Visceral fat accumulation determines postprandial lipemic response, lipid peroxidation, DNA damage, and endothelial dysfunction in nonobese Korean men

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Abstract **Visceral fat has been associated with multiple cardiovascular disease (CVD) risk factors. The aim of this study was to identify anthropometrical measures most closely associated with some well-known CVD risk factors. Because most Asians at risk have normal body mass index (BMI) according to Western standards, we studied healthy nonobese Korean males (n = 102; age: 36.5 ± 0.8 years, BMI: 23.8 ± 0.2 kg/m^2). Visceral fat area (VFA) at the fourth lumbar vertebra was associated with increased postprandial triglyceride (TG) response ($r = 0.53$, $P < 0.001$) and with plasma malondialdehyde (MDA) ($r = 0.36$, $P < 0.01$) and $PGF2_\alpha$ ($r = 0.24$, $P < 0.05$). When matched for BMI and age, men with high VFA (HVFA) (≥100 cm^2; n = 27) had higher blood pressure ($P < 0.01$), increased consumption of cigarettes ($P < 0.01$), and lower ratio of energy expenditure to calorie intake ($P < 0.01$) as compared with low VFA men (<100 cm^2; n = 27). Men with HVFA showed higher TG, glucose, and insulin responses following fat and oral glucose tolerance tests respectively higher plasma concentrations of MDA ($P < 0.001$), urinary $PGF_{2\alpha}$ ($P < 0.05$), and lymphocytes deoxyribonucleic acid tail moments ($P < 0.01$). Conversely, HVFA was associated with lower testosterone, insulin-like growth factor-1, and brachial artery flow-mediated dilation ($P < 0.001$). In conclusion, our data indicate that visceral fat accumulation, even in nonobese men, is a major factor contributing to increased CVD risk.**—Jang, Y., O. Y. Kim, H. J. Ryu, J. Y. Kim, S. H. Song, J. M. Ordovas, and J. H. Lee. **Visceral fat accumulation determines postprandial lipemic response, lipid peroxidation, DNA damage, and endothelial dysfunction in nonobese Korean men.** ***J. Lipid Res.*** **2003.** 44: **2356–2364.**

Supplementary key words flow-mediated dilation • body fat distribution • glucose tolerance

The most recent figures about the epidemic of obesity and overweight in the US and other industrialized countries are appalling. Thirty-four percent of US adults are considered overweight, and an additional 31 percent are obese. The consequences of this are far beyond the esthetics of the population. Being overweight or obese increases the risk of hypertension, heart disease, stroke, diabetes, and some cancers. In the US alone, 300,000 people die each year due to obesity-related causes, making it the second-leading cause of death after smoking. Asians experience similar risk of obesity-related diseases, but the problem is more insidious, as the risk threshold at which body mass index (BMI) appears to trigger the disease may be much lower for Asians (~23 kg/m^2) than for white populations (30 kg/m^2) (1). This may result from differences in body frame that affect the relation between body fat and BMI.

The population of Korea, similar to other industrialized countries in Asia, is experiencing dramatic and fast changes in dietary and physical activity habits that are the driving force behind the increase in obesity and obesity-related diseases. Korean adults have increased the percent of calories from fat in their diets from 6% in 1969 to 19% in 1998. Despite the greater than 3-fold increase in the dietary fat intake, their daily calorie intake (about 1,950 kcal/d) and BMI (about 22.5 kg/m^2) have changed little over the same period (2). However, these lifestyle changes have dramatically affected the central adipose tissue distribution pattern as reflected by the global increase of waist-hip ratio (WHR). Among Koreans, the mortality rate (per 100,000 of the population) as a result of ischemic heart disease has rapidly increased from 6.8 in 1988 to 13.8 in

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1997 (3). The increase in cardiovascular disease (CVD) rates is partially related to the rapid increase in the number of elderly subjects; however, the increase of fat intake in Korean parallels also the disease trends (2, 3). These temporal changes suggest that the increase in fat intake and central fat distribution may be partially responsible for the increasing CVD rates in Korea and other neighboring countries.

Previous research has shown that adipose tissue distribution is more closely associated with risk of diabetes, hypertension, and hyperlipidemia than total body fat or BMI (4). Thus, visceral fat values above 100–130 cm^2 represent a strong risk for these metabolic disturbances (5, 6). Moreover, both obesity and visceral fat accumulation have been reported to be related with increased postprandial lipemia (7), an underestimated CVD risk factor. However, it remains uncertain which measure of obesity represents the best predictor of postprandial lipemia response. Moreover, other behavioral factors, such as alcohol consumption, cigarette smoking, or poor eating patterns such as excessive calorie or high fat intake, are also significant determinants of postprandial lipemia, as well as lipid peroxidation, and deoxyribonucleic acid (DNA) damage (8–13).

