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Influence of high-fat and high-carbohydrate meals on lipids, apolipoproteins, and coagulation and anticoagulation factors

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ABSTRACT

Background: Nonfasting triglyceride levels have an equal or greater impact on predicting cardiovascular disease events than fasting levels.
Objectives: To examine the effects of consuming a high-fat or a high-carbohydrate meal on the plasma levels of lipids, apolipoproteins, and coagulation and anticoagulation factors.
Methods: In a randomized cross-over study, 12 young non-obese women were served isocaloric meals, predominantly fat (69 E% fat) or carbohydrate (91 E% carbohydrates), on two separate days, and blood samples were taken before and 2.5 and 5.5 h after the meal.
Results: After consuming a high-fat meal, there were significant increases in triglycerides, small dense LDL (sdLDL) cholesterol, and factor VII activity. However, levels of total, LDL, and HDL cholesterol, and apolipoproteins decreased; and levels of total protein S antigen and its activity, free protein S antigen, protein C antigen, and fibrinogen remained unchanged. On the other hand, all these parameters, except for apoC-II and total protein S antigen, decreased after eating a high-carbohydrate meal. Insulin increased postprandially, while glucose levels remained unchanged. Strong positive relationships between triglycerides and the levels of sdLDL cholesterol and apoC-II were observed both at fasting and after consuming meals. However, no correlation was found between triglycerides and coagulation and anticoagulation factors such as factor VII activity and total protein S antigen and its activity.
Conclusions: Consuming a high-fat meal increased factor VII activity during the postprandial elevation of triglycerides and sdLDL cholesterol. However, the activated protein C-dependent anticoagulant system may not regulate this postprandial hypercoagulable state.

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Key Words
High-fat or high-carbohydrate meal, triglycerides, factor VII, protein S, small dense LDL cholesterol

I. Introduction

Plasma lipid profiles, including lipids, lipoproteins, and apolipoproteins, are affected by food intake and show a 24-h cycle. Therefore, they are evaluated in blood drawn after fasting for at least 8 h, which normally only occurs a few hours before breakfast [1]. Elevated low-density lipoprotein (LDL) cholesterol is a well-established independent risk factor for coronary heart disease (CHD) [2]; however, the importance of elevated triglycerides remains to be elucidated. Triglyceride-rich lipoproteins and their remnant lipoproteins are increased in plasma postprandially and may be atherogenic [3] [4]. Prospective cohort studies have reported that elevated levels of nonfasting triglycerides have an equal or greater impact on predicting cardiovascular disease (CVD) events and mortality than fasting levels [5-9].

Postprandial triglyderidemia, particularly after eating

high-fat meals, leads to increased levels of plasma factor VII activity and activated factor VII [10]. In vitro, large very-low-density lipoproteins (VLDL) rather than small VLDL enhance tissue factor (TF)-independent activation of factor VII by factor Xa and factor Xa/Va [11]. In addition, vitamin K-dependent coagulation and anticoagulation factors, such as factors VII, IX, X, prothrombin, protein C, and protein S, as well as C4b-binding protein (C4BP), a complement regulatory protein, have been reported to be associated with triglyceride-rich lipoproteins, chylomicrons, chylomicron remnants, and VLDL, especially after consuming high saturated fat meals; however, there is no association with LDL or high-density lipoprotein (HDL) [12] [13].

Protein S plays an important role in anticoagulation as a cofactor for the activated protein C (APC)-dependent inactivation of factors Va and VIIIa. It also functions as a cofactor for the tissue factor pathway inhibitor (TFPI)-dependent inactivation of factors Xa and TF-VIIa complex [14] [15]. In human plasma, approximately 40% of protein S circulates in its free form, with the remaining 60% of protein S non-covalently binding to C4BP and losing its cofactor activities. Using a cross-sectional study, we previously reported that apolipoprotein (apo) C-II, a critical cofactor of lipoprotein lipase [16], is a significant predictor of fasting total protein S antigen levels in middle-aged obese women, about half of whom exhibited dyslipidemia, as well as in nonobese young women with normolipidemic profiles [17].

