

RESEARCH ARTICLE

# Influence of the definition of “metabolically healthy obesity” on the progression of coronary artery calcification

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## Abstract

### Objectives

Debates whether metabolically healthy obesity (MHO) increases the cardiovascular risk might be due to the metabolic instability of MHO or the absence of a perfect definition of MHO. Therefore, we aimed to investigate the influence of the MHO phenotype on the coronary artery calcium score (CACS) progression according to definition of MHO.

### Methods

We analyzed a retrospective cohort with a CACS of 0 at baseline and available serial CACS measurements taken  $\geq 12$  months apart ( $n = 1,218$ ). Obesity was defined as BMI  $\geq 25$  kg/m<sup>2</sup>, and MHO was defined as obesity accompanied by  $\leq 1$  (MHO class I) or 0 (MHO class II) components of metabolic syndrome (MetS).

### Results

During a median follow-up of 45 months, 32.2% of MHO class I and 10.2% of MHO class II subjects developed MetS. Compared to non-obese/metabolically healthy subjects (reference group), hazard ratios (HR) for development of MetS were 2.174 (95% confidence interval [CI]: 1.513–3.124) and 1.166 (95% CI: 0.434–3.129) for MHO class I and II subjects, respectively. The MHO class I subjects showed a significantly increased risk of CACS progression as compared to the reference group (HR: 1.653; 95% CI: 1.144–2.390), whereas MHO class II subjects did not (HR: 1.195; 95% CI: 0.514–2.778). Among subjects with MHO class I, no significant CACS progression was observed in the subjects who maintained metabolic health during follow-up (HR: 1.448; 95% CI: 0.921–2.278).

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## Conclusions

The risks of metabolic deterioration and CACS progression were significant in subjects with MHO class I, but not in those with MHO class II.

## Introduction

The prevalence of obesity has increased rapidly in recent years [1] and is associated with various metabolic abnormalities that lead to cardiovascular morbidity and mortality [1]. However, obesity is not always associated with metabolic abnormality. Obesity without metabolic disturbance has been reported in 20–40% of obese people [2,3]: This phenotype of obesity is defined as metabolically healthy obesity (MHO) and is not associated with the risk of atherosclerosis [4]. Previous studies have reported several possible mechanisms underlying MHO, such as difference in fat distribution [4], subclinical inflammation [5], expansion capacity of adipose tissue [6], adiponectin level [7,8] and adipose carbohydrate responsive element binding protein  $\beta$  [9]. However, longitudinal studies have shown that MHO is an unstable state and it progresses to a metabolically unhealthy and obese (MUHO) status in a considerable proportion of patients [10,11]. Furthermore, although individuals categorized as MHO have demonstrated increased cardiovascular events compared to non-obese individuals during long-term follow-up [12], the data are conflicting among studies [13,14].

Considering that the Asian population has a higher metabolic risk increase for the same increase in body mass index (BMI) compared to Caucasians [15], the influence of obesity on the risk of coronary artery disease (CAD) in the Asian population should be elucidated on the basis of the metabolic status. However, only a few studies have been conducted on MHO and CAD risk in the Asian population thus far.

Among methods for assessment of cardiovascular risk, the coronary artery calcium score (CACS), which is determined by computed tomography (CT), is an excellent tool for clinical measurement of the burden of CAD risk. Quantitative pathologic analysis has shown that segments with greater calcification tend to have a larger burden of atherosclerotic plaque and percent lumen stenosis [16]. Thus, CACS reflects the presence and extent of coronary atherosclerosis [17]. Furthermore, after serial assessment, CACS has been proposed as a useful predictor of cardiac outcome [18–21]. Therefore, in this study, we aimed to investigate the influence of the MHO phenotype on the development of subclinical coronary atherosclerosis by evaluating CACS in a retrospective cohort.

## Materials and methods

### Study population

This was a retrospective cohort study. Among people who underwent routine general health examinations at the Healthcare System Gangnam Center, Seoul National University Hospital ( $n > 10,000/\text{year}$ ), we shortlisted 17,390 subjects who underwent coronary artery CT evaluation for screening purpose on their demand from October 2003 and December 2013. Of these, 2,473 subjects underwent baseline and follow-up coronary artery CT for assessment of CACS. Of those, we selected 2,435 subjects whose second CACS evaluation was at least 12 months after the initial evaluation. Thereafter, we excluded subjects who had (1) experienced coronary revascularization including coronary artery bypass surgery ( $n = 15$ ) or (2) insufficient clinical data to define obesity and metabolic syndrome ( $n = 62$ ). Further, we excluded subjects with

baseline CACS > 0. Finally, 1,218 subjects (882 men and 336 women) with baseline CACS of 0 were included in the analysis.

