ORIGINAL

Investigation of dose-dependent effects of fat on blood glucose, serum insulin, and appetite sensation

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Abstract : Humans have a high preference for fat, and its excessive intake leads to obesity. This study aimed to investigate the effects of dose-dependent fat intake on biological responses and postprandial appetite sensation in healthy adult subjects. Age and body mass index were 29 ± 1 years and 21.1 ± 0.4 kg/m², respectively. We conducted a randomized, crossover trial and measured laboratory data and appetite sensation via the visual analog scale. Each participant was provided with four different test meals. They consisted of common, basic foods and contained 75 g liquid glucose and 4 slices of crackers to which 0 g butter (control), 10 g butter (B10), 20 g butter (B20), and 40 g butter (B40) were added, respectively. The results indicated that single ingestion of butter did not influence laboratory values of glucose, insulin, glucose-dependent insulinotropic polypeptide (GIP), total bile acids, or high-sensitivity CRP (hs-CRP). Regarding postprandial appetite sensation, appetite ratings for fullness were the highest after the B40 meal ($p < 0.05$) ; however, satisfaction ratings were not significantly different after the ingestion of this meal. Ratings were significantly different after the B20 meal. In conclusion, healthy adult subjects experienced fullness and satisfaction after ingesting 20-40 g of butter. J. Med. Invest. 65 : 203-207, August, 2018

Keywords **:** *VAS, appetite, fat intake, fullness, satisfaction*

INTRODUCTION

Overweight and obesity have increased rapidly worldwide. It is generally agreed that fatty foods are consumed preferably due to their pleasant taste and flavor and that fat intake is strongly addictive (1). Previous research suggests that fat intake influences laboratory data and sensory responses. First, fat evokes an effect on incretin that is similar to that observed for glucose and stimulates postprandial insulin release via the secretion of glucose-dependent insulinotropic polypeptide (GIP) (2-4). The fact that incretin stimulates insulin release is now widely accepted. In addition, one study reported that GIP promoted the efficient storage of ingested fat (5). In other words, fat induced GIP release and stored dietary fat via GIP and insulin release. Nevertheless, the studies are inconclusive, and insulin levels after the ingestion of dietary fat have been found to increase (6) or decrease (7). Furthermore, previous studies have reported that dietary fat intake induced the release of cholecystokinin (CCK) (8) and glucagon-like peptide-1 (GLP-1) (9). Second, it has been reported that lipids induced fullness (10) and suppressed hunger and prospective demand (11). Fullness and satisfaction influence prospective demand, which regulates appetite (12). In addition, our prior work found that postprandial appetite sensation is associated with habits of dietary fat intake (13), and we reported that fat increases satiety when a single vegetable is included in a meal (14). Some studies have examined laboratory data and appetite sensation after the intake of fatty test meals. However, many researchers altered the amount of carbohydrate and protein in test meals to maintain energy intake. The effects of dietary fat on postprandial lipemia and lipoproteins have been previously reported (15), but few studies have examined the dose-dependent effect of dietary fat on postprandial glucose, insulin, and appetite sensation. The current study thus investigated the effects of dose-dependent fat intake on biological responses and postprandial appetite sensation in healthy adult subjects.

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MATERIALS AND METHODS

Subjects. Eight healthy participants (4 men and 4 women) were recruited for this study. The sample size calculation was based on a 2-sided with 5% type I error, ensuring the statistical power of 80% in paired t test. It was assumed as a mean difference of 35 (visual analog scale (VAS)) and a standard deviation of 20 (VAS). It resulted in a sample size of 7, so we recruited 8 subjects in this study. Written informed consent was obtained from all subjects and approval was granted by the Ethics Committee of the University of Tokushima. Participants' clinical and biological characteristics are shown in Table 1. The mean [±standard error of the mean (SEM)] age and body mass index (BMI) were 29 ± 1 years and 21.1 ± 0.4 kg/m², respectively. Exclusion criteria consisted of a history of chronic disease such as diabetes, hypertension, or hyperlipidemia.