Plasma LDL comprises multiple distinct subspecies and has been grouped into four major subclasses based on density, LDL-I through -IV, from the largest, most buoyant to the smallest, most dense [18]. These subspecies differ in their metabolic behavior and pathogenic roles. An elevated level of small dense LDL (sdLDL) particles is accepted as an emerging risk factor for CHD [2] [19]. They are largely formed from the delipidation of triglyceride-rich VLDL catalyzed by lipoprotein lipase and hepatic lipase [18]. Recently, an automated homogeneous assay was developed that accurately measures sdLDL cholesterol [20]. A systematic review and meta-analysis reported a positive association between fasting level of sdLDL cholesterol and CHD [21].

The aim of this study was to investigate the impact of consuming a high-fat meal on the levels of lipids and apolipoproteins, including triglycerides, sdLDL cholesterol, and apo C-II, as well as coagulation and anticoagulation factors such as factor VII and protein S, and to compare the findings with those of an isoenergic high-carbohydrates (low-fat) meal used as a control.

II. Methods

Study subjects and study design

Thirteen healthy Japanese female university students were enrolled in this study. The students were not current smokers, did not have menstruation disorders, nor use oral contraceptives. Gene analysis showed that all subjects were homozygous for the wild-type of protein S Tokushima (p.Lys196Glu, rs121918474), a protein S gene polymorphism prevalent among the Japanese population [22]. One subject dropped out of the study due to feeling sick while consuming a test meal. The basic characteristics (mean ± SD) of the final 12 participants were as follows: age, 21.3 ± 0.5 years; height, 160.8 ± 5.6 cm; weight, 56.6 ± 6.5 kg; BMI, 21.9 ± 2.2 kg/m^2. The present study was performed after approval of the Ethics Committee of Nakamura Gakuen University and written informed consent was obtained from all participants.

The trial was a cross-over study with the order of the meals randomized. The day before each study, the participants were directed to refrain from eating high-fat meals, drinking alcohol, and carrying out intense activity. On the day of the study, all participants consumed an experimental isoenergic high-fat or high-carbohydrate meal after an overnight fast. The meals were served on two separate days (seven to twenty days apart; average: 11 days) at admission to the Health Promotion Center of Nakamura Gakuen University. After measuring weight and height and taking a blood sample, the participants consumed test meals with water under observation between 8:45 and 9:00 in the morning. High-fat meals (total energy 705 kcal: 69 E% fat, 27 E% carbohydrate, and 4 E% protein) consisting of bread, butter, margarine, and pudding, and high-carbohydrate meals (total energy 701 kcal: 2 E% fat, 91 E% carbohydrate, and 5 E% protein) consisting of rice and nonfat agar jelly were consumed. Then the participants were allowed to rest in a sitting position and were instructed not to consume anything except for water (< 500 mL during the study). Blood samples were drawn at 2.5 and 5.5 h after the meal based on a standardized oral fat tolerance test [10], with a slight modification.

Analyses of blood samples

Plasma and serum samples were prepared and stored as reported previously [17]. The analysis of the antigen level and APC-cofactor activity of total protein S was performed by using a total protein S-assay system (Shino-Test Co., Sagamihara, Japan) [23]. Free protein S and protein C antigen were measured using latex agglutination methods (Diagnostica Stago, Inc., New Jersey, USA and LSI Medience. Co., Tokyo, Japan, respectively),

factor VII activity was by a clotting assay (Instrumentation Laboratory Co., Bedford, USA), and fibrinogen by a thrombin-time method (Sysmex Co., Kobe, Japan). Total, LDL, and HDL cholesterol, triglycerides, apoA-I, B (the sum of B-48 and B-100), C-II, C-III, E, glucose, and insulin were measured using previously reported methods [17]. The level of sdLDL cholesterol was assayed by an automated homogeneous assay (Denka Seiken Co., Tokyo, Japan) [20]. Genomic DNA was purified from the buffy coat of citrated blood samples, and the genotype of protein S Tokushima mutation was assessed, as reported previously [23].

