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Circulating PCSK9 concentrations are increased in postmenopausal women with the metabolic syndrome

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ABSTRACT

Background: High PCSK9 concentrations are associated with an increased risk of cardiovascular disease (CVD). We investigated PCSK9 concentrations and their association with metabolic parameters in Thai subjects and to compare PCSK9 concentrations in pre- and postmenopausal women with and without metabolic syndrome (MetS).

Methods: Anthropometric data, serum lipids, fasting blood glucose (FBG), and PCSK9 concentrations were measured in 436 Thai subjects (152 men, 143 premenopausal, and 141 postmenopausal women).

Results: PCSK9 concentrations were significantly higher in women than in men ($p = .002$) and increased in subjects with an increasing number of MetS components (p for trend = .011). PCSK9 concentrations were significantly higher in postmenopausal women than in premenopausal women ($p < .001$), in the MetS group than in the non-MetS group ($p = .037$), and in postmenopausal women with MetS than in premenopausal women without MetS ($p < .001$). Serum PCSK9 concentrations were positively correlated with several metabolic parameters, including age, BMI, systolic blood pressure (SBP), total cholesterol, triglyceride, LDL-C, and FBG.

Conclusion: PCSK9 concentrations are influenced by age, gender, MetS status, and menopausal status among Thai subjects. These findings suggest that an elevation in PCSK9 concentrations may increase cardiovascular risk in postmenopausal women with MetS.

1. Introduction

Proprotein convertase subtilisin/kexin 9 (PCSK9) is a serine protease that plays a key role in cholesterol homeostasis [1]. PCSK9 is highly expressed in the liver, intestine, kidney, and brain [2]. Its catalytic domain binds to the EGF-A domain of low-density lipoprotein (LDLR) receptors and subsequently targets LDLR for lysosomal destruction within the hepatocyte [3]. This process results in reduced LDLR, which decreases the clearance of LDL-C, and consequently, results in an accumulation of circulating LDL-C [4,5]. Gain-of-function (GOF) and loss-of-function (LOF) mutations of the *PCSK9* gene result in hypercholesterolemia and hypocholesterolemia, respectively [6].

PCSK9 has been found to be associated with an increased risk of cardiovascular disease (CVD). PCSK9 may accelerate atherosclerosis and CVD by several mechanisms beyond the degradation of hepatic LDLR [7,8]. Recent studies have demonstrated that PCSK9 might directly promote inflammation, scavenger receptor upregulation, and endothelial dysfunction in atherosclerosis [9–12]. Increased PCSK9 concentrations are associated with the presence and severity of coronary artery calcification in statin-treated asymptomatic familial hypercholesterolemia (FH) patients [13], an increased risk of subclinical carotid atherosclerosis [14], subclinical vascular changes in obese subjects [15], and an increased carotid intima media thickness (IMT) in hypertensive patients [16]. Increased concentrations of PCSK9 were also observed in patients with type 1 diabetes [17], type 2 diabetes [18], obesity [18], metabolic syndrome (MetS) [19,20], postmenopause [21–24], and chronic kidney disease [25]. Moreover, PCSK9 concentrations were found to be positively correlated with age, BMI, total cholesterol, triglyceride, LDL-C, and fasting blood glucose (FBG) concentrations [21,22,26–28]. Serum PCSK9 concentrations in humans among various populations were widely distributed and varied from 11 ng/ml to 10,000 ng/ml [29]. There are many influencing factors related to PCSK9 concentrations, e.g., genetics, ethnicity, diurnal rhythm, exercise, diet, hormones, pregnancy, menopausal status, fasting, and pharmacologic factors [30]. In the Thai population, PCSK9 concentrations and their relationship with metabolic parameters have still not been elucidated.

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Table 1
Anthropometric and biochemical characteristics of the study subjects.