Test meal. Participants were provided with four different test meals. They consisted of common, basic foods and contained 75 g of liquid glucose (225 mL) and 4 slices of crackers to which 0 g butter (control), 10 g butter (B10), 20 g butter (B20), and 40 g butter (B40) were added, respectively (Table 2).

Study protocol. This study followed a randomized, crossover design. All subjects were asked to avoid heavy exercise and intake of alcohol three days prior to study commencement and to maintain their regular lifestyle. After an overnight fast, subjects

Table 1. Characteristics of subjects.

		Subjects (n=8)
Age	(years)	29 ± 1
Sex	(M/F)	4 / 4
BMI	(kg/m^2)	21.1 ± 0.4
Fasting blood glucose	(mg/dL)	92 ± 1
Fasting serum insulin	(μU/mL)	4.9 ± 0.3
Triglyceride	(mg/dL)	74 ± 7
Total cholesterol	(mg/dL)	187 ± 11
LDL cholesterol	(mg/dL)	103 ± 11

Table 2. Composition of the test meal.

Test meal	Control meal (Control)	Butter 10 g meal (B10)	Butter 20 g meal (B20)	Butter 40 g meal (B40)
75 g glucose liquid (bottle)	1	1	1	1
Crackers (slice)	4	4	4	4
Butter (g)	—	10	20	40
Energy (kcal)	356	429	502	648
Protein (g)	1.3	1.4	1.5	1.7
Fat (g)	1.6	9.7	17.8	34.0
Carbohydrate (g)	84.1	84.1	84.1	84.1

ingested each test meal at 9 : 00. Subjects were instructed to consume the meal within 10 min and to chew the same number of times when eating each test meal. Venous blood samples were drawn before (0 min) and after (15, 30, 60, and 120 min) each of four test meals for the analysis of glucose, insulin, total bile acids, and high-sensitivity CRP (hs-CRP). We measured GIP at 0, 30, and 60 min in the control and B40 meals. Before each blood test, each subject was asked to complete a short questionnaire rating their appetite using a VAS.

VAS. Subjects were asked to rate their fullness, satisfaction and desire for sweet, savory, salty, and fatty foods. A VAS (100 mm in length with words anchored at each end to express the most positive and negative ratings) was used to assess palatability at the aforementioned time points. For example, fullness and satisfaction were rated on 100-mm lines and preceded by the questions : "How full do you feel right now?" and "How much satisfaction do you feel right now?" and anchored on the left and right by "Not at all" and "Very much," respectively. Prospective demand and palatability desire for savory, sweet, salty, and fatty were rated on 100-mm lines and preceded by the questions : "How much do you think you could eat right now?", "How much sweet food do you think you could eat right now?", "How much savory food do you think you could eat right now?", "How much salty food do you think you could eat right now?", and "How much fatty food do you think you could eat right now?" and anchored on the left and right by "Nothing at all" and "A large amount," respectively. Participants completed the ratings before (0 min) and after (15, 30, 60, and 120 min) viewing the test meals. Subjects did not discuss or compare their ratings with one another and could not refer to their previous ratings when marking the VAS.

Laboratory analysis. Blood samples were centrifuged at 3,500 ×g for 10 min at 4°C and then separated into plasma and serum. Plasma and serum samples were stored at -80°C until use. Plasma glucose concentration was measured by the enzymatic method. Serum insulin concentration was measured by chemiluminescent enzyme immunoassay. The serum total bile acid concentration was measured by the enzymatic method. GIP concentration was measured by enzyme-linked immunosorbent assay. hs-CRP concentration was measured by Nephelometric immunoassay.