Statistical analysis

Kolmogorov-Smirnov test was used to test the normality of data distribution, and all data were found to be normally distributed. Paired Student's t test was used to evaluate the fasting levels of all measures on two different study visits and there was no significant difference. A one-way repeated measures ANOVA followed by multiple comparisons was used to test for differences across time. We performed univariate linear regression analyses to determine whether there were any correlations between fasting values (averaged of two visits), postprandial values, and changes in values during blood sampling, and Pearson's correlation coefficients were calculated. Statistical analyses were performed using PASW Statistics ver. 18 (SPSS Inc., Chicago, IL, USA), and a P value < 0.05 was considered significant.

III. Results

After consuming a high-fat meal, triglycerides significantly increased at 2.5 h and decreased afterward (**Table 1**, **Figure 1**). The level of sdLDL cholesterol increased after 5.5 h; however, total, LDL, and HDL cholesterol, as well as apo A-I, B, C-II, C-III, and E decreased postprandially. Accordingly, the ratio of sdLDL cholesterol to LDL cholesterol showed a significant increase 5.5 h after the meal. A significant increase in factor VII activity was found over time after the meal, whereas the levels of fibrinogen, total PS antigen and its activity, free protein S antigen, and protein C antigen remained unchanged. Insulin levels peaked 2.5 h after meal consumption, while glucose levels remained unchanged. On the other hand,

Table 1 Fasting and postprandial levels of lipids, apolipoproteins, and coagulation and anticoagulation factors.

	High-fat meal				High-carbohydrate meal			
	Fasting	Postprandial		P value[b]	Fasting	Postprandial		P value
		2.5 h	5.5 h			2.5 h	5.5 h	
Triglycerides (mg/dL)	70.7 ± 39.4[a]	124.0 ± 68.4***	97.9 ± 69.6	<0.001	76.2 ± 47.0	67.8 ± 48.7	52.7 ± 37.6***	<0.001
