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ORIGINAL ARTICLE

Transcription factor 7-like 2 single nucleotide polymorphisms are associated with lipid profile in the Balinese

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Abstract

Transcription factor 7-like 2 (TCF7L2) protein plays an important role in glucose and lipid metabolisms. Single nucleotide polymorphisms (SNPs) in the TCF7L2 gene contribute to increased fasting plasma glucose (FPG) and body mass index (BMI), and altered lipid concentrations in various population. We investigated whether the TCF7L2 SNPs were associated with obesity, high FPG and altered lipid profile in the Balinese. A total of 608 Balinese from rural and urban Bali, Indonesia, were recruited. Triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and FPG were measured, and BMI was calculated. Ratios of TG/HDL-C, LDL-C/HDL-C, and TC/HDL-C were determined. Genotyping of SNPs rs7903146, rs10885406, and rs12255372 were done in all samples. Genetic association analyses under a dominant model showed that the rs7903146 (OR 5.50, 95% CI 2.34–12.91, $p = 8.5 \times 10^{-5}$), rs12255372 (OR 4.15, 95% CI 1.66–10.33, $p = 0.003$) and rs10885406 (OR 2.43, 95% CI 1.39–4.25, $p = 0.003$) were significantly associated with high TC/HDL-C ratio. The rs10885406 also presented a significant association with high TG (OR 2.21, 95% CI 1.29–3.81, $p = 0.004$) and low HDL-C (OR 3.02, 95% CI 1.58–5.80, $p = 0.001$) concentrations, as well as high TG/HDL-C ratio (OR 1.95, 95% CI 1.16–3.27, $p = 0.013$). None of the SNPs exhibited significant association with obesity or high FPG. SNPs in the TCF7L2 gene are associated with altered lipid profile in the Balinese.

Keywords TCF7L2 · SNPs · Genetic association · Lipid profile

Introduction

Transcription factor 7-like 2 (TCF7L2) gene has been consistently reported as a significant genetic determinant conferring the risk of type 2 diabetes mellitus (T2DM) [1], cardiovascular

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diseases (CVDs) [2], and metabolic syndrome [3]. This gene encodes a high mobility group (HMG) box-containing transcription factor that mediates activation of canonical Wingless-type (Wnt) signaling pathway [4]. Wnt signaling regulates the expression of target genes involved in metabolic process, including glucose and cholesterol metabolisms [5], adipogenesis [6], and lipid homeostasis [7].

Given the crucial roles of Wnt pathway/TCF7L2 in metabolisms, single nucleotide polymorphisms (SNPs) in the TCF7L2 gene located on chromosome 10q25.2-q25.3 have been associated with several metabolic traits. The rs7903146 and rs12255372 were associated with elevated fasting plasma glucose (FPG) and T2DM in different populations [8, 9]. Associations between the rs12255372 and body mass index (BMI) and lipid concentrations were confirmed in Mexicans [10]. In North Indian population, it is also confirmed that the rs7903146, rs12255372, and rs10885406 contributed to obesity and lipid concentrations [11]. Nevertheless, the other studies demonstrated conflicting results and heterogeneity between populations [12, 13]. Therefore, more investigations on the SNPs effects across different populations are required to uncover their contributions on altered metabolic profile.

Population of Bali Islands, Indonesia, has undergone a transition from subsistence farming towards the adoption of western dietary pattern (excessive intake of red and processed meats and refined carbohydrates), and a reduction of physical activities, due to the growing tourism sector over the last 3 decades [14]. Increased prevalences of obesity, metabolic syndrome, and T2DM have been reported in this population, particularly in the urban and tourism destination areas [15–17]. In this study, we investigated the genetic association between TCF7L2 SNPs (rs7903146, rs12255372, and rs10885406) and obesity, increased FPG, and altered lipid profile in the Balinese. Obesity, impaired fasting glucose and altered lipid profile are closely linked to one another, which collectively contribute to increased cardio-metabolic risks [18, 19]. Identification of genetic risk factors associated with these metabolic traits may provide important insights into underlying pathological mechanisms and diagnosis of non-communicable diseases in the Balinese.

