High Protein Milk Significantly Lowered Desire to Eat in Obese Subjects: A Pilot Study

Kamalita Pertiwi, Susana and Astri Kurniati

***Abstract*—One of the proposed ways to address the problem of obesity is by modifying components of food to provide a sustained satiety. Protein is considered to be the most satiating macronutrients. Therefore, three nutritional formulas differed in protein contribution to energy were developed to assess the satiating properties of higher protein content using subjective satiety ratings in obese subjects. In a randomized, single-blind design, subjects received breakfast, either balanced protein formula (12.4%E), moderate protein formula (23.5%E), or high protein formula (40.6%E) in three different sessions. To assess short-term satiety, subjective satiety ratings using VAS and energy intake at *ad libitum lunch* were measured. In this pilot study, high and moderate protein formula showed the tendency to induce higher satiety than balanced protein formula. It was found that desire to eat at 15 min was significantly lower after high protein formula than balanced protein formula (P<0.05). But, the higher satiety ratings produced by high protein formula were not translated as a reduction in energy intake at *ad libitum* lunch.**

***Index Terms*—Ad libitum intake, high protein, obese, satiety, weight management.**

I. Introduction

Obesity rate is increasing not only in developed Western countries, but also in developing countries, with even faster changes in developing countries. Compared with the United States and European countries, where the annual increase in the prevalence of overweight and obesity among adult men and women is about 0.25 each, Asia experienced very high rate - two to five times greater rate of change [1].

Obesity is considered as a consequence of modern obesogenic environment, where energy-dense, high-fat and/or sugar foods are freely available and heavily promoted [2], [3]. Meanwhile, these fast foods of high energy density are reported to have low satiating power [2], [4], [5], therefore people tend to overeat.

The solution for the problem of overweight and obesity is body weight management through weight loss and/or weight maintenance. Weight management requires a multi-factorial approach since body weight regulation involves several pathways [6]. It is also of importance that hunger, satiety and sensory signals are the main regulatory factors of meal size, meal frequency, and food selection [7].

One of conditions for successful weight management is sustained satiety despite a negative energy balance [8]. Satiety itself is defined as process that leads to inhibition of further eating, decline in hunger, increase in fullness after a meal has finished [9]. Several components of food have been known to influence satiety, e.g macronutrients and fiber.

Macronutrients have different satiating effect, with protein being the most satiating, and fat the least. Dose dependent satiating effect of protein has been shown, with quite a range of concentrations of protein offered acutely, in a single meal, to subjects who are in energy balance and weight stable [10], [11]. Whereas, when subjects are not in energy balance, it may be considered that the relative percentages of protein intake will shift [8]. Although not conclusive, the body of evidence from studies of dietary protein and perceived hunger and satiety suggests that higher-protein meals have the potential to suppress hunger to a greater degree and result in enhanced sensations of satiety [12].

Satiety-inducing effect of protein is regarded as multifactorial and nutrient-specific, but the mechanism is hypothesized to consist mainly of synchronization with elevated amino acid concentrations. Several studies have shown some evidence that a relatively high increase in concentrations of anorexigenic hormones (Glucagon-like peptide-1, cholecystokinin, PYY) was observed after high protein preload [8].

The present study aims to compare short-term satiety profile of three nutritional formulas developed which differ in protein contributions to energy in obese subjects. Specifically, this study will investigate whether higher protein formula produces higher subjective satiety compared to lower protein formula, resulting in lower energy intake in subsequent meal.

II. Subjects and Methods

A. Subjects

Four Indonesian female volunteers (BMI 25-33 kg/m^2; age 27-35 years) were recruited. They were selected on being in good health, having stable weight (no more than 4 kg change in weight) in the last 6 months, and not breastfeeding or being pregnant, not in a restricted diet or consuming supplements or drugs to lose weight, not allergic to milk products and other foods provided in this study and having the same level of activity (being sedentary, working in front of computer all day). They underwent a screening including measurement of body composition and medical history. A written informed consent was obtained from these participants.

We chose obese person as participant because we regarded that the result of the study will be most beneficial to this group of people.

