [21]. They furthermore show that in addition to rodents, Protozoa, Nematoda, Mollusca, Arthropoda, Vertebrata including fish, birds and mammals have the same indispensable amino acids, except humans, which do not require arginine [21]. 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 [22]. A general amino acid control system which is activated by deprivation via deacylated tRNA showed conservation of amino acid sensory mechanisms across eukaryotic species [21].

Also, when in our previous experiment, gelatin was added to the diet over 36 h VAS ratings on the appetite profile showed a stronger hunger suppression in comparison with casein [13], possibly through

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increased gluconeogenesis [13]. Evidence for this 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 (PEPCK), an enzyme involved in gluconeogenesis, is increased in rat 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 signaling to the brain.

Moreover, over 24 h it was 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 this moment it is not clear whether the beneficial short-term effects of gelatin on hunger and energy expenditure may play a role in the long term during a weight maintenance period after weight loss.

Since gelatin is an incomplete protein, 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. Then the hunger suppression effect of gelatin being an incomplete protein may have disappeared, but a possible hunger suppression effect due to improved appetite homeostasis through gluconeogenesis still may sustain. Therefore, the aim of this study was to investigate if the addition of gelatin to a milk protein diet would improve weight maintenance during a 4-months weight maintenance period after weight loss. To investigate this during weight loss and weight maintenance, 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 effect of the three diets on body weight (BW), body composition, respiratory quotient (RQ), resting energy expenditure, eating behavior, physical activity, postabsorptive appetite profile and relevant blood parameters were determined at the start, after 8 weeks and after 16 weeks of the weight maintenance period following weight loss.

## 2. Methods and procedures

### 2.1. Subjects

Eighty-one subjects aged 18–65 years with a body mass index (BMI) of  $\geq 25$  kg/m<sup>2</sup> were recruited by advertisements on notice boards of Maastricht University and in local newspapers. Subjects underwent a medical screening and all were in good health, were non-smokers, did not use medication (except for contraceptives), did not have a cow milk allergy and were at most moderate alcohol users. Nine subjects did not complete the weight loss period due to inability to comply with the diet or for personal reasons. From the 72 subjects who finished the weight loss period, 65 subjects started the weight maintenance period after a weight loss of  $6 \pm 2\%$  of initial body weight. Subjects signed an informed consent before participating in the study. The study protocol was approved by the Medical Ethical Committee of the Maastricht University Medical Centre.

### 2.2. Experimental design

The study had a single blind parallel design. Subjects were randomly assigned to one of three treatment groups: 1) sustained milk protein diet (SMP, control group 1), 2) supra-sustained milk protein diet (SSMP, control group 2), 3) supra-sustained gelatin–milk protein diet (GMP, intervention group). After following an 8-week weight loss period, based on an energy intake of 33% of individual energy re-

quirements, subjects started a 4-month weight maintenance period. Subjects visited the university for measurements at the end of week 8, after 2 months (end week 16) and after 4 months (end week 24) of the weight maintenance period.

### 2.3. Energy intake

During the complete weight maintenance period (weeks 9–24) subjects from all 3 diet groups consumed a diet that was 100% of their individual energy requirements for energy balance. This was calculated as 67% from 100% of energy requirements at the start of the weight loss period. The energy content of the diet at the start of the weight loss period was based on subject-specific average daily energy requirements and calculated as the Basal Metabolic Rate (BMR) multiplied with a Physical Activity Level (PAL) of 1.5. BMR was calculated with the Harris–Benedict formula [24].

### 2.4. Diets

During the complete 4-months weight maintenance period subjects from all 3 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. During the first 2 months of weight maintenance, the complete diet (100% of energy requirements) was provided. During the last 2 months of weight maintenance half of the diet (50% of energy requirements) was provided, while subjects were able to eat ad libitum, without counselling of the research team, for the other 50% of energy requirements. To keep protein intake the same during the complete weight maintenance period, each diet differed in percentage of energy from protein between weeks 9–16 and weeks 17–24 (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 similar between the 3 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 3 diets were provided as meal replacements and contained all necessary vitamins, minerals, fatty acids and carbohydrates. The protein content of the sustained and supra-sustained milk protein diets consisted of 100% milk protein, while the protein content of the supra-sustained gelatin–milk protein diet consisted of 50% milk protein and 50% gelatin. In addition, subjects were instructed to eat four portions of fruit and vegetables each day and drink at least 1.5 L of water.

#### 2.4.1. Compliance with protein intake

Before every test day subjects collected their urine for 24 h, which was analyzed for nitrogen to check the compliance with protein intake. Protein intake was calculated from the 24 h nitrogen output as follows: protein intake (g/d) = nitrogen output in 24 h urine (g/d)  $\times$  6.25.

**Table 1**

Macronutrient compositions of the SMP, SSMP and GMP diets during weeks 9–16 and weeks 17–24 of the weight maintenance period.

