Original Article

Plasma phospholipid fatty acid composition and estimated desaturase activity in heart failure patients with metabolic syndrome

Seungmin Lee,[1] Hyun Ju Do,[1] Seok-Min Kang,[2] Ji Hyung Chung,[2] Eunju Park[3,*] and Min-Jeong Shin[1,*]

[1]Department of Food and Nutrition and Institute of Health Science, Korea University, Seoul, Republic of Korea
[2]Cardiovascular Research Institute and Cardiology Division, Yonsei University College of Medicine, Seoul, Republic of Korea
[3]Department of Food and Nutrition, Kyungnam University, Changwon, Republic of Korea

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Metabolic syndrome is one of the major factors to increase the incidence of heart failure. In our study, we compared plasma fatty acid compositions among heart failure patients with and without Metabolic syndrome. Fatty acid (FA) composition of plasma phospholipids was analyzed and the activities of desaturase were estimated as the ratio of substrate and product fatty acids in 85 stable heart failure patients. Fatty acid and estimated desaturase activities were further examined for their associations with Metabolic syndrome components. Heart failure patients with Metabolic syndrome showed significant changes in fatty acid composition in comparison to those without Metabolic syndrome, which had a decreased proportion of lauric acid (C12:0) and an increased proportion of dihomo-γ-linolenic acid (C20:3n-6). Also, estimated desaturase activities (D5D and D6D) were closely related to Metabolic syndrome condition among heart failure patients. The content of dihomo-γ-linolenic acid showed positive correlations with BMI, waist circumference, and plasma triglyceride levels. D6D were positively associated with plasma triglyceride levels, whereas D5D showed a negative correlation with plasma triglyceride levels and waist circumferences. The content of dihomo-γ-linolenic acid as well as estimated D6D and D5D were altered in heart failure patients with Metabolic syndrome.

***Key Words*: heart failure, fatty acid, metabolic syndrome, dihomo-γ-linolenic acid, desaturase**

As one of the most common chronic diseases, heart failure (HF) has become prevalent over time in both developing and developed countries.[1,2] Even after HF diagnosis, the HF incidence barely diminished according to a population-based cohort study.[3] Along with an increasing prevalence of HF, it has become evident that the mortality and morbidity of HF is adversely affected by one of metabolic syndrome (MetS) risk factor including insulin resistance, obesity, and dyslipidemia.[4,5] Accumulating data have indicated that the importance of consideration of MetS in HF patients.[6–8] Several studies have reported that MetS was a significant predictor of HF, independent of established risk factors for HF.[9,10] These indicate that individual risk factors such as insulin resistance, dyslipidemia, obesity and hypertension reflected by MetS have involved in the underlying mechanisms explaining association between MetS and HF mortality. Besides the components of MetS, it was also reported that alterations in FAs composition in plasma and red blood cells have been related to metabolic diseases.[11] Numerous studies have studied on the roles of dietary intakes and blood compositions of FAs in various metabolic disorders such as diabetes,[12] coronary heart disease[11,13] or risk factors for MetS.[11–13] For example, elevated saturated fatty acid (SFA) intake is associated with the development of insulin resistance and thereby increased chances of progression to MetS.[14] Also, decreased plasma levels of polyunsaturated fatty acids (PUFAs) especially n-3 PUFAs were observed with individuals with MetS, compared to the healthy subjects.[15] Among newly diagnosed insulin resistance patients, differential fatty acid composition was detected, shown as a decrease in linoleic acid (18:2) and an increase in gamma-linolenic (18:3), dihomo-γ-linolenic acid (DGLA) (20:3) and arachidonic acid (20:4).[12,16] In addition, recent studies have proposed estimated desaturase activities using the ratio of a product to precursor fatty acids as an emerging risk factor for MetS.[13,17] In the case of Japanese men with MetS, FA composition from plasm cholesteryl esters indicates higher delta 9 desaturase activity and lower delta 5 desaturase activity.[17] Also, delta 5 desaturase activity has been reported to be lower in the condition of myocardial infarction.[13] These suggest that FA composition and estimated desaturase activity might have pathological role in chronic diseases either independently or as part of MetS.