	All subjects (n = 436)	Men (n = 152)	Women (n = 284)	p-value
Age (y)	52.50 ± 13.56	53.52 ± 13.75	51.95 ± 13.46	0.188
BMI (kg/m^2)	23.98 ± 4.39	23.14 ± 3.47	24.42 ± 4.77	0.014
SBP (mmHg)	130.83 ± 19.63	129.74 ± 17.88	131.41 ± 20.50	0.373
DBP (mmHg)	79.36 ± 11.91	78.47 ± 10.00	79.83 ± 12.79	0.224
TC (mg/dl)	205.81 ± 41.30	204.50 ± 39.01	206.51 ± 42.53	0.628
TG (mg/dl)	125.83 ± 59.36	127.20 ± 58.71	125.10 ± 59.79	0.742
HDL-C (mg/dl)	60.58 ± 17.73	59.74 ± 18.68	61.03 ± 17.22	0.571
LDL-C (mg/dl)	120.05 ± 37.76	119.34 ± 36.61	120.43 ± 38.42	0.775
FBG (mg/dl)	104.15 ± 25.70	103.84 ± 24.69	104.32 ± 26.27	0.757
PCSK9 (ng/ml)	84.51 ± 26.42	79.15 ± 25.51	87.38 ± 26.49	0.002
Metabolic syndrome (%)	172 (39.45%)	27 (17.76%)	145 (51.06%)	0.000

TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose; PCSK9, proprotein convertase subtilisin/kexin 9.

2. Materials and methods

2.1. Subjects and sample collection

Four hundred thirty-six participants were randomly selected from the Kiriwong and Bansakha districts of the Nakhon Si Thammarat in Southern Thailand. The participants were healthy volunteers aged 18 to 94 years. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured. The exclusion criteria for subjects included the presence of chronic disease and the use of medicines, such as hormone replacement therapy (HRT), oral contraceptives, antihypertensive agents, lipid-lowering agents, antidiabetic agents, and drug abuse, as well as the use of omega-3 fatty acids. Postmenopause was defined as presenting an absence of menstruation for a preceding 12 months at minimum. Metabolic syndrome (MetS) was diagnosed according to National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) criteria if three of the following five factors were present [31]: (a) Central obesity: waist circumference (WC) ≥ 80 cm in women, WC ≥ 90 cm in men, (b) high triglycerides: triglycerides ≥150 mg/dl, (c) Low HDL-C: HDL-C < 50 mg/dl in women, HDL-C < 40 mg/dl in men, (d) hypertension: SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg, and (e) increased fasting blood glucose: FBG ≥ 100 mg/dl. Written informed consent was obtained from all subjects before being included in the study. The study protocol was approved by the Ethics Committee of Walailak University.

2.2. Laboratory analysis

Blood samples were collected from the subjects after 12 h of fasting. Serum and plasma were separated by centrifugation at 3000 rpm for 10 min. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations were measured using standard enzymatic methods. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula. Fasting blood glucose (FBG) was measured using the glucose oxidase method. All tests were performed by using a Konelab analyzer (Konelab 20). The intra-assay and interassay CVs for TC, TG, HDL-C, and FBG were 2.54% to 4.04%, 1.80% to 4.33%, 2.54% to 4.04%, and 1.40% to 3.90%, respectively. For PCSK9 measurement, serum was collected, aliquoted after overnight fasting and stored at −80 °C. PCSK9 concentrations in serum were measured using a commercially available quantitative sandwich ELISA assay following the manufacturer's instructions (Biolegend). The intra-assay and interassay CVs of PCSK9 concentrations were 5.9% and 7.5%, respectively.

2.3. Statistical analysis

All data were analyzed using SPSS (ver 17). Continuous variables were expressed as the mean and SD. Differences between the two groups were tested using Student's t-test for parametric factors and the Mann–Whitney U test for nonparametric factors. For multiple comparisons of means among groups, one-way ANOVA or the Kruskal–Wallis test was performed. The relationship between PCSK9 and lipid profiles was analyzed by Spearman's correlation. A $p < .05$ was considered statistically significant.

