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Effects of Co-ingestion of Amino Acids with Fat on Postchallenge Glycemia and Lipidemia in Healthy Young Women

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Abstract: Aim: To investigate the effects of co-ingestion of amino acids with fat on postchallenge glycemia and lipidemia. Methods: Nine healthy young Japanese women were enrolled. At each session, the subjects ingested one of three beverages containing fat cream (OFTT™ cream, 1 g/kg body weight, 0.35 g/kg as fat) and/or amino acid mixture (Amizet B™, 600 mg/kg body weight, 60 mg/kg as amino acids) in a randomized crossover design. The three beverages were as follows: fat cream (F trial), amino acid mixture (A trial), or both (FA trial). Venous blood samples were obtained before (0) and 0.5, 1, 2, and 3 h in the A trial, and at 0, 0.5, 1, 2, 4, and 6 h after ingestion in the F and FA trials. Results: Fat ingestion increased the secretion of incretins (GIP and GLP-1), but only a weak effect on insulin secretion was observed. Amino acids ingestion had only a weak effect on the secretion of incretins, but synergistically stimulated the insulin secretion induced by fat ingestion. Glucagon showed behavior similar to that of insulin. There were no significant effects of the co-ingestion of amino acids on lipid or lipoprotein metabolism compared to the ingestion of fat alone. All of these phenomena were observed without a change in blood glucose levels. Conclusion: Fat ingestion stimulated mainly the secretion of incretins via intestinal cells, whereas the amino acid ingestion stimulated mainly insulin secretion by directly acting on pancreatic β cells. The co-ingestion of amino acids with fat may improve postchallenge glucose metabolism, but it had little effect on postchallenge lipid or lipoprotein metabolism.

Keywords: Amino Acids, Postchallenge Glycemia, Postchallenge Lipidemia, Young Women, GIP, GLP-1, Insulin, Glucagon

1. Introduction

Postprandial dysmetabolism such as postprandial hyperglycemia and hyperlipidemia have been reported to be risk factors for cardiovascular disease, even in non-diabetic subjects [1]. Our studies have revealed that in healthy young women, postchallenge lipidemia was markedly delayed and exacerbated when fructose or high-fructose corn syrup was ingested with fat [2–4]. In addition, postprandial hyperglycemia had been observed even in healthy young women, indicating that postprandial dysmetabolism can occur even at young ages [5].

Postprandial metabolism is known to be affected by meal timing [6], the food intake order [7], the prior meal's composition [8], diet composition [9], skipping a meal [10], gastric emptying [11], and other factors. Postprandial hyperglycemia and hyperlipidemia induce oxidative stress, insulin resistance, and impaired glucose tolerance, leading to cardiovascular disease and atherosclerosis [12, 13]. These phenomena have been reported to occur even in normoglycemic subjects [14]. The dietary management of postprandial glycemia and lipidemia may thus be beneficial for the prevention of diabetes and dyslipidemia.

The diet that is usually consumed every day contains macronutrients: protein, fat, and carbohydrate together. We have shown that the absorption and metabolism of fat and glucose are mutually inter-related [15], and thus both direct and indirect interactions can occur among the macronutrients.

Although many studies have examined the effects of the consumption of carbohydrate with fat or protein on postprandial metabolism, the effects of co-ingestion of fat with protein on postprandial lipidemia remain to be understood. There have also been few investigations of the effects of amino acids on postprandial metabolism in humans. We conducted the present study to evaluate the effects of co-ingestion of amino acids with fat on postchallenge glycemia and lipidemia in healthy young women, by using an amino acid mixture, components of protein.

2. Subjects and Methods

2.1. Subjects

Nine healthy young Japanese women with a normal ovarian cycle and apolipoprotein E phenotype not containing E2 or E4 were enrolled. All subjects were non-smokers, had no apparent acute or chronic illness, and were not taking any medications or dietary supplements. This study was approved by the Institutional Review Board of Sugiyama Jogakuen University School of Life Studies (No. 2019-23), and each subject provided written informed consent for participation. The procedures were conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983.

2.2. Methods

2.2.1. Anthropometric and Body Composition Measurement

The subjects' body weight and height were measured according to standard methods. Waist circumference was assessed as the abdominal girth at the level of the umbilicus, and hip circumference was measured at the level of the greater trochanters. The waist-to-hip (W/H) ratio was calculated. Body composition, including the visceral fat area (VFA), was analyzed by an eight-polar bioelectrical impedance method (InBody720, Biospace, Tokyo, Japan).

2.2.2. Test Beverages

Each subject underwent three test trials, and they ingested one of the three beverages: (1) for the fat (F) trial: 1.0 g/kg body weight (BW) of fat cream (OFTT cream, Jomo, Takasaki, Japan, containing 0.35 g/kg BW as fat); (2) for the amino acids (A) trial: 600 mg/kg BW of an amino acid mixture (Amizet B, Terumo, Tokyo, containing 60 mg/kg BW as amino acids); or (3) for the fat and amino acids (FA) trial: the amino acid mixture + the fat cream. We added 170 mL of water to all three of the above beverages, and 20 mg of sucralose (San-Ei Gen F. F. I., Osaka, Japan) was also added to the beverages in order to improve the taste in the trials that contained amino acids (trials A and FA). The formulas of the OFTT cream and Amizet B are provided in Tables 1 and 2, respectively.