Methods

Subjects and biochemical measurements

We conducted a cross-sectional study and recruited a total of 608 subjects from rural (327 subjects) and urban (281 subjects) areas of Bali, Indonesia, during 2008 until 2011, with informed consent. Ethical approvals for this study were granted by the Eijkman Institute Research Ethics Commission (number 32 on October 27, 2008), and by the Faculty of Medicine Ethic Committee, Udayana University (number 690a/SKRT/X/2010 on October 28, 2010) [15, 16]. The rural populations were from Penglipuran, Pedawa and Nusa Ceningan villages, while the urban population was from Legian village (Fig. 1). According to Badan Pusat Statistik (BPS/Statistics Indonesia), an urban village should have administrative areas which satisfies certain criteria of population density, percentage of agricultural households, and a number of urban facilities such as roads, formal education facilities, public health services, telephones, public electricities, cinemas, markets, etc. [20].

Demographic and anthropometric data were collected, including age, sex, height and weight. BMI was calculated as body weight in kilograms divided by the square of height in meters. Fasting plasma glucose (FPG) and serum lipid concentrations [triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C) and total cholesterol (TC)] were examined from peripheral blood, drawn after at least 10 h of overnight fasting. FPG was measured using the standard hexokinase method, while fasting serum lipids were measured by employing standard spectrophotometric methods, as previously described [21]. Ratios of TG to HDL-C (TG/HDL-C), LDL-C to HDL-C (LDL-C/HDL-C) and TC to HDL-C (TC/HDL-C) were calculated.

Metabolic parameters were categorized into binary traits (yes/no) according to previously published cut-offs, as follows: obesity (BMI $\geq$ 25 kg/m^2) [22], high FPG ($\geq$ 100 mg/dL) [23], high TG ($\geq$ 150 mg/dL) [24], high LDL-C ($\geq$ 130 mg/dL) [24], low HDL-C ($<$ 40 mg/dL) [24], high TC ($\geq$ 200 mg/dL) [24], high TG/HDL-C ratio ($>$ 3.5 for male or $>$ 2.5 for female) [25], high LDL-C/HDL-C ratio ($>$ 3.5 for male or $>$ 3.0 for female) [26] and high TC/HDL-C ratio ($>$ 5.0 for male or $>$ 4.5 for female) [26].

DNA extraction and genotyping

Genomic DNA was extracted as previously described [16]. The rs7903146, rs12255372, and rs10885406 were detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Detection of rs7903146 was performed using a previously published PCR-RFLP method [27], while genotyping assays of rs12255372 and rs10885406 were performed using novel PCR-RFLP methods. The primer pairs were designed using PerlPrimer® [28] and BioEdit® Sequence

