The effect of high protein milk supplementation on satiety in normal weight subjects

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1. Introduction

Nowadays, people are living in the modern era, where the improvement of technology is getting massive and hastens humans to fulfil their needs. One of the facilities offered is the ease of obtaining food. People need only a small effort using mobile technology to order the food they want, then the food will be delivered to them. However, this phenomenon leads to a higher complexity of health problems, such as increased overweight and obesity (Purnamasari, 2018).

Obesity is a condition when adipose tissue or fat is accumulated excessively. Even though there is no specific cut-off for a fat percentage to be classified as obese, there are some parameters such as body mass index and waist circumference (central obesity) (Purnell 2018). This accumulation occurred because calorie intake is higher than expenditure which causes imbalance (Jih et al., 2014). A sedentary lifestyle and poor consumption behaviour become the most affecting factor in causing obesity (González-Muniesa et al., 2017). Especially with all convenience that the world offers nowadays, those two factors, without a doubt are quite possible to be reached in no time. While humans are carried away by the euphoria of being comfortable, silent threats always lurk. This condition makes even healthy people become more prone to excessive calorie intake if they do not take full responsibility to improve their lifestyle.

Obesity emerges as a root cause of other non-communicable diseases and for suffering further chronic complication disease (Purnamasari, 2018). Based on the updated data related to obesity by Basic Health Research, the prevalence of obesity in adults in Indonesia is increasing from 10.5% (2007) to 14.8% (2013) and reaching up to 21.8% in 2018 (Kementerian Kesehatan Republik Indonesia, 2018).

To date, there are many efforts done to prevent calorie excess, such as increasing energy expenditure and limiting food intake. One important factor in limiting food intake is to increase satiety. Thus, consuming suitable food to increase satiety becomes crucial. Protein is a macronutrient whose satiating property has been widely studied. Several studies showed that high protein consumption; in the form of skim milk, high-protein milk, and a high-protein meal had contributed to the increase in satiety followed by decreasing in energy intake (Harper et al., 2007; Dove et al., 2009; Lorenzen...
However, these researches employed overweight and obese subjects, and none has been found on healthy adults with normal body mass index (BMI 18.5 – 22.9 kg/m², Asia-Pacific region) (WHO, 2000). The satiety response between the normal and obese groups was different. Meyer-Gerspach et al. (2014) observed that obese people tend to have delayed gastric emptying which leads to decreased secretion of the satiation peptides, GLP-1 and PYY. They tend to reach maximal satiation in a shorter time than normal weight but consumed more calories to get full. Tsani et al. (2021) also mentioned that obese and normal-weight people have different satiety profile due to some reasons. Normal-weight people have a lower score in fullness right after eating yet a higher score in the next 3 hours compared with obese people. This could occur because of different levels of leptin between obese and normal people. Normal people have higher leptin so that they could preserve longer fullness than obese people.

Therefore, this study aimed to evaluate the satiating effect of high protein milk on healthy adults with normal BMI and whether it will give the same beneficial effect as overweight or obese subjects.

2. Materials and methods

2.1 Subjects

Male subjects were recruited for this study at PT. Nutrifood Indonesia (Jakarta, Indonesia). The inclusion criteria were: male age 19-40 years, normal weight (BMI 18.5-22.9 kg/m²), not being on a restricted diet and not having an allergy to dairy products, had been weight-stable (no more than 6% change in weight in the last 3 months), and regularly have breakfast.

Subjects completed the International Physical Activity Questionnaire (IPAQ) before the test. Weight, body composition, and basal metabolic rate (BMR) (InBody720, Biospace, Korea) were measured. IPAQ was used to measure the activity level of the subject. Daily energy requirements (DER) were assumed to be equal to total daily energy expenditure (as subjects reported to be weight stable) (Astbury et al., 2010). DER was calculated by multiplying measured BMR by a physical activity level, which was estimated using the IPAQ (Craig et al., 2003). The purpose was to calculate the calorie needed by each subject for breakfast.