Previously, it has been reported that abnormally higher or lower composition of specific FAs were associated with the risks for HF.[18–23] Also healthy subjects with MetS and without MetS were examined for their FA compositions.[17,24] However, the differences in FA composition and desaturase activities in HF patients with the MetS from those without the MetS have not been studied. MetS is a major factor accelerating HF pathological conditions in patients and their FA metabolism could be differentially affected by HF in conjunction with MetS. Therefore, in this study we aimed to compare plasma phospholipid FA compositions and estimated desaturase activities among HF patients with and without MetS to identify specific FA aberration in HF patients with MetS. We compared stearoyl-CoA desaturase (SCD), delta 6 desaturase (D6D) and delta 5 desaturase (D5D) between the groups which have been previously reported to be related to the MetS.[25–27] Correlations of MetS components with FAs or desaturase activities in HF patients were further evaluated.

Subjects and Methods

Study subjects. The total study population consisted of 177 eligible consecutive ambulatory HF patients enrolled at 2 separate sessions (91 subjects from the first and 87 subjects from the second session) from HF outpatient clinic at Yonsei Cardiovascular Center.[28] Inclusion criteria were (1) ages no greater than

*To whom correspondence should be addressed.
E-mail: mjshin@korea.ac.kr (M-J Shin), pej@kyungnam.ac.kr (E Park)

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80 years (2) systolic HF diagnosed with a left ventricular ejection fraction (LVEF) lower than 50% (3) stable HF on medication for at least 1 month prior to the study. We omitted patients (1) with HF with preserved ejection fraction greater than 50%, (2) who had acute myocardial infarction 3 months prior to the study, or (3) who had a severe cognitive impairment. All patients provided written informed consent. The Institutional Review Board at Yonsei University Medical Center approved the study protocol. Hospital database was accessed to obtain medical history including diagnosis, underlying disease, etiology of HF and drug use. Among the total 177 subjects, plasma FA composition data were obtained only in the 85 subjects whose samples were available for the measurement and also, those who were not taking special dietary substitutes such as omega 3 and conjugated linoleic acid supplementations. Fasting venous blood samples were collected from the forearm of the patients in EDTA-treated and plain tubes. MetS was defined when three or more of the following criteria were met, which is a modified NCEP ATP III definition (ATP III criteria and the WHO Western Pacific Region obesity criteria). (1) abdominal obesity is defined when waist circumference is greater than 90 cm in men and 80 cm in women; (2) hypertriglyceridemia is greater than 150 mg/dl; (3) hypertension is either systolic blood pressure greater than 130 mmHg or diastolic pressure greater than 85 mmHg, or on anti-hypertensive medication; (4) low HDL-cholesterol is less than 40 mg/dl in men and 50 mg/dl in women; (5) high fasting glucose is greater than 110 mg/dl or under treatment for diabetes. Waist circumference was measured twice to the nearest 0.1 cm at the level of the navel. Body weight and height were measured and BMI was calculated as the ratio of body weight (kg) to height (m^2).

Blood lipid profiles and glucose concentrations. Plasma cholesterol, LDL-cholesterol, and HDL-cholesterol from fasting blood samples were measured with enzymatic methods using commercially available kits (Choongwae, Seoul, Korea). Plasma triglyceride (TG) levels were assessed by a total glycerol test kit (Roche, Basel, Switzerland). All determinants were obtained on a Hitachi 747 analyzer (Tokyo, Japan). Fasting plasma glucose levels were analyzed by the glucose oxidase method using a Beckman Glucose Analyzer (Irvine, CA).

Plasma phospholipid FA composition and estimation of desturase activities. Plasma total lipids were extracted according to the Folch method[29] and the phospholipid fraction was isolated by thin-layer chromatography using a development solvent composed of hexane, diethyl ether, and acetic acid (80:20:2). The phospholipid fractions were then methylated to FA methyl esters (FAMEs) by the Lepage and Roy method.[30] The FAMEs of individual FAs of phospholipids were separated by gas chromatography (model 6890, Agilent Technologies Inc., Palo Alto, CA), using a capillary column (Omegawaz TM 250; 30 m, Supelco, Bellefonte, PA), as previously described.[31] Peak retention times were obtained by comparison with known standards (37 component FAME mix and PUFA-2, Supelco, Bellefonte, PA; GLC37, NuCheck Prep., Elysian, MN) and analyzed with ChemStation software (Agilent Technologies). Plasma phospholipid FAs were expressed as the percentage of total FAs. The desaturase activities were calculated as the ratio of product to precursor fatty acids. SCD activities are defined by C16:1 n-7/C16:0 (SCD n-7) and C18:1 n-9/C18:0 (SCD n-9). D6D is C20:3 n-6/C18:2 n-6 and D5D is C20:4 n-6/C20:3 n-6.[25–27]