The objectives of this study were to identify the anthropometrical factor most closely associated with variability on the postprandial lipid response to a fat load challenge and to investigate the relationships between this variability, lipid peroxides, and DNA damage of lymphocytes in groups of healthy nonobese men categorized according to the relevant anthropometrical factor emerging from our research. Our findings will contribute to a better understanding of the metabolic alterations and behavioral factors responsible for the rapid increase in CVD mortality in Korea and to provide guidance for preventive and therapeutic interventions.

METHODS

Subjects

Study subjects were recruited from volunteers who responded to advertisements for a nutrition study conducted by the Clinical Nutrition Research Team at Yonsei University in 2001. All subjects had normal glucose tolerance tests and electrocardiograms. None of them was taking any medication or had clinical evidence of CVD or cancer. Finally, 102 healthy men were selected as study subjects. The ranges for age and BMI were 21–53 years and 18.2–27.6 kg/m^2, respectively. Written informed consent was obtained from all subjects and the protocol was approved from the Ethical Committee of the Yonsei University.

Meal tolerance test

A 6 h postprandial lipemia response test was carried out starting at 8:30 AM after an overnight fast of greater than 12 h. A standardized test meal was prepared in the metabolic ward using common food items. It consisted of a sandwich containing white bread, lettuce, ham, and soybean oil-based mayonnaise. The energy content, calculated from the computerized database Korean food-code based on food composition tables from the National Rural Living Science Institute (6th edition, 2000) in Korea, was 608 kcal (2.54 mJ), representing the average calorie intake from a traditional breakfast. Fat represented 41.4% (28 g) of the calories, carbohydrates made up 45.4% (69 g), and 13.2% of calories (20 g) were derived from protein. In contrast, the macronutrient composition of the subjects' usual diet was that of a typical diet with cooked refined rice, consumed by a substantial number of Koreans, that is, about 57% of energy from carbohydrate, 22% from fat, 16% from protein, and 5% from others (mainly alcohol).

Anthropometrical and blood pressure measurements

Body weight and height were measured in the morning, unclothed and without shoes. BMI was calculated as body weight in kilograms divided by height in square meters. Waist and hip circumferences were combined into the WHR representing a commonly used surrogate of body fat distribution. Blood pressure was read from the left arm while subjects remained seated. An average of three measurements was recorded for each subject.

Regional fat and muscle areas

We performed computerized tomography (CT) scanning using a General Electric (GE) High Speed Advantage 9800 scanner (Milwaukee, WI) to measure fat and muscle areas. Four cross-sectional images were obtained from each subject. Two abdominal ones at the level of the first lumbar (L1) and fourth lumbar (L4) vertebras, one from the thigh (midway between patella and pubis), and one from the calf (at the most protruding area). Each CT slice was analyzed for the cross-sectional area of fat using a density control program available in the standard GE computer software. Parameters for total abdominal fat density at the levels of L1 and L4 were selected between the range of −150 and −50 Hounsfield units (HU). Total abdominal fat area was divided into visceral and subcutaneous fat areas to calculate specific fat areas. Parameters for thigh and calf muscle areas were selected as between the range of −49 and +100 HU and for fat areas between −150 and −50 HU.

Blood collection

Venous blood samples were obtained from the forearm and collected into EDTA-treated and plain tubes during fasting (baseline), and at 2 h, 3 h, 4 h, and 6 h after breakfast for assessment of glucose, insulin, free fatty acids (FFAs), and triglycerides (TGs). Tubes were immediately covered with aluminum foil and placed on ice until they arrived at the analytical laboratory (within 1–3 h) and were stored at −70°C. Subjects were asked to refrain from performing strenuous exercise or drinking alcoholic beverages 24 h prior to the fat tolerance test. They were also instructed to avoid eating or drinking anything except water during the test period.

Serum lipid profile and apolipoprotein A-I and B

Fasting serum concentrations of total cholesterol and TG were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). After precipitation of serum chylomicron, LDL, and VLDL with dextran sulfate-magnesium, HDL left in the supernatant was measured by an enzymatic method. LDL cholesterol concentrations were estimated indirectly using the Friedwald formula for subjects with serum TG < 4.52 mmol/l (400 mg/ml), or otherwise directly using commercially available kits on a Hitachi 7150 Autoanalyzer. Serum apolipoprotein A-I (apoA-I) and apoB were determined by turbidometry at 340 nm using a specific anti-serum (Roche, Switzerland).

Oral glucose tolerance test and homeostasis model assessment

At a different time than the fat tolerance test, we carried out a glucose load test in order to investigate glucose tolerance. Each subject received a 75 g glucose solution after an overnight fast.