All subjects gave their written consent after the experimental procedure had been explained to them. The study was approved by the Institute of Research and the Community Service Atma Jaya Catholic University of Indonesia with register number 0908/III/LPPM-PM.10.05/07/2019.

2.2 Study design

A single-blind, controlled, cross-over trial was performed. All subjects were involved on two separate occasions with a washed-out period of 7 days between each study. Subjects were advised to refrain from doing vigorous exercise for 24 hours before the trial. They were instructed not to consume any other foods or drinks (except a maximum of 200 mL water) after 10.30 pm on the day before the trial until 8.00 am on the next morning.

Subjects were instructed to arrive at the place of the experiment at 8.00 am. On arrival, subjects were seated in a room for 15 mins in order to adapt to the room condition. At 08.15 am (t = 0 min), the subjects’ appetite profile was assessed using a visual analogue scale (VAS) questionnaire. After the assessment, subjects were given a standardized breakfast with preloads (high protein milk (HPM) or standard protein milk (SPM)) at approximately 08.30 am, which they had 15 mins to consume. Appetite ratings were repeated 15 mins after breakfast (08.45 am) and then keep recorded every 30 mins. At 09.45 am (t = 90 mins) and 11.15 am (t = 180 mins), subjects were provided with 100 mL of water before they recorded the appetite rating. At approximately 12.15 pm (t = 240 mins), Subjects were then provided with an ad libitum meal to consume. They were instructed to eat as much as they wished of the meal, within 20 mins, until they felt comfortably full. Subjects completed the appetite VAS questionnaire immediately.

2.2.1 Breakfast

The standardized breakfast was a strawberry sandwich, which consisted of double soft white bread (Sari Roti, Indonesia) and strawberry spread (Morin Brand, Indonesia) as well as preloads (HPM or SPM). This meal was made equivalent to 20% of individual DER and the proportion of energy from protein, fat, and carbohydrate was 18, 18, and 64% (from the breakfast’s total energy), respectively with HPM preloads and 11, 18, and 71%, respectively with SPM preload.

2.2.2 Preloads

Preloads were two-fixed energy, chocolate-flavoured milk beverages. Preloads were two iso-energetic 120 kcal milk beverages containing 12 g protein for HPM preloads and 4 g protein for SPM preloads (Table 1).

2.2.3 Lunch

The food which was provided in ad libitum lunch consisted of fried rice with a fried egg. Subjects were initially provided with 200 g of fried rice and 50 g of...
fried egg. A new portion of food (100 g of fried rice and 25 g of fried egg) was offered after the dish became empty and subjects chose to eat another portion. This process was repeated until the subject indicated that they wished to terminate the meal. Each portion (200 g of fried rice and 50 g of fried egg) consisted of 16% energy from protein, 26% from fat, and 58% from carbohydrates.

2.2.4 Appetite and taste ratings

Visual analogue scale (VAS), 10 cm in length with words anchored at each end, expressing the most positive and the most negative rating, was used to assess hunger, satiety, and desire to eat (Figure 1). Subjects did not discuss or compare their ratings with each other (Flint et al., 2000).

3. Results and discussion

3.1 Subjects

Seventeen healthy adult men (age 27±5 years; BMI 21.4±1.13 kg/m²) completed this study and there was no dropout. To detect a 1.6 mm difference in VAS, with an 80% power and SD of 1.42 mm based on a similar previous study by Pertwi et al. (2012), a minimum of 15 participants were required for the study. Table 2 displays subject characteristics.

3.2 Appetite profile

The appetite ratings during the experiment were assessed to evaluate the effect of high-protein milk on subjective appetite ratings. Before the breakfast (t = 0), the parameters for fullness, hunger, and desire to eat between HPM and SPM treatments were not significantly different (Table 3).