2.2.3. Experimental Design

The subjects abstained from consuming caffeine or alcohol on the day before the experiment. Each subject was studied on three occasions in a randomized single-blinded crossover design and ingested a beverage after a 12-h overnight fast.

Table 1. *Formula of OFTT cream.*

Energy (kcal)	342
Composition (%)	
Butter fat	35.0
Proportion in the fat (%)	
Saturated fatty acids	64.3
Monounsaturated fatty acids	32.2
Polyunsaturated fatty acids	3.5
Casein Na	1.3
Sucrose ester of fatty acids	0.6
Lecithin	0.4
Glycerin fatty acids	0.3
Stevia sweetener	0.02
Water	62.4

Values are per 100 g of OFTT cream.

Table 2. *Formula of Amizet B.*

Ingredients (mg/dL)	L-Isoleucine	850
	L-Leucine	1,350
	Lysine malate (L-Lysine)	1,216 (800)
	L-Methionine	390
	L-Phenylalanine	770
	L-Threonine	480
	L-Tryptophan	160
	L-Valine	900
	Cysteine malate (L-Cysteine)	155 (100)
	L-Tyrosine	50
	L-Arginine	1,110
	L-Histidine	470
	L-Alanine	860
	L-Aspartic acid	50
	L-Glutamic acid	50
	Glycine	550
	L-Proline	640
	L-Serine	420
Additive	Succinic acid	Appropriate amount
Composition	Total amino acids (mg/dL)	10,000
	Branched chain amino acids (%)	31.0
	Total nitrogen (mg/dL)	1,560
	Essential amino acids/non-essential amino acids	1.53

Values are per 100 mL of Amizet B.

Venous blood samples were collected while the subject was in a supine position, before (0) and 0.5, 1, 2, and 3 h after ingesting the beverage in the A trial, and at 0, 0.5, 1, 2, 4, and 6 h after ingestion in the F and FA trials. During the test period, the subjects avoided exercise and eating but had free access to water from 1 h after ingestion. There was a 4-week interval between the trials in order to minimize the confounding effects of menstrual cycles on metabolism.

2.2.4. Biochemical Analysis

The serum or plasma samples were immediately refrigerated (4°C) or frozen (−20°C or −80°C) until analysis. The serum glucose level was measured by a mutarotase-glucose oxidase method (Wako, Osaka). The serum insulin was measured using a chemiluminescent enzyme immunoassay (CLEIA) (Fujirebio, Tokyo). The plasma glucagon level was measured by an enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden).

The plasma glucose-dependent insulinotropic polypeptide (GIP) (active form) and glucagon-like peptide-1 (GLP-1) (active form) levels were measured by an ELISA (Immuno-Biological Laboratories, Gunma, Japan). The hemoglobin A1c (HbA1c) level was measured with an enzymatic method (Minaris Medical, Tokyo). The level of free fatty acids (FFA) was measured by an acyl-CoA synthetase-acyl-CoA oxidase method (Wako). The serum C-peptide immunoreactivity (CPR) level was measured in a CLEIA (Fujirebio).

The level of total cholesterol (TC) was measured using a cholesterol oxidase-peroxidase method (Sekisui Medical, Tokyo). The level of high-density lipoprotein-cholesterol (HDL-C) and that of low-density lipoprotein-cholesterol (LDL-C) were measured by a direct method (Sekisui Medical). The small, dense LDL (sdLDL) level was measured by a direct method (Denka, Tokyo). The triglyceride (TG) level was enzymatically measured (Sekisui Medical). The level of remnant lipoprotein-cholesterol (RLP-C) was measured by an enzymatic method (Minaris Medical). A latex agglutination method (Sekisui Medical) was used to measure the serum lipoprotein(a) [Lp(a)] level. The level of apoproteins (Apo) A-I, A-II, B, C-II, C-III, and E were measured by an immunoturbidimetric method (Sekisui Medical). ApoB48 values were determined by a CLEIA (Fujirebio). The level of ApoB100 was calculated by subtracting the value of ApoB48 from the value of ApoB. The total ketone bodies (TKB), β-hydroxybutyrate (β-HB), and acetoacetate (AA) were measured by an enzymatic method (Kainos Laboratories, Tokyo). The apoE phenotype was examined using the isometric electrophoresis method (Joko, Tokyo).

2.2.5. Quantification of Postchallenge Metabolism

The postchallenge changes in the concentrations of insulin, glucagon, GIP, GLP-1, TG, RLP-C, and ApoB48 were calculated as the difference from the baseline mean value (as 0 at 0 h) and are shown as *Δ*insulin, *Δ*glucagon, *Δ*GIP, *Δ*GLP-1, *Δ*TG, *Δ*RLP-C, and *Δ*ApoB48. We quantified the postchallenge metabolism by calculating the incremental area under the curve (*Δ*AUC), which was defined as the area determined by the trapezoidal method minus the area below the baseline (0 h) from 0 to 2 h in the A trial and from 0 to 6 h in the F and FA trials.

2.2.6. Statistical Analyses

All data are expressed as mean ± standard error of the mean (SEM). The statistical analyses were performed using SPSS ver. 26 (IBM, Tokyo). Normal distributions of data were verified using the Shapiro-Wilk test. Non-normally distributed data were examined for normal distribution when logarithmically transformed and were analyzed using parametric statistics. We analyzed the differences in the time course compared with the fasting values by performing a repeated measures analysis of variance (ANOVA), followed by the Dunnett test. The differences in the measured values at each time point in the three trials were assessed by a repeated measures ANOVA followed by the Bonferroni test. For all data, $p<0.05$ was considered significant.