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TABLE I: PARTICIPANTS CHARACTERISTICS
(MEAN VALUES AND STANDARD DEVIATIONS FOR FOUR SUBJECTS)

	Mean ± SD
Body mass index (BMI) (kg/m^2)	28.1 ± 3.22
Age (years)	31.5 ± 3.42
Percentage body fat (PBF) (%)	43.2 ± 4.97
Basal metabolism rate (BMR) (Kcal)	1170 ± 81.93
Skeletal muscle mass (SMM) (kg)	20.0 ± 2.22
Body weight (kg)	65.4 ± 5.24

B. Design

A randomized, single-blind, crossover study was performed. All subjects came to the center on 3 occasions, separated by at least 3 days. On each test day, upon coming to the center, subjects filled the appetite rating and received breakfast. Four hours after breakfast, subjects were offered lunch. Within this period, appetite ratings were obtained.

1) Breakfast

Test sample was offered as breakfast, which is chocolate-flavoured milk beverage differed in macronutrient composition (Table II). The test samples were prepared by diluting the formula into 200 ml warm water. The protein content of the formula consisted of casein and whey. The breakfast contained 200 Kcal each serving. BMR was provided by InBody (InBody Co Ltd, Korea) body composition measurement.

2) Lunch

Lunch consisted of white rice (180 kcal/100 g), *cap cay* (sautéed mixed vegetables) (97 kcal/100 g), roasted chicken fillet (298 kcal/100 g), tofu (80 kcal/100 g), and boiled eggs (77 kcal/100 g).

TABLE II: MACRONUTRIENT CONTRIBUTIONS ON ENERGY CONTENT OF EACH TEST SAMPLE

	Balanced protein formula (BPF)	*Moderate protein formula (MPF)*	*High protein formula (HPF)*
Protein	12.4%	23.5%	40.6%
Whey: Casein ratio	20:80	46:54	74:26
Carbohydrate	68.2%	56.9%	40.2%
Fat	22.6%	22.7%	22.4%
Energy density (kJ/gram)	3.54	3.59	3.56

3) Study Protocol

The day before the test, subjects were instructed to fast from 22.00 h, and on the test day, subjects came to the centre at 08.00 h. The test started at 08.00 h with scoring appetite ratings. Breakfast was offered (t=0 min) and completed within 10 minutes. Appetite ratings were completed at 0 (before breakfast), 15, 30, 60, 90, 120, 180, and 240 minutes. Hedonic rating is rated after drinking the test sample. After completing the questionnaire at 240 minutes, subjects were offered an *ad libitum* lunch and were instructed to eat just as much until they were comfortably satiated. Subjects were allowed to drink maximally 500 ml of water spread over the entire test period. Subjects were instructed to maintain the same pattern for diet and exercise during the entire study period and confirmed by food diary. The timeline of this study can be seen in Fig. 1.

4) Measurements

Appetite profile To determine appetite profile, 100 mm visual analogue scales (VAS) anchored with describing the extremes (that is, `I have never been more hungry'/`I am not hungry at all') were used during the test day. Subjective appetite ratings measured were hunger, fullness, desire to eat, satiety, appetite, and thoughts of food. Subjects were instructed to rate the appetite dimensions by marking the scale at the point that was most appropriate to their feeling at that time. Visual analogue scales are valid and reproducible tools widely used to measure subjective appetite [13].

Energy intake at lunch To explore the effect of different protein content to subsequent energy intake, subjects were provided with lunch. Lunch was a buffet-style meal, presented 4 hours after taking breakfast, that allowed subjects *ad libitum* self-selection of a variety of foods. The food presented in the lunch had varying amounts of fat, carbohydrate, and protein to allow subjects to vary not only energy intake but also proportions of macronutrients. All foods served were weighed before being eaten by the subjects and reweighed after subjects finished eating to obtain the net amount consumed (in grams) of each food. Energy intakes were calculated by using nutritional information for the foods obtained from Indonesian food composition table (*Tabel Komposisi Pangan Indonesia*).

Hedonic ratings Hedonic aspect of the three test samples were rated using 7-point hedonic scale from 'like very much' to 'dislike very much'.

5) Statistical analysis

Data are presented as mean changes from baseline standard error to the mean (SEM), unless otherwise indicated. Differences between samples are tested for significance by using ANOVA with post hoc Tukey test. A p-value <0.05 was regarded as statistically significant. Statistical procedures were performed using SPSS version 13.0 (SPSS, Inc., Chicago IL).

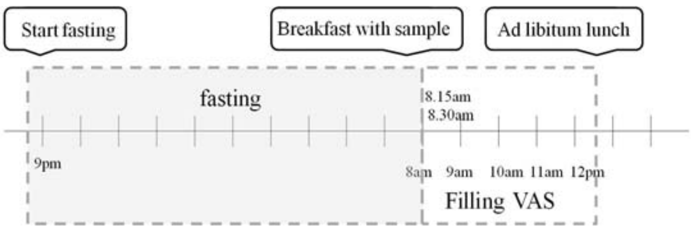

Fig. 1. Study timeline

III. Results

A. Subjects

Characteristics of subjects are displayed at Table I.