	Weeks 9–16: 100% of energy requirements			Weeks 17–24: 50% of energy requirements (+ ad libitum)		
	SMP	SSMP	GMP	SMP	SSMP	GMP
Milk protein (En%)	15	30	15	30	60	30
Gelatin (En%)			15			30
Fat (En%)	40	25	25	35	5	5
Carbohydrate (En%)	45	45	45	35	35	35

Abbreviations: En% = % of energy; GMP = supra-sustained gelatin–milk protein diet; SMP = sustained milk protein diet; SSMP = supra-sustained milk protein diet.

## 2.5. Measurements

Subjects visited the university for the following measurements at the end of week 8, after 2 months (end week 16) and after 4 months (end week 24) of the weight maintenance period. 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.

### 2.5.1. Body weight and height

BW was measured on a digital scale (BOD POD, Life Measurement Inc., 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, Hamburg, Germany). BMI was calculated as BW divided by height<sup>2</sup> (kg/m<sup>2</sup>).

### 2.5.2. Waist and hip circumference

Waist circumference was measured at the site of the smallest circumference between the rib cage and the iliac 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.

### 2.5.3. Body composition

Body composition was determined according to the 3-compartment model based on BW, body volume as measured with the air displacement plethysmograph [26], and total body water as measured with the deuterium dilution (<sup>2</sup>H<sub>2</sub>O) technique [27,28], and was calculated by using the combined equation of Siri [29].

### 2.5.4. Resting energy expenditure (REE) and RQ

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 oxygen analyzer (Servomex, type 500A, Crowborough, Sussex, UK) and an infrared carbon dioxide analyzer (Servomex, type 500A, Crowborough, Sussex, UK). Calculation of REE was based upon Weir's formula [30]. RQ was calculated as CO<sub>2</sub> produced/O<sub>2</sub> consumed. This measurement was not performed at week 24.

### 2.5.5. 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.

### 2.5.6. Eating behavior

The Dutch translation of the Three Factor Eating Questionnaire (TFEQ) [31] was used to determine if attitude towards food intake changed during the weight loss period. The first factor of the TFEQ (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.

### 2.5.7. Physical activity

To determine if physical activity was kept constant during the weight maintenance period, subjects filled in the Baecke questionnaire [32]. From this questionnaire 1) physical activity at work, 2) sport during leisure time, and 3) physical activity during leisure time excluding sport, could be determined.

### 2.5.8. Postabsorptive appetite profile

In the morning, after an overnight fast, appetite was scored by 100 mm anchored Visual Analogue Scales (VAS). 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?".

### 2.5.9. 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), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triacylglycerol (TAG) and free fatty acid (FFA) concentrations. The blood for GLP-1 was collected into EDTA-containing tubes to which dipeptidyl peptidase IV inhibitor (10 µL/mL blood) was added. For PYY analysis, blood was collected into EDTA-containing tubes in which dipeptidyl peptidase IV inhibitor (10 µL/mL blood) and aprotinin (500 KIU/mL blood) was added. The blood for insulin, glucose, HDL, LDL, TAG and FFA was collected into EDTA-containing tubes. The blood for creatinine was collected in serum separator tubes. After the collection of blood into the tubes, blood samples were immediately centrifuged for 10 min (3000 rpm at 4 °C), except for the creatinine tube, which was centrifuged after being kept 60 min at room temperature. Plasma and serum samples were immediately frozen in liquid nitrogen and stored at -80 °C until analyzed further. Plasma active GLP-1 concentrations were analyzed by enzyme-linked immunosorbent assay (EGLP-35K; Linco Research Inc, St Charles, MO). Plasma concentrations of PYY and insulin were measured by radioimmunoassay (Linco Research Inc, St Charles, MO). Plasma glucose concentrations were determined by using the hexokinase method (Glucose HK CP kit; ABX diagnostics, Montpellier, France). The HOMA index was calculated as (fasting glucose [mmol/L] × fasting insulin [microU/mL]) / 22.5. Serum creatinine concentrations were analyzed by means of the Jaffe rate method on the Synchron LX20 Pro (Beckman Coulter, Nyon, Switzerland). Plasma total cholesterol and HDL concentrations were analyzed using CHOD-PAP reagent (Roche Diagnostics GmbH, Mannheim, Germany). LDL was calculated using the Friedewald formula [33]. Plasma TAG concentrations were analyzed with the GPO-Trinder kit (Sigma, Missouri, USA). Plasma FFA concentrations were analyzed by using the ACS-ACOD-MEHA method in the Wako-NEFA-C kit (Wako Chemicals GmbH, Neuss, Germany). No blood measurements were performed at week 24.

## 2.6. Statistical analysis

Data are presented as mean ± standard deviation (SD), unless otherwise indicated. For each diet group, a repeated-measures ANOVA was carried out for determination of possible differences between week 8 and week 16, and between week 16 and week 24 in all measured parameters. To determine possible differences between the supra-sustained gelatin-milk protein diet group and the two control diet groups, a factorial ANOVA was carried out. Post-hoc analyses were made with Fisher's PLSD. To determine relationships between variables, simple linear regression analyses were performed. The level of statistical significance was set at *P* < 0.05. Statistical analyses were performed by using StatView 5.0 (SAS Institute Inc., Cary, NC).