Data analysis. We calculated incremental (0-2 h) area under the curve (IAUC). These values are expressed as variations in concentration over baseline. Laboratory data and VAS tests for each subject's samples were analyzed and compared between meals using the Friedman test. The Friedman test was followed by Holm (Holm-Bonfferoni) post hoc test. P values < 0.05 were considered statistically significant. All statistical calculations were made using "EZR" open-source statistical software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) (16). More precisely, it is a modified version of the R commander, designed to add statistical functions frequently used in biostatistics. All values are expressed as mean ± SEM.

RESULTS

Laboratory data after test meals. Fig. 1 shows changes in laboratory data across the time points examined. Plasma glucose, serum insulin, total bile acid, and hs-CRP did not differ significantly after ingestion of any of the four test meals (Fig. 1). GIP was measured for 60 min. There was no significant difference between the control and B40 meals. Peak glucose level occurred 30 min after each test meal. Peak insulin level occurred at 30 min in the control, B10, and B20 meals, but at 60 min in the B40 meal. Finally, IAUC for glucose, insulin, and total bile acids were not significantly different across test meals (Fig. 2).

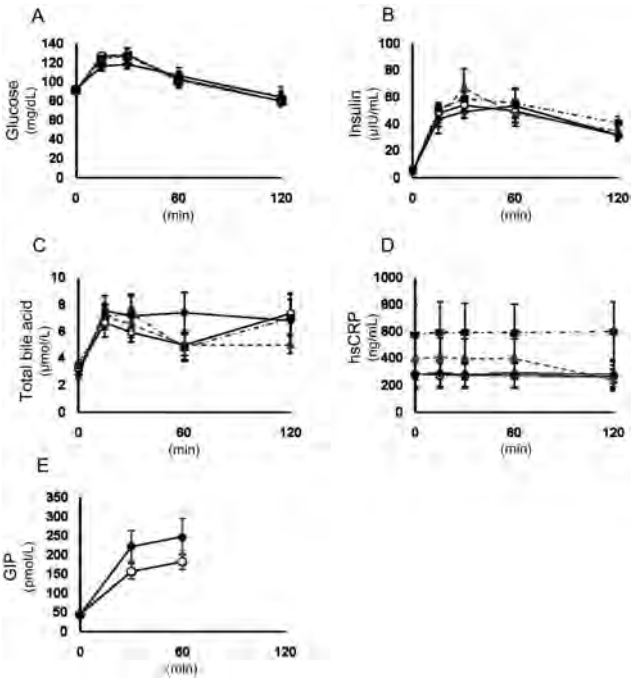

Fig. 1. Acute response to the test meal in healthy subject.
Values are means, with their standard errors represented by vertical bars of (A) plasma glucose, (B) serum insulin, (C) total bile acid, (D) high sensitivity CRP, and (E) GIP after test meals.
control meal (open circle) ; B10 meal (open triangle) ; B20 meal (closed square) ;
B40 meal (closed diamond).

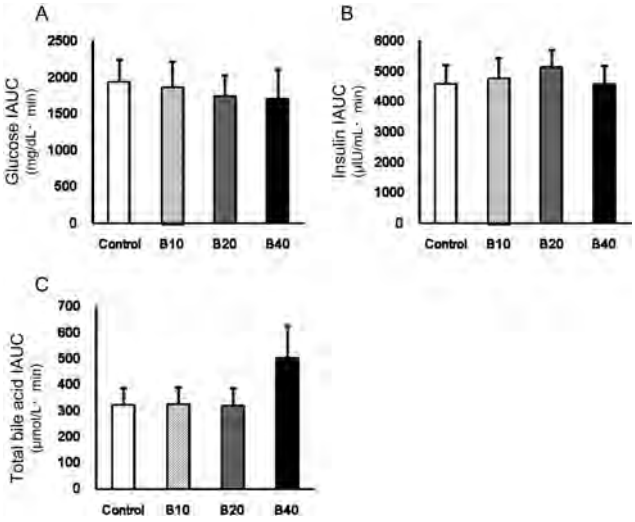

Fig. 2. Incremental area under the curve (IAUC) after test meal in healthy subject.
Values are means, with their standard errors represented by vertical bars of IAUC for (A) plasma glucose, (B) serum insulin, and (C) serum total bile acid calculated over 120 min time periods after test meals.

