Fermented soybean enhances post-meal response in appetite-regulating hormones among Indonesian girls with obesity

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Fermented soybean enhances post-meal response in appetite-regulating hormones among Indonesian girls with obesity

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ABSTRACT

Objective: To assess the post-meal response in appetite-regulating hormones acyl-ghrelin and insulin after fermented soybean (*tempeh*) consumption in girls with obesity.
Methods: A randomized counter-balanced crossover study was conducted using a breakfast (307 kcal, protein: 28%, fat: 23%, and carbohydrate: 55%) containing fermented soybean or isocaloric non-fermented soybean among 13 females (aged 18–20 y; BMI 25–30) after an overnight fast. The outcome variables were plasma acyl-ghrelin, insulin, arginine and score of the visual analog scale (VAS) appetite questionnaire.
Results: While no change was observed after the non-fermented soybean meal, plasma acyl-ghrelin decreased by 35% at 30 min and remained below baseline until 120 min after the fermented soybean meal ($P < 0.05$). Plasma insulin increased after consumption of both meals and fermented soybean meal-induced 30% greater response in insulin at 120 min than non-fermented soybean meal ($P < 0.05$). Circulating arginine levels were slightly greater (24%) at 120 min after the fermented soybean meal than the non-fermented soybean meal ($P < 0.05$). No difference in subjective appetite was observed between the fermented soybean meal and the non-fermented soybean meal.
Conclusions: Fermented soybean meal induced greater response in appetite-regulating hormones compared with non-fermented soybean meal. No difference in post-meal satiety feeling between fermented and non-fermented soybean meal suggests poor sensitivity of the brain to the appetite-regulating hormones among girls with obesity.

Introduction

Ghrelin and insulin are primary hormonal regulators of appetite, which modulate hunger and satiety feeling in the brain after a meal [1–4]. Ghrelin, known as a "hunger hormone", is produced by X/A-like cells within gastric oxyntic glands in the stomach. Acyl-ghrelin (AG) is a bioactive form of ghrelin, which stimulates appetite in the brain via circulation [5–7]. Circulating AG increases during fasting and decreases after a meal [8,9]. In obese children, pre-meal total ghrelin levels were inversely associated to insulin levels and the severity of insulin resistance [10]. Insulin releases from beta-cells of pancreas after meal is known to inhibit appetite [11]. However, AG levels are usually lower and insulin levels are usually higher in people with obesity compared with lean people [12,13]. This observation implicates a link between an individual variation of brain sensitivity to these appetite regulating hormones and the development of obesity.

Protein is known to have a relatively stronger satiety effect compared to other macronutrients [14,15]. Therefore, high protein meals have been widely adopted as a dietary strategy for fat loss [16,17]. Fermented soybean, known as *tempeh*, is a widely consumed protein source in the daily life of Indonesia. This dietary protein source is fermented using fungus *Rhizopus oligosporus* to improve the digestibility of soybeans [18]. The effects of fermented soybean versus non-fermented soybean on the satiety-associated appetite regulator in girls who developed obesity have not been reported in the past. In this study, we hypothesized an enhanced post-meal response in the appetite-regulating hormones AG and insulin following consumption of fermented soybean. Furthermore, we examined whether the post-meal changes in the appetite-regulating

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Table 1
Nutritional content of non-fermented and fermented meals (Energy: 307 kcal; Protein: 22 g; Fat: 8 g; and Carbohydrate: 42 g, Fiber: 4.5 g).

Ingredients	Non-fermented	Fermented
Fermented soybean, g	–	100
Non-fermented soybean, g	100	–
Potato, g	50	50
Carrot, g	50	50
Green peas, g	50	50

hormones are consistent with the subjective hunger feeling among Indonesian girls with obesity.

Materials and methods

Study design

A 2-way, crossover, randomized, double-blind controlled trial with a 1-week washout period for each meal test was conducted. The isocaloric meal used in the study consisted of a fermented soybean steak (*tempeh*) or non-fermented soybean steak. The dietary composition of both meals are shown in Table 1. For the first trial, half of the participants consumed the non-fermented soybean meal, and the other halves consumed the fermented soybean meal as a breakfast in the laboratory at 06:30 am. For the crossover trial, participants switched to the alternative meal one week after the first trial. A visual analog scale (VAS) questionnaire was used to rate participants' appetite sensation [19] and blood sampling was collected each testing day. The flow chart study is shown in Fig. 1.