3. Results

All subjects completed the trials and ingested the test beverages without problems. The physical characteristics and fasting blood chemical data of the subjects are summarized in Table 3. There were no significant differences in any of these data among the three trials (data not shown). The fasting and postchallenge chemical data in the three trials are presented in Table 4 (glucose, insulin, glucagon, GIP, and GLP-1) and Table 5 (TG, RLP-C, ApoB48, FFA, and TKB). The time courses of *Δ*insulin, *Δ*glucagon, *Δ*GIP, and *Δ*GLP-1 are shown in Figure 1, and those of *Δ*TG, *Δ*RLP-C, and *Δ*ApoB48 are shown in Figure 2. The *Δ*AUC of insulin, glucagon, GIP, GLP-1, TG, RLP-C, and ApoB48 are also shown in the figure inset.

Table 3. *Physical characteristics and fasting blood chemical data of the subjects.*

Height (cm)	159.0	±	1.7
Weight (kg)	50.9	±	1.8
BMI (kg/m^2)	20.1	±	0.6
Waist (cm)	67.3	±	2.5
W/H	0.76	±	0.02
VFA (cm^2)	22.9	±	5.1
SBP (mmHg)	110.1	±	2.5
DBP (mmHg)	64.4	±	1.6
PR (beats/min)	79.0	±	3.2
CPR (ng/mL)	1.4	±	0.1
HbA1c (%)	5.1	±	0.1
HOMA-IR	1.4	±	0.2
TC (mg/dL)	183.0	±	11.6
HDL-C (mg/dL)	67.6	±	3.3
LDL-C (mg/dL)	102.1	±	9.3
sdLDL (mg/dL)	19.9	±	2.7
Lp(a) (mg/dL)	11.9	±	2.5
ApoA-I (mg/dL)	154.8	±	6.8
ApoA-II (mg/dL)	24.6	±	1.1
ApoB100 (mg/dL)	68.8	±	5.6
ApoC-II (mg/dL)	2.9	±	0.2
ApoC-III (mg/dL)	7.8	±	0.7
ApoE (mg/dL)	4.2	±	0.3

Values are mean ± SEM. SBP: systolic blood pressure, DBP: diastolic blood pressure, PR: pulse rate.

3.1. Glucose, Insulin and Glucagon

The subjects' serum glucose concentrations did not rise significantly during the experiment in all three trials (Table 4). There were no significant differences in the *Δ*AUC(0–2h)-glucose among the three trials (data not shown).

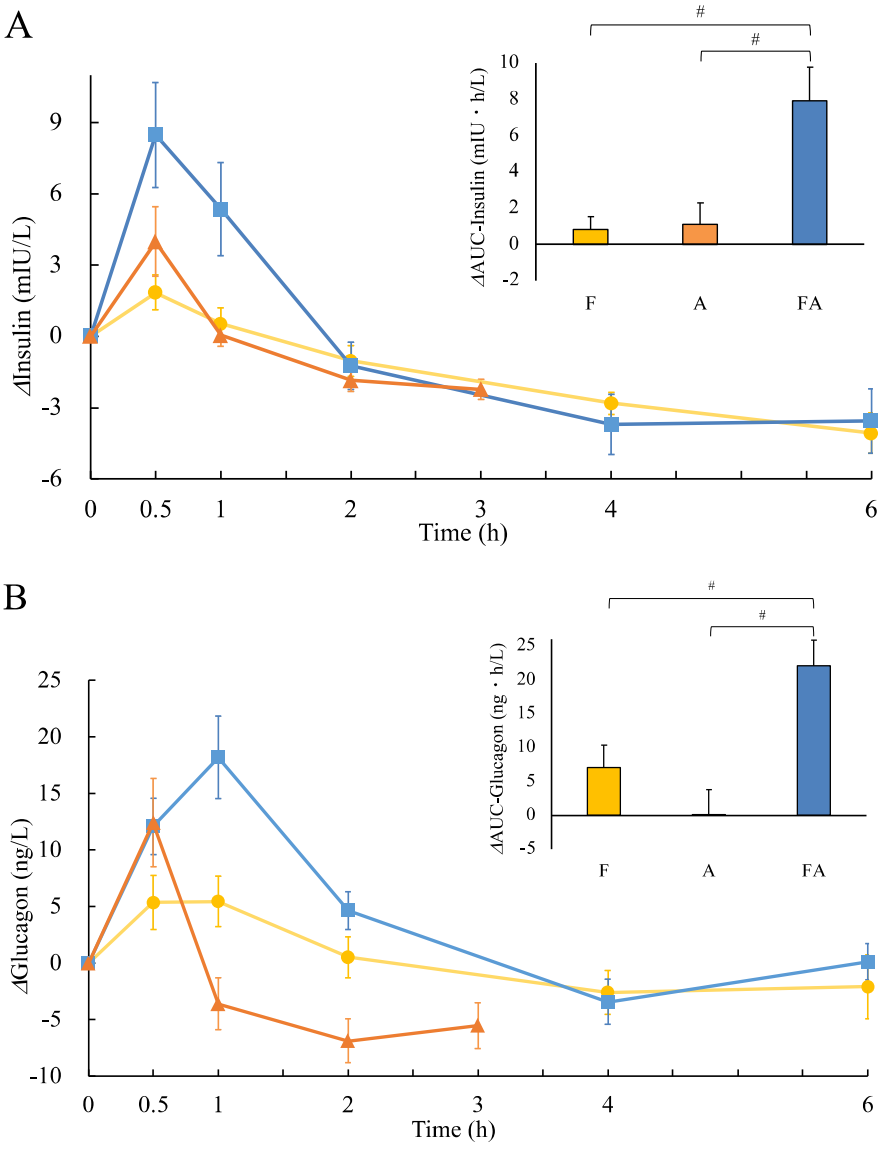
The serum insulin concentration peaked at 0.5 h in the A and FA trials, but no significant increase was observed in the F trial (Table 4, Figure 1A). At 1 and 2 h, the insulin concentration in the FA trial was significantly higher than that in the A trial. In the FA trial, the *Δ*AUC(0–2h)-insulin was significantly larger than those in the F and A trials. The plasma glucagon concentration was significantly increased at 0.5 h in the A trial and at 0.5 and 1 h in the FA trial compared to each fasting value (Table 4, Figure 1B). At 1 and 2 h, the glucagon concentration in the FA trial was significantly higher than that in the A trial. In the FA trial, the *Δ*AUC(0–2h)-glucagon was