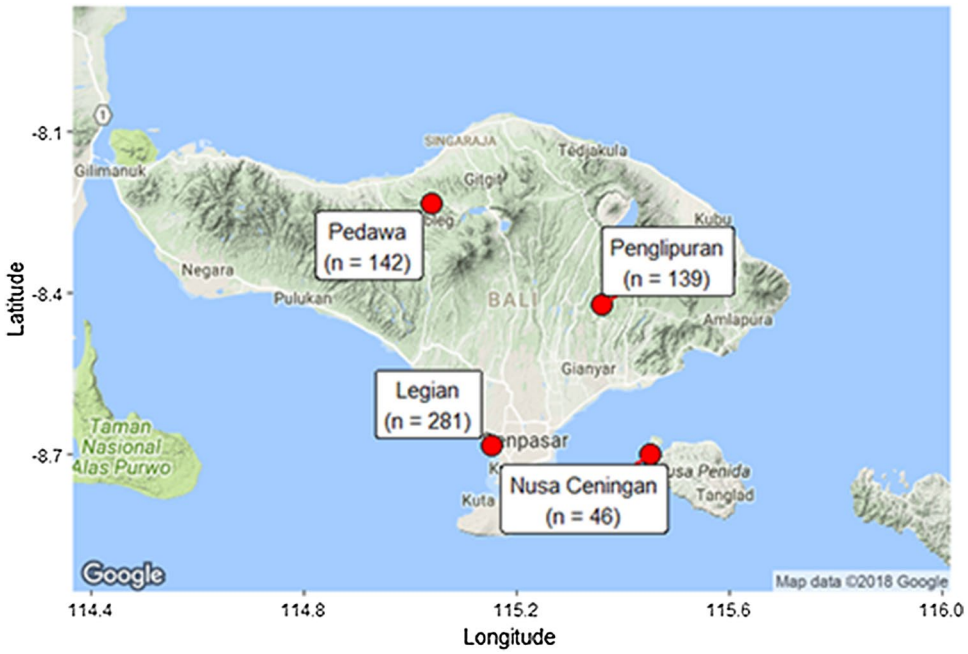

Fig. 1 Map of sampling sites across Bali, Indonesia. Samples were collected from rural (Pedawa, Penglipuran, and Nusa Ceningan) and urban (Legian) villages. The map was generated from Google Static Maps using the "ggmap" and "ggrepel" packages in RStudio

Alignment Editor (Ibis Bioscience, Carlsbed, CA, USA). Primer sequences and restriction enzymes are provided in Additional Table. PCR was performed using GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA), and followed by restriction enzyme digestions (New England BioLabs, Ipswich, MA, USA) at 37 °C for rs7903146 and rs10885406 assays or at 65 °C for rs12255372 assay. Restriction products were resolved on 2.5% agarose gel electrophoresis (Lonza, Basel, Switzerland). Variant alleles were confirmed by DNA sequencing using BigDye® Terminator v.3.1 Cycle Sequencing Kits, with ABI 3130xl Genetic Analyzer (Applied Biosystem).

Statistical analyses

Statistical analyses were carried out in R version 3.4.3 (http://www.r-project.org) with RStudio version 1.0.423 (http://www.rstudio.com). A map of sampling sites was generated from the Google Static Maps using the "ggmap" and "ggrepel" packages. Continuous variables are presented as median (interquartile range) and compared using the Wilcoxon–Mann Whitney U test. All SNPs were tested for departure from Hardy–Weinberg equilibrium (HWE) using the Fisher's exact test. The similar test was employed to compare the genotype distribution between populations. Linkage disequilibrium (LD) between SNP pairs was calculated using D' and r^2 statistics, as implemented in the "genetics" package.

Genetic associations with metabolic traits were assessed using the likelihood ratio test and presented in adjusted odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs), as implemented in the "SNPassoc" package [29]. Analyses were adjusted for age, sex (male/female), population (rural/urban), with the addition of high FPG when obesity was the outcome, or adjusting for obesity when high FPG was the outcome. Genetic association analyses for lipid profile were adjusted for age, sex, population, obesity and high FPG. The significant level was set at 0.017, following the Bonferroni correction (p value = 0.050/three SNPs) [30]. Due to the low frequency of the minor alleles, only dominant model was considered for genetic association analyses. Power calculation was performed using the "GeneticsDesign" package [31].

Results

General characteristics of the subjects

Table 1 shows the distinctive characteristics of the rural and urban Balinese. Subjects in rural areas were significantly older in age and had a higher FPG concentration compared with subjects in urban areas (all $p < 0.001$). Whereas, urban population had a significantly higher BMI ($p < 0.001$) and TG concentration ($p = 0.003$), a lower HDL-C concentration ($p = 0.004$), as well as more elevated ratios of TG/