Participants

Thirteen Indonesian girls (aged 18–20 y) with obesity (BMI ranged 25–30 kg/m^2) has completely participated in the study (Table 2). A minimum sample size of 13 participants was required to achieve a power of 0.7 with a probability of error < 0.05 [20]. All participants were weight stable (within ±3 kg) 2 months before study recruitment. They were non-smokers and had no daily prescribed medication or special nutritional supplementation. The exclusion criteria are participants with history of chronic diseases (such as cardiovascular disease, diabetes, dyslipidemia, or hypertension) and users of weight-related medication/food, heavy alcohol/drug consumption, and those with unintentional weight loss $>$ 10% initial body weight 2 months before interventions. None of the participants had thyroid diseases and depressive disorders. All participants assessed height and body weight before the study started. All participants received the information of the benefit, risks of the treatments, and then signed a written consent form.

Randomization

Participants were randomly assigned following simple randomization procedures (computerized random numbers) to 1 of 2 treatment groups. The allocation of participants into either group was performed

Table 2
Baseline characteristics of participants.

Variable	N = 13
Female/male	13/0
Age, y	19.2 ± 0.6
Body weight, kg	72.2 ± 7.7
BMI, kg/m^2	29.3 ± 2.5
Waist circumference, cm	83.5 ± 3.9
Acyl-ghrelin, pg/mL	43.8 ± 5.6
Insulin, μIU/mL	4.8 ± 1.1
Arginine, ng/mL	22.9 ± 4.0

Dara is expressed as mean ± SE.
BMI = Body Mass Index.

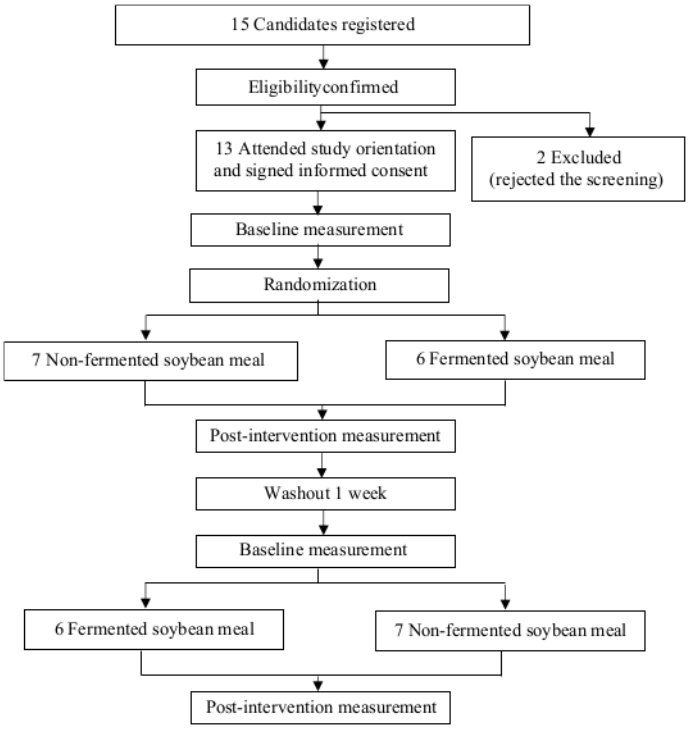

Fig. 1. Flow diagram of participants through study.

blindly by a research coordinator. During the intervention of supplement, both participants and investigator were kept blind. The intervention was delivered by a research coordinator.

Anthropometric measurements

Anthropometric variables were assessed by an experienced research coordinator at baseline and the end of the study. A medical stadiometer with an accuracy to 0.1 cm (Quick Medical, Issaquah, WA) was used to measure height. Weight, fat, and lean mass were assessed on a self-calibrating bioelectric impedance analyzer (Tanita DC 360, Japan). BMI was calculated as body weight in kilograms divided by height in meters squared (kg/m^2). Waist Circumference (WC) was measured with the inelastic measuring tape (WIN Tape 205 cm; accurate to 0.1 cm) around the mid-section between the last rib and the iliac crest.