Total cholesterol (mg/dL)	194.4 ± 32.8	187.0 ± 35.2*	188.8 ± 32.9	0.038	191.0 ± 36.9	185.6 ± 31.0	177.9 ± 28.9***	<0.001
LDL cholesterol (mg/dL)	109.8 ± 27.5	102.3 ± 26.4***	104.3 ± 25.9**	<0.001	101.4 ± 28.7	98.5 ± 25.0	94.1 ± 23.6***	<0.001
sdLDL cholesterol (mg/dL)	25.9 ± 12.8	25.6 ± 13.3	28.6 ± 15.3*	0.003	24.6 ± 12.6	22.5 ± 11.2*	20.1 ± 10.0***	<0.001
sdLDL cholesterol/ LDL cholesterol (%)	22.6 ± 6.4	23.9 ± 7.3	26.2 ± 8.4***	<0.001	23.6 ± 7.8	22.2 ± 7.7*	20.6 ± 7.0***	<0.001
HDL cholesterol (mg/dL)	70.7 ± 12.7	65.5 ± 14.2***	65.8 ± 12.2***	<0.001	72.5 ± 15.1	70.7 ± 14.7	68.4 ± 14.7***	<0.001
ApoA-I (mg/dL)	160.8 ± 23.9	154.9 ± 27.7*	156.1 ± 25.1	0.033	167.2 ± 33.6	164.0 ± 33.1	158.8 ± 32.3***	<0.001
ApoB (mg/dL)	79.0 ± 18.7	75.7 ± 19.5*	76.7 ± 18.6	0.012	75.0 ± 18.4	73.2 ± 16.5	69.9 ± 15.8***	<0.001
ApoC-II (mg/dL)	3.2 ± 1.0	3.0 ± 1.0	2.7 ± 0.8**	<0.001	3.2 ± 1.1	3.4 ± 1.1*	3.3 ± 1.1	0.021
ApoC-III (mg/dL)	8.3 ± 2.2	8.1 ± 2.3	7.4 ± 1.9***	<0.001	8.9 ± 2.4	9.2 ± 2.5	8.4 ± 2.3**	<0.001
ApoE (mg/dL)	4.3 ± 0.9	4.1 ± 0.8	3.8 ± 0.8***	<0.001	4.2 ± 0.9	3.9 ± 0.8*	3.5 ± 0.7***	<0.001
Factor VII activity (%)	88.8 ± 19.7	96.1 ± 22.8*	97.9 ± 18.8**	0.005	87.9 ± 21.9	85.7 ± 19.1	77.7 ± 21.5**	0.001
Fibrinogen (mg/dL)	254.8 ± 41.0	251.3 ± 37.5	252.1 ± 38.4	0.598	276.1 ± 41.2	274.5 ± 43.5	264.9 ± 43.4*	0.009
Total protein S antigen (IU/dL)	92.6 ± 7.2	89.8 ± 6.2	90.4 ± 8.1	0.051	90.0 ± 9.2	89.8 ± 8.8	88.3 ± 9.8	0.347
Total protein S activity (IU/dL)	96.1 ± 6.6	94.3 ± 6.7	95.1 ± 8.5	0.321	94.6 ± 10.0	94.3 ± 9.2	92.7 ± 10.0	0.160
Free protein S antigen (%)	103.1 ± 10.9	101.3 ± 12.7	101.3 ± 12.5	0.526	101.0 ± 15.4	98.8 ± 13.7	97.1 ± 12.8*	0.034
Protein C antigen (%)	115.1 ± 26.7	112.3 ± 26.8	113.0 ± 26.1	0.154	113.1 ± 28.5	110.9 ± 27.7	107.2 ± 26.0**	0.008
Insulin (mIU/mL)	5.7 ± 2.0	12.7 ± 6.6**	5.1 ± 2.5	<0.001	6.4 ± 2.4	51.9 ± 21.9***	20.0 ± 9.9	<0.001
Glucose (mg/dL)	89.6 ± 2.8	90.5 ± 13.7	89.0 ± 7.3	0.929	90.7 ± 5.6	90.7 ± 11.5	89.8 ± 11.7	0.967