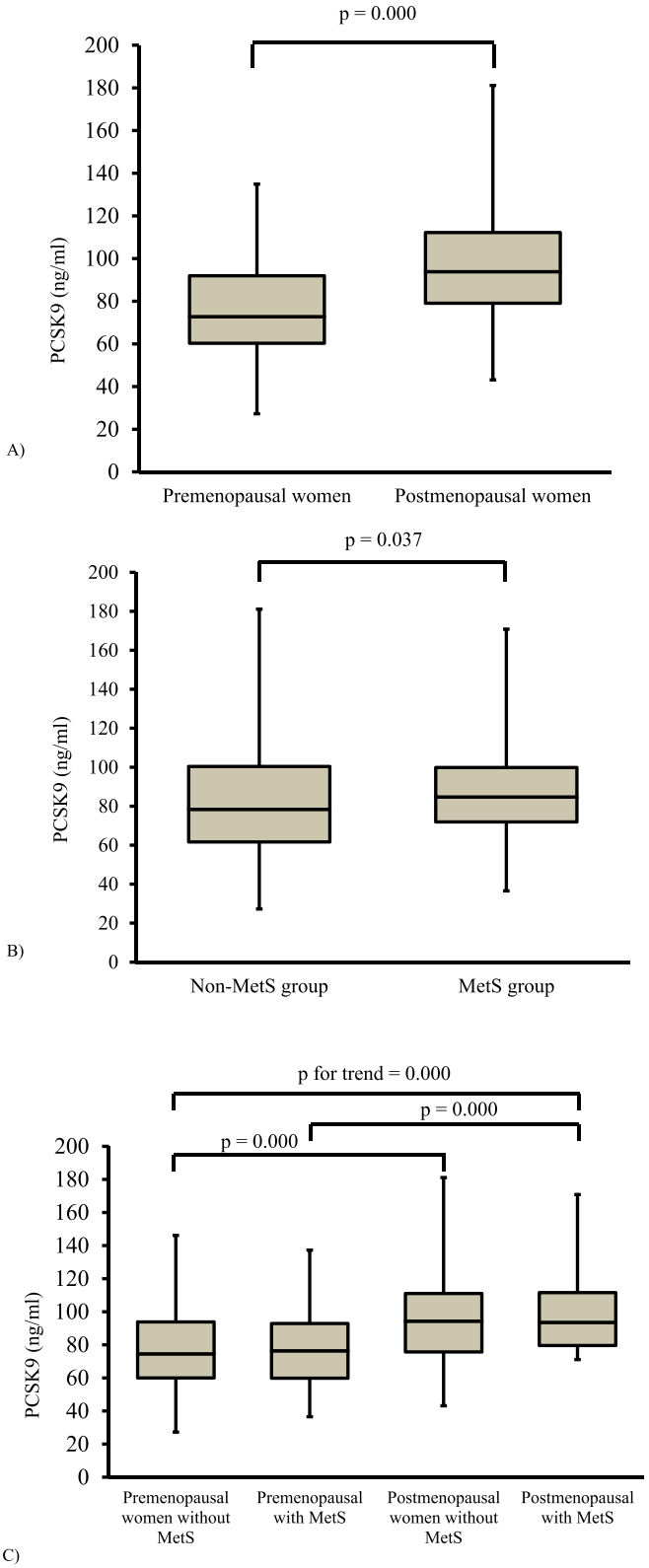
3. Results

3.1. Baseline characteristics

The anthropometric and biochemical characteristics of the study subjects are summarized in Table 1. Serum PCSK9 concentrations ranged from 12.85 to 222.50 ng/ml in the study population. PCSK9 concentrations were significantly higher in women than in men. The prevalence of MetS in men, women, premenopausal women, and postmenopausal women was 17.76%, 51.06%, 46.85%, and 55.32%, respectively (Table 1, Table S1). PCSK9 concentrations were significantly higher in postmenopausal women and the MetS group than in premenopausal women and the non-MetS group, respectively. Moreover, postmenopausal women with and without MetS had significantly higher PCSK9 concentrations than premenopausal women with and without MetS, respectively. Additionally, postmenopausal women with MetS had a significantly higher PCSK9 concentration than premenopausal women without MetS. Furthermore, PCSK9 concentrations were significantly increased in subjects with an increasing number of MetS components (Fig. 1, Table S1–S4). PCSK9 concentrations according to the presence of metabolic components are shown in Fig. 1. The mean PCSK9 concentrations of those with central obesity and high blood pressure were significantly higher than those in subjects with a normal WC and normal blood pressure, respectively. However, the mean PCSK9 concentrations between other normal and abnormal metabolic components were not significantly different.