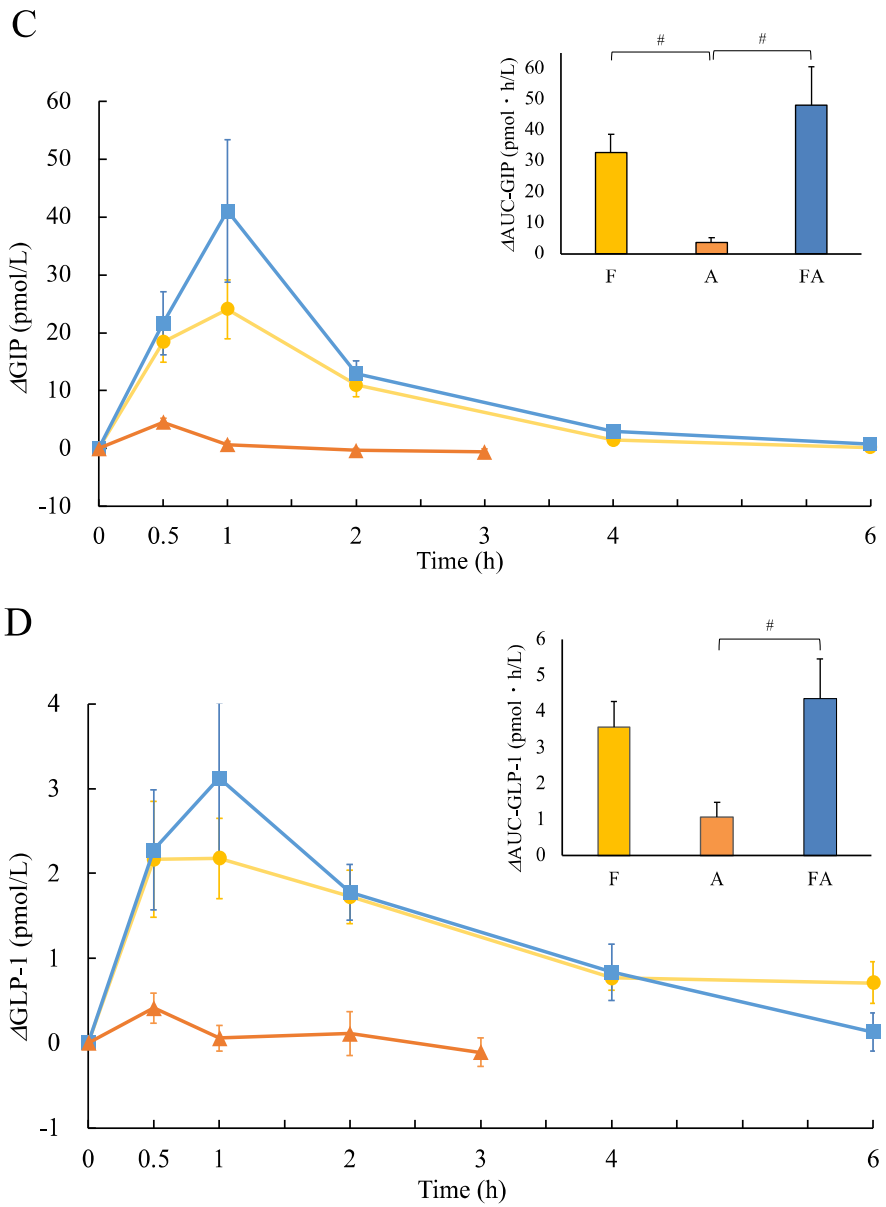
significantly larger than those in the F and A trials.