[a]Mean ± SD, [b]one-way repeated measures ANOVA to test for differences across time. *P<0.05, **P<0.01, ***P<0.001 vs. fasting by multiple comparisons.
Abbreviations, LDL: low-density lipoprotein, sdLDL, small dense LDL; HDL, high-density lipoprotein; Apo, apolipoprotein.

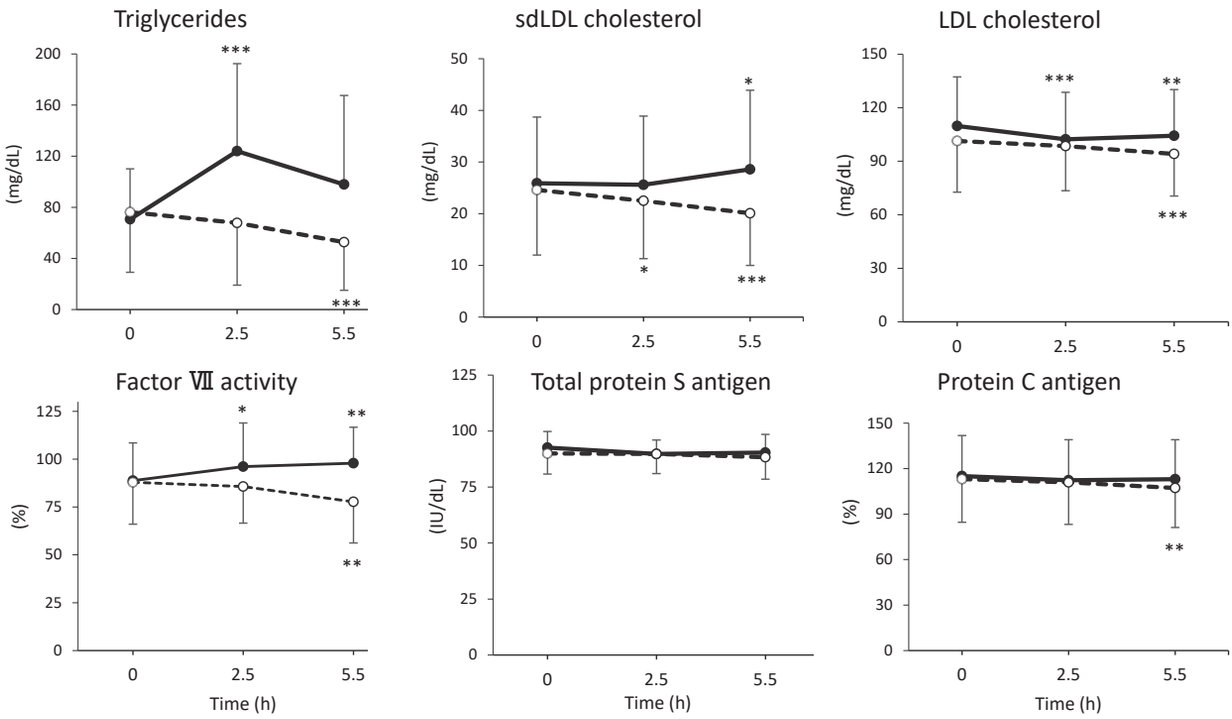

Figure 1 Levels of triglycerides, sdLDL cholesterol, LDL cholesterol, factor VII activity, total protein S antigen, and protein C antigen before and after consuming a high-fat meal (—●—) or a high-carbohydrate meal (--○--). The mean and SD are shown. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. fasting (0 h-value) by multiple comparison.

after consuming a high-carbohydrate meal, all the levels of lipids, apolipoproteins, and coagulation and anticoagulation factors decreased, except for apo C-II and total protein S antigen and its activity. Apo C-II increased 2.5 h after the meal, while total protein S antigen and its activity remained unchanged. Insulin peaked 2.5 h after the meal and the peak was higher than after consuming a high-fat meal, but glucose remained unchanged. The postprandial decrease in total, LDL, HDL cholesterol, and most apolipoprotein may be due to hemodilution [24], because the participants consumed approximately 500 mL of water during the study.

We then conducted univariate linear regression analyses to identify the factors associated with triglycerides (**Table 2**). During fasting, there were strong positive relationships between triglycerides and the levels of sdLDL cholesterol, sdLDL cholesterol/LDL cholesterol ratio, and apo C-II, and these relationships became stronger after consuming a high-fat or high-carbohydrate meal. In contrast, triglyceride levels were positively correlated with total and LDL cholesterol only after consuming a high-fat meal, and with apoB and apo C-III after consuming both high-fat and high-carbohydrate meals. No correlation was found between triglycerides and any other variables, including coagulation and anticoagulation factors such as factor VII activity, protein S antigen and its activity, and protein C antigen in both fasting and postprandial states. Furthermore, correlation analyses did not reveal any statistically significant associations between factor VII activity and the levels of triglycerides or sdLDL cholesterol (data not shown), nor between the postprandial changes in factor VII activity and those in triglycerides and sdLDL cholesterol (**Table 3**).

IV. Discussion

This study demonstrated that consuming a high-fat meal but not a high-carbohydrate (low-fat) meal increased factor VII activity during the postprandial elevation of triglycerides and sdLDL cholesterol. However, total protein S antigen and its APC-cofactor activity, free protein S antigen, and protein C antigen levels did not change postprandially. Strong positive relationships were observed between triglycerides and the levels of sdLDL cholesterol and apoC-II both at fasting and after consuming meals.

Activated factor VII (VIIa) and VIIa-antithrombin complex are reported to significantly increase after eating high-fat meals, indicating postprandial activation of factor VII [10) 25) 26)]. An in vitro study using purified systems reported that large VLDL, rather than small VLDL, enhances TF-independent activation of factor VII by factor Xa and factor Xa/Va [11)]. However, we did not find any significant associations between factor VII activity and the

Table 2 Relationships between triglycerides and the levels of other lipids, apolipoproteins, and coagulation and anticoagulation factors.