The study protocol conformed to the ethical guidelines of the 2013 Declaration of Helsinki, and was reviewed and approved by the institutional review board (IRB) of Seoul National University Hospital (IRB No. H-1401-099-549) and the IRB of Boramae Medical Center (IRB No. 26-2013-105). As the current study was retrospective in nature and involved the use of a database and medical records, informed consent was waived by the board. All participants were informed of the possible risk of radiation exposure by CT scanning and agreed to undergo the examination.

## Clinical and laboratory assessments

Clinical and laboratory assessments were performed at baseline and follow-up. Height and body weight were measured to the nearest 0.1. BMI was calculated as body weight (kg) divided by height squared ( $m^2$ ). Waist circumference (WC) was measured mid-way between the iliac crest and the lower rib margin by a trained nurse. Systolic and diastolic blood pressures were also measured. Blood samples were taken > 12 h after fasting, and the levels of fasting blood glucose, high-density lipoprotein (HDL) cholesterol, and triglycerides (TG) were measured using Architect Ci8200 (Abbott Laboratories, Abbott Park, IL, USA). Hemoglobin A1c (HbA1c) concentration was measured using COBAS INTEGRA 400 (Roche Diagnostics GmbH, Mannheim, Germany). The low-density lipoprotein (LDL) cholesterol level was calculated by using the Friedewald equation in subjects with a TG level < 4.5 mmol/L. In subjects with a TG level  $\geq$  4.5 mmol/L, the measured LDL cholesterol level was used for analysis. Fasting insulin level was measured with an immunoradiometric assay kit (INS-IRMA kit; Biosource, Belgium) in part of the subjects ( $n = 640$ ); and insulin resistance was indirectly evaluated using the homeostasis model assessment of insulin resistance (HOMA-IR), as described previously [22]. Subjects were asked to complete a structured questionnaire that included medical history, current medications, and smoking history at the time of the medical examinations. Hypertension was defined as blood pressure  $\geq$  140/90 mmHg or intake of antihypertensive medications. Diabetes was defined as a fasting glucose level  $\geq$  7 mmol/L or HbA1c concentration  $\geq$  6.5%, or intake of anti-diabetic medications. Hypercholesterolemia was defined as an LDL cholesterol level  $\geq$  4.1 mmol/L or intake of lipid-lowering agents.

Overweight and obesity were defined as BMI 23–24.9  $kg/m^2$  and  $\geq$  25  $kg/m^2$ , respectively according to the World Health Organization Asia–Pacific criteria [23]. Abdominal obesity was defined as WC  $\geq$  90 cm for men and  $\geq$  80 cm for women according to the International Obesity Task Force criteria for the Asian-Pacific population [23]. MHO was defined according to the most frequently used definition in recent studies (MHO class I) [24–27]. Briefly, obese participants who met  $\leq$  1 of the following National Cholesterol Education Program–Adult Treatment Panel III (NCEP–ATP III) criteria for metabolic syndrome [28] were considered to have MHO class I: (a) TG level  $\geq$  1.7 mmol/L (150 mg/dL); (b) HDL cholesterol level < 1.0 mmol/L (40 mg/dL) in men and < 1.3 mmol/L (50 mg/dL) in women; (c) blood pressure  $\geq$  130/85 mmHg or intake of antihypertensive medication; and (d) fasting glucose level  $\geq$  5.6 mmol/L (100 mg/dL) or intake of anti-diabetic medication. The WC criterion was not used in the definition of metabolic health as in the previous studies [24–27,29,30], because of collinearity with BMI. Additionally, we defined MHO using stricter criteria following the Healthy Obese Project, which was a population-based cohort study conducted in 7 European countries (MHO class II) [31]: MHO class II was established when subjects with obesity had none of the metabolic syndrome components. Also, the WC criterion was not used in the definition of MHO

class II. Smoking status was classified as current smokers and non-smokers. Subjects were classified as physically active if they performed regular exercise more than once per week.