B. Satiety

Effect of the test samples on subjective ratings of appetite was assessed by analyzing the ratings collected before lunch meal was served. There were no significant differences in baseline among the test samples.

From six appetite ratings assessed, it was observed that only one parameter showed statistical significance. Desire to eat after consumption of test sample (t 15min) rated by subjective VAS scores was statistically significant (p<0.05). At 120 min, ratings for hunger, prospective consumption, appetite and thoughts of food of balanced protein formula (BPF) were higher than moderate (MPF) and high protein formula (HPF), although, by statistic, this difference was not significant. It was also observed that fullness rating of BPF was lower than MPF and HPF, but also not significant (P=0.38). Overall, BPF showed lower subjective satiety ratings than MPF and HPF, while HPF has the highest subjective satiety ratings for all the parameters.

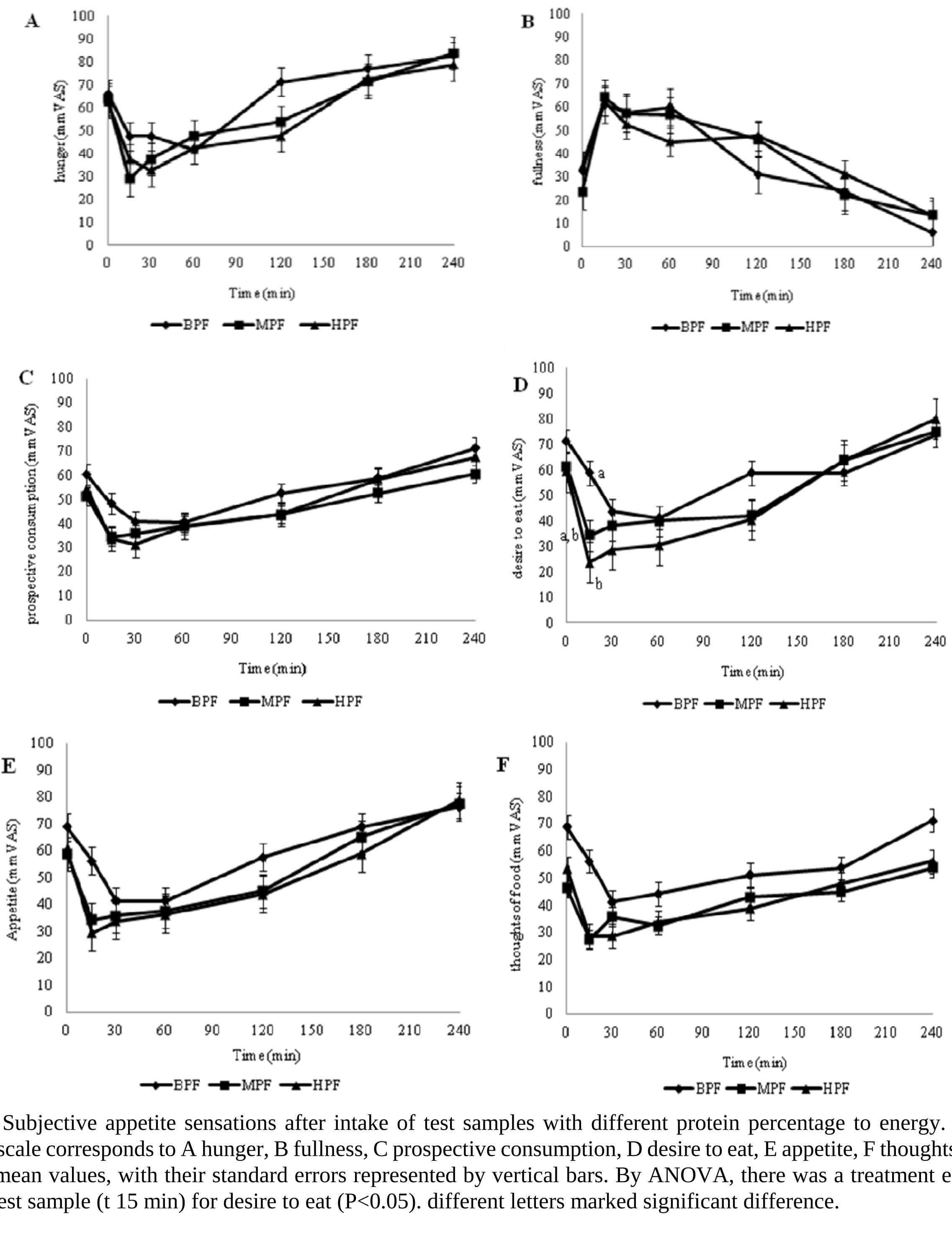

Fig. 2. Subjective appetite sensations after intake of test samples with different protein percentage to energy. A visual analogue scale corresponds to A hunger, B fullness, C prospective consumption, D desire to eat, E appetite, F thoughts of food. Data are mean values, with their standard errors represented by vertical bars. By ANOVA, there was a treatment effect just after the test sample (t 15 min) for desire to eat (P<0.05). different letters marked significant difference.

C. Energy Intake at Lunch

Subjects' energy intake after moderate protein formula (MPF) was lower than after consuming balanced protein formula (BPF) and high protein formula (HPF). Energy intake at lunch did not differ significantly (p=0.31) when compared among the three formulas with different protein percentage to energy.

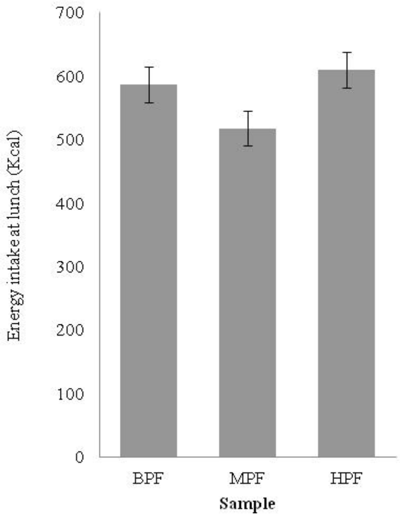

Fig. 3. Energy intake at an ad libitum lunch 240min after consumption of test sample. Mean energy intakes at lunch were 587 Kcal after the balanced protein formula, 517 Kcal after moderate protein formula and 610 Kcal after high protein formula. There was no significant difference in energy intake at lunch (p=0.31)