			High-fat meal				High-carbohydrate meal			
	Fasting[a]		2.5 h		5.5 h		2.5 h		5.5 h	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value	r	*P* value
Total cholesterol	0.136	0.674	0.559	0.059	0.738	0.006**	0.432	0.161	0.381	0.221
LDL cholesterol	0.140	0.664	0.627	0.029**	0.700	0.011*	0.492	0.104	0.417	0.177
sdLDL cholesterol	0.678	0.015**	0.820	0.001**	0.853	<0.001***	0.876	<0.001***	0.826	<0.001***
sdLDL cholesterol/LDL cholesterol	0.745	0.005**	0.779	0.003**	0.823	0.001**	0.827	<0.001***	0.849	<0.001***
HDL cholesterol	-0.193	0.549	-0.280	0.378	-0.169	0.600	-0.305	0.335	-0.317	0.316
ApoA-I	0.011	0.972	0.057	0.861	0.395	0.204	0.030	0.927	0.002	0.994
ApoB	0.423	0.170	0.746	0.005**	0.743	0.006**	0.746	0.005**	0.670	0.017**
ApoC-II	0.837	<0.001***	0.865	<0.001***	0.824	<0.001***	0.774	0.003**	0.851	<0.001***
ApoC-III	0.518	0.085	0.581	0.048*	0.718	0.009**	0.655	0.021**	0.647	0.023**
ApoE	0.115	0.723	0.430	0.163	0.374	0.232	0.310	0.327	0.078	0.811
Factor VII activity	0.064	0.842	-0.344	0.274	-0.002	0.995	0.042	0.896	-0.072	0.825
Fibrinogen	-0.412	0.183	-0.381	0.222	-0.066	0.838	-0.380	0.223	-0.306	0.333
Total protein S antigen	0.418	0.176	0.493	0.104	0.351	0.263	0.235	0.462	0.122	0.707
Total protein S activity	0.348	0.268	0.432	0.161	0.324	0.304	0.330	0.295	0.238	0.457
Free protein S antigen	0.103	0.750	0.314	0.319	0.316	0.317	0.419	0.175	0.285	0.370
Protein C antigen	0.246	0.440	0.327	0.299	0.463	0.130	0.441	0.152	0.421	0.173
Insulin	0.290	0.361	-0.480	0.115	0.298	0.348	0.243	0.447	-0.029	0.929
Glucose	0.043	0.895	-0.461	0.132	0.452	0.210	0.210	0.512	-0.020	0.951

[a]Fasting levels of all measures were the means of the data obtained at two visits. r, Pearson's correlation coefficient: *P<0.05, **P<0.01, ***P<0.001
Details of the abbreviations used are shown in **Table 1**.

Table 3 Relationships between changes of factor VII activity and those of triglycerides and sdLDL cholesterol during food intakes

	High-fat meal				High-carbohydrate meal			
	FVII:C △ 2.5-0		FVII:C △ 5.5-2.5		FVII:C △ 2.5-0		FVII:C △ 5.5-2.5	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value
Triglycerides △ 2.5-0	-0.396	0.202			0.485	0.110		
Triglycerides △ 5.5-2.5			-0.130	0.687			0.430	0.163
sdLDL cholesterol △ 2.5-0	-0.236	0.460			-0.454	0.138		
sdLDL cholesterol △ 5.5-2.5			0.371	0.236			0.550	0.064

FVII:C, factor VII activity; △ 2.5-0, changes between fasting and 2.5 h postprandial: △ 5.5-2.5, changes between 2.5 h postprandial and 5.5 h postprandial: sdLDL, small dense LDL; r, Pearson's correlation coefficient.

levels of triglycerides or sdLDL cholesterol, nor between changes in factor VII activity and changes in the levels of triglycerides and sdLDL cholesterol during food consumption. Some in vivo studies have shown that factor IX [10] or kallikrein [27] is essential for the postprandial activation of factor VII; however, the detailed mechanisms are not yet understood.