Table 1 Characteristic of studied subjects stratified by population

Variable	All (n = 608)	Rural (n = 327)	Urban (n = 281)	p^a (rural versus urban)
Male/female	343/265	168/159	175/106	
Age (years)	46.0 (40.0–57.3)	50.0 (40.0–63.0)	44.0 (39.0–52.0)	**< 0.001**
BMI (kg/m^2)	23.5 (20.9–26.5)	22.0 (19.2–24.8)	25.3 (22.9–27.8)	**< 0.001**
FPG (mg/dL)	92.0 (85.0–100.0)	94.0 (87.0–102.0)	89.0 (83.0–96.0)	**< 0.001**
TG (mg/dL)	119.0 (87.0–171.2)	112.0 (85.5–158.0)	131.0 (88.0–184.0)	**0.003**
LDL-C (mg/dL)	118.0 (99.0–142.0)	121.0 (101.0–141.5)	115.0 (96.0–142.0)	0.227
HDL-C (mg/dL)	51.0 (44.0–58.0)	52.0 (45.0–61.0)	50.0 (42.0–57.0)	**0.004**
TC (mg/dL)	198.5 (173.0–223.2)	200.0 (175.0–221.5)	197.0 (172.0–225.0)	0.786
TG/HDL-C ratio	2.9 (1.5–3.7)	2.2 (1.4–3.4)	2.7 (1.6–4.0)	**0.001**
LDL-C/HDL-C ratio	2.3 (1.8–2.9)	2.3 (1.8–2.9)	2.4 (1.9–2.9)	0.303
TC/HDL-C ratio	3.9 (3.2–4.7)	3.7 (3.1–4.6)	4.0 (3.3–4.8)	**0.008**

Data are presented in median (interquartile range)

BMI body mass index, *FPG* fasting plasma glucose, *TG* triglyceride, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TC* total cholesterol.

[a]Wilcoxon–Mann Whitney U test. The significant *p* values after Bonferroni correction are indicated in bold ($p < 0.017$)

HDL-C ($p = 0.001$) and TC/HDL-C ($p = 0.008$) than rural population.

Genotypic and allelic distributions, Hardy–Weinberg disequilibrium, and linkage disequilibrium

The minor allele frequency (MAF) for rs7903146, rs12255372, and rs10885406 SNPs was 0.02, 0.02, and 0.06, respectively. The homozygous genotypes of rs7903146 and rs12255372 were more prevalent in urban than in rural (all $p < 0.050$). The distributions of all SNPs were in HWE for total, urban, and rural populations (all $p > 0.050$) (Table 2). High LD ($D' > 0.75$) was detected for the following TCF7L2 SNPs: rs7903146 and rs10885406 ($D' = 0.96$ and $R^2 = 0.54$), rs12255372 and rs10885406 ($D' = 0.89$ and $R^2 = 0.47$), and rs7903146 and rs12255372 ($D' = 0.76$ and $R^2 = 0.69$).

Genetic associations with metabolic traits

The three SNPs did not show any significant associations with obesity and high FPG concentration (Table 3). All SNPs presented significant associations with high TC/HDL-C ratio (Table 4). The adjusted ORs for minor allele carriers were 5.50 (95% CI 2.34–12.91, $p = 8.5 \times 10^{-5}$, power = 0.70) for rs7903146, 4.15 (95% CI 1.66–10.33, $p = 0.003$, power = 0.55) for rs12255372, and 2.43 (95% CI 1.39–4.25, $p = 0.003$, power = 0.22) for rs10885406. The rs10885406 was also associated with high TG (OR 2.21, 95% CI 1.29–3.81, $p = 0.004$, power = 0.20) and low HDL-C (OR 3.02, 95% CI 1.58–5.80, $p = 0.001$, power = 0.24) concentrations, as well as high TG/HDL-C ratio (OR 1.95, 95% CI 1.16–3.27, $p = 0.013$, power = 0.17). None of the SNPs showed any significant associations with high LDL-C, high TC, or high LDL-C/HDL-C ratio (Table 4).

Discussion

The present study found that the urban Balinese presented more altered metabolic profile related to obesity and lipid profile, which could be contributed by their lifestyle shifts toward more intakes of processed food and sedentary lifestyle. Regardless of the difference in environmental and genetic factors between rural and urban populations, our study demonstrated the contributions of TCF7L2 SNPs on high TG and low HDL-C concentrations, as well as high ratios of TG/HDL-C and TC/HDL-C in the Balinese.