Table 4. *Fasting and postchallenge concentrations of serum glucose, insulin, plasma glucagon, GIP, and GLP-1.*

		0	0.5	1	2	3	4	6
Glucose (mg/dL)	F	87.6 ± 2.2	82.4 ± 1.7	84.0 ± 1.6	83.3 ± 1.8		86.3 ± 2.3	80.8 ± 2.5*
	A	84.4 ± 2.1	84.4 ± 2.1	86.0 ± 2.1	85.5 ± 2.0	86.5 ± 2.7		
	FA	87.1 ± 2.1	87.5 ± 2.7	82.4 ± 2.4	87.0 ± 2.4		85.6 ± 1.7	81.8 ± 2.1*
Insulin (mIU/L)	F	6.5 ± 0.7	8.4 ± 1.2	7.1 ± 0.8	5.5 ± 0.7		3.7 ± 0.4*	2.5 ± 0.4*] #
	A	5.3 ± 0.4	9.3 ± 1.5*	5.4 ± 0.5] #	3.5 ± 0.4] #	3.1 ± 0.3		
	FA	7.6 ± 1.5	16.2 ± 3.4*	13.4 ± 2.9*	6.4 ± 0.7		3.8 ± 0.3	3.3 ± 0.4*
Glucagon (ng/L)	F	22.0 ± 1.7	27.4 ± 2.4	27.5 ± 3.1	22.6 ± 2.8		19.5 ± 2.9	19.9 ± 4.0
	A	20.2 ± 2.6	32.6 ± 4.1*	16.6 ± 1.5] #	13.3 ± 1.4] #	14.6 ± 1.8		
	FA	18.9 ± 2.9	31.0 ± 4.1*	37.1 ± 6.0*	23.6 ± 3.6		15.5 ± 2.0	19.0 ± 3.1
GIP (pmol/L)	F	3.3 ± 0.3	21.8 ± 3.5*] #	27.4 ± 5.1*] #	14.3 ± 1.9*] #		4.8 ± 0.9	3.5 ± 0.6
	A	3.5 ± 0.7	7.7 ± 0.6*] #	3.8 ± 0.5] #	2.8 ± 0.4] #	2.5 ± 0.4		
	FA	2.5 ± 0.4	24.2 ± 5.6*	43.6 ± 12.5*	15.5 ± 2.3		5.5 ± 1.1	3.8 ± 0.7
GLP-1 (pmol/L)	F	1.3 ± 0.2	3.5 ± 0.7*	3.5 ± 0.6*	3.0 ± 0.4*] #		2.1 ± 0.3	2.0 ± 0.4
	A	1.4 ± 0.2	1.7 ± 0.2	1.4 ± 0.2] #	1.4 ± 0.3] #	1.2 ± 0.2		
	FA	1.3 ± 0.2	3.6 ± 0.8*	4.4 ± 0.9*	3.1 ± 0.4*		2.2 ± 0.4	1.6 ± 0.3

Values are mean ± SEM. *p<0.05 vs. the fasting value. #p<0.05 between the trials.





F trial (●), A trial (▲), FA trial (■). Values are mean ± SEM.

Figure 1. *Δinsulin (A), Δglucagon (B), ΔGIP (C), and ΔGLP-1 (D), and ΔAUC (0–2h) for each parameter (inset).*

3.2. GIP and GLP-1

The plasma GIP concentration was significantly increased at 0.5 h in the A trial and at 0.5 and 1 h in the F and FA trials compared to each fasting value, but the increase was only slight in the A trial (Table 4, Figure 1C). At 0.5, 1, and 2 h in the F and FA trials, the GIP concentration was significantly higher than that in the A trial. In the F and FA trials, the *Δ*AUC(0–2h)-GIP was significantly larger than that in the A trial.

The plasma GLP-1 concentration was significantly increased at 0.5, 1, and 2 h in the F and FA trials compared to each fasting value (Table 4, Figure 1D). In the A trial, a non-significant increase in the GLP-1 concentration was observed at 0.5 h. At 2 h in the F trial and at 1 and 2 h in the FA trial, the GLP-1 concentration was significantly higher than that in the A trial. In the FA trial, the *Δ*AUC(0–2h)-GLP-1 was significantly larger than that in the A trial.

3.3. TG, RLP-C, ApoB48, and FFA

The serum TG concentration was significantly increased at 2 h in the F and FA trials compared to each fasting level and returned to baseline at 6 h (Table 5, Figure 2A). At 2 h in the F and FA trials, the TG concentration was significantly higher than that in the A trial. The TG concentration in the FA trial was slightly but not significantly higher than in the F trial and had a tendency to be delayed. In the FA trial, the *Δ*AUC(0–6h)-TG was slightly but not significantly larger than that in the F trial.

The serum RLP-C concentration was significantly increased at 2 and 4 h in the F and FA trials compared to each fasting level and returned to baseline at 6 h (Table 5, Figure

2B). In the A trial, it was slightly increased at 0.5 h, but not significantly. The RLP-C concentration was not significantly different between the F and FA trials, but the values in the FA trial were slightly higher than those in the F trial and had a tendency to be delayed. In the FA trial, the *Δ*AUC(0–6h)-RLP-C was slightly but not significantly larger than that in the F trial.

The serum ApoB48 concentration was significantly increased at 2 and 4 h in the F and FA trials and did not return to baseline at 6 h in the F trial (Table 5, Figure 2C). The concentration was not significantly different between the F and FA trials, but the concentration in the FA trial was slightly higher than that in the F trial and tended to be delayed. In the FA trial, the *Δ*AUC(0–6h)-ApoB48 was slightly but not significantly larger than that in the F trial.