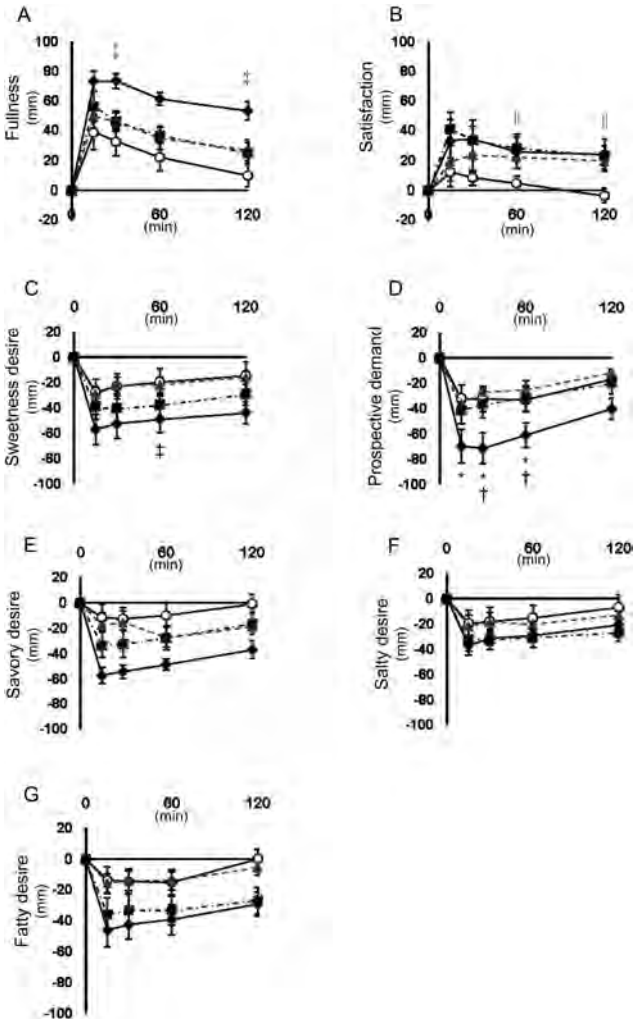

Fig. 3. Visual analogue scale (VAS) ratings for (A) fullness, (B) satisfaction, and desire of (C) sweet, (D) prospective demand, (E) savory, (F) salty, and (G) fatty. Each time point change from baseline (0min).
*$p < 0.05$ B10 vs B40, †$p < 0.05$ B20 vs B40, ‡$p < 0.05$ B0 vs B40, §$p < 0.05$ B10 vs B20, ||$p < 0.05$ B0 vs B20
control meal (open circle) ; B10 meal (open triangle) ; B20 meal (closed square) ; B40 meal (closed diamond).

Sensory response after test meals. Results of the VAS for the indication of fullness, satisfaction, and prospective demand, and for savory, sweetness, salty, and fatty desire are shown in Fig. 3. Appetite ratings for fullness were significantly higher with the B40 than with the control meal at 30 and 120 min. Satisfaction ratings were significantly higher with the B20 than with the control meal at 60 and 120 min. However, there was no significant difference in satisfaction between the control and B40 meals. Ratings for prospective demand were significantly lower with the B40 than with the B10 meal at 15, 30, and 60 min. Prospective demand was also significantly lower with the B40 than with B20 meal at 30 and 60 min. Ratings for sweetness, savory, salty, and fatty desire were not significantly different at any time points. The IAUC for fullness was higher with the B40 than with the control, B10, and B20 meals (Fig. 4A). However, the IAUC for satisfaction was not significantly different across test meals (Fig. 4B). The IAUC for sweetness desire was lower with the B40 than with the control meal (Fig. 4C). Finally, the IAUC for prospective demand was lower with the B40 meal than the control, B10, and B20 meals (Fig. 4D). The IAUC for savory, salty, and fatty desire were not significantly different across test meals. (Fig. 4E-G).