The rs7903146, rs12255372, and rs10885406 SNPs were reported as common polymorphisms in Europeans and Asian Indians, with the MAFs ranging from 0.46 to 0.57 [11, 32]. In the Balinese, rs7903146 and rs12255372 were found in much lower frequencies (both 0.02), but their MAFs were comparable with Japanese (0.03–0.05) [33] and Chinese (0.02–0.06) [34] populations, confirming that these SNPs were not commonly found in East-Asian descendants. To date, the rs10885406 has not been extensively characterized

Table 2 Genotypes, allele frequencies and Hardy–Weinberg equilibrium of TCF7L2 SNPs

SNPs	All (n = 608)	Rural (n = 327)	Urban (n = 281)	p^a (rural versus urban)
rs7903146				
CC	0.96	0.98	0.93	**0.003**
CT	0.04	0.02	0.07	
TT	NA	NA	NA	
MAF	0.02	0.01	0.03	
p value of HWE test[a]	1.000	1.000	1.000	
rs12255372				
GG	0.96	0.98	0.94	**0.006**
GT	0.04	0.02	0.06	
TT	NA	NA	NA	
MAF	0.02	0.01	0.03	
p value of HWE[a]	1.000	1.000	1.000	
rs10885406				
AA	0.88	0.89	0.87	0.339
AG	0.12	0.11	0.12	
GG	0.003	NA	0.01	
MAF	0.06	0.06	0.09	
p value of HWE[a]	1.000	0.611	0.362	

MAF minor allele frequency, *HWE* Hardy–Weinberg equilibrium, *NA* not available

[a]Fisher's exact test. The significant *p* values after Bonferroni correction are indicated in bold ($p < 0.017$)

in Asian populations. However, considering that rs10885406 exists in high LD with the two other SNPs, it is expected that its MAF was also low in our population (0.06).

Among the three SNPs, the rs10885406 was found to be associated with high TG and low HDL-C concentrations. The rs10885406 association with elevated TG concentrations has been previously reported in Khatri Sikhs population of North India [11]. However, they found no notable impact of rs10885406 on HDL-C concentration [11], which was in contrast to our finding. Differences in genetic backgrounds, lifestyles, diets, and risk factors for dyslipidemia may have accounted for these discrepancies between populations.

Our findings added new information about the contribution of TCF7L2 SNPs on elevated ratios of TG/HDL-C and TC/HDL-C. We found that the rs10885406 was associated with high ratios of TG/HDL-C and TC/HDL-C, which were likely influenced by the SNP association with increased TG and lowered HDL-C concentrations. Interestingly, despite their lack of association with both TC and HDL-C concentrations, the rs7903146 and rs12255372 SNPs were associated with high TC/HDL-C ratio. Considering that these SNPs were in a strong LD, they might have similar effects on TC/HDL-C ratio. TG/HDL-C and TC/HDL-C ratios are atherogenic indices that associate with insulin resistance and increased cardio-metabolic risks [26]. The impacts of these TCF7L2 SNPs on lipid ratios suggest their involvements in increasing atherogenic potential, which may further link to their established associations with increased CVD risk [2].

To date, the underlying mechanism of the influence of TCF7L2 SNPs on lipid profile has not been fully understood. Studies have indicated the role of TCF7L2 protein in regulation of gene expressions important for lipid metabolisms in the hepatocytes [35], and cholesterol biosynthesis in the oligodendrocytes [36]. In addition, rs7903146 was found to be associated with altered postprandial TG response that was independent of insulin [37]. These SNPs located within the intronic regions are thought to modify alternative splicing mechanisms, resulting in the changes of mRNA variants which may excrete distinct physiological roles in activation of Wnt signaling [38]. Kaminska et al. identified an mRNA transcripts lacking of exon 12, 13 and 13a was associated with increased free fatty acid (FFA) and impaired insulin action in adipose tissue. Nevertheless, they found no notable impact of rs7903146 on the changes of TCF7L2 splicing pattern [39]. Elucidation of the role of the TCF7L2 SNPs in splicing mechanisms is further complicated by the presence