Blood samples

Forearm venous blood concentrations for AG, insulin, and arginine were determined from samples collected at fasting, 30 min, and 120 min. The specimen placed in the tubes containing EDTA were centrifuged at 3000 *g* for 10 min at 4 °C, and was stored at −80 °C until analysis. Plasma concentrations of AG, insulin, and arginine were measured by an enzyme-linked immunosorbent assay (ELISA) using the human ELISA kit (Elabscience, EL-H2002; H2665, EL-0042, Houston, Texas, USA). The sensitivity for the AG KIT was 9.38 ng/mL, and the intra-assay coefficient of variation (CV) was 5.1–6.6%. For insulin, the sensitivity of the test was 0.4 μIU/mL, and the intra-assay CV 4.9–5.4%. For arginine, the sensitivity of the test was 9.38 ng/mL, and the intra-assay CV was 5.2–6.9%.

Subjective satiety

VAS motivation score to intake food was measured with a 10 cm line. Participants completed VAS to measure hunger/fullness at the same time points of blood samples collection during the study protocol (30 and 120 min). The score of VAS questionnaire presents subjective appetite-related sensations [21], and was used to correlate plasma hormone markers and dietary intake data. On each of the test days, participants were asked to rate the following components relating to hunger and satiety using various questions, which included: (1) hunger; (2) desire to eat; (3) fullness; (4) motivation to eat. The overall composite score of appetite is calculated using the following formula:

$$[\text{Satiety} + \text{hunger} + (100 - \text{fulness}) - (100 - \text{prospective food consumption})]/4$$

[22].

Statistical analysis

Two-way ANOVA with repeated measure was used to analyze the differences in mean values for the main effect and interactive effect of meal and time. A level of $P < 0.05$ was set for significance for all tests, and values are expressed as means ± SE. SPSS software for IBM 27.0 version was used for statistical analysis.

Results

Participants' characteristics

Of the 15 potential participants, 2 participants were excluded due to rejection of the screening assessment (Fig. 1). Thirteen girls with obesity participated in this study were randomly assigned to fermented soybean meal (n = 6) and non-fermented soybean meal (n = 7). Baseline characteristics of the participants (aged 18–20 y) are summarized in Table 2. All participants showed apparent abdominal obesity with waist

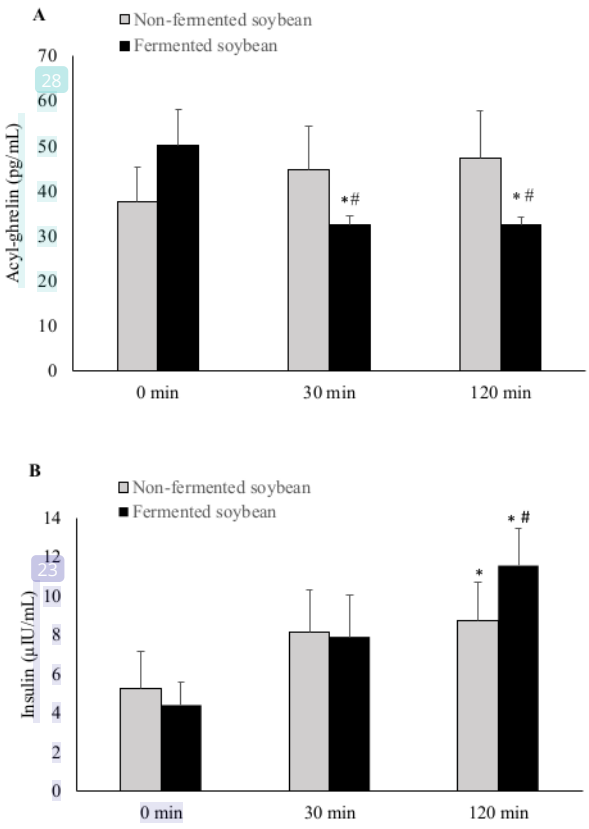

Fig. 2. Appetite-regulating hormone response to fermented and non-fermented soybean meal consumption. Acyl-ghrelin (A) decreased, and insulin (B) increased 120 min after fermented soybean meal. Fermented soybean meal lowered plasma acyl-ghrelin and produced higher plasma insulin response at 120 min compared with non-fermented soybean meal. * Significant difference against 0 min, $P < 0.05$; # Significant interactive effect between meal and time, $P < 0.05$.