## 3. Results

Subject characteristics for the 3 diet groups at the start of the weight maintenance period are presented in Table 2.

### 3.1. Compliance to the diets

A good compliance was reached during the complete weight maintenance period. At weeks 8 and 16 the daily protein intake (DPI) was significantly higher in both supra-sustained protein diet groups compared with the sustained milk protein diet group (Table 2).

**Table 2**  
Subject characteristics and measured variables of the sustained milk protein (SMP), supra-sustained milk protein (SSMP) and supra-sustained gelatin–milk protein (GMP) diet groups at the start, at week 16, and at the end of weight maintenance.

	SMP			SSMP			GMP		
	Week 8	Week 16	Week 24	Week 8	Week 16	Week 24	Week 8	Week 16	Week 24
N (M/F)	26 (4/22)	–	–	19 (6/13)	–	–	20 (5/15)	–	–
Age (year)	43 ± 11	–	–	43 ± 10	–	–	44 ± 10	–	–
Height (m)	1.67 ± 0.06	–	–	1.74 ± 0.07	–	–	1.67 ± 0.12	–	–
DPI (g/kg per day)	0.77 ± 0.23	0.78 ± 0.27	0.97 ± 0.39	1.15 ± 0.32 <sup>a</sup>	1.16 ± 0.25 <sup>a</sup>	1.31 ± 0.37 <sup>a</sup>	1.17 ± 0.48 <sup>a</sup>	1.21 ± 0.43 <sup>a</sup>	1.26 ± 0.67
Waist:hip ratio <sup>#</sup>	0.89 ± 0.05	0.87 ± 0.06 <sup>b</sup>	0.87 ± 0.06	0.90 ± 0.07	0.89 ± 0.07	0.90 ± 0.07	0.91 ± 0.05	0.88 ± 0.07 <sup>b</sup>	0.88 ± 0.07
RQ <sup>#</sup>	0.81 ± 0.03	0.84 ± 0.04 <sup>b</sup>	–	0.80 ± 0.03	0.83 ± 0.05 <sup>b</sup>	–	0.82 ± 0.03	0.83 ± 0.05	–
Diastole <sup>#</sup> (mmHg)	75 ± 9	77 ± 9	75 ± 9	72 ± 9	74 ± 7	74 ± 7	75 ± 11	75 ± 12	72 ± 11
Systole <sup>#</sup> (mmHg)	118 ± 10	120 ± 13	118 ± 12	116 ± 11	117 ± 10	119 ± 9	116 ± 14	118 ± 15	116 ± 15
Heart rate <sup>#</sup> (beats/min)	64 ± 9	65 ± 10	69 ± 8 <sup>c</sup>	64 ± 8	66 ± 8	71 ± 10 <sup>c</sup>	66 ± 10	65 ± 10	71 ± 11 <sup>c</sup>
TFEQ1 <sup>d,e,f</sup> (dietary restraint)	11 ± 5	11 ± 5	12 ± 5	11 ± 4	12 ± 4	12 ± 5	12 ± 5	12 ± 4	12 ± 5
TFEQ2 <sup>e,f</sup> (disinhibition)	5 ± 2	5 ± 2	4 ± 2	5 ± 3	5 ± 3	5 ± 3	5 ± 3	4 ± 2	5 ± 2
TFEQ3 <sup>e,f</sup> (hunger)	3 ± 3	3 ± 2	3 ± 3	4 ± 3	3 ± 2	3 ± 2	3 ± 3	2 ± 2	3 ± 4
Baecke <sup>#</sup> (work)	2.77 ± 0.53	2.69 ± 0.58	2.70 ± 0.58	2.55 ± 0.62	2.56 ± 0.62	2.55 ± 0.63	2.90 ± 0.49	2.85 ± 0.47	2.90 ± 0.46
Baecke <sup>#</sup> (sport)	2.69 ± 0.91	2.66 ± 1.00	2.40 ± 0.93	2.63 ± 0.82	2.67 ± 0.82	2.58 ± 0.73	2.69 ± 0.91	2.62 ± 0.96	2.57 ± 0.79
Baecke <sup>#</sup> (leisure)	3.17 ± 0.63	3.19 ± 0.56	3.33 ± 0.65	3.07 ± 0.67	3.05 ± 0.60	2.96 ± 0.55	3.16 ± 0.56	3.19 ± 0.64	3.18 ± 0.68
Baecke <sup>#</sup> (total)	8.63 ± 1.36	8.54 ± 1.43	8.44 ± 1.49	8.25 ± 1.45	8.28 ± 1.47	8.09 ± 1.42	8.74 ± 1.48	8.66 ± 1.34	8.65 ± 1.30
VAS <sup>#</sup> (satiety)	44 ± 16	46 ± 16	46 ± 21	39 ± 23	40 ± 16	40 ± 20	37 ± 16	38 ± 17	40 ± 20
VAS <sup>#</sup> (fullness)	43 ± 16	47 ± 12	49 ± 17	41 ± 23	37 ± 18	40 ± 13	34 ± 14	32 ± 14	42 ± 22
VAS <sup>#</sup> (hunger)	33 ± 17	35 ± 17	28 ± 18	25 ± 19	26 ± 18	32 ± 17	34 ± 20	33 ± 18	34 ± 20
VAS <sup>#</sup> (desire to eat)	37 ± 19	39 ± 15	33 ± 20	24 ± 17	30 ± 21	36 ± 25	37 ± 19	38 ± 21	39 ± 22