The serum FFA concentration gently increased to 2 h and was significantly increased at 6 h in the F and FA trials (Table 5). There were no significant differences in the timepoints' values among the three trials, or between the *Δ*AUC(0–6h)-FFA values of the F and FA trials (data not shown).

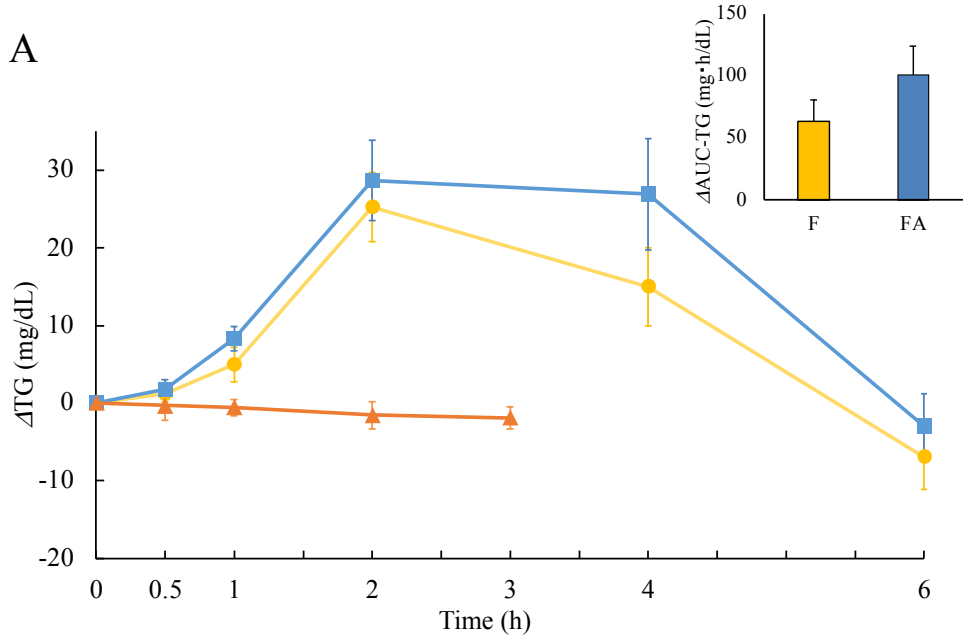
3.4. TKB

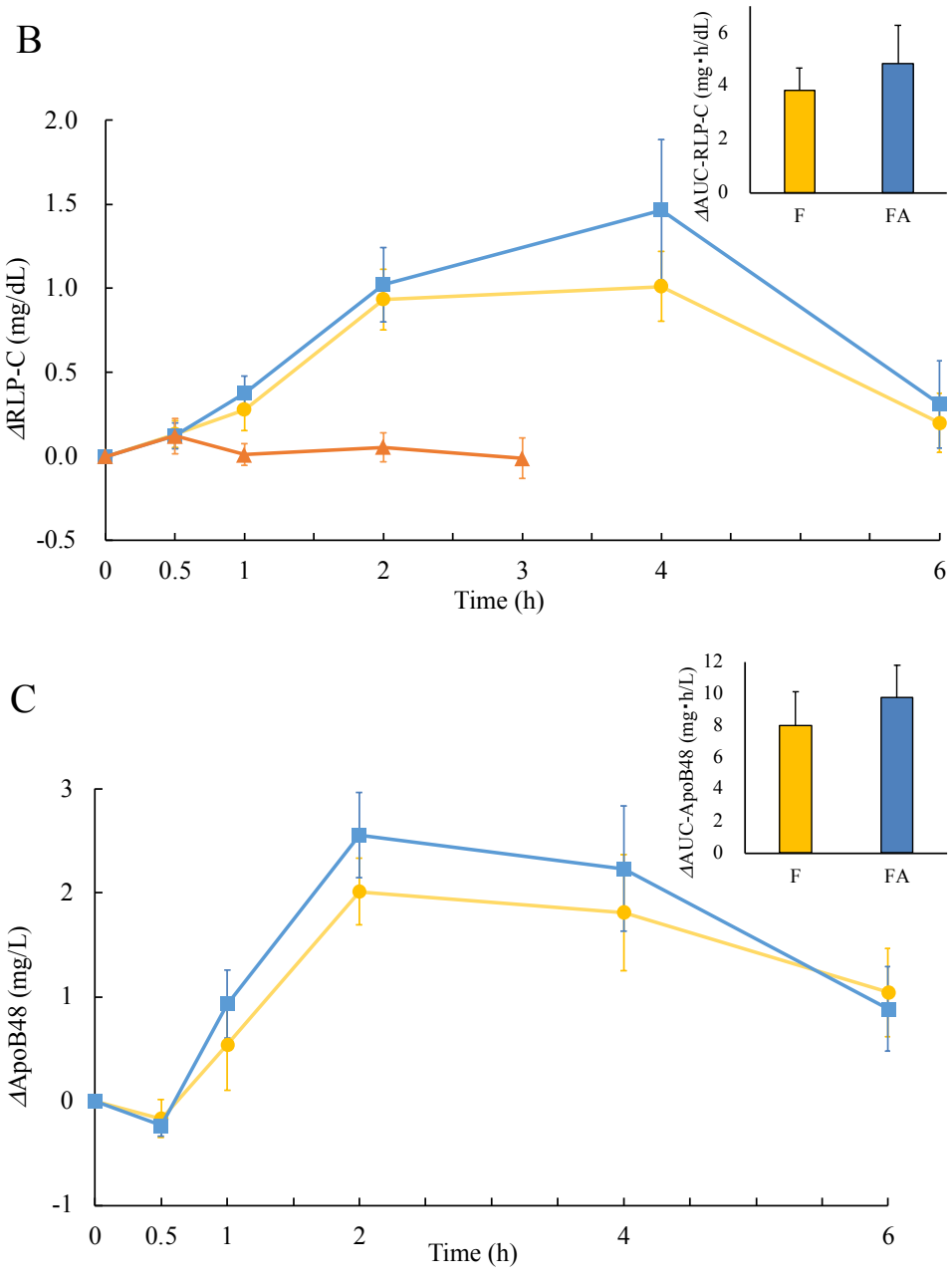
The serum TKB concentration gradually increased after ingestion in the F and FA trials (Table 5). In the A trial, the TKB concentration was significantly decreased at 1 h and then returned to baseline by 3 h. There were no significant differences in the values at each timepoint, or between the *Δ*AUC(0–6h)-TKB values of the F and FA trials (data not shown). The β-HB and AA concentrations showed patterns that were similar to that of TKB (data not shown).