DISCUSSION

This study was conducted to investigate the effects of dose-dependent fat intake on biological responses and postprandial appetite sensation in healthy adult subjects. This was investigated by comparing four test meals with the same amounts of protein and carbohydrate and contained different amounts of butter (from 0 to 40 g). Our results revealed two main findings : 1) single ingestion of butter did not influence laboratory data such as glucose, insulin, GIP, total bile acids, and hs-CRP ; and 2) healthy young subjects felt fullness and satisfaction after ingesting 20 to 40 g of butter.

Fat evoked an incretin effect similar to than known for glucose 4 and stimulated GIP release at about 10 to 20 g ingestion (3, 7). A previous study reported that saturated fatty acids increased insulin secretion (6). We used butter as a fat source because it is rich in saturated fatty acids and easy to eat. A previous study in rats also reported that GIP was released after infusion of lipid emulsion in the duodenal feeding tube (17). However, postprandial insulin levels were increased (6) or decreased (7) in humans following fat ingestion. Some studies have reported that acute postprandial effects differed among subjects that were healthy, obese, or had type 2 diabetes (18, 19). Further, in the study reporting a decrease in postprandial insulin release, test meals did not contain equal amounts of carbohydrate and protein (7). We anticipated that GIP and insulin release would increase when test meals had constant amounts of carbohydrate and protein. In addition, previous studies indicated that peak GIP secretion occurred 30-60 min after fat intake (20). Therefore, we measured GIP for 60 min. However, our results showed that GIP did not increase after ingestion of 40 g butter. Postprandial insulin levels did not significantly differ across the four test meals. A study in human pancreatic islet reported that insulin secretion was induced more by monounsaturated fatty acids than saturated fatty acids (21). Further studies are needed in order to investigate these effects of fat.

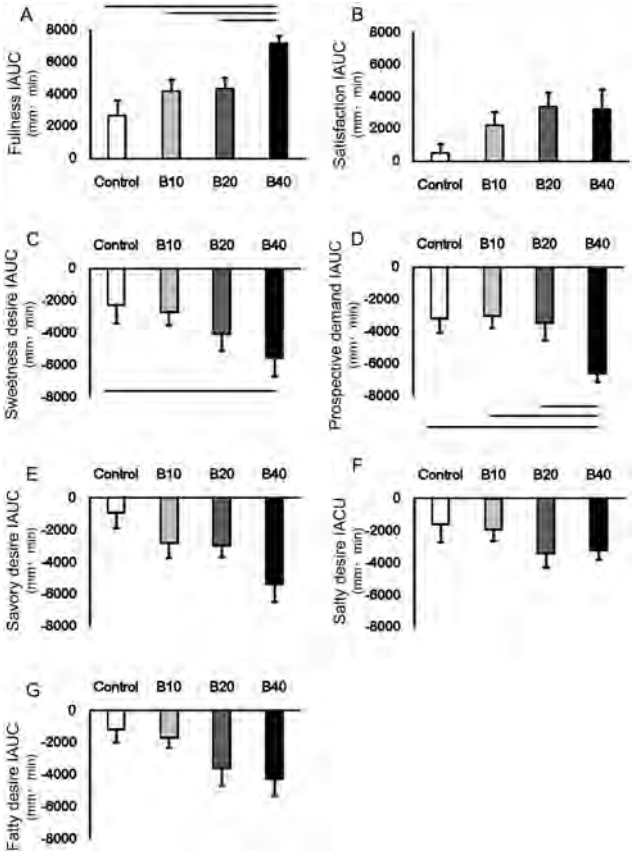

Fig. 4. Incremental area under the curve (IAUC) for VAS test after test meal in healthy subject.
Values are means, with their standard errors represented by vertical bars of IAUC for palatability calculated over 120 min time periods after test meals. IAUC for (A) fullness,
(B) satisfaction, and desire of (C) sweet, (D) prospective demand, (E) savory, (F) salty, and (G) fatty. $p < 0.05$