Values are means ± SD. <sup>#</sup> No significant difference between the GMP diet and the two control diets for changes in the variable from weeks 8 to 16, and weeks 16 to 24. <sup>a</sup> Significantly different from SMP diet group at same week,  $P < 0.05$ . Significantly different from <sup>b</sup> week 8, <sup>c</sup> week 16 within a diet group,  $P < 0.05$ . <sup>d,e,f</sup> Factors 1, 2 and 3, respectively, of the TFEQ. Abbreviations: DPI = daily protein intake; REE = resting energy expenditure; RQ = respiratory quotient; TFEQ = Three Factor Eating Questionnaire; VAS = visual analogue scale.

During the last 2 months of the weight maintenance period, when subjects were allowed to eat ad libitum in addition, protein intake increased in all 3 diet groups, indicating a good compliance during this period.

### 3.2. Body weight and BMI

At the end of the complete 4-month weight maintenance period, no significant weight regain occurred in all 3 diet groups. Weight loss at week 8 was  $-6.1 \pm 2.2$  kg,  $-5.3 \pm 1.5$ , and  $-4.7 \pm 1.5$  kg in the SMP, SSMP and GMP diet groups respectively, while weight loss at week 24 was  $-7.5 \pm 5.3$  kg,  $-6.4 \pm 2.6$ , and  $-4.5 \pm 3.3$  kg respectively. In all 3 diet groups BW and BMI significantly decreased from weeks 8 to 16 (Fig. 1,  $P < 0.05$ ); after that BW and BMI were sustained. The changes in BW and BMI were similar between the GMP diet group and both control groups during the first 2 months and during the last 2 months of the weight maintenance period. Moreover, the changes between the SMP and SSMP group were similar.

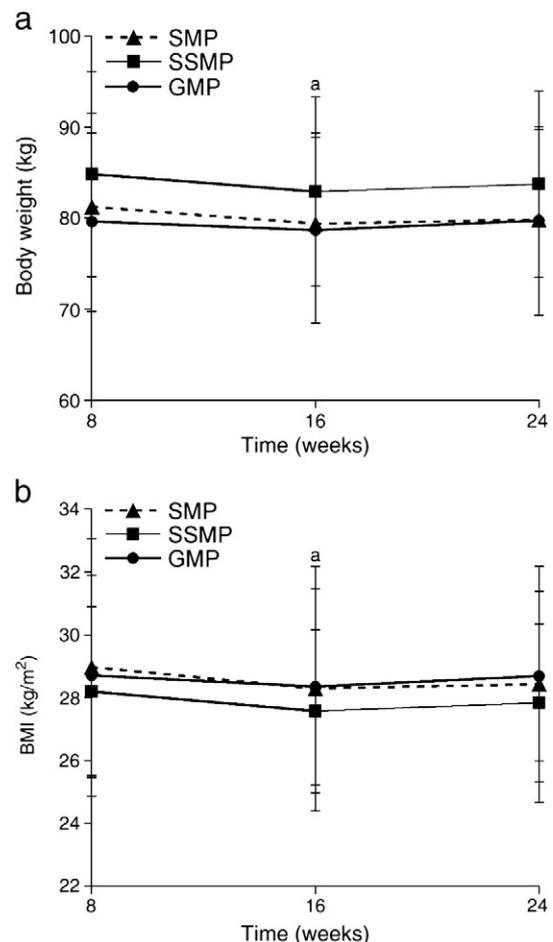
### 3.3. Waist:hip ratio

Waist:hip ratio significantly decreased from weeks 8 to 16 in the SMP and GMP diet group ( $P < 0.05$ , Table 2), and remained the same from weeks 16 to 24 in all 3 diet groups. The changes in waist:hip ratio were similar between the GMP diet group and both control groups during the first 2 months and during the last 2 months of the weight maintenance period.