Table 5. *Fasting and postchallenge concentrations of serum TG, RLP-C, ApoB48, FFA, and TKB.*

		0	0.5	1	2	3	4	6
TG (mg/dL)	F	65.1 ± 7.0	66.3 ± 6.6	70.1 ± 6.7	90.3 ± 7.8*]#		80.1 ± 8.2*	58.2 ± 6.5
	A	55.7 ± 5.5	55.3 ± 6.0	55.1 ± 5.9	54.1 ± 5.9]#	53.8 ± 5.6		
	FA	60.3 ± 6.4	62.1 ± 6.5	68.7 ± 6.5	89.0 ± 10.6*		87.2 ± 12.2*	58.8 ± 6.9
RLP-C (mg/dL)	F	3.3 ± 0.5	3.4 ± 0.5	3.5 ± 0.5	4.2 ± 0.5*		4.3 ± 0.5*	3.5 ± 0.4
	A	2.7 ± 0.3	2.9 ± 0.3	2.7 ± 0.3	2.8 ± 0.3	2.7 ± 0.3		
	FA	3.2 ± 0.4	3.3 ± 0.5	3.6 ± 0.4	4.2 ± 0.6*		4.6 ± 0.8*	3.7 ± 0.6
ApoB48 (mg/L)	F	2.6 ± 0.4	2.4 ± 0.3	3.1 ± 0.4	4.6 ± 0.5*		4.4 ± 0.5*	3.6 ± 0.4*
	FA	2.4 ± 0.2	2.2 ± 0.3	3.4 ± 0.4	5.0 ± 0.6*		4.7 ± 0.8*	3.3 ± 0.6
FFA (mmol/L)	F	0.37 ± 0.05	0.35 ± 0.06	0.24 ± 0.02	0.47 ± 0.07		0.50 ± 0.05	0.64 ± 0.08*
	A	0.60 ± 0.26	0.37 ± 0.06	0.32 ± 0.06	0.41 ± 0.07	0.53 ± 0.12		
	FA	0.38 ± 0.06	0.32 ± 0.05	0.26 ± 0.03	0.33 ± 0.05		0.51 ± 0.06*	0.69 ± 0.08*
TKB (μmol/L)	F	98.8 ± 19.1	139.9 ± 31.1*	154.1 ± 32.3*	163.9 ± 39.7*		271.4 ± 61.7*	368.4 ± 85.4*
	A	295.3 ± 173.8	174.7 ± 88.7	132.3 ± 70.7*	215.9 ± 74.6	326.6 ± 110.6		
	FA	59.0 ± 10.7	117.2 ± 25.3*	111.7 ± 15.4*	109.6 ± 24.5*		240.2 ± 38.7*	309.2 ± 42.0*

Values are mean ± SEM. *p<0.05 vs. the fasting value. #p<0.05 between the trials.





F trial (●), A trial (▲), FA trial (■). Values are mean ± SEM.

Figure 2. *ΔTG (A), ΔRLP-C (B), and ΔApoB48 (C), and ΔAUC(0–6h) for each parameter (inset).*

4. Discussion

The major findings of this study are as follows. Although the subjects' ingestion of fat significantly increased the secretion of incretins (GIP and GLP-1), only a weak effect of fat ingestion on insulin secretion was observed. In contrast, although the amino acids themselves had little or only a weak effect on the secretion of incretins, they synergistically stimulated the insulin secretion induced by fat. These results indicate that fat stimulates mainly the secretion of incretins via intestinal cells, but amino acids stimulate mainly insulin secretion by directly acting on pancreatic β cells. The pattern shown by glucagon was similar to that shown by insulin. In addition, there were no significant effects of the co-ingestion of amino acids together with fat on the lipid or lipoprotein metabolism compared to the ingestion of fat only. All of these phenomena occurred without a change in blood glucose levels.

The concentration of glucose was not significantly increased by the ingestion of fat and/or amino acids, in agreement with reports that glucose concentrations were not significantly increased or were unchanged by the ingestion of fat [4, 16] or amino acids [17, 18].