Table 3 Results of adjusted likelihood ratio test for associations of TCF7L2 SNPs with obesity and high fasting plasma glucose

Traits	rs7903146					rs12255372					rs10885406				
	CC (%)	CT (%)	OR (95% CI)	*p*	Power[c]	GG (%)	GT (%)	OR (95% CI)	*p*	Power[c]	AA (%)	AG + GG (%)	OR (95% CI)	*p*	Power[c]
Obesity[a]															
Yes (n = 221)	93.2	6.8	1.90 (0.80–4.51)	0.140	0.31	94.6	5.4	1.70 (0.67–4.30)	0.257	0.25	89.1	10.9	0.80 (0.46–1.38)	0.419	0.06
No (n = 387)	97.4	2.6				97.7	2.3				87.6	12.4			
High FPG[b]															
Yes (n = 156)	94.2	5.8	2.20 (0.89–5.41)	0.097	0.12	95.5	4.5	1.99 (0.74–5.38)	0.187	0.09	86.5	13.5	1.39 (0.78–2.47)	0.273	0.06
No (n = 452)	96.5	3.5				96.9	3.1				88.7	11.3			

Criteria Obesity (BMI ≥ 25 kg/m^2) [22], high FPG (≥ 100 mg/dL) [23]

OR odds ratio, *95% CI* 95% confidence intervals, *BMI* body mass index, *FPG* fasting plasma glucose

[a]Adjusted for age, sex, population (rural/urban), and high FPG

[b]Adjusted for age, sex, population (rural/urban), and obesity

[c]Statistical power

of tissue-specific pattern of alternative splicing [40]. Further study is warranted to understand the mechanism of action of these SNPs on lipid metabolism.

The current study had several limitations. Considering the relatively small sample size that is obtained from one population, the results need be interpreted with cautions and are limited to the Balinese population only. As we did not detect the significant associations with obesity and high FPG, the influences of TCF7L2 SNPs on these traits have to be confirmed in further studies involving larger sample size to improve the statistical power. Our study only considered BMI but did not include waist circumference and waist-to-hip ratio (WHR), although these are also important obesity-related phenotypes that have been previously studied in genetic association studies [41, 42], which also limit the comprehensive interpretation of the result. As for T2DM, we only measured FPG, but did not include fasting insulin level as a T2DM-related continuous phenotype, which has been previously studied [43, 44], therefore the lack of association should be interpreted cautiously. Furthermore, the present study only examined blood glucose and lipid profile in the fasting state, thus the impacts of the SNPs on postprandial response were not evaluated. We previously reported that the rs7903146 was associated with elevated postprandial blood glucose concentration in a limited urban Balinese from Legian village [45]. However, this finding could not be confirmed in this study, since the measurement was not performed in the rural populations. More investigations are warranted to reveal the role of TCF7L2 gene polymorphisms on alteration of metabolic profiles on both fasting and postprandial conditions.

Another limitation of this study is the lack of dietary intakes assessment. Dietary factors such as dietary fat intake could also play an important role in T2DM etiology, and gene-diet interaction could be present in T2DM pathogenesis, as reported by Bodhini et al. which showed a significant interaction between rs12255372 and fat intake on HDL-C concentration [46]. Therefore, further studies are needed to investigate gene-diet interaction that may contribute to pathogenesis of obesity and T2DM.

In summary, our study demonstrated the association of TCF7L2 SNPs (rs7903146, rs12255372, and rs10885406) with high TC/HDL-C ratio. We also showed that the rs10885406 was associated with high TG and low HDL-C concentrations, as well as high TG/HDL-C ratio. Our findings highlight the influence of TCF7L2 SNPs on altered lipid profile in the Balinese, which may further link to the risk of CVDs.