## Cardiac computed tomography and analysis for CACS

Coronary CT was performed using either a 256-slice multidetector CT scanner (Brilliance iCT 256; Philips Medical Systems, Cleveland, Ohio) or a 16-slice scanner (Somatom Sensation 16; Siemens Medical Solutions, Forchheim, Germany). A standard scanning protocol was applied, with  $128 \times 0.625$  mm section collimation, 0.27 ms rotation time, 120 kV tube voltage, and 800 mA tube current. All scans were performed with electrocardiogram-gated dose modulation. The CACS was calculated quantitatively according to the method described by Agatston et al. [32] and using a software program (Rapidia 2.8; INFINTT, Seoul, Republic of Korea). Progression of CACS was defined as any increase in CACS at follow-up according to the previous study [33]. We included only the subjects with CACS = 0 at baseline; progression was defined as CACS >0 at follow-up.

## Statistical analysis

To compare the clinical characteristics according to obesity and metabolic health status, ANOVA was used for continuous variables and  $\chi^2$  tests for categorical variables. The Bonferroni post-hoc analysis was performed for ANOVA. Cox proportional hazard regression was used to assess the risk of developing metabolic syndrome or CACS progression during the follow-up according to the baseline obesity and metabolic status. *P*-values <0.05 were considered statistically significant. All statistical analyses were conducted using SPSS 19 (SPSS Inc., Chicago, IL, USA).

## Results

### Clinical characteristics according to metabolic abnormalities

We analyzed the cohort data from 1,218 subjects (median follow-up duration, 45 months; interquartile range, 28–59 months). Prevalence of MHO class I and class II was 15.6% (17.8% in men and 9.8% in women) and 4.8% (5.0% in men and 4.2% in women) in the study subjects, respectively. Among obese subjects, 38.9% had MHO class I and 11.9% had MHO class II.

The clinical characteristics of subjects according to the baseline obesity status and metabolic abnormalities were shown in Table 1. Irrespective of the definition of MHO, subjects with MHO group showed a significantly higher BMI, WC, blood pressure, and serum triglyceride level than metabolically healthy non-obese (MHNO) group (Table 1). However, in the case of HOMA-IR and hsCRP level, only the subjects defined as MHO class I had significantly higher levels compared to MHNO ( $P < 0.001$  in both). There was no difference in HOMA-IR or hsCRP level between MHO class II and MHNO (Table 1).

To elucidate the difference between MHO class I and II, we compared obesity with no metabolic abnormalities and obesity with one metabolic abnormality. Compared to MHO subjects without any metabolic abnormality (that is, MHO class II), MHO subjects with one metabolic abnormality showed a higher HOMA-IR level ( $P = 0.015$ ; S1 Table).

### Risk for the development of metabolic syndrome during follow-up

The further analysis for the risk of developing metabolic syndrome during the follow-up period, only the subjects with metabolic health at the baseline were included. Among obese subjects without any metabolic abnormality at baseline (MHO class II), 10.2% developed metabolic syndrome during the follow-up period, which was not significantly different as compared