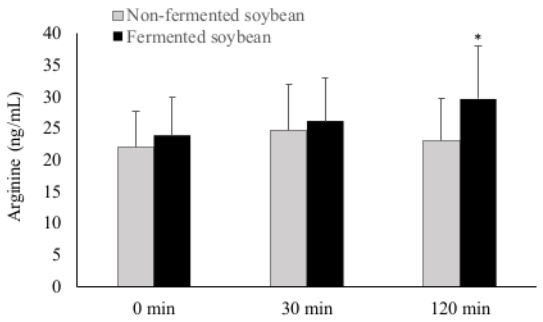

Fig. 3. Arginine levels after consumption of fermented and non-fermented soybean meals. Fermented soybean meal induced small elevation in plasma arginine level. * Significant difference against 0 min, $P < 0.05$; # Significant interactive effect between meal and time, $P < 0.05$.

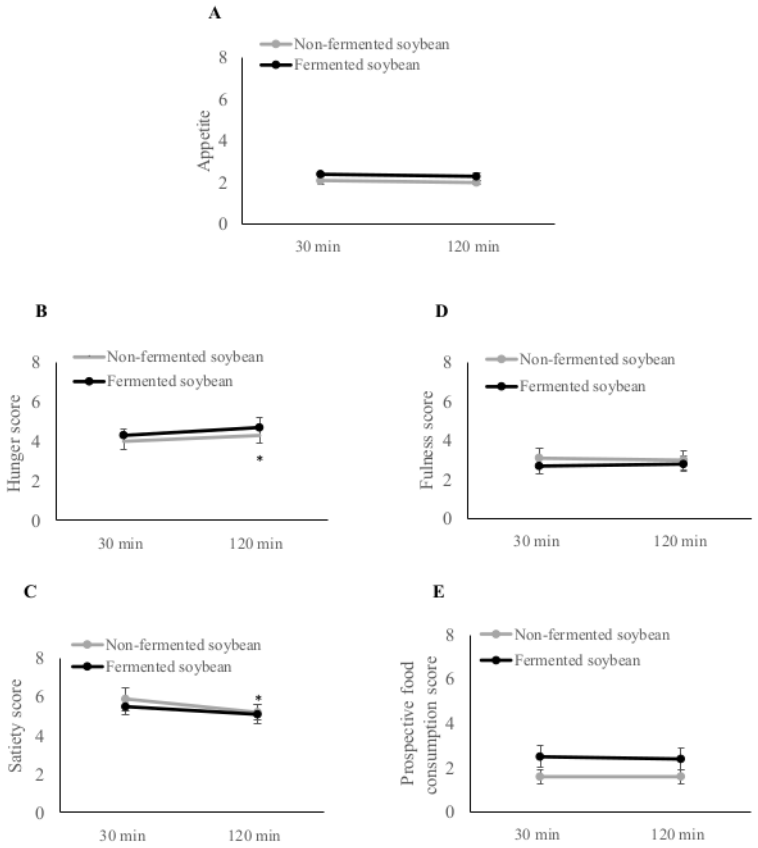

Fig. 4. Visual analog scale (VAS) appetite score after consumption of fermented and non-fermented soybean meals. Appetite score decreased after both meals. No difference in VAS scores for hunger (A), satiety (B), fullness (C), prospective food consumption (D) was found between non-fermented and fermented soybean meals. Significant difference against 0 min, $P < 0.05$; # Significant interactive effect between meal and time, $P < 0.05$.

circumference in a range of 78–91 cm.

Appetite-regulating hormones

Plasma AG response is shown in Fig. 2A. Following the non-fermented meal, plasma AG was unaltered within 120 min post-meal. However, fermented meal decreased plasma AG by ~35% during the same period. In a contrary, insulin (shown in Fig. 2B) increased after both fermented and non-fermented soybean meals. Approximately 30% greater insulin level was observed 120 min after fermented soybean meal compared with non-fermented soybean meal ($P < 0.05$). Fig. 3 shows post-meal arginine concentration in plasma. Fermented soybean meal consumption slightly increased plasma arginine level compared with non-fermented soybean meal at 120 min.

Rating of subjective appetite sensation

No difference in appetite VAS score (Fig. 4A) was observed between fermented soybean meal and non-fermented soybean meal among the girls with obesity.

Both fermented and non-fermented soybean meals showed a modest effect on subjective hunger (Fig. 4B) and satiety feeling (Fig. 4C). Two-hour ratings of fullness (Fig. 4D) and prospective food consumption (Fig. 4E) shows no difference between the meals. Multiple linear regression to predict appetite score using plasma insulin and acyl-ghrelin shows low coefficient of determination ($R^2 = 0.1$).