D. Hedonic Ratings

There was no significant difference between samples in hedonic ratings rated by the subjects.

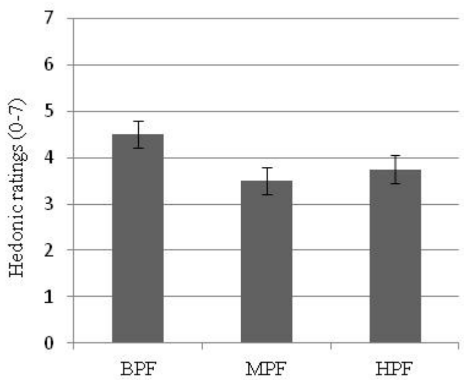

Fig. 4. Hedonic evaluation of each test sample (p=0.28). values represent means with their standard errors.

IV. Discussions

The World Health Organization recommends that dietary protein should account for 10–15% of energy when in energy balance and weight stable [1]. Given the range of the 'normal' protein intake, meals with on average 20% to 30% of energy from protein are representative for high protein meals, when consumed in energy balance [6].

Protein-induced satiety has been shown acutely, with single meals, with contents of 25% to 81% of energy from protein in general or from specific proteins, while subsequent energy intake reduction was significant [8]. While on a review by Halton and Hu, eight of 15 studies showed that consuming foods high in protein, at least 30% of total calories will cause the subjects to consume fewer calories at the next meal intake [14].

Three samples were developed to be high protein formula, moderate protein formula and balanced protein formula, having different contributions to energy as shown in Table 2. With the high protein formula having 40% protein contribution to energy, therefore we hypothesized that the high protein formula (HPF) will give a higher satiety compared to lower protein formulas.

It was observed that the subjective satiety ratings for balanced protein formula (12.4% E) were consistently lower than moderate and high protein formula, although this difference was later proved to be not statistically significant for parameters of hunger, fullness, appetite, prospective consumption, and thoughts of food.

It was also shown that the effect of protein formula as preload on appetite ratings has occurred as early as 15 minutes after consumption of the preload drink. This finding was also observed by a study by Chungchunlam *et al* [15].