**Table 1. Clinical characteristics according to baseline metabolic abnormality and obesity.**

	MHO class I				P-value <sup>d</sup>	P-value <sup>e</sup>	MHO class II				P-value <sup>d</sup>	P-value <sup>e</sup>	
	MHNO	MHO	MUHNO	MUHO			MHNO	MHO	MUHNO	MUHO			
N	492	190	238	298			218	58	512	430			
Male, %	62.4	82.6	70.6	83.9	<0.001	<0.001	59.2	75.9	67.6	84.4	<0.001	0.022	
Age, years	54.6±6.4	54.6±6.8	55.9±6.7	54.2±7.0	0.023	0.907	54.3±6.6	54.6±6.8	55.4±6.5	54.3±6.9	0.055	0.758	
BMI*, kg/m <sup>2</sup>	22.4±1.7	26.5±1.3	23.1±1.5	27.1±1.9	<0.001	<0.001	22.3±1.8	26.4±1.2	22.8±1.6	27.0±1.7	<0.001	<0.001	
WC*, cm	M	84.2±4.7	92.3±4.4	85.5±4.7	93.6±5.6	<0.001	<0.001	84.3±5.1	92.4±4.7	84.8±4.6	93.2±5.2	<0.001	<0.001
	F	80.1±6.0	91.1±5.8	83.6±5.5	92.7±5.4	<0.001	<0.001	79.1±5.8	89.1±4.1	82.1±6.0	92.6±5.7	<0.001	<0.001
Systolic BP, mmHg	113.7±13.4	119.3±13.0	125.2±13.7	127.6±13.2	<0.001	<0.001	109.1±10.2	112.6±8.7	121.0±14.7	125.9±13.5	<0.001	0.019	
Diastolic BP, mmHg	74.7±10.5	78.1±9.6	82.1±10.0	85.0±10.9	<0.001	<0.001	70.5±8.3	72.9±6.8	79.9±10.6	83.6±10.7	<0.001	0.020	
FPG, mmol/L	5.3±0.9	5.3±0.6	6.2±1.1	6.2±1.1	<0.001	0.561	5.0±0.3	5.1±0.3	5.8±1.2	6.0±1.1	<0.001	0.011	
HbA1c, %	5.7±0.6	5.7±0.4	6.0±0.7	6.0±0.8	<0.001	0.335	5.6±0.3	5.6±0.3	5.9±0.7	5.9±0.7	<0.001	0.366	
LDL-C, mmol/L	3.23±0.77	3.25±0.83	3.15±0.80	3.26±0.85	0.397	0.763	3.16±0.75	3.48±0.82	3.22±0.79	3.23±0.84	0.064	0.005	
HDL-C, mmol/L	M	1.37±0.28	1.35±0.29	1.27±0.32	1.19±0.28	<0.001	0.283	1.39±0.26	1.39±0.27	1.32±0.31	1.24±0.29	<0.001	0.800
	F	1.61±0.34	1.44±0.26	1.36±0.38	1.28±0.26	<0.001	0.002	1.66±0.33	1.48±0.17	1.47±0.38	1.32±0.28	<0.001	0.003
Triglyceride, mmol/L*	1.04±0.50	1.21±0.59	1.73±1.13	1.92±1.00	<0.001	<0.001	0.92±0.33	1.04±0.33	1.41±0.93	1.73±0.96	<0.001	0.012	
AST*, IU/L	24.1±10.0	26.3±10.8	25.0±10.6	27.9±12.2	<0.001	0.002	24.4±12.7	26.3±12.5	24.9±9.0	27.4±11.6	<0.001	0.105	
ALT*, IU/L	23.7±12.3	31.1±19.3	28.0±16.6	34.8±22.1	<0.001	<0.001	22.3±9.8	30.2±21.4	26.3±15.2	33.8±21.1	<0.001	<0.001	
GGT*, IU/L	29.2±30.0	40.2±30.7	41.1±37.7	54.1±55.4	<0.001	<0.001	26.3±24.1	31.5±16.5	35.9±34.4	51.0±50.1	<0.001	0.005	
HOMA-IR*	1.85±0.87	2.41±1.39	2.77±1.29	3.36±1.61	<0.001	<0.001	1.70±1.76	2.06±1.39	2.37±1.19	3.13±1.58	<0.001	0.162	
hs-CRP*, mg/L	1.1±3.4	1.8±4.0	1.1±1.7	2.0±4.3	<0.001	<0.001	1.1±2.5	1.4±2.4	1.1±3.1	2.0±4.3	<0.001	0.249	
Diabetes mellitus, % <sup>a</sup>	6.5	3.2	24.4	22.1	<0.001	0.096	0.0	0.0	17.6	16.7	<0.001	NA	
Hypertension, % <sup>b</sup>	16.5	22.6	53.4	58.1	<0.001	0.076	0.0	0.0	40.6	50.2	<0.001	NA	
Abdominal obesity, % <sup>c</sup>	26.8	76.8	36.3	79.9	<0.001	<0.001	27.1	78.2	31.1	78.7	<0.001	<0.001	
Current smoker, %	14.0	17.8	17.8	21.6	0.028	0.311	15.2	28.2	15.4	19.0	0.553	0.098	
Exercise, %	29.1	30.0	34.9	33.2	0.121	0.851	25.7	22.4	33.2	33.3	0.026	0.733	

\*Log-transformed when comparing among groups

<sup>a</sup>Subjects with FPG ≥ 7.0 mmol/L, HbA1c ≥ 6.5%, or taking anti-diabetic medication

<sup>b</sup>Subjects with SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, or taking anti-hypertensive medication

<sup>c</sup>Subjects with waist circumference ≥ 90 cm in men and ≥ 80 cm in women.