Statistical analysis. The SPSS 12.0 software package was used for statistical analysis. Data are presented as the mean ± SD. Each variable was examined for normal distribution, and abnormally distributed variables such as TG and HDL-cholesterol were log-transformed. Differences in variables between the groups were evaluated using Student's *t* test and χ^2 test. General Linear Model (GLM) was used to test the differences of parameters between the groups after adjusting age and BMI *p* value less than 0.05 was considered statistically significant.

Results

Characteristics of HF subjects with MetS or without MetS. Among the total of 85 HF subjects, 26 subjects were classified as having the MetS. Gender difference and ejection fraction were similar between subjects with MetS and without MetS. As for an average age, subjects with MetS demonstrated older average age of 66.4 ± 9.7 years than those without MetS showing 58.9 ± 13.2 years ($p<0.05$). Proportion of the subjects with HF-related cariomyopathy or valvular disease was not different between the two groups. Also, medical treatments for HF except ACEI and/or ARB were similar for all HF subjects. The prevalence of stroke and chronic renal failure between the two groups was similar, whereas that of type 2 diabetes mellitus was comparable between the groups (Table 1).

MetS risk factors and blood lipids in HF subjects. As expected, HF subjects with MetS displayed significantly different measurements in the criteria for MetS compared to those without MetS. HF subjects with MetS showed significantly elevated plasma levels of fasting glucose (HF without MetS 100.1 ± 17.2 mg/dl vs with MetS 131.4 ± 41.0 mg/dl, $p<0.001$) and TG (HF without MetS 129.9 ± 80.9 mg/dL vs with MetS 199.0 ± 76.6 mg/dl, $p<0.001$). Also reduced levels of HDL-cholesterol (HF without MetS 49.5 ± 11.6 mg/dl vs with MetS 35.8 ± 7.7 mg/dl, $p<0.001$) were observed in HF subjects with MetS compared to those without MetS. On the other hand, total cholesterol (HF without MetS 169.7 ± 35.5 mg/dl vs with MetS 168.4 ± 4.09 mg/dl, ns) and LDL-cholesterol levels (HF without MetS 94.3 ± 31.1 mg/dl vs with MetS 92.7 ± 34.1 mg/dl, ns) were not significantly different between the two groups. These data confirm that HF subjects with MetS were differentiated from those without MetS and also indicate that the prevalence of metabolic syndrome among HF patients.

Plasma phospholipid fatty acid composition in HF subjects with MetS and without MetS. When FA compositions in plasma phospholipids were analyzed between HF patients with

Table 1. Age, gender, EF, anthropometric measurements and medical treatment between HF patients without MetS and HF patients with MetS

	HF without MetS (*n* = 59)	HF with MetS (*n* = 26)
Age (yrs)	58.9 ± 13.2	66.4 ± 9.7*
Gender (M:F)[1]	34:25	11:15
BMI (kg/m^2)	22.9 ± 3.3	24.6 ± 3.7*
Waist circumferences (cm)	80.7 ± 8.0	86.6 ± 8.7**
EF (%)	32.6 ± 13.1	36.1 ± 11.4
Etiology of HF[1]		
CAD (%)	22	38.5
Cardiomyopathy (%)	55.9	42.3
Valvular disease (%)	13.6	11.5
Hypertension (%)	16.9	73.1**
Underlying disease[1]		
Type 2 diabetes mellitus (%)	5.1	30.8**
Chronic renal failure (%)	3.4	11.5
Stroke (%)	6.8	3.8
Medical treatment[1]		
Diuretics (%)	74.6	73.1
Digitalis (%)	28.8	34.6
β-blocker (%)	50.8	57.7
ACEI and/or ARB (%)	72.9	96.2*
Anti-platelet agents (%)	62.7	69.2
Hypolipidemic agents (%)	33.9	42.3

Values are Mean ± SD. [1] χ^2 test. EF: ejection fraction; CAD: coronary artery disease; ACEI: angiotensin converting enzyme inhibitors; ARB: angiotensin II receptor blocker. *$p<0.05$, **$p<0.005$.