Venous specimens were collected before and 30 min, 60 min, 90 min, and 120 min after the glucose load test. We used criteria, newly developed and modified by the National Diabetes Data Group and the World Health Organization Expert Committee on Diabetes Mellitus, to categorize subjects according to their glucose status (14). Glucose was measured by a glucose oxidase method using the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA). Insulin was measured by radioimmuno assays with commercial kits from Immuno Nucleo Corporation (Stillwater, MN). FFA was analyzed with a Hitachi 7150 autoanalyzer (Hitachi Ltd., Tokyo, Japan). Each response of glucose, insulin, and FFA was calculated with the area under each response curve. Insulin resistance (IR) was also calculated with the homeostasis model assessment (HOMA) (15) using the following equation: IR = [fasting insulin (μU/ml) $\times$ fasting glucose (mmol/l)]/22.5.

Other measurements

Immunoradiometric assays (IRMAs) were used to measure serum total testosterone using RIA-mat 280 (Byk-Sangtec Diagnostica, Germany) with Cost-A-Count total testosterone. Serum insulin like growth factor-1 (IGF-1) was measured using the IRMA kit from Diagnostic System Laboratories. Serum leptin was measured using Packard Cobra II 5005 R-Counter with the human leptin RIA kit from Linco.

Urine collection and 8-epi-prostaglandin $F_{2\alpha}$ and plasma malondialdehyde

Urine was collected after a 12 h fast in polyethylene bottles containing 1% butylated hydroxytoluene before blood collection. The tubes were immediately covered with aluminum foil and stored at −70°C until extraction. Urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) was quantified with gas chromatography (Hewlett Packard 6890, Wilmington, DE) and mass selective detector (Hewlett Packard 3973), according to the modified method of Pratico, Lawson, and Fitzgerald (16) and Mori et al. (17). Urinary creatinines were determined by the alkaline picrated (Jeffe) reaction (18), and urinary 8-epi-$PGF_{2\alpha}$ levels were expressed as picogram per milligram creatinine (pgl/mg of creatinine). Plasma malondialdehyde (MDA) was assayed according to the fluorometric method described by Buckingham (19).

Alkaline comet assay for DNA damage

For the comet assay, 120 μl whole blood was mixed with 900 μl PBS and poured gently over 150 μl lymphocyte separation solution (HISTOPAQUE-1077). After centrifugation at 1,450 rpm for 4 min, lymphocytes were pipetted out and transferred to another tube. DNA damage was analyzed basically as described by Green et al. (20). All steps were performed under dimmed light and the electrophoresis tank was covered with black paper to avoid additional light-induced DNA damage.

Vasodilatation response of brachial artery

Using high-resolution ultrasound (GE Vigmed Ultrasound, Horten, Norway), we assessed brachial arterial vasoreactivity to reactive hyperemia (flow-mediated dilation; FMD) and sublingual nitroglycerin (nitroglycerin-mediated dilation; NMD) with the method described by Kang et al. (21). A 10 MHz linear phased array ultrasound transducer (GE Vigmed Ultrasound, Horten, Norway) was used to image the dominant arm branchial artery longitudinally 3–5 cm just above the antecubital fossa. All subjects rested in the supine position for 10 min in a quiet room. The straight segment of the artery was chosen. After the depth and gain setting were optimized to identify the vessel wall, lumen interface and baseline brachial artery diameter were obtained. Brachial artery diameter was measured from the anterior to the posterior interface between the media and the adventitia and determined at end diastole on 2D image. Reactive hyperemia was induced by inflation and then deflation of a pneumatic cuff placed around the forearm portion. The blood pressure cuff was inflated to 250 mm Hg for 5 min, creating distal limb ischemia. After release of the cuff, brachial artery diameter was measured within the first 15 s of reactive hyperemia. FMD was then used as a measure of endothelium-dependent vasodilation. The brachial artery was allowed to return to the initial baseline level 10 min after cuff release. A further baseline brachial artery diameter was obtained. A 0.6 mg of nitroglycerin was then given sublingually, and the brachial artery diameter was then measured for the ensuing 3 min. The NMD was used as a measure of endothelium-independent vasodilation. The percent change in diameter caused by reactive hyperemia was calculated by dividing the difference from baseline end-diastolic diameter by the baseline value (FMD%). The percent change in diameter caused by nitroglycerin administration was also calculated in the same way (NMD%). Blood pressure and heart rate were measured before the examination. All data were calculated as an average from four consecutive cardiac cycles.

Assessment of food intake and physical activity level

Usual food intake was assessed with a 24 h recall method and a semi-quantitative food frequency questionnaire. Nutrient intake data were calculated as mean values from the same data base as referred above. Total calorie expenditure (kcal/day) was calculated from activity patterns including basal metabolic rate, physical activity for 24 h (22), and specific dynamic action of food. Basal metabolic rate for each subject was calculated with the Harris-Benedict equation (23).