3.2. Serum PCSK9 concentrations correlate with cholesterol and other metabolic parameters

The Spearman correlation analysis between PCSK9 concentrations and cholesterol and other metabolic parameters is shown in Table 2. PCSK9 concentrations were positively correlated with age, SBP, TC, and FBG in women, but no such relationships were found in men. In addition, PCSK9 concentrations were positively correlated with age, SBP, TC, TG, and/or LDL-C in the MetS group and premenopausal women. PCSK9 concentrations were positively correlated with age and DBP, and/or negatively correlated with BMI in the non-MetS group and postmenopausal women.



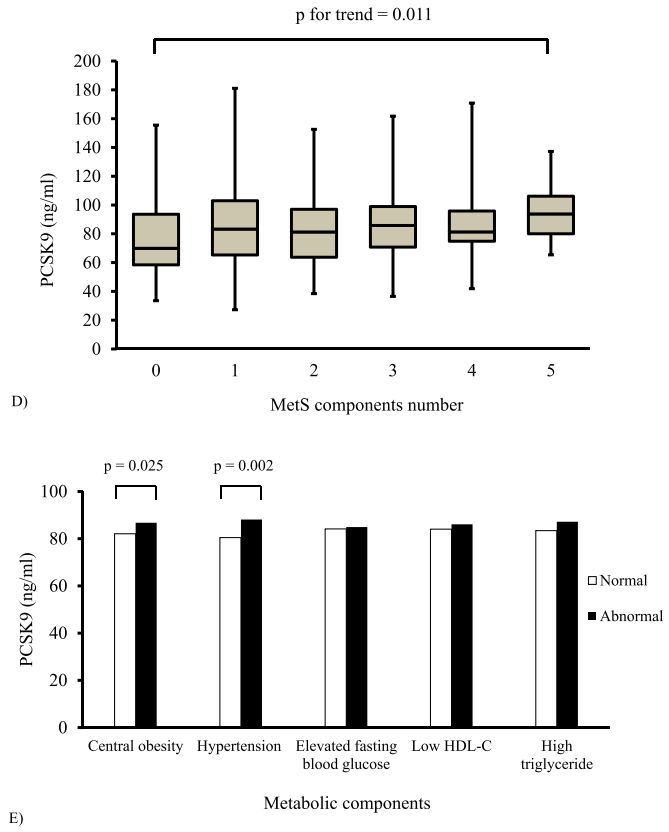


Fig. 1. Serum PCSK9 concentrations in (A) premenopausal and postmenopausal women, (B) the MetS group and non-MetS group, (C) premenopausal and postmenopausal women with and without MetS, (D) the increasing number of metabolic components, and (E) normal and abnormal metabolic components.

Fig. 1. (*continued*)

4. Discussion

The PCSK9 concentrations in this study varied from 12.85 to 222.5 ng/ml, and the mean PCSK9 concentration was 84.51 ± 26.42 ng/ml. The range of PCSK9 concentrations in this study was similar to those in Han Chinese (12.80–222.5 ng/ml) [22], French Canadian (17.6–211.7 ng/ml) [26], and Canadian (35–225 ng/ml) populations [27]. Nevertheless, the range of PCSK9 concentrations was widely different when compared with those in Canadian (420–13,390 ng/ml) [32], U.S. (11–115 ng/ml, 33–2988 ng/ml) [21,33], Australian (100–9300 ng/ml) [34], and France (96–758 ng/ml) populations [35].

In this study, PCSK9 concentrations were significantly higher in women than in men. This finding was consistent with a large Han Chinese population study [22], multiethnic groups from the Dallas Heart Study [21], and a Swedish volunteer study [36]. The gender effect on PCSK9 concentrations was also observed in 1739 French Canadian youth aged 9, 13, and 16 years [26]. Plasma PCSK9 concentrations declined in boys 9–16 years of age, but it peaked at age 13–14 years in girls [26]. In contrast, PCSK9 concentrations were not significantly different in men and women in Canadian subjects [32]. The discrepancy in PCSK9 concentrations among various studies may be due to differences in ethnicity, age, gender, number of study subjects, as well as the different types of PCSK9 antibodies in the detection of PCSK9 concentrations.

Moreover, postmenopausal women had higher PCSK9 concentrations than premenopausal women in this study. This result agreed with a large Han Chinese population study [22], a multiethnic study in the

Table 2
Correlation analyses between PCSK9 concentrations and lipid profiles.