<sup>d</sup>P from ANOVA among 4 groups (MHNO, MHO, MUHNO, and MUHO)

<sup>e</sup>Compared between MHNO and MHO

Abbreviations: MHO, metabolically healthy obesity; MHNO, metabolically healthy non-obesity; MUHNO, metabolically unhealthy non-obesity; MUHO, metabolically unhealthy obesity; BMI, body mass index; WC, Waist circumference; M, male; F, female; BP, blood pressure; FPG, fasting plasma glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HOMA-IR, homeostatic model assessment of insulin resistance; CRP, c-reactive protein.

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to 10.8% of non-obese subjects with no metabolic abnormality (age- and sex-adjusted hazard ratio [HR]: 1.166, 95% confidence interval [CI]: 0.434–3.129,  $P = 0.761$ ; Table 2). In contrast, following the MHO class I definition, 31.6% of MHO and 16.7% of MHNO developed metabolic syndrome during the follow-up. The risk of developing metabolic syndrome in MHO class I was significantly higher than that in MHNO (age- and sex-adjusted HR: 2.174, 95% CI: 1.513–3.124,  $P < 0.001$ ; Table 2). The prevalence of metabolic syndrome at follow-up increased linearly with the number of metabolic abnormalities at baseline ( $P$  for trend  $< 0.001$ ; S1 Fig).

**Table 2. Risk of developing metabolic syndrome during follow-up according to baseline metabolic abnormalities and obesity status.**

	Metabolic syndrome <sup>a</sup> at follow-up			
	Hazard ratio <sup>b</sup>	P-value <sup>b</sup>	Hazard ratio <sup>c</sup>	P-value <sup>c</sup>
MHO definition I				
MHNO ( $\leq 1$ abnormality)	(reference)	–	(reference)	–
Obesity with $\leq 1$ abnormality (MHO class I)	2.359(1.650–3.374)	<0.001	2.174(1.513–3.124)	<0.001
MHO definition II				
MHNO (no abnormality)	(reference)	–	(reference)	–
Obesity with no abnormality (MHO class II)	1.197 (0.449–3.195)	0.719	1.166 (0.434–3.129)	0.761
Obesity with one abnormality	4.182 (2.482–7.045)	<0.001	4.030 (2.338–6.947)	<0.001
Obesity with $\leq 1$ abnormality (MHO class I)	3.401 (2.033–5.690)	<0.001	3.238 (1.901–5.515)	<0.001

<sup>a</sup>those who met  $\geq 2$  of the following National Cholesterol Education Program–Adult Treatment Panel III criteria except abdominal obesity criterion

<sup>b</sup> without adjustment

<sup>c</sup> with adjustment for age and sex

Abbreviations: MHO, metabolically healthy obesity; MHNO, metabolically healthy non-obesity

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To differentiate the risk from MHO class I and II, we subsequently analyzed the risk of developing metabolic syndrome in MHO subjects according to the number of baseline metabolic abnormality, that is, 0 or 1. We showed that there was no difference in the risk of developing metabolic syndrome between obese subjects with no metabolic abnormality (MHO class II; Table 2) and non-obese subjects with no metabolic abnormality. By contrast, obese subjects with one metabolic abnormality showed about 4 time higher risk of developing metabolic syndrome compared to MHNO subjects even after adjustment for age and sex (HR: 4.030, 95% CI: 2.338–6.947,  $P < 0.001$ ; Table 2). Additional adjustment for HOMA-IR did not attenuated the statistical significance in that (HR: 4.186, 95% CI: 1.699–10.314,  $P = 0.002$ ).

Subsequently, as current definition of MHNO includes overweight, which might underestimate the risk of MHO class II, we redefined the reference group as subjects with both of normal weight and metabolic health. Redefinition of reference group also confirmed a difference between MHO class I and II: only MHO class I showed a significant risk of future metabolic syndrome compared to reference group (S2 Table). MHO class II still showed no difference in the risk of developing metabolic syndrome compared to metabolically healthy and normal weight group (age and sex-adjusted HR: 1.509, 95% CI: 0.487–4.681; S2 Table).