Subjects were shown to respond in higher subjective satiety ratings to moderate (23.5%E) and high protein formula (40.6%E) than to balanced protein formula, although this difference was later proven to be not statistically significant in most satiety ratings: hunger, fullness, appetite, prospective consumption and thoughts of food. However, at t=15 minutes, desire to eat as response after high protein formula was significantly lower than balanced protein formula ($P<0.05$). This result might suggest that balanced protein might not be enough to give satiety to obese subjects, whereas high protein formula showed the potential to reduce desire to eat in obese people, who have been suggested to experienced altered regulation of satiety [16]. Meanwhile, there was no significant difference between balanced protein formula and moderate protein formula. The result of this study showed the trend that the higher protein content of a food, a higher satiety was also observed. Although this was only observed in one parameter, this result supported the notion that high protein food may produce higher satiety [10], [14], [17], [18].

The weight of evidence suggests that higher protein intakes cause a decreased subsequent energy intake although the results are not entirely consistent [14]. In this study, however, the tendency to show a higher satiety for high protein intake was not shown in *ad libitum* lunch. It was observed that energy intake was highest after high protein formula, and the lowest after moderate protein formula, although this difference was not statistically significant. It was shown by Veldhorst *et al* [17] that differences in appetite ratings need to be at least larger than 15 mm VAS in order to have a significant effect on subsequent energy intake. In this study, although high protein formula was rated as more satiating than balanced protein formula, the difference was not large enough to be translated as a reduction in energy intake.

Studies on satiety and protein itself do not always show consistent result between the subjective satiety ratings and *ad libitum* energy intake. Specifically, as also shown by Chungchunlam *et al*, one of methodological issues explaining inconsistent findings on satiating effects of protein is the time interval between preload and test meal. Based on the study, a time delay of 120 min would provide a more sensitive test for satiety than other times tested [15]. Differences in timing may explain different results; as shown by Anderson *et al*, timing is essential in studying *ad libitum* energy intake after a preload or a meal [18]. It is also suggested by another study that one may use the satiating power of a high protein meal optimally when timing of the meal interval synchronizes with timing of the amino acid profiles [19].

There is some suggestion that different protein will differentially affect satiety. Thus, difference in whey:casein ratio of the test sample may also play a role. Several studies have highlighted the potential of whey having higher satiating capacities than casein [20].

The digestion and absorption of whey and casein differ in that casein, unlike whey, coagulates in the stomach due to its precipitation by gastric acid. As a result, overall gastric emptying time for casein appears to be longer and a smaller postprandial increase in plasma amino acids was observed compared with the non-coagulating whey protein [7].

Although the high protein formula (with the highest whey:casein ratio) showed the tendency to have the highest satiating capacity, however, the result at *ad libitum* lunch did not support that notion. With regard to the result of this study, it is of interest to further explore the effect of different whey:casein ratio in nutritional high protein formula to satiety ratings and/or satiety hormones and energy intake and also the timing effect.

There are several limitations of this pilot study. First is the small numbers of subjects, thus there is a possibility that this study may not have sufficient power to detect small difference in satiety ratings, and, for the second limitation, there was no adjustment in calorie of the samples into individual needs (subject-specific).

Assessing independent effects of macronutrient manipulations is difficult while trying to control all the factors having been reported to affect satiety, such as energy content, weight, volume, energy density, sensory and hedonic properties of a food or drink. Therefore to minimize the effects of energy, volume, energy density of protein-containing test sampel, we match test sample for energy, volume, and sensory as also done by Astbury *et al* [21]. Furthermore, to ensure all test samples had similar energy densities, we replaced carbohydrate with protein. Exchanging the increasing protein with carbohydrate meant that an increase in the amount of protein in the preload was accompanied by a reduction in carbohydrate. Thus, we cannot rule out the resulting difference in carbohydrate content may have contributed to our findings. Related to the hedonic aspect, it was also shown that our subjects rated three test samples having no significant difference in hedonic ratings (see Fig.4), therefore, we concluded that hedonic aspect did not influence our observed findings.

In conclusion, high protein formula has been shown to be able to reduce desire to eat in obese subjects compared to moderate and balanced protein formula, but not significantly in other satiety parameters. Overall, high protein formula showed the highest satiety ratings, but in this pilot study the difference was not large enough to induce a reduction in energy intake.

Studies investigating satiety were often conducted in non-Asian, but less in Asian subjects, therefore, this study may contribute to existing literature on protein and satiety, especially in Asian subjects. This study has promising result to be continued into a larger study, involving more subjects and more specific satiety parameters, such as satiety hormones.

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