Discussion

Appetite-suppressing effect of dietary protein has been well established [22]. The major finding of this study is the superior effect of fermented soybean on altering appetite-regulating hormones than non-fermented soybean for girls with obesity. Nevertheless, the current study was unable to detect a significant effect of fermented soybean on subjective appetite feeling. The discrepancy between the appetite-regulating hormone response and the subjective appetite may be associated with the obesity nature of participants in the study. A blunted appetite regulating hormones in response to meals has been found in people with obesity [23]. It has been widely reported that individuals with obesity have dysregulation in appetite [11,24]. Effects of dietary protein meal on subjective appetite sensation have been described in the past [22,25–27]. The inconsistency among studies on the association between appetite hormones and subjective appetite is probably due to genetic variation of the participants [28].

Previous studies have shown that amino acid composition of dietary protein can influence post-meal response in appetite-regulating hormone levels [3,29,30]. In particular, arginine increases insulin release into circulation under intravenous glucose-challenged condition. Glucose-induced response increased from 426 pM to 1,516 pM when arginine hydrochloride (5 g) was infused in 11 healthy 58-year-old female participants [31]. The increased arginine concentration in plasma is probably reflecting the accelerated digestion of dietary soybean protein into amino acids during fermentation [18,32]. Arginine has been

found to significantly decrease food intake in rodents suggesting its direct effect on appetite [33]. However, no similar effect on total energy intake has been shown in humans [34].

The possible mechanism to explain the treatment effect of fermented soybean remains incomplete. Several fermented soybean products are traditionally consumed in Asia. In Indonesia, a special strain of fungus *Rhizopus oligosporus* is commonly used for soybean fermentation. In Japan, similar fermentation methods are also used to produced "natto", which has been shown to produce bioactive components including fibrinolytic enzyme and polyamine. Both components have been shown to lower risk of heart disease, which is closely associated with insulin resistance and obesity [35]. Another major differences between fermented and non-fermented soybean is the bioavailability of isoflavone algycones and small peptides released during fermentation [36]. Compared with unfermented soybean, fermented soybean shows greater insulinotropic effect which possibly due to faster degradation of the protein.

The major limitations of this study are: 1) the significant effect of fermented soybean on appetite-suppressing hormones cannot be generalized into the knowledge that fermented soybean is a better choice for treating obesity than non-fermented soybean. Genetic variation in the sensitivity of brain to the appetite regulating hormones may confound the actual outcomes in feeding behavior and obesity; 2) We cannot conclude that arginine from fermented soybean is the only factor of the observed suppressive effect on appetite regulating hormones. If soybean fermentation helps to release digested micronutrients in soybean, the role of other amino acids and soybean ingredients on suppressive effect of appetite-regulating hormone remains to be examined. Another limitation of the study is the small number of participants. Therefore, the result of this study should be considered as a preliminary data to encourage randomized-controlled trial with inclusion of more participants for further confirmation.

Conclusion

Soybean is widely used as a dietary protein source in preventing obesity based on its satiety effect. The present study provides the novel evidence which demonstrates a greater response in appetite-regulating hormones after consumption of a fermented soybean meals in girls with obesity, compared with a non-fermented control meal. However, the fermented soybean meal failed to produce an noticeable effect on suppressing subjective appetite for Indonesian girls with obesity.

Author statements

Chia-Hua Kuo: Conceptualization, Methodology, Software Etika Ratna Noer.: Data curation, Writing- Original draft preparation. Luthfia Dewi: Visualization, Investigation. Etika Ratna Noer: Supervision.: Luthfia Dewi: Software, Validation.: Chia-Hua Kuo: Writing- Reviewing and Editing.

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Clinical trial registration

ClinicalTrials.gov identifier: NCT04425109.

Ethics approval and consent to participate

The study protocol has been reviewed and approved by Institutional Review Board at the University of Diponegoro with number 427/EC/FK-RSDK/VII/2018.

Consent for publication

Not applicable.

Availability of data and material

The datasets from this study will be available from Dr. Etika Ratna Noera (e-mail: etikaratna@fk.undip.ac.id) on written request.

Conflict of interest

This study was sponsored by Indonesian Endowment Fund. The authors have no financial relationship or conflict of interest relevant to this article to disclose.

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