Among subjects with MHO class I at baseline, those who showed deteriorated metabolic health at the follow-up had a significantly higher systolic and diastolic blood pressures, triglyceride, and gamma-glutamyl transferase (GGT) levels ( $P = 0.002$ ,  $P < 0.001$ ,  $P = 0.040$ , and  $P = 0.006$ , respectively; S3 Table). Prevalence of abdominal obesity, serum CRP level and HOMA-IR were not significantly different between two groups in MHO class I (S3 Table).

### Risk for the progression of CACS

The prevalence of CACS progression during the follow-up period was 11.5%, 19.3%, 28.6%, 32.7%, and 33.3% in non-obese subjects with 0, 1, 2, 3, and 4 metabolic abnormalities, respectively ( $P$  for trend  $< 0.001$ ), and 12.1%, 29.5%, 25.3%, 37.9%, and 28.0% in obese subjects with 0, 1, 2, 3, and 4 metabolic abnormalities, respectively ( $P$  for trend = 0.018; S2 Fig). For the analysis assessing the risk of CACS progression according to MHO status, we only used the subjects with metabolic health at the baseline.

After adjustment for age and sex, MHO class I increased the risk of CACS progression during the follow-up period (age- and sex-adjusted HR: 1.653, 95% CI: 1.144–2.390,  $P = 0.007$ ;



**Table 3. Risk of CACS progression during follow-up period according to baseline metabolic abnormalities and obesity status.**

	N	CACS progression, %	Hazard ratio (HR)			
			HR <sup>a</sup>	P-value <sup>a</sup>	HR <sup>b</sup>	P-value <sup>b</sup>
MHO definition I						
MHNO ( $\leq 1$ abnormality)	492	15.9	(reference)	–	(reference)	–
Obesity with $\leq 1$ abnormality (MHO class I)	190	24.2	1.809 (1.255–2.609)	0.001	1.653 (1.144–2.390)	0.007
MHO definition II						
MHNO (no abnormality)	218	11.5	(reference)	–	(reference)	–
Obesity with no abnormality (MHO class II)	58	12.1	1.334 (0.576–3.090)	0.501	1.195 (0.514–2.778)	0.678
Obesity with one abnormality	132	29.5	2.694 (1.622–4.475)	<0.001	2.247 (1.342–3.763)	0.002
Obesity with $\leq 1$ abnormality (MHO class I)	190	24.2	2.324 (1.422–3.800)	0.001	1.972 (1.198–3.248)	0.008

<sup>a</sup> without adjustment

<sup>b</sup> with adjustment for age and sex

Abbreviations: CACS, coronary artery calcium score; MHO, metabolically healthy obesity; MHNO, metabolically healthy non-obesity

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Table 3). However, MHO class II did not increase the future risk of CACS progression as compared to the non-obese subjects without any metabolic abnormality (age and sex-adjusted HR: 1.195, 95% CI: 0.514–2.778,  $P = 0.678$ ). Compared with MHNO without any metabolic abnormality, even obese subjects with only one metabolic abnormality showed a significantly increased the risk of CACS progression (age- and sex-adjusted HR: 2.247, 95% CI: 1.342–3.763,  $P = 0.002$ ). Additional adjustment for HOMA-IR did not attenuated their risk of CACS progression (HR: 2.514, 95% CI: 1.052–6.006,  $P = 0.038$ ). Redefinition of reference group as subjects with normal weight and metabolic health also showed that MHO class II did not increased the risk of CACS progression compared to reference group (S4 Table).

Among subjects with MHO class I, the risk of CACS progression differed depending whether the subjects maintain or deteriorated the metabolic health during follow-up. The HR of CACS progression was 1.448 (95% CI: 0.921–2.278,  $P = 0.109$ ) in those who maintain metabolic health, but was 2.175 (95% CI: 1.338–3.537,  $P = 0.002$ ) in those who develop metabolic syndrome.