Table 2. Proportions of FA composition in plasma phospholipids and estimated desaturase activities between HF patients with MetS and without MetS

	HF without MetS (n = 59)	HF with MetS (n = 26)	p
12:0 lauric acid	0.24 ± 0.15	0.18 ± 0.09	<0.05
14:0 myristic acid	0.7 ± 0.17	0.66 ± 0.19	ns
16:0 palmitic acid	27.45 ± 2.93	27.85 ± 2.28	ns
16:1 palmitoleic acid	0.77 ± 0.23	0.83 ± 0.26	ns
18:0 stearic acid	14.99 ± 1.94	14.49 ± 1.69	ns
18:1 ω9 oleic acid	8.06 ± 1.45	8.04 ± 1.34	ns
18:1 t ω9 elaidic acid	2.24 ± 0.58	2.15 ± 0.68	ns
18:2 ω6 linoleic acid	14.11 ± 2.79	13.11 ± 2.77	ns
18:2 tt linolelaidic acid	0.69 ± 0.3	0.66 ± 0.25	ns
18:3 ω6 γ-linolenic acid	0.27 ± 0.12	0.26 ± 0.05	ns
18:3 ω3 α-linolenic acid	0.59 ± 0.26	0.66 ± 0.25	ns
20:0 arachidic acid	0.97 ± 0.23	1.0 ± 0.33	ns
20:1 ω9 gondoic acid (cis-11-eicosenoic acid)	0.39 ± 0.15	0.35 ± 0.1	ns
20:2 cis-11,14-eicosadienoic acid	1.24 ± 0.77	1.15 ± 0.27	ns
20:3 ω6 dihimo-γ-linolenic acid	2.5 ± 0.62	2.9 ± 0.74	<0.05
20:3 ω3 5-8-11-eicosatrienoic acid	0.45 ± 0.25	0.38 ± 0.14	ns
20:4 ω6 arachidonic acid	7.02 ± 1.61	7.04 ± 1.47	ns
20:5 ω3 EPA	2.7 ± 1.2	3.26 ± 1.28	ns
22:0 behenic acid	1.88 ± 0.5	1.91 ± 0.38	ns
22:1 erucic acid	0.6 ± 0.21	0.54 ± 0.16	ns
22:2 docosadienoic acid	0.37 ± 0.27	0.33 ± 0.12	ns
24:0 lignoceric acid	1.96 ± 0.72	1.9 ± 0.64	ns
22:6 ω3 DHA	8.26 ± 2.17	8.88 ± 1.81	ns
SCD16 (16:1n-7/16:0)	0.028 ± 0.009	0.03 ± 0.009	ns
SCD18 (18:1n-9/18:0)	0.54 ± 0.12	0.56 ± 0.11	ns
D6D (20:3n-6/18:2n-6)	0.18 ± 0.06	0.23 ± 0.09	<0.01
D5D (20:4n-6/20:3n-6)	2.96 ± 0.98	2.53 ± 0.63	<0.05

Mean ± SD. Expressed as percentage of total fatty acids.

MetS and without MetS, significant differences in DGLA and lauric acid between the two groups were observed (Table 2). HF subjects with MetS displayed a higher proportion of C12:0 (lauric acid) (p<0.05) compared to HF without MetS. In addition, a significantly higher proportion of C20:3n-6 (DGLA) was shown in HF subjects with MetS than those without MetS (p<0.05). C20:5n-3 (EPA) showed a tendency to have a higher proportion in HF with MetS even if it was not statistically significant (p = 0.055). With respect to D5D and D6D, their activities were estimated by calculating corresponding FA product-to-precursor ratios. Estimated D5D activities were markedly lower in HF with MetS than in HF without MetS (p<0.05). However, estimated D6D activities were significantly increased in HF with MetS compared to HF without MetS (p<0.01). Overall significant elevation of DGLA (C20:5n-6) value from HF with MetS could result from the combination of decreased activity of D5D and increased activity of D6D. In GLM analysis (Fig. 1), DGLA and estimated D6D activity were proven to be higher with MetS in HF patients after adjusting for age (p<0.05 and p<0.05, respectively). In contrast, estimated D5D activity was similar between the two groups after age adjustment.