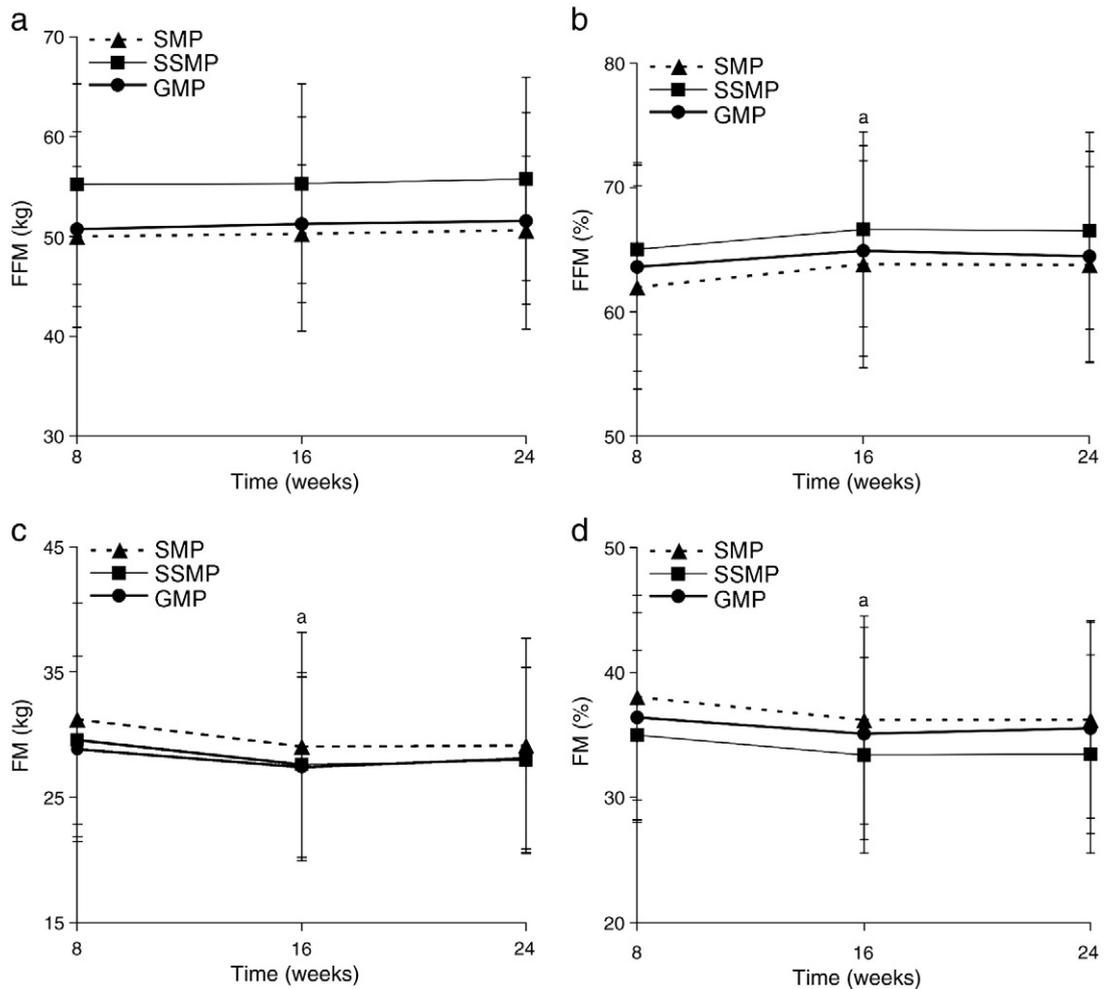
### 3.4. Body composition

Fat free mass (FFM) in kg did not significantly change from weeks 8 to 16, and from weeks 16 to 24, in all 3 diet groups (Fig. 2a). FFM% significantly increased, and fat mass (FM) in kg and expressed as % of BW were significantly decreased from weeks 8 to 16 in all 3 diet groups ( $P < 0.05$ , Fig. 2b–d), while sustained from week 16 to week 24. The changes in FFM (kg or %) and FM (kg or %) were similar between the GMP diet group and both control groups during the first 2 months and during the last 2 months of the weight maintenance period.

Post-hoc comparisons between the SMP and SSMP diets show that the decrease in body mass and fat mass over time is similar, yet fat free



**Fig. 1.** Body weight (a) and body mass index (BMI) (b) for the sustained milk protein (SMP), supra-sustained milk protein (SSMP) and supra-sustained gelatin–milk protein (GMP) diet group before (week 8), during (week 16) and after (week 24) the weight maintenance period. Values are means ± SD. <sup>a</sup> Significantly different from week 8 for all 3 diet groups,  $P < 0.05$ . Changes in body weight and BMI from week 8 to week 16, and from week 16 to week 24, were not significantly different between the GMP diet and the two control diets.



**Fig. 2.** Fat free mass (FFM) in kg (a) and expressed as % of body weight (b), and fat mass (FM) in kg (c) and expressed as % of body weight (d) for the sustained milk protein (SMP), supra-sustained milk protein (SSMP) and supra-sustained gelatin–milk protein (GMP) diet group before (week 8), during (week 16) and after (week 24) the weight maintenance period. Values are means  $\pm$  SD. <sup>a</sup> Significantly different from week 8 for all 3 diet groups,  $P < 0.05$ . Changes in FFM (kg or %) and FM (kg or %) from week 8 to week 16, and from week 16 to week 24, were not significantly different between the GMP diet and the two control diets.

mass did not decrease in the SSMP group, but slightly decreased in the SP group. Since the research is part of a larger study, that part is submitted elsewhere by Soenen et al.

### 3.5. RQ

RQ significantly increased from weeks 8 to 16 in the SMP and SSMP diet group ( $P < 0.05$ , Table 2), and changes in RQ were similar between the GMP diet group and both control groups.