## Discussion

In the current study, we aimed to investigate the influence of MHO on the development of subclinical coronary artery disease using CACS, and the risk of CACS progression by MHO differed according to the definition. MHO class II without any metabolic abnormality did not significantly increase the risk of CACS progression even after adjusting for possible confounders. By contrast, MHO class I, which has been frequently defined in previous studies [24–27,29,30], showed a 1.7-fold higher risk for CACS progression than non-obese metabolically healthy subjects. A stratified analysis of this MHO class I group according to the number of metabolic abnormality (that is, 0 or 1) showed that there was a significant difference in the risk of CACS progression between MHO subjects with no metabolic abnormality and one metabolic abnormality at the baseline. The number of metabolic abnormality at the baseline in MHO class I subjects also determined the risk of developing metabolic syndrome during the follow-up period. Considering the difference in the following risk of CACS progression and developing metabolic syndrome in MHO subjects according to the presence of any metabolic abnormality at the baseline, strict definition of MHO might be needed to differentiate the benign form of obesity from general obese population.

Difference in the definition of MHO might influence the conflicting results on the association between cardiovascular events and MHO. A recent meta-analysis showed that MHO is not a fully benign condition [34]; however, this finding was not consistent [35,36]. There is also conflicting evidence regarding the association between MHO phenotype and subclinical CAD [37–39]. Recently, Chang et al. used a strict definition for MHO, that is, obesity with no metabolic abnormality and low insulin resistance, in a large sample of healthy subjects, and showed that MHO exhibits higher coronary calcification prevalence [37], which is in contrast to ours. Our study showed that, with strict definition, MHO does not increase the risk of subclinical CAD. The difference between two studies might be due to the difference in the baseline cardiovascular risk in both study subjects. Chang et al. included participants with coronary artery calcium deposition (6.8% of study subjects had CACS > 0); by contrast, we include only the subjects with no coronary artery calcium deposition (baseline CACS = 0), which suggests low cardiovascular risk [40]. In our preliminary analysis, baseline CACS was not only one of the risk factors for CACS progression, but also correlated to the CT follow-up interval. Therefore, we excluded subjects with baseline CACS > 0 to avoid the confounding effect of baseline CACS and to focus on the development of subclinical CAD. In addition, Chang et al. reported the cross-sectional association between MHO and CACS, which might be considered carefully to compare their result with ours. We assessed the effect of MHO on CAD risk longitudinally through the assessment of change of serial CACS. A dichotomous classification of body weight in our study may have underestimated the risk of MHO; however, a similar result was obtained when the non-obese subject was subdivided into normal weight and overweight.

In our study, during the 45 months of follow-up, 32.2% of MHO class I subjects and 10.2% of MHO class II subjects developed metabolic syndrome. MHO is considered an unstable condition that may deteriorate and transit to MUHO over time. In previous studies that investigated the natural course of MHO, approximately 30–40% of subjects with MHO at baseline developed a metabolically unhealthy state at follow-up [10,29], which was similar to that of MHO class I group in our study. By contrast, using more strict definition of MHO, that is, MHO class II, showed that MHO did not increase the prevalence of metabolic syndrome at follow-up compared to those in non-obese metabolically healthy subjects. Furthermore, MHO class II did not increase the risk of CACS progression, either. Even in the subjects with MHO class I, those maintaining metabolic health during follow-up period did not show an increased risk of CACS progression compared to MHNO. Previous study also showed that among MHO subjects, those who maintained long-term metabolic health did not have higher risk of diabetes and cardiovascular diseases compared to normal weight with no metabolic abnormality [31]. Studies that demonstrated an increased cardiometabolic risk in subjects with the MHO phenotype, including our study, may be partially affected by this metabolic instability of MHO.

There has been no consensus on the definition of MHO, and various definitions have been proposed thus far [2,12,24–27,41]. It is unclear whether the metabolic instability of MHO stems from the intrinsic characteristics of MHO or the absence of a perfect definition of MHO. In the current study, we defined MHO class I following the most frequently used definition in the recent studies [24–27], and confirmed the metabolic instability of MHO class I. The mere absence of metabolic syndrome may not guarantee metabolic health, which contributes to the unstable nature of MHO and higher risk of CAD progression seen in this study. Among subjects with MHO class I, those who showed deteriorated metabolic health at follow-up had significantly higher systolic and diastolic blood pressures and triglyceride level, which are the factors associated with insulin resistance. Ectopic fat [25,42,43] has been suggested as the factor determining the fate of MHO. In our study, there was no difference in the prevalence of abdominal obesity at the baseline according to development of metabolic syndrome at the follow-up. However, among MHO class I subjects, those who developing metabolic syndrome



showed a significantly higher level of GGT level compared to those maintaining metabolic health. Although we had no liver fat data of study subjects, GGT level can reflect the liver fat [44]. Liver fat might be more important to the risk of metabolic health than abdominal obesity [42]. High inflammation [24] as well as large ectopic fat [25] also has been reported to be associated with a poor future outcome among MHO subject; however there was no difference in CRP level according to metabolic instability among MHO subjects. The lack of unique criteria for definition is the main barrier to understanding the MHO phenotype, its clinical implications, and the benefits of therapeutic intervention.