After breakfast, for all treatments, the VAS score for fullness decreased while hunger and desire to eat increased (Figure 2). There were significant differences between time for fullness (F (4.166,66.665) = 40.781, P < 0.001), hunger (F (4.321,69.143) = 37.273, P < 0.001), and desire to eat (F (3.854,61.657) = 32.177, P < 0.001). Subsequently, there were significant differences between treatment for fullness (F (1,16) = 5.578, P = 0.031) and hunger (F (1,16) = 4.811, P = 0.043) while there was an increase in desire to eat in SPM compared with HPM (even though statistically it did not significant) as well as no significant differences in interaction between time and treatment for all parameters. Because significant differences between treatment in fullness and hunger parameters were achieved, a two-tailed paired t-test was conducted. For the fullness parameter, the time when the differences were significant were at t = 60 (P = 0.044), 120 (P = 0.046), 150 (P = 0.028), and 240 (P = 0.011) while for the hunger parameter at t = 150 (P = 0.030) and 240 (P = 0.022). For the AUC result, there were significant differences for fullness (F (1,16) = 6.016, P = 0.026) and hunger (F (1,16) = 5.579, P = 0.031). However, there were no significant differences in the
desire to eat although SPM gave a higher score than HPM.

3.3 Energy intake at test meal

Despite significant differences in fullness and hunger between high protein and standard preloads, no significant differences were observed in energy intake during *ad libitum* lunch for the two groups (Figure 3).

4. Discussion

When it came to evaluating the satiating effect of protein, previous researches mostly focus on overweight or obese subjects. As far as we know, this is the first
study that investigates the effect of high-protein milk on satiety in normal BMI subjects, especially for the Asia-Pacific Region. Besides, most studies used a high amount of protein (>20% protein) (Dhillon et al., 2016). This study investigated whether a smaller amount of protein, near the bottom line in the high protein meal category (15%) (Foltz et al., 2010), would be sufficient to give satiating effect to the subjects.

In this study, two controlled, fixed-energy milk beverages were given as preload. These two beverages contained the same energy content, with different ratios between protein and carbohydrate. HPM contained 12 g protein (40.5% energy from preloaded drink), around two times higher protein compared with cow milk (Pereira 2014). The other milk, SPM only contained 4 g of protein (13.5% energy from the preloaded drink). All iso-caloric preloads were consumed together with standardized breakfast resulting in 18.4% energy from the protein in the treatment final meal and 11.2% in the control final meal. This followed the criteria for treatment to be considered as high protein meal compared with control meal (meals and diets with an average >15% of energy from protein are representative of high protein meals and diets) (Foltz et al., 2010).

In order to achieve valid data from the VAS score for measurement of appetite ratings and to show the detectable difference of appetite scores, there is a minimum subject needed to have participated. There were 17 subjects in this present study. This number was sufficient to cover a test of an effect size of 10% with a study power of 0.9 of the appetite ratings in a paired design (Flint et al., 2000).

Satiety is the feeling of fullness that persists after eating, potentially suppressing further energy intake until hunger returns, which could not be recorded by one kind of rating alone. Usually, several parameters are used to see this feeling, such as fullness, hunger, and desire to eat (Benelam, 2009). This study showed high protein milk gave a better satiating effect significantly, especially for fullness and hunger ratings. The appetite rating on the desire to eat for SPM was higher than HPM which indicated that on this parameter, HPM also gave a better satiating effect even though statistically insignificant. These results confirmed our hypothesis about the satiating effect of protein. Protein has been known for its ability to prolong satiety compared with other macronutrients. Some studies also showed that a higher intake of protein (above 15%) gave a stronger effect on satiety than equivalent quantities of energy from carbohydrates or fat on overweight and obese subjects (Benelam, 2009). In our study, this satiating effect was also observed in normal-weight subjects. This finding was in line with a study involving normal BMI subjects (even though the BMI cut-off was different from our study; the cut-off for overweight was 25) conducted by Fernández-Raudales et al. (2018). This study conclude that higher protein intake (50% energy of preload from plant-based protein) also showed a higher satiating effect.