### 3.6. REE as a function of FFM

REE is plotted as a function of fat free mass at the start (week 8) and after 2 months of the weight maintenance period (week 16) for the SMP (Fig. 3a), SSMP (Fig. 3b) and GMP (Fig. 3c) diet groups. In all 3 diet groups a significant linear relation was present between REE (MJ/d) and FFM (kg) at week 8 and week 16 ( $P < 0.0001$ ). To determine for each diet group if REE as function of FFM changed significantly over time, as shown by the regression lines at week 8 and week 16, the FFM (kg) values from week 16 were filled in the slope equation of week 8 to obtain a calculated REE of week 16. The calculated and measured REE of week 16 were analyzed with ANOVA repeated-measures to determine any changes in REE as function of FFM. In all 3 diet groups, REE as a function of FFM appeared not to have changed significantly from weeks 8 to 16, and possible small but not

significant differences in these relationships did not differ between the GMP diet and both control diet groups.

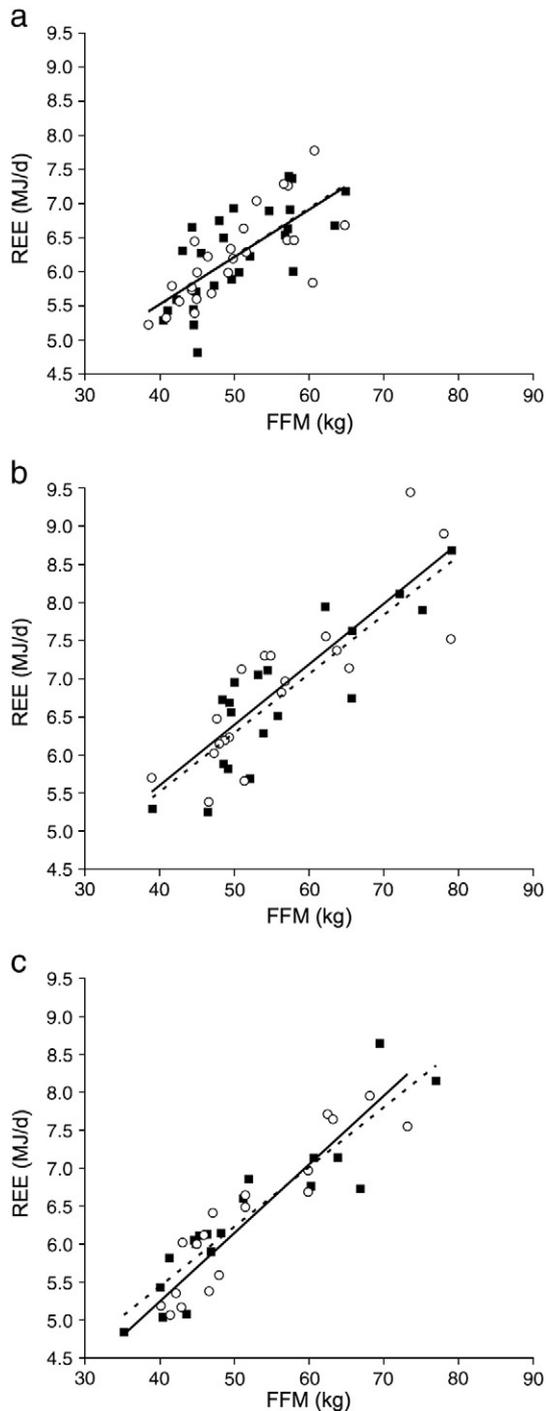
Similarly, REE as a function of fat free mass did not decrease in the SSMP group, but decreased slightly in the SMP group after 4 months. Since the research is part of a larger study, that part is submitted elsewhere by Soenen et al.

### 3.7. Blood pressure and heart rate

Diastole and systole were similar between weeks 8 and 16, and weeks 16 and 24 in all 3 diet groups (Table 2). Heart rate was similar between weeks 8 and 16, and significantly increased from weeks 16 to 24 ( $P < 0.05$ ) in all 3 diet groups (Table 2). The changes in blood pressure and heart rate were similar between the GMP diet group and both control groups during the first 2 months and during the last 2 months of the weight maintenance period.

### 3.8. Eating behavior

Dietary restraint (factor 1 of the TFEQ), disinhibition (factor 2) and hunger (factor 3) did not significantly change from weeks 8 to 16, and from weeks 16 to 24 in all 3 diet groups (Table 2). The changes in dietary restraint, disinhibition and hunger from weeks 8 to 16 and from weeks 16 to 24 were similar between the GMP diet group and both control groups.