Correlations between DGLA/EPA/estimated desaturase activites and components of MetS. Among HF subjects, we evaluated correlations between DGLA and estimated desaturase indexes (D5D and D6D) and the components of MetS (BMI, waist circumference, plasma TG, HDL-cholesterol levels, and fasting glucose) (Table 3). DGLA (C20:3n-6) was positively correlated with BMI (r = 0.274, p<0.05), waist circumference (r = 0.295, p<0.01) and plasma TG levels (r = 0.390, p<0.001). There were correlations of plasma TG levels positively with estimated D6D activity (r = 0.295, p<0.01) whereas negatively with estimated D5D activity (r = –0.299, p<0.001).

Discussion

In the present study, we observed significant differences in the FA compositions and estimated desaturase activities between HF patients with and without MetS, providing new information on the altered FA metabolisms in these conditions. Specifically, we found that increased proportion of DGLA and estimated D6D activity and decreased estimated D5D activity were associated with the presence of MetS among HF patients.

A few studies have reported about the associations between FA levels in plasma and the risk and severity for HF without distinguishing the MetS status.[18–23] For instance, intake of saturated fats is known to increase the risk of HF.[18,19] This was in part supported by a study showing the association of the elevated plasma levels of SFA and reduced plasma levels of DHA with the risks of HF.[20] Moreover, Oie *et al.*[21] and Zhao *et al.*[22] compared the changes in FA composition between the normal control and HF subjects and found out the roles of monounsaturated fatty acids (MUFAs) and some PUFA in HF subjects. They demonstrated that high levels of MUFA and linoleic acid and low levels of arachidonic acid were associated with those subjects.[21,22] In addition, it was reported that EPA and DHA are negatively correlated with heart rate, indicating that low proportion of either EPA or DHA increases a chance to elevate heart rate.[23]

While the evidence on the importance of FA metabolism has been accumulated, there has been limited information on the altered FA metabolism of HF patients in the presence of MetS. In this study, we have shown that MetS condition in HF patients had a positive association with elevated DGLA levels in plasma. Previously, DGLA has been implicated in the heart function by its positive association with diastolic blood pressure.[32,33] In addition, high levels of DGLA detected together with increased heart rate

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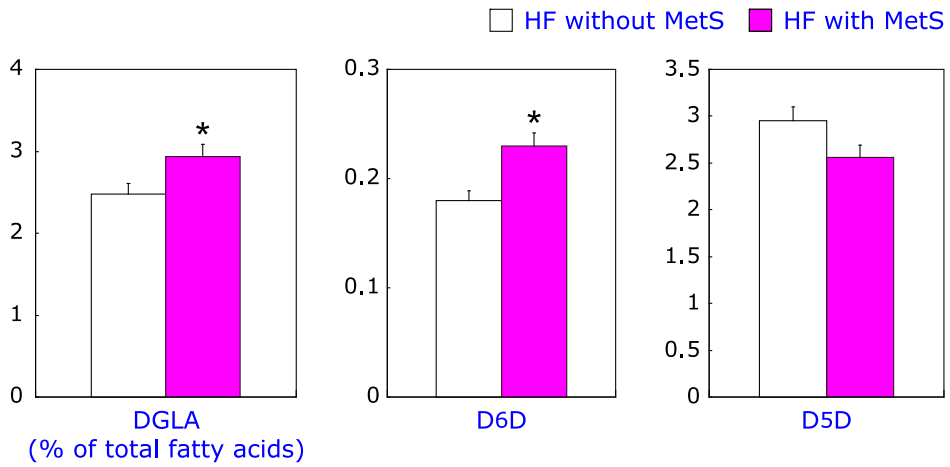

Fig. 1. Comparison of the proportions of DGLA, estimated D6D and D5D after age adjustment between HF patients with and without MetS (*p<0.01).