Statistical analysis

We used SPSS version 11.0 for Windows (Statistical Package for the Social Science, SPSS Inc., Chicago, IL) for all our statistical analyses. Each variable was examined for normal distribution and significantly skewed variables were log transformed. For descriptive purposes, mean values were presented on untransformed and unadjusted variables. Results were expressed as mean ± SE. We used Pearson correlation coefficient to evaluate the correlation of the variables, and multiple regression analysis to investigate main factors influencing postprandial lipid response.

Following identification of the main factor, subjects in upper 25th percentile of the factor were selected and individually matched by age (within a 2 year difference) and BMI (within a 1 kg/m^2 difference) to subjects within the remaining 75th percentile. Selected subjects were grouped for subsequent analyses. Chi-squared tests were used to compare differences in frequencies for categorical variables. A two tailed value of $P < 0.05$ was considered statistically significant.

RESULTS

Basal anthropometrical, clinical, and behavioral characteristics of the participants

Table 1 shows the basal characteristics of the 102 subjects initially selected to participate in this study. Their mean BMI was 23.8 ± 0.21 with a range of 18.2 to 27.6 years. Seventy-four men reported consumption of alcoholic beverages and 39 were current smokers. These subjects had similar habitual dietary fat intake, physical activity, and socioeconomic status.

TABLE 1. General characteristics of the sample of 102 nonobese healthy men

		Min–Max
Age (years)	36.5 ± 0.82	(21–53)
BMI (kg/m^2)	23.8 ± 0.21	(18.2–27.6)
WHR	0.86 ± 0.00	(0.75–0.97)
Body fat (%)	23.5 ± 0.56	(11.0–38.0)
Blood pressure		
SBP (mmHg)	123.2 ± 1.30	(102–171)
DBP (mmHg)	77.0 ± 1.09	(55–119)
Current smoking (%)	38	
Tobacco consumption (cigarettes/day)	7.90 ± 0.95	(0–40)
Current drinking (%)	72	
Alcohol intake (g/day)	18.6 ± 2.48	(0.0–153.9)
Hypertension (%)	16	
Total energy expenditure (kcal/day)	2465.8 ± 36.7	(1862–2943)
Total calorie intake (kcal/day)	2402.8 ± 51.7	(1112–3104)
% fat from total calorie intake	21.9 ± 0.88	(11.9–33.8)
Adipose tissue areas (cm^2) at first lumbar vertebra		
Subcutaneous	72.1 ± 3.23	(12.8–182.7)
Visceral	98.7 ± 4.89	(13.3–227.0)
Adipose tissue areas (cm^2) at fourth lumbar vertebra		
Subcutaneous	76.2 ± 2.92	(17.3–139.4)
Visceral	126.2 ± 4.47	(44.4–266.6)
Postprandial TG area	972.4 ± 59.0	(225–3174)
MDA (nmol/ml)	3.11 ± 0.17	(0.40–7.31)
8-epi-PGF$_{2\alpha}$ (pg/mg creatinine)	251.8 ± 24.0	(20.2–1156.4)

BMI, body mass index; DBP, diastolic blood pressure; 8-epi-PGF$_{2\alpha}$, 8-epi prostaglandin F$_{2\alpha}$; MDA, malondialdehyde; SBP, systolic blood pressure; TG, triacylglycerol; WHR, waist-hip ratio. Mean ± SE.

Relationship of anthropometrical parameters to postprandial lipemia and lipid peroxidation

The average mean values of subcutaneous and visceral fat areas (VFAs) at L1 and L4 vertebrae, postprandial TG area, MDA, and 8-epi-PGF$_{2\alpha}$ of the 102 subjects are shown in Table 1. **Table 2** shows that some anthropometrical parameters such as body fatness and abdominal adipose tissue distribution (WHR and VFA at L1 and L4 vertebrae) were positively correlated with postprandial lipemia, whereas all variables reflecting body fatness and adipose tissue distribution were positively associated with plasma MDA concentrations. BMI, WHR, subcutaneous fat areas at L1 and L4 levels, and VFA at L4 showed positive correlation with urinary excretion of 8-epi-PGF$_{2\alpha}$. Postprandial lipemia showed positively significant correlation with plasma MDA concentration (R = 0.332, P = 0.005, data was not shown in Table 2). Age was associated with neither postprandial lipemia nor lipid peroxides.

TABLE 2. Pearson correlations of age, body fat distribution, total triglyceride response area, and lipid peroxides in 102 nonobese healthy men

	Postprandial TG Area[a]		MDA		8-epi-PGF$_{2\alpha}$[a]	
	R	P	R	P	R	P
Age	0.166	0.138	−0.032	0.771	−0.102	0.348
SBP	0.421	0.000	0.264	0.015	0.160	0.138
DBP	0.424	0.000	0.244	0.021	0.115	0.289
BMI	0.197	0.078	0.308	0.004	0.248	0.021
WHR	0.346	0.002	0.292	0.007	0.243	0.023
L1VF	0.461	0.000	0.327	0.002	0.201	0.064
L1SF	0.083	0.464	0.321	0.003	0.414	0.000
L4VF	0.586	0.000	0.356	0.001	0.242	0.025
L4SF	0.036	0.754	0.315	0.003	0.359	0.001

L1VF, visceral fat area at first lumbar vertebra; L1SF, subcutaneous fat area at first lumbar vertebra; L4VF, visceral fat area at fourth lumbar vertebra; L4SF, subcutaneous fat area at fourth lumbar vertebra.
[a] LN, log transformed.