There are several possible mechanisms. First, protein might influence the satiety hormone. A number of hormones are produced from the gut to indicate that food has been consumed, which gives signals directly or indirectly to the brain, and promotes satiety. High protein consumption increases glucagon-like peptide-1 (GLP-1), cholecystokinin, and PYY which cause an increase in satiety as well as a decrease in ghrelin hormone, which is responsible for decreasing hunger (Veldhorst, Smeets, Soenen et al., 2008; Morell and Fiszman, 2017). A study carried out by Ghazzawi and Mustafa in 2019 confirmed this mechanism, even though it was conducted on overweight subjects. The finding showed that PYY levels were significantly higher when subjects were given high protein breakfast (51% protein, 13% CHO, 36% fat) compared to a high carbohydrate breakfast (0% protein, 60%CHO, 30% fat) as well as the satiety VAS score. Second, higher protein intake can increase body temperature and oxygen consumption. This increase may lead to feeling deprived of oxygen and thus promote satiety. Not only increase energy expenditure, but protein also decreases energy intake through mechanisms that influence appetite control (Benelam, 2009; Morell and Fiszman, 2017).

Third, there is a hypothesis saying that the brain has a satiety centre. This centre is sensitive to serum amino acid levels, and once reaching a certain point, hunger would decrease. Fourth, is gluconeogenesis. The satiating effect could be related to the improvement of glucose homeostasis through the modulation of hepatic gluconeogenesis and subsequent glucose metabolism (Veldhorst, Smeets, Soenen et al., 2008). Fifth, is the brain reward system. The central mesolimbic reward system generates a sensation of pleasure and promotes the motivation for food consumption through its activation. In a study, a high-protein diet could reduce reward-driven eating behaviour through the activation of specific brain regions in the corticolimbic system, which decreases the hunger sensation (Cuenca-Sánchez et al., 2015).

Whilst appetite rating showed significant improvement, the tendency to show higher satiety for high protein intake was not shown in ad libitum lunch. Veldhorst, Nieuwenhuizen, Hochstenbach-Waelen et al. (2008) in their research concluded 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, even though the differences between treatments for appetite ratings were statistically significant, the average score was lower than 15 mm for all locations, which means the difference was not large enough to be translated as a reduction in energy intake. The other possibility was the ad libitum lunch implementation. In this study, the subjects were asked to eat more subjectively by raising their hand and it occurred when their dishes were empty. This condition can make them terminate their meal even though they are still not comfortable full because people tend to finish the meal and would not leave leftovers. The researcher should add a new portion of food before the dish became empty and the subject continued to eat. This ensured the cue of an empty dish did not prompt meal termination (Marriott, 1995; Astbury et al., 2010).

A limitation of this study is we did not do further confirmation from the VAS score by measuring hormones level. VAS score is a subjective parameter that is affected by many factors, such as psychological issues in individuals to choose on which scale, they will measure their appetite ratings (tendency to avoid extreme score, etc.) (Benelam, 2009). The other limitation was that we did not control H-1 dinner to homogenize the baseline level of satiety in the subjects. It could affect the variation of baseline level in the morning before subjects started the experiment, such as the second meal effect (Ibrügger et al., 2014).

In conclusion, our study found that high protein milk supplementation has been shown to be able to increase fullness and reduce hunger ratings in healthy adults with normal body mass index (BMI 18.5 – 22.9 kg/m², Asia-Pacific region). However, this difference in satiety level was not sufficient to reduce energy intake during the subsequent meal. This finding could trigger deeper research possibilities in the satiating effect of protein on healthy normal BMI subjects (Asia-Pacific cut-off).

Conflict of interest

The authors are employees of PT Nutrifood Indonesia.

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