**Fig. 3.** Resting energy expenditure (REE) as a function of fat free mass (FFM) plotted for week 8 (start weight maintenance, ○, trendline —) and for week 16 (after 8 weeks of weight maintenance, ■, 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 SMP diet, week 8:  $REE = 0.069FFM + 2.747$  ( $R^2 = 0.559$ ,  $P < 0.0001$ ). Regression equation SMP diet, week 16:  $REE = 0.071FFM + 2.68$  ( $R^2 = 0.499$ ,  $P < 0.0001$ ). Regression equation SSMP diet, week 8:  $REE = 0.079FFM + 2.425$  ( $R^2 = 0.717$ ,  $P < 0.0001$ ). Regression equation SSMP diet, week 16:  $REE = 0.077FFM + 2.42$  ( $R^2 = 0.740$ ,  $P < 0.0001$ ). Regression equation GMP diet, week 8:  $REE = 0.09FFM + 1.631$  ( $R^2 = 0.864$ ,  $P < 0.0001$ ). Regression equation GMP diet, week 16:  $REE = 0.079FFM + 2.292$  ( $R^2 = 0.838$ ,  $P < 0.0001$ ).

### 3.9. Physical activity

Physical activity (Baecke Work, Sport, Leisure or Total, Table 2) did not significantly change from weeks 8 to 16, and weeks 16 to 24 in all 3 diet groups. The changes in physical activity were similar between

the GMP diet group and both control groups during the first 2 months and during the last 2 months of the weight maintenance period.

### 3.10. Postabsorptive appetite profile

Postabsorptive scores for satiety, fullness, hunger and desire to eat were similar between weeks 8 and 16, and weeks 16 and 24 in all 3 diet groups. The changes in these scores were similar between the GMP diet group and both control groups during the first 2 months and during the last 2 months of the weight maintenance period.

### 3.11. Blood parameters

Fasting blood concentrations were measured at weeks 8 and 16 (Table 3). Plasma GLP-1, PYY, insulin and TAG concentrations, serum creatinine concentrations and HOMA index did not significantly change from weeks 8 to 16 in all 3 diet groups. Plasma glucose concentrations significantly decreased in the GMP diet group ( $P < 0.05$ ), and did not significantly change in the SMP and SSMP diet groups. Plasma HDL concentrations significantly increased in the GMP diet group ( $P < 0.05$ ), and did not significantly change in the SMP and SSMP diet groups. Plasma LDL concentrations significantly increased in all 3 diet groups ( $P < 0.05$ ). Plasma FFA concentrations significantly decreased in the SMP and GMP diet groups ( $P < 0.05$ ), and did not significantly change in the SSMP diet group. The changes in all blood variables were similar between the GMP diet group and both control groups.

## 4. Discussion

In this study we investigated whether the addition of gelatin to a milk protein diet results in a better weight maintenance during a 4-months weight maintenance period after weight loss. All 3 diets resulted in a successful weight maintenance period, as no weight was significantly regained after weight loss at the end of the 4-months weight maintenance period. No significant differences between the GMP diet group and the SMP and SSMP diet groups were observed in changes over the first and last 8 weeks of the weight maintenance period in BW, BMI, waist:hip ratio, body composition, RQ, REE as a function of FFM, blood pressure, heart rate, dietary restraint, disinhibition, subjective feeling of general hunger, physical activity, postabsorptive appetite profile, plasma/serum GLP-1, PYY, insulin, glucose, creatinine, HDL, LDL, TAG, and FFA concentrations, and HOMA index. For successful weight maintenance, a sustained milk protein diet is sufficient, while addition of gelatin has no additional effects on weight maintenance. Compliance in this study was confirmed with the 24 h urinary nitrogen results. The DPI of the sustained milk protein diet group at weeks 8 and 16 was 0.8 g/kg per day, which is the required minimum amount of daily protein intake as recommended by the World Health Organization [34]. The DPI of the supra-sustained protein diet groups, being 1.2 g/kg per day at weeks 8 and 16, were significantly higher compared with the sustained protein diet group, while protein intake was similar between both supra-sustained protein diet groups. During the last 8 weeks of weight maintenance 50% of energy requirements was provided as a diet, which contained the same absolute amount of protein they had to consume during the first 8 weeks of weight maintenance. As subjects were allowed to eat ad libitum in addition, from the increases in protein intake in all 3 diet groups can be concluded that subjects were also compliant during the last 8 weeks of the weight maintenance period.

The beneficial short-term effect of gelatin on hunger suppression [8,13] did not play a role in the long term during weight maintenance. As expected the hunger suppression effect of an incomplete protein had disappeared by adding the complete milk-protein to the gelatin in a sufficient amount, and the supposed gluconeogenesis did not

**Table 3**

Fasting blood variables of the sustained milk protein (SMP), supra-sustained milk protein (SSMP) and supra-sustained gelatin–milk protein (GMP) diet groups at the start and at week 16 of weight maintenance<sup>a</sup>.