Identification of main influencing factor on postprandial lipid response

Based on the information generated from the correlation analyses between body fat measures and postprandial TG area, we carried out stepwise multiple regression analysis in order to identify the most significant anthropometrical predictor of postprandial lipid response. Measures of body fatness, alcohol consumption, and cigarette smoking were used as independent variables, and postprandial TG area was the dependent variable. The data presented in **Table 3** show that the most significant predictor of postprandial lipemic response was VFA at L4 level followed by total fat area at L4 level.

Comparison of postprandial lipemia, lipid peroxidation, DNA damage, and endothelial cell function between the two groups categorized according to VFA at L4 level

Figure 1 shows the distribution of VFA at L4 level in all 102 nonobese healthy men. Subjects in upper 25th percentile of the main factor (VFA at L4 level) were categorized as high VFA (HVFA, n = 27). The lowest VFA in this group was over 100 cm^2 (100.3 cm^2) and the average area was 114 ± 2 cm^2. Subjects with HVFA were age- (within a 1 year difference) and BMI- (within a 1 kg/m^2 difference) matched to control subjects selected for having low VFA (LVFA; <100 cm^2, n = 27). The average area of the control group was 74 ± 3 cm^2.

Blood pressure and body fat distribution

Men within the HVFA were characterized by higher WHR and blood pressure and higher consumption of cigarettes than those within the LVFA group (**Table 4**). Among subjects within the HVFA, 38.5% had hypertension. No differences were found in subcutaneous fat area at both L1 and L4 vertebrae (Table 4). Nonobese men with HVFA showed higher ratio of visceral to subcutane-

TABLE 3. Stepwise multiple regression for 102 nonobese healthy men using age, blood pressure, anthropometric parameter, fat areas at different levels of body, alcohol consumption, and cigarette smoking as independent variables and postprandial TG area as dependent variable

Dependent Variable	Step	Independent Variable	R^2	Adjusted R^2	P
Postprandial TG area[a]	1 step	L4VF	0.344	0.336	0.000
	2 step	L4TF	0.396	0.381	0.000

L4TF, total fat area at 4th lumbar vertebra. Fat areas at different levels of body: total, visceral, and subcutaneous fat areas at both 1st and 4th lumbar vertebra.
[a] LN, log transformed.

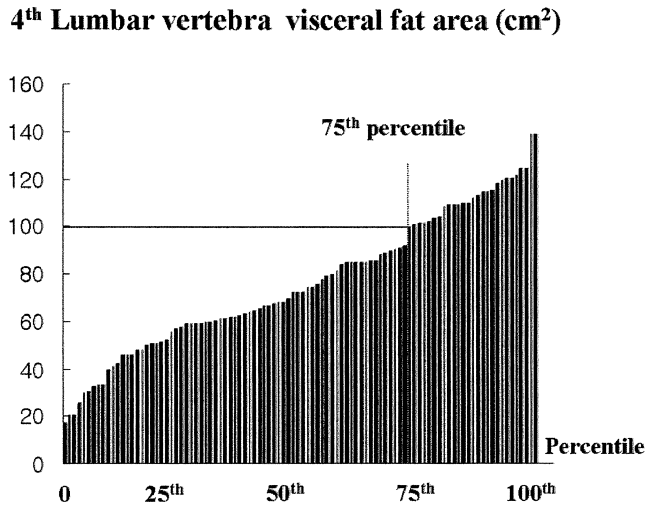

Fig. 1. Distribution of visceral fat area (VFA) at 4th lumbar vertebra in 102 nonobese healthy men.

ous fat area and lower muscle area of midthigh compared with those with LVFA.

Serum lipid, hormone, and oral glucose tolerance test

No differences were found in HDL cholesterol and apoA-I; however, men characterized by a HVFA showed higher serum concentrations of TG, total cholesterol, LDL cholesterol, and apoB (**Table 5**). Moreover, they had lower serum concentrations of testosterone and IGF-1 and higher fasting FFA levels than those with LVFA. Oral glucose tolerance test (OGTT) area under the curve for glucose, insulin, and FFA were higher in nonobese men with HVFA than in those with LVFA (Table 5).