In general, insulin is secreted in response to an increased glucose concentration. Amino acids increase the insulin secretion when ingested with carbohydrate [19]; however, it was reported that the insulin concentration increased slightly

after the ingestion of amino acids alone [20]. In the present study, the subjects' insulin levels significantly increased when amino acids were ingested alone or with fat, although their glucose levels did not change. On the other hand, the subjects' insulin levels were not significantly increased by the ingestion of fat only. It can thus be inferred that the insulin secretion after the co-ingestion of amino acids with fat is strongly influenced by amino acids, and that the intake of amino acids may stimulate insulin secretion without affecting the glucose concentration. The *Δ*AUC(0–2h)-insulin was synergistically greater when amino acids were ingested with fat compared to the ingestion of fat alone or amino acids alone.

We observed that our subjects' glucagon concentration was significantly increased after the ingestion of amino acids alone or when amino acids were co-ingested with fat, similar to the time course of insulin. It was reported that the secretion of not only insulin but also that of glucagon is stimulated after the ingestion of a mixed meal [21]. Accordingly, we speculate that our subjects' glucose concentration was maintained because glucagon was secreted together with insulin. It has also been reported that amino acids stimulate glucagon secretion [18, 22], which is consistent with our present results. Protein intake was also reported to increase the secretion of glucagon regardless of the glucose concentration [16].

In the present study, the time courses of GIP and GLP-1 were similar in each trial. The ingestion of fat [16, 23] and/or protein [16] was reported to promote incretin secretion. In the ingestion of fat, FFA1 [24], FABP5 [25], and GPRs (i.e., GPR40 and GPR120) function as sensors and may mediate the release of GIP or GLP-1 [24–29]. Amino acids also have been reported to promote the release of GLP-1 via amino acid transporters and several receptors on the cell membrane [30, 31]. However, in the present study, the subjects' ingestion of amino acids did not increase or only slightly increased the secretion of incretins, which is consistent with a report that GIP secretion was mainly by carbohydrate and fat, with only slight secretion by amino acids [22].

Monounsaturated fatty acids (MUFA) were reported to secrete more incretins than saturated fatty acids [32], suggesting that MUFA in the fat cream used in the present investigation stimulated the secretion of incretins. In addition, although it is widely accepted that incretins stimulate insulin secretion, we observed no significant increase in insulin with the intake of fat alone, which resulted in higher incretins compared to the ingestion of amino acids alone. The subjects' glucagon concentration did not increase, as well. Richard et al. reported that they observed no correlation between incretin levels and islet hormone levels after fat intake and no significant change in the glucagon concentration [16]. We thus suggest that the ingestion of fat stimulates incretin secretion but has no effect on insulin or glucagon secretion.

Based on previous findings that incretins stimulate insulin secretion, we speculated that the peak of insulin would be observed after the peak of incretins, but this was not observed in the present study. We thus suggest that amino acids stimulated insulin secretion by a mechanism other than via incretins. Fat intake alone stimulated incretin secretion but did not affect insulin secretion. Our results suggest the following: the mechanism in which the ingestion of amino acids promotes insulin secretion without affecting incretins may be a direct stimulation of pancreatic β cells. Several amino acids (e.g., alanine, arginine, glutamine, glutamate, and leucine) have been reported to activate metabolism in mitochondria, increase the ATP concentration, and induce the exocytosis of insulin by increasing the intracellular Ca^{2+} concentration due to depolarization of the cell membrane [33, 34]. Arginine, as a result of its electrical transport into the cell via mCAT2A, causes an increase in Ca^{2+} that triggers insulin secretion [35]. Based on these studies, we suspect that the increase of insulin by amino acids in our present subjects was due to a direct stimulation of pancreatic β cells, not through incretins.

We also observed that the concentrations of TG, RLP-C, and ApoB48 showed a similar tendency in the trials containing fat. With the ingestion of amino acids alone, there was no significant increase in TG or RLP-C. Although there is a report that the addition of whey to a high-fat diet improved postprandial lipidemia by decreasing chylomicron formation or by increasing its removal [36], the present results suggest that an intake of amino acids has no effect on improving postchallenge lipid metabolism. However, since the research regarding the relationship between amino acids and lipid metabolism is limited, further investigations are needed.

This study has some limitations. Because of the small number of subjects, the results should be interpreted with caution. Although postchallenge dysmetabolism (glycemia and lipidemia) can be used as a surrogate for postprandial dysmetabolism, it is not equivalent to it; therefore, postprandial dysmetabolism should be examined after the consumption of a usual meal. Finally, because only healthy young Japanese females were enrolled, the results may not be applicable to other populations.

5. Conclusion

We investigated the effects of co-ingestion of amino acids with fat on postprandial glycemia and lipidemia in healthy young women. The co-ingestion of amino acids with fat synergistically stimulated the secretions of GIP and GLP-1 and the secretion of insulin by amino acids through a direct stimulation of β cells, without affecting the blood glucose concentration. No significant differences were observed in lipid metabolism compared to the ingestion of fat only. Based on these results, we conclude that co-ingestion of amino acids with fat may improve postchallenge glucose metabolism but has little effect on postchallenge lipid or lipoprotein metabolism. The effects of nutritional combinations on postchallenge metabolism observed in this study may provide a basis for considering dietary prevention and treatment of non-communicable diseases, such as diabetes mellitus and dyslipidemia.

Conflicts of Interest

The authors declare that they have no competing interests.

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