The most important strength of our study is that the effect of MHO on CAD risk was assessed longitudinally through the assessment of change of serial CACS. Furthermore, we performed the analysis with homogenous subjects who had a low risk of CAD, that is, CACS = 0 at the baseline, which might reinforce the causal-relationship between MHO and CACS progression shown by our study. Furthermore, the homogenous study population can reduce the confounding effect of the baseline CAD risk in the association between CACS progression and baseline obesity phenotype. It is well-known that a high baseline CAD risk increases future CAD events [40]. The second strength of our study is that we compared the MHO phenotype using comprehensive evaluation during structured health check-ups including medical histories and anthropometric and biochemical measurements. We were able to assess the change in metabolic status during a follow-up period and investigate the influence of metabolic change on the progression of CAD in MHO. Lastly, we applied a stricter definition of MHO, i.e., obesity without any metabolic abnormality (MHO class II). We compared the risk of CACS progression in subjects with MHO class I and class II, and found that the future CAD risk of MHO could be categorized according to the number of metabolic abnormalities combined with obesity.

Despite our important findings, there are some limitations in this study. First, because the study was designed as a retrospective study, confounding factors and bias could be more common than seen in a prospective study. Second, the subjects in our study underwent CACS on request, which might be a source of selection bias. Time interval between baseline and follow-up CACS measurement might be also partly dependent on individuals' demand. Lastly, this study was performed in Korea, and therefore, its results cannot be generalized to other ethnicities.

In conclusion, the definition of MHO is of importance when evaluating the influence of MHO on the risk of CAD progression. With the more commonly used definition (MHO class I), MHO seemed to be harmful and exhibited a significant risk of subclinical CAD development. The risks of metabolic deterioration and CACS progression were significant in MHO class I subjects, and metabolic instability seems to partly contribute to CACS progression in these population. On the other hand, MHO class II was not associated with the risk of developing metabolic syndrome and CACS progression. A stricter definition of MHO might help differentiate the benign phenotype of obesity from the other phenotypes; however, longer term effect should be explored in the future study.

## Supporting information

**S1 Fig. The prevalence of metabolic syndrome during the follow-up.** The prevalence of metabolic syndrome at the follow-up was CACS progression during the follow-up period was 10.8%, 21.6%, 48.9%, 62.5%, and 83.3% in non-obese subjects with 0, 1, 2, 3, and 4 metabolic abnormalities, respectively (black bars;  $P$  for trend < 0.001), and 10.2%, 41.0%, 61.3%, 67.1%, and 86.4% in obese subjects with 0, 1, 2, 3, and 4 metabolic abnormalities, respectively (gray bars;  $P$  for trend < 0.001).

(TIF)

**S2 Fig. The prevalence of coronary artery calcium score (CACs) progression during the follow-up period.** The prevalence of CACS progression during the follow-up period was 11.5%, 19.3%, 28.6%, 32.7%, and 33.3% in non-obese subjects with 0, 1, 2, 3, and 4 metabolic abnormalities, respectively (black bars;  $P$  for trend  $< 0.001$ ), and 12.1%, 29.5%, 25.3%, 37.9%, and 28.0% in obese subjects with 0, 1, 2, 3, and 4 metabolic abnormalities, respectively (gray bars;  $P$  for trend = 0.018).

(TIF)

**S1 Table. Clinical characteristics according to baseline metabolic abnormality and obesity.**

(DOCX)

**S2 Table. Risk of developing metabolic syndrome during follow-up according to BMI categories.**

(DOCX)

**S3 Table. Comparison of the characteristics between metabolically stable and deteriorated subjects in MHO class I.**

(DOCX)

**S4 Table. Risk of CACS progression during follow-up period according to BMI categories.**

(DOCX)

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