TABLE 4. General characteristics, fat, and muscle areas in two groups of nonobese men matched on the basis of age and BMI but with low versus high visceral fat area

	Low VFA	HVFA
Age (years)	40.2 ± 1.71	40.1 ± 1.07
BMI (kg/m²)	25.2 ± 0.25	25.3 ± 0.30
Waist-hip ratio	0.86 ± 0.01	0.91 ± 0.01[c]
Body fat (%)	24.1 ± 0.87	26.0 ± 0.80
Blood pressure		
SBP (mmHg)	122.7 ± 1.94	132.7 ± 2.79[b]
DBP (mmHg)	75.0 ± 1.78	85.3 ± 2.23[c]
Current drinking (%)	75.0	88.9
Alcohol (g/day)	17.1 ± 5.49	25.2 ± 5.11
Current smoking (%)	39.3	70.4[a]
Tobacco (cigarettes/day)	3.50 ± 1.08	13.4 ± 2.02[b]
Hypertension (%)	10.7	38.5[a]
First lumbar vertebra		
Total fat (cm²)	177.6 ± 8.68	237.0 ± 10.5[b]
Visceral fat (cm²)	102.6 ± 5.92	149.8 ± 7.57[b]
Subcutaneous fat (cm²)	75.1 ± 4.06	87.2 ± 5.92
Visceral-subcutaneous fat ratio	1.40 ± 0.08	1.87 ± 0.14[a]
Fourth lumbar vertebra		
Total fat (cm²)	209.6 ± 7.83	259.8 ± 8.30[b]
Visceral fat (cm²)	73.7 ± 3.03	114.1 ± 2.03[b]
Subcutaneous fat (cm²)	135.8 ± 6.60	146.6 ± 7.86
Visceral-subcutaneous fat ratio	0.57 ± 0.03	0.83 ± 0.04[b]
Calf		
Fat (cm²)	16.3 ± 0.49	16.2 ± 0.80
Muscle (cm²)	82.8 ± 2.20	80.1 ± 3.25
Mid thigh		
Fat (cm²)	44.3 ± 2.19	43.0 ± 2.44
Muscle (cm²)	159.0 ± 1.78	147.7 ± 2.66[b]

HVFA, high visceral fat area (VFA ⩾ 100 cm², n = 27); VFA, visceral fat area. Low VFA, VFA < 100 cm², n = 27. Mean ± SE.
[a] $P < 0.05$ compared with low VFA.
[b] $P < 0.01$ compared with low VFA.
[c] $P < 0.001$ compared with low VFA.

TABLE 5. Serum lipids, hormones, and glucose and responses to a 75 g oral glucose tolerance test in two subgroups of nonobese men matched on the basis of age and BMI but with low versus high visceral fat area

	Low VFA	HVFA
TG (mg/dl)	148.2 ± 16.0	233.4 ± 22.6[a]
Total cholesterol (mg/dl)	192.2 ± 6.22	229.4 ± 7.29[b]
HDL cholesterol (mg/dl)	49.2 ± 2.30	45.5 ± 1.71
LDL cholesterol (mg/dl)	113.7 ± 5.28	137.7 ± 7.47[c]
Total/HDL cholesterol	4.08 ± 0.19	5.21 ± 0.23[b]
ApoA-I (mg/dl)	131.9 ± 4.50	127.9 ± 4.01
ApoB (mg/dl)	84.8 ± 2.82	105.7 ± 4.17[b]
Testosterone (ng/ml)	6.22 ± 0.23	5.33 ± 0.22[a]
IGF-1 (ng/ml)	289.2 ± 22.1	230.9 ± 10.3[c]
Leptin (ng/ml)	3.33 ± 0.26	3.94 ± 0.50
Fasting level		
Glucose (mg/dl)	83.0 ± 1.46	89.5 ± 2.80[d]
FFA (μEq/l)	434.7 ± 31.0	559.8 ± 43.8[c]
Insulin (μIU/ml)	8.35 ± 0.76	9.30 ± 0.72
Response area		
Glucose (mg/dl × h)	226.5 ± 8.16	259.0 ± 9.34[a]
FFA (μEq/l × h)	508.7 ± 43.9	654.4 ± 46.9[c]
Insulin (μIU/l × h)	77.9 ± 4.14	107.9 ± 7.24[a]
HOMA insulin resistance	1.70 ± 0.16	2.09 ± 0.19

Apo, apolipoprotein; FFA, free fatty acid; IGF-1, insulin-like growth factor-1; HOMA, homeostasis model assessment. n = 27. Mean ± SE.
[a] $P < 0.01$ compared with low VFA.
[b] $P < 0.001$ compared with low VFA.
[c] $P < 0.05$ compared with low VFA.
[d] $P < 0.1$ compared with low VFA.