		Age	BMI	SBP	DBP	TC	TG	HDL-C	LDL-C	FBG
All subjects	r	0.267	0.050	0.166	−0.024	0.077	0.120	−0.019	0.069	0.090
	p	0.000	0.302	0.001	0.623	0.107	0.013	0.687	0.152	0.059
Men	r	−0.019	0.077	−0.027	−0.077	0.005	0.130	−0.139	0.041	0.009
	p	0.813	0.347	0.743	0.350	0.950	0.110	0.088	0.616	0.914
Women	r	0.460	0.007	0.257	−0.002	0.118	0.113	0.046	0.088	0.138
	p	0.000	0.908	0.000	0.969	0.046	0.057	0.438	0.138	0.020
MetS group	r	0.212	0.050	0.157	0.042	0.147	0.145	−0.041	0.141	−0.005
	p	0.001	0.417	0.011	0.503	0.017	0.018	0.511	0.022	0.934
Non-MetS group	r	0.326	−0.145	0.079	−0.203	−0.072	−0.037	0.096	−0.098	0.133
	p	0.000	0.058	0.310	0.008	0.345	0.627	0.211	0.202	0.082
Premenopausal women	r	0.240	0.207	0.195	0.123	0.196	0.174	−0.002	0.158	0.093
	p	0.004	0.013	0.021	0.145	0.019	0.038	0.982	0.059	0.267
Postmenopausal women	r	0.213	−0.177	0.132	−0.062	−0.062	0.019	0.050	−0.088	0.005
	p	0.011	0.036	0.123	0.471	0.462	0.826	0.552	0.297	0.953

SDB, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; FBG, fasting blood glucose.

Dallas Heart Study [21], and Canadian [23], Chinese [24], and Swedish populations [36]. This may be related to the decreased estrogen during menopause. Previous studies demonstrated a negative correlation between estrogen and PCSK9 in women in Sweden [36] and Canada [23]. Additionally, high-dose ethinylestradiol was shown to decrease PCSK9 gene expression by 45% in rats [37]. An increased endogenous estrogen was also associated with reduced serum PCSK9 in women scheduled for *in vitro* fertilization [38]. In contrast, PCSK9 concentrations were not correlated with estrogen concentrations in 727 healthy women [24], and estrogen replacement therapy did not have any effect on circulating PCSK9 [21,23]. In addition, estradiol did not significantly alter PCSK9 expression in HepG2 [24] and HuH7 cells [39]. Nevertheless, estradiol treatment of HuH7 cells resulted in decreased phosphorylation of secreted PCSK9 [39]. This estradiol-induced posttranslational modification of PCSK9 may affect PCSK9 function. High concentrations of phosphorylated PCSK9 have also been correlated with insulin resistance [40]. Therefore, the correlation between PCSK9 and estrogen remains controversial. The underlying mechanism of the regulation of PCSK9 by estrogen should be further investigated.

In this study, PCSK9 concentrations were higher in the MetS group than in the non-MetS group. PCSK9 concentrations were also increased in subjects with an increasing number of MetS components. These findings agreed with studies in Kenyan [19] and Spanish [20] populations. In addition, central obesity and abnormal blood pressure had an effect on PCSK9 concentrations compared with the other components in the present study. Previous evidence has supported that PCSK9 may be either a cause or an effect of MetS. Although the molecular mechanism of PCSK9 on MetS is still not completely elucidated, several plausible explanations have been proposed. An *in vivo* study demonstrated that PCSK9 was regulated by a proinflammatory cytokine, TNF-α, in a SOCS3-dependent manner [41]. PCSK9 concentrations were positively correlated with C-reactive protein (CRP) concentrations in women but not in men [21]. Resistin and leptin, adipokines that are increased in obesity, were found to suppress LDLR and LDL uptake and elevate PCSK9 expression through enhanced HNF1α expression and the increased activation of the p38MAPK pathway [42]. These results suggest that PCSK9 may be related to MetS through the regulation by inflammatory cytokines and adipokines.