After consuming high saturated fat meals, protein S, protein C, and C4BP as well as vitamin K-dependent coagulation factors such as factors VII have been reported to be associated with triglyceride-rich lipoproteins, chylomicrons, chylomicron remnants, and VLDL [12) 13)]. However, our study found no significant changes in the levels of total protein S antigen and its APC-cofactor activity, free protein S antigen, and protein C antigen levels after a high-fat meal. Protein S plays a critical role in anticoagulation as a cofactor for the APC-dependent and TFPI-dependent pathways [14) 15)]. Previous research has shown that the consumption of high-fat meals has no effect on plasma levels of free TFPI [26) 27)]. Taken together, the APC-dependent and TFPI-dependent anticoagulation pathways may not regulate the postprandial activation of factor VII after consuming high-fat meals.

Our earlier report demonstrated that apoC-II, a critical cofactor of lipoprotein lipase [16)], is a significant predictor of fasting total protein S antigen levels [17)]. In the pres-

ent study, we observed a weak correlation ($r = 0.522$, $P = 0.082$) between total protein S antigen and apoC-II during fasting; however, this correlation disappeared after consuming meals (data not shown). We also found that of all the apolipoproteins, only apoC-II had a significant correlation with triglycerides, both during fasting and after consuming meals. This may explain why apoC-II has become a potential target for developing new drugs to treat hypertriglyceridemia [16].

In line with a previous report [27], we observed that insulin levels were significantly higher after eating a high-carbohydrate meal compared to a high-fat meal; however, factor VII activity decreased after eating a high-carbohydrate meal, suggesting that there was little or no factor VII activation. A study conducted on healthy individuals who were exposed to 24 h of hyperinsulinemia with or without hyperglycemia reported that factor VII activity decreased the most during high glucose/high insulin and to a lesser degree during selective hyperinsulinemia and selective hyperglycemia [28].

Ogita et al. [29] have reported that sdLDL cholesterol of young adults remains unchanged for up to 4 h after consuming a fat-rich test material (17 g fat/m^2 of body surface area), while triglycerides peak at 2 h after the meal. In our study, the amount of fat consumed was an average of 35 fat/m^2 of body surface area, which was twice as much as their study. As a result, consuming meals containing a high amount of fat tends to raise the levels of sdLDL cholesterol. The homogeneous assay of sdLDL cholesterol measures cholesterol in LDL particles with a density of 1.044-1.63 g/mL [20], which corresponds with very small LDL-IV [18]. The sdLDL particles are formed from the lipolysis of large triglyceride-rich VLDL by lipoprotein lipase and hepatic lipase [18]. This may explain why the increase in sdLDL cholesterol was observed 3 h later than the increase in triglycerides after consuming a high-fat meal. As expected, significant correlations were found between triglycerides and sdLDL cholesterol during fasting and after consuming a high-fat or a high-carbohydrate meal. The atherogenic potential of sdLDL includes prolonged plasma residence, increased susceptibility to oxidative modification, and a greater tendency to penetrate into the arterial wall [18, 30]. Therefore, the postprandial elevation of sdLDL cholesterol may contribute to the relationship between elevated levels of nonfasting triglycerides and the prediction of CVD events and mortality [5-9].

The present study had several limitations. First, the study had a small sample size, which may reduce the ability to detect significant changes from fasting to nonfasting conditions and postprandial changes. Second, the study only included young women as subjects. We previously found that the relationship between protein S and apoC-II is much stronger in middle-aged obese women, about half of whom exhibited dyslipidemia, than in young nonobese women [17]. Third, we analyzed only factor VII and fibrinogen as coagulation factors; however, previous studies reported that prothrombin, factors VII, IX, X are associated with triglyceride-rich lipoproteins in both fasting and postprandial plasma [12]. Fourth, we did not analyze TFPI-cofactor activity of protein S.

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Authorship Contributions

H. Tsuda and K. Noguchi designed the research. H. Tsuda, K. Noguchi, and M. M. Kawae performed the study and analyzed the data. H. Tsuda wrote the manuscript. All authors read and approved the final version of the paper.

Disclosure of Conflicts of Interest

The authors declare that they have no conflict of interest.

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