Lipid peroxides, DNA damage in lymphocytes, and vasoreactivity of brachial artery

Nonobese men with HVFA showed higher concentrations of urinary excretion of $PGF_{2\alpha}$ and plasma MDA than those with LVFA (**Fig. 2**). Tail moments on the DNA in lymphocytes were also significantly higher in nonobese men with HVFA as compared with those in the LVFA group (Fig. 2). In brachial artery diameters at baseline to test for FMD% and NMD%, there were no significant differences between LVFA group and HVFA group (for FMD%; 4.46 ± 0.13 mm vs. 4.72 ± 0.14 mm, $P = 0.177$, and for NMD%: 4.48 ± 0.13 mm vs. 4.67 ± 0.14 mm, $P = 0.678$). Both groups had no significant differences in NMD% (LVFA group: 15.5 ± 1.8%; and LVFA group: 16.5 ± 1.7%); however, the HVFA group had significantly lower FMD% compared with the LVFA group (Fig. 2).

Postprandial responses of TG, glucose, FFA, and insulin

Figure 3 illustrates concentrations of TG, glucose, FFA, and insulin before and after the mixed-meal tolerance test among subjects with HFVA and LVFA. Subjects within the HVFA showed higher concentrations of TG at the 0 h, 2 h, 3 h, 4 h, and 6 h time points and 82% greater TG AUC compared with men in the LVFA group. TG concentra-

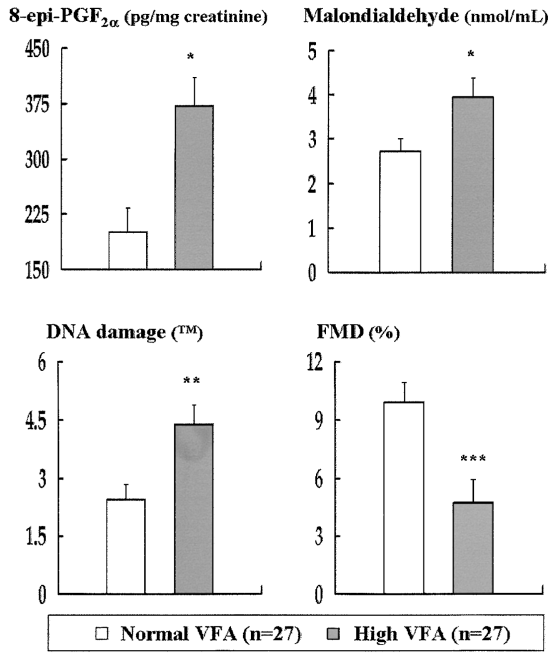

Fig. 2. Lipid peroxides, deoxyribonucleic acid (DNA) damage of lymphocytes and flow-mediated dilation of brachial artery in two subgroups of nonobese men matched on the basis of age and body mass index (BMI) but with low versus high VFA, mean ± SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.01$ compared with low VFA subgroup. Low VFA, VFA < 100 cm^2; high VFA, VFA ⩾ 100 cm^2; TM, tail moment = % of DNA in the tail × tail length (μm). 8-epi-$PGF_{2\alpha}$, 8-epi-prostaglandin $F_{2\alpha}$; FMD, flow-mediated dilator.

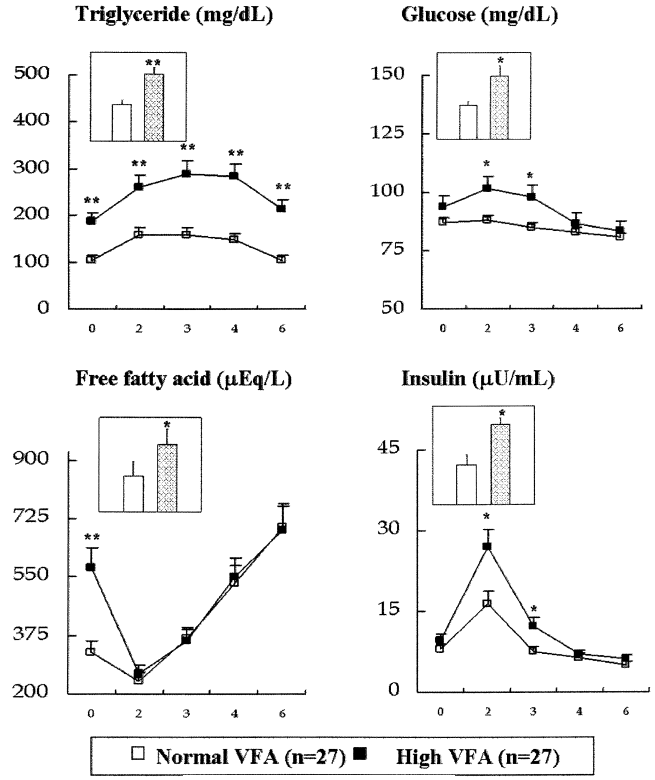

Fig. 3. Postprandial responses of triglyceride, glucose, free fatty acid, and insulin to a high-fat meal in two subgroups of nonobese men matched on the basis of age and BMI but with low versus high VFA mean ± SE. * $P < 0.05$, ** $P < 0.001$ compared with low VFA subgroup. Low VFA, VFA < 100 cm^2; high VFA, VFA ⩾ 100 cm^2.

tions at 6 h were back to fasting values among men with LVFA; however, among men with HVFA, TG concentrations measured at the 6 h time point remained 14% higher than fasting values ($P = 0.032$). Glucose and insulin concentrations at the 2 h and 3 h time points and fasting FFA were higher in the HVFA than in the LVFA group. Postprandial AUCs for FFA, glucose, and insulin were significantly increased by 28%, 14%, and 38%, respectively (Fig. 3), as compared with LVFA group.