	SMP		SSMP		GMP	
	Week 8	Week 16	Week 8	Week 16	Week 8	Week 16
GLP-1 (pmol/L)	1.3 ± 0.9	1.5 ± 1.3	2.1 ± 2.2	1.9 ± 2.2	1.3 ± 0.7	1.3 ± 0.7
PYY (pg/mL)	19 ± 11	16 ± 16	17 ± 17	15 ± 9	18 ± 18	25 ± 30
Insulin (μU/mL)	11.31 ± 3.92	11.03 ± 3.84	9.84 ± 3.91	10.51 ± 3.68	10.31 ± 4.31	10.31 ± 3.08
Glucose (mmol/L)	5.10 ± 0.51	4.99 ± 0.35	5.13 ± 0.48	5.08 ± 0.41	5.21 ± 0.52	4.94 ± 0.34 <sup>b</sup>
HOMA index	2.60 ± 1.06	2.47 ± 0.98	2.27 ± 1.06	2.40 ± 0.94	2.41 ± 1.08	2.27 ± 0.68
Creatinine (μmol/L)	83 ± 16	84 ± 21	83 ± 16	75 ± 13	82 ± 15	83 ± 21
HDL (mmol/L)	1.36 ± 0.34	1.41 ± 0.40	1.30 ± 0.22	1.36 ± 0.28	1.38 ± 0.29	1.45 ± 0.32 <sup>b</sup>
LDL (mmol/L)	2.91 ± 0.59	3.26 ± 0.82 <sup>b</sup>	2.93 ± 0.99	3.44 ± 0.73 <sup>b</sup>	3.05 ± 0.75	3.39 ± 0.85 <sup>b</sup>
TAG (mmol/L)	1.15 ± 0.33	1.29 ± 0.45	1.00 ± 0.29	1.10 ± 0.29	1.06 ± 0.27	1.09 ± 0.31
FFA (mmol/L)	576 ± 198	440 ± 150 <sup>b</sup>	542 ± 154	476 ± 115	514 ± 164	410 ± 166 <sup>b</sup>

Values are means ± SD.

<sup>a</sup> No significant difference in change over time between the GMP diet and the two control diets in all variables.

<sup>b</sup> Significantly different from week 8 within a diet group,  $P < 0.05$ .

support appetite homeostasis in a way that satiety would be increased [19–22]. This was confirmed by the postabsorptive appetite profiles and fasting plasma concentrations of the so-called satiety hormones GLP-1 and PYY. These variables did not significantly change over time and were not different between the GMP diet and both control diets. In addition, no significant changes in dietary restraint, disinhibition and general feelings of hunger as determined by the TFEQ were observed over time in all 3 diet groups, and as these changes over time were not different between the GMP diet and both control diets they could not have contributed to possible differences in weight change during this period. In all 3 diet groups the decrease in body weight from weeks 8 to 16 was the result of a loss in absolute fat mass, while absolute fat free mass did not change. This resulted in an improved body composition, as in all 3 diet groups FFM% increased, and FM% decreased. From weeks 16 to 24 the improved body composition was maintained in all 3 diet groups. No changes in FFM (kg or %) and FM (kg or %) were observed in all 3 diet groups. As physical activity did not change over time in all 3 diet groups, physical activity was not involved in the improvement in body composition due to sparing of fat free mass. Although from weeks 8 to 16, protein intake was 0.43 and 0.05 g/kg per day higher in the GMP diet group compared with the SMP and SSMP diet groups, this did not result in a higher preservation of fat free mass. Thus, the addition of gelatin to a sustained milk protein diet, or the exchange of energy from milk protein with energy from gelatin in a supra-sustained protein diet did not affect body composition differently. Accordingly, resting energy expenditure as a function of fat free mass did not change from weeks 8 to 16 in all 3 diet groups. In addition, changes in RQ over time were not different between the GMP diet group and both milk protein diet groups. Although we focus here on the effect of the additional gelatin to the diet, the question on possible differences between the SMP and SSMP diets also is relevant. Post-hoc comparisons between those diets show that the decrease in body mass and fat mass over time is similar, yet fat free mass did not decrease in the SSMP group, but slightly decreased in the SP group. It appears that once subjects keep a sustained milk protein diet at the level of 0.8 g protein/kg body mass intake, the main effect on body weight is reached. The SSMP diet only adds to this by sparing more fat free mass resulting in a better body composition. Accordingly REE as a function of fat free mass is preserved in that group throughout the phases.

Regarding health benefits, during the first 8 weeks of the weight maintenance period beneficial reductions in waist:hip ratio and FFA were observed. Although after weight loss plasma LDL concentrations were decreased [35], from weeks 8 to 16 of weight maintenance non-favourable increases in plasma LDL concentrations were observed in all 3 diet groups. The beneficial decreases in diastole, systole and heart rate after weight loss [35] were maintained throughout the 4 months

weight maintenance period, except from an increase in heart rate during the last 8 weeks of weight maintenance in all 3 diet groups.

In summary, this study shows that effects of a diet observed in the short-term do not necessarily result in similar effects in the long term, partly because it was not possible to give just gelatin as a protein over the long term, partly because the possible additional effect due to additional gelatin intake, namely gluconeogenesis, did not support body weight maintenance in a distinguishable way. The new aspect of this study was that the supposition that additional gelatin used in the long term could have a weak effect was ruled out.

We conclude that a supra-sustained gelatin–milk protein diet did not induce more beneficial effects on body weight maintenance and related variables during a 4-months weight maintenance period after weight loss compared with a sustained and supra-sustained milk protein diet.

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