Table 4 Results of adjusted likelihood ratio test for associations of TCF7L2 SNPs with lipid profile

Traits	rs7903146					rs12255372					rs10885406				
	CC (%)	CT (%)	OR (95% CI)	*p*	Power[a]	GG (%)	GT (%)	OR (95% CI)	*p*	Power[a]	AA (%)	AG + GG (%)	OR (95% CI)	*p*	Power[a]
High TG															
Yes (n = 200)	92.5	7.5	2.14 (0.90–5.11)	0.084	0.40	94.5	5.5	1.66 (0.65–4.24)	0.291	0.24	83.0	17.0	2.21 (1.29–3.81)	**0.004**	0.20
No (n = 408)	97.5	2.5				97.5	2.5				90.7	9.3			
High LDL-C															
Yes (n = 232)	95.3	4.7	1.23 (0.54–2.80)	0.628	0.07	95.7	4.3	1.50 (0.62–3.66)	0.371	0.11	88.4	11.6	1.01 (0.60–1.69)	0.968	0.05
No (n = 376)	96.3	3.7				97.1	2.9				88.0	12.0			
Low HDL-C															
Yes (n = 77)	88.3	11.7	2.96 (1.19–7.38)	0.025	0.43	90.9	9.1	2.98 (1.08–8.24)	0.045	0.36	76.6	23.4	3.02 (1.58–5.80)	**0.001**	0.24
No (n = 531)	97.0	3.0				97.4	2.6				89.8	10.2			
High TC															
Yes (n = 297)	94.9	5.1	1.58 (0.68–3.68)	0.280	0.12	96.0	4.0	1.46 (0.59–3.62)	0.408	0.09	87.2	12.8	1.30 (0.79–2.16)	0.302	0.06
No (n = 311)	96.8	3.2				97.1	2.9				89.1	10.9			
High TG/HDL-C ratio															
Yes (n = 209)	93.3	6.7	1.90 (0.82–4.41)	0.135	0.27	93.8	6.2	2.65 (1.04–6.78)	0.038	0.38	83.7	16.3	1.95 (1.16–3.27)	**0.013**	0.17
No (n = 399)	97.2	2.8				98.0	2.0				90.5	9.5			
High LDL-C/HDL-C ratio															
Yes (n = 82)	91.5	8.5	2.36 (0.92–6.02)	0.089	0.23	95.1	4.9	1.42 (0.45–4.46)	0.560	0.08	84.1	15.9	1.61 (0.82–3.13)	0.178	0.08
No (n = 526)	96.6	3.4				96.8	3.2				88.8	11.2			
High TC/HDL-C ratio															
Yes (n = 123)	87.8	12.2	5.50 (2.34−12.91)	$\mathbf{8.5 \times 10^{-5}}$	0.70	91.1	8.9	4.15 (1.66−0.33)	**0.003**	0.55	80.5	19.5	2.43 (1.39−4.25)	**0.003**	0.22
No (n = 485)	97.9	2.1				97.9	2.1				90.1	9.9			

Criteria High TG (≥ 150 mg/dL) [24], high LDL-C (≥ 130 mg/dL) [24], low HDL-C (< 40 mg/dL) [24], high (TC ≥ 200 mg/dL) [24], high TG/HDL-C ratio (> 3.5 for male or > 2.5 for female) [25], high LDL-C/HDL-C ratio (> 3.5 for male or > 3.0 for female) [26], high TC/HDL-C ratio (> 5.0 for male or > 4.5 for female) [26]. All analyses were adjusted for age, sex, population (rural/urban), high BMI, and high FPG

OR odds ratio, *95% CI* 95% confidence intervals, *TG* triglyceride, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TC* total cholesterol

[a]Statistical power. The significant *p* values after Bonferroni correction are indicated in bold ($p < 0.017$)

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical approvals for this study were granted by the Eijkman Institute Research Ethics Commission (Number 32 on October 27, 2008), and by the Faculty of Medicine Ethic Committee, Udayana University (Number 690a/SKRT/X/2010 on October 28, 2010).

Informed consent Informed consent was obtained from all individual participants included in the study.

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