Table 3. Correlates between DGLA, estimated desaturase activities and components of MetS

	BMI	Waist	TG	HDL-cholesterol	Fasting glucose
DGLA	0.274*	0.294**	0.390***	–0.089	0.078
D6D	0.146	0.208	0.295**	–0.146	0.043
D5D	–0.163	–0.220*	–0.299***	0.193	0.003

*p<0.05, **p<0.01, ***p<0.001. DGLA: dihimo-r-linolenic acid, D5D: delta-5 desaturase, D6D: delta-6 desaturase.

and insulin levels was suggested to be responsible for the development of left ventricular dysfunction.[34] These findings indicate that elevated levels of DGLA in HF patients with MetS could burden the function of the heart more aggressively than in HF patient without MetS. On the other hand, Ebbesson *et al.*[23] showed inconsistent results with no association of DGLA with heart rate. Therefore, further studies are needed to elucidate the exact role of DGLA in heart function. Besides the implication of DGLA in the function of heart, several previous studies have reported the associations between the proportion of DGLA and insulin resistance. The elevated level of DGLA in non-insulin diabetes mellitus (NIDDM) patients was found compared to healthy subjects.[12] Similar observation was described in old men with negative correlation of DGLA with insulin sensitivity.[35] Another report by Maruyama *et al.*[24] indicated higher DGLA level in subjects with MetS compared to without MetS. In line with this, our data provide additional information on MetS specific elevation of DGLA in HF patients, suggesting the relation of DGLA with MetS. It may be that MetS condition where hyperinsulinemic state could stimulate D6D activity, could further elevate proportion of DGLA in plasma phospholipids in HF patients. Alternatively, higher contents of DGLA in FA composition, in turn, could indicate the risk of insulin resistance.

Along with the changes in DGLA proportion, we observed significant differences in estimated activities of desaturases with elevated D6D activity and decreased D5D activity with MetS among HF patients, although the association with D5D disappeared after age adjustment. Similar to our results, Maruyama *et al.*[24] observed higher DGLA level as well as lower estimated D5D activity in subjects with MetS compared to those without MetS. Also, lower estimated D5D activity and higher estimated D6D activity were shown in Japanese men with MetS.[17] In addition, young Japanese women harboring metabolic risk factors showed similar relationship of desaturase activities.[29] Furthermore, in a population-based study, the authors suggested indices for high D6D activity and low D5D activity along with increased delta 9 desaturase activity be predictors of the development of MetS,[36] indicating the close relationship between abnormal activities of desaturase and MetS. In our study, estimated D6D activity was calculated based on the ratio of DGLA to linoleic acid and estimation of D5D activity was obtained by the ratio of arachidonic acid to DGLA. Considering that DGLA is converted to arachidonic acid by D5D, diminished D5D activity could result in increased level of DGLA among HF subjects with MetS. At the same time, DGLA is a product of D6D activity from linoleic acid (C18:2n-6). Taken together, it is likely that high contents of DGLA in plasma phospholipid in MetS subjects with HF could also be due to dysregulated activitites of D5D and D6D and act as indicators of abnormal regulation of desaturase activities. Also, this might indicate that desaturases affect metabolic processes either via their product DGLA or by their own potential capacity to influence metabolism independently.[37] Furthermore, D6D could be linked to inflammation through inflammation where PUFAs synthesized by D6D may affect the permeability and function of cell membranes.[38] Alternatively, PUFAs synthesized by D6D participate in synthesizing eicosanoids[39] which act as inflammatory mediators and also, they can be used as ligands for transcriptional factors[40] that are involved in inflammatory pathways.

In summary, we provide evidence that DGLA as well as estimated desaturase activities (D5D and D6D) were closely related to MetS condition in HF patients. Study limitations include a relatively small number of patients and no healthy control group, which discourages us to generalize these results to a population with no HF. In addition, the degrees of MetS of subjects may not be enough to detect subtle changes in FA compositions, and also the pathological condition of HF might mask the differences induced by MetS which could be easily detected only when compared with the healthy control. Despite these limitations, this is the first study to evaluate associations between various desaturase activities along with serum individual FAs and the risk for MetS in HF patients.

Conflict of Interest

No potential conflicts of interest were disclosed.

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Abbreviations

BMI	body mass index
CVD	cardiovascular disease
DGLA	dihomo-γ-linolenic acid
FA	fatty acid
HF	heart failure
LVEF	left ventricular ejection fraction
MetS	metabolic syndrome
TG	triglyceride

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