Additionally, a previous study showed that PCSK9 induced the degradation of epithelial sodium channels (ENaC) *in vitro* in kidney cells *via* the proteasome pathway [43], and this may inhibit ENaC-mediated Na^+ absorption and alter blood pressure [43]. Some rare PCSK9 variants were found to influence blood pressure among African Americans [44]. In contrast, PCSK9 KO mice displayed normal sodium balance and blood pressure regulation [45]. Thus, the mechanism of PCSK9 concentrations on hypertension should be further elucidated. Finally, the Dallas Heart Study reported a significant correlation between PCSK9 and FBG, insulin concentration, and a homeostasis model assessment of insulin resistance (HOMA-IR) [21]. A gain-of-function mutation of *PCSK9* resulted in the overproduction of apoB100, VLDL, IDL, and LDL in humans [46]. Likewise, overexpression of PCSK9 was found to increase hepatic VLDL production during fasting [47] and increased apoB100, apoB48, and TG in plasma in mice [48]. This indicates that PCSK9 may alter TG and glucose metabolism.

In the present study, PCSK9 concentrations were positively associated with age, SBP, and TG in all subjects. However, PCSK9 concentrations were positively correlated with age, SBP, and serum lipids in women, those with MetS, and premenopausal women but not in men, those without MetS, and postmenopausal women. This suggests that the relationship between PCSK9 and anthropometric and biochemical data may be different among various metabolic conditions. The results of this study appeared to contradict previous studies in which PCSK9 concentrations were positively correlated with serum lipids in men in Canada [32] and in both men and women in Sweden [36]. Moreover, several previous studies have shown that PCSK9 concentrations were significantly correlated with age, BMI, SBP, DBP, LDL-C, TG, FBG, and fasting insulin in 3138 participants in the Dallas Heart Study [21]. Serum PCSK9 concentrations were also positively correlated with age, BMI, TC, LDL, TG, and FBG in a large population of 2719 Han Chinese [22]. Moreover, there were positive associations between PCSK9 and TC, LDL-C, TG, HDL-C, apoA1, and apoB in 1739 French Canadian youths in the Quebec Child and Adolescent Health and Social Survey [26]. In addition, plasma PCSK9 correlated positively with age, BMI, TC, LDL-C, TG, and FBG in 254 healthy Canadians [27]. Finally, positive correlations of plasma PCSK9 with TC, LDL-C, non-HDL-C, and TG concentrations were also observed in 52 Caucasian participants in the Netherlands [28].

There was no association between PCSK9 and LDL-C in all subjects in this study. Our results were consistent with adult females in the UAE [49], healthy subjects and T2DM patients in Tunisia [50], and the subjects in Canada [32], in which there was no association between PCSK9 and LDL-C. This may be explained by the degradation of LDLR-mediated PCSK9 in both intracellular and extracellular pathways [51]. However, only the secreted PCSK9 in the extracellular pathway correlates with the concentrations of plasma cholesterol [33]. In addition, the PCSK9 concentrations in this study were detected by sandwich ELISA, which was measured in both mature and furin-cleaved PCSK9 forms. Thus, the total forms of PCSK9 were not surrogated by active PCSK9 [52]. A previous study demonstrated that the furin-cleaved form, a biologically inactive form, was not significantly correlated with oxLDL-C [53]. Moreover, other forms of PCSK9, such as mutated and posttranslational modifications [40] that could not be discriminated by ELISA, may alter PCSK9 function and eliminate the correlation between PCSK9 and LDL-C. Furthermore, the interaction between PCSK9

concentrations and LDL-C may be influenced by dietary intake. Cariou et al. reported that the increased concentrations of PCSK9 under a high fructose diet were not associated with an increase in LDL-C [54]. Similarly, Rodriguez-Perez et al. demonstrated no correlation between PCSK9 concentrations and LDL-C in subjects who consumed Canola oil, CanolaDHA, or CanolaOleic [55].

There were some limitations to this study. LDL-C was not directly measured but it was calculated using the Friedewald formula. A previous study showed that the accuracy of the Friedewald formula decreases when triglyceride concentrations are > 200 mg/dl [56]. In addition, dietary intake was not assessed, and estrogen concentrations were not measured. In conclusion, PCSK9 concentrations are influenced by age, gender, MetS, and menopausal status among Thai subjects. These findings also suggest that an elevation of PCSK9 concentrations may increase cardiovascular risk in postmenopausal women with MetS.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.04.067.

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