Sensory properties of food, including appetite factors and desire for palatability, play a major role in dietary selection, thus influencing food intake. Fullness and satisfaction influence prospective demand, which regulates appetite (12). It has been reported that lipids induce fullness (10) and suppress hunger and prospective demand (11). Fatty foods are quite palatable and consumed preferably because of their pleasant taste and flavor. Previous studies have reported a high preference for fat (1, 22, 23). However, there have been no reports on the dose-dependent effects of fat on healthy adults' level of fullness and satisfaction. The current study suggested that the IAUC for fullness was significantly higher with the B40 meal than with the control, B10, and B20 meals. In other words, ingestion of 40 g butter induced greater fullness than smaller amounts of butter or no butter. Our study indicated that fullness from the B40 meal was highest at 30 and 120 min. In addition, satisfaction ratings for the B20 meal were highest at 60 and 120 min. Fullness increased proportionally to the amount of butter, whereas satisfaction did not. We concluded that 40 g butter is excessive because subjects did not report satisfaction despite experiencing greater fullness.

Prospective demand was significantly lower with the B40 than with the B10 and B20 meals. Further, the IAUC for prospective demand was significantly lower with the B40 than with the control, B10, and B20 meals. Taking into account the results of IAUC for fullness suggests that subjects may have experienced a loss of appetite after ingesting 40 g butter. Sweetness desire was significantly lower with the B40 than with the control meal at 60 min. In other words, subjects did not desire something sweet when they ingested 40 g butter compared to 0 g butter.

This study had certain limitations. First, while some studies reported that laboratory data differed between healthy and obese subjects, the current sample consisted only of healthy, young subjects. As such, we did not examine whether the effects of butter differ in obese or elderly compared to healthy individuals. Secondly, test meals consisted of simple, common foods which may differ in taste and type from common breakfasts. As such, butter might affect digestion, absorption, and hormone secretion differently when ingested as part of a composite meal. Because energy and volume of the test meals used herein were lower and smaller than those typically consumed, participants may have required a larger dose of butter to experience fullness and satisfaction. Accordingly, individuals might experience fullness and satisfaction after ingesting less than 20 g butter if it was consumed as part of a larger, more energy-dense meal. Thirdly, the current experiment did not measure hormones to estimate related markers of appetite such as CCK, ghrelin, peptide YY, or GLP-1, although a previous study reported that appetite ratings for fullness and hunger correlated with insulin, ghrelin, and leptin (24). These hormones might be related to the suppression of hunger and desire for sweetness.

In summary, we conducted a randomized, crossover trial in order to investigate the effects of dose-dependent fat intake on biological responses and postprandial appetite sensation in healthy adult subjects. Our data indicated that intake of 75 g of liquid glucose and crackers with 40 g butter led to higher fullness than with 0, 10, or 20 g butter. However, satisfaction was higher after consumption of 20 g than 0 g of butter. We concluded that ingestion of 40 g of butter is excessive because subjects did not report higher satisfaction despite feeling more full. Overall, study results indicate that young healthy adults experience fullness and satisfaction after ingesting 20 g to 40 g of butter.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to declare.

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AUTHOR CONTRIBUTIONS

Chise Yamaguchi : wrote the manuscript ; Hisami Yamanaka-Okumura : contributed to the study design ; Chise Yamaguchi and Haruka Esumi : collected the samples and analyzed the data ; Masashi Masuda, Takafumi Katayama and Yutaka Taketani : provided helpful comments about the study. All authors read and approved the final manuscript.

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