Total calorie intake and total energy expenditure

There were no significant differences between the two groups in the proportion of energy intake derived from macronutrients and alcohol. However, men with HVFA showed higher total calorie intake, lower total energy expenditure, and lower ratio of total energy expenditure to total calorie intake than those with LVFA (**Table 6**).

Adjusted effects of visceral fat at L4 level on postprandial TG area, lipid peroxides, DNA damage, and endothelial cell function

The HVFA group had the increased levels of postprandial TG area, MDA, 8-epi-$PGF_{2\alpha}$, and DNA damage, and decreased levels of FMD compared with LVFA group (**Table 7**). However, because the significant differences in smoking status between the LVFA and HVFA groups we carried the analyses after adjustment for cigarette smoking. The results of these analyses provided a similar pattern to those described for unadjusted results.

DISCUSSION

Our data show that abdominal body fat distribution, more specifically VFA at the L4 level, an index of metabolic complications in obese subjects (24), was the major predictor of postprandial lipemia response (measured as TG AUC) in our population consisting of nonobese subjects. Moreover, we demonstrated tight associations between HVFA at the L4 level, peroxidation (measured as urinary 8-epi-$PGF_{2\alpha}$, plasma MDA, and DNA damage), and glucose tolerance (measured as glucose, insulin, and FFA concentrations). Most interestingly, even for subjects

TABLE 6. Usual total calorie intake and total energy expenditure in two subgroups of nonobese men matched based on age and BMI but with low versus high visceral fat area

	Low VFA	HVFA
Total energy expenditure	2555.8 ± 67.4	2364.8 ± 28.5[a]
Total calorie intake	2391.2 ± 67.5	2541.6 ± 43.7[a]
% protein	15.8 ± 0.43	16.4 ± 0.77
% carbohydrate	58.2 ± 1.18	54.7 ± 1.56
% fat	21.2 ± 1.14	22.8 ± 1.37
% alcohol	4.83 ± 0.57	6.10 ± 0.75
Total energy expenditure/ total calorie intake	1.06 ± 0.04	0.93 ± 0.01[b]

Mean ± SE.
[a] $P < 0.05$ compared with low VFA.
[b] $P < 0.01$ compared with low VFA.

TABLE 7. Cigarette smoking-adjusted effects of VFA at 4th lumbar vertebra on lipid peroxides, deoxyribonucleic acid damage, and endothelial cell function in two subgroups of nonobese men matched on the basis of age and BMI but with low versus high VFA

	Unadjusted *P*	Adjusted *P*
Postprandial TG area[a]	0.000	0.000
MDA	0.000	0.003
8-epi-$PGF_{2\alpha}$[a]	0.011	0.000
DNA damage[a]	0.000	0.000
FMD	0.000	0.000
Fasting glucose[a]	0.061	NS
Fasting FFA[a]	0.044	0.000
Fasting insulin	NS	NS
HOMA insulin resistance[a]	NS	NS

DNA, deoxyribonucleic acid; FMD, flow-mediated dilation; NS, not significant by ANCOVA.

[a] LN, log transformed.

with normal BMI, HVFA was associated with unhealthy lifestyle factors such as higher consumption of cigarettes and lower ratio of total energy expenditure to total calorie intake compared with subjects with LVFA.

Metabolic abnormalities, such as high blood pressure, high lipid concentrations, and increased areas of glucose, FFA, and insulin following an OGTT, in subjects with high visceral fat are consistent with a "visceral fat syndrome" proposed by Yamashita et al. (24). As visceral adipose tissue, not subcutaneous adipose tissue is drained by the portal venous system and has a direct connection with the liver, the higher lipolytic activity in visceral adipocytes leads to IR by causing FFA levels to increase in the liver (25, 26). In glucose metabolism, response areas of glucose, FFA, and insulin were higher in HVFA group compared with LVFA group; however, as $HOMA_{IR}$ was not significantly different between the two groups, this is probably due to the fact that changes in fasting glucose level might interfere with the consistency of fasting insulin levels when fasting insulin level is not high. Therefore, it might be difficult to show IR with only $HOMA_{IR}$ using only fasting levels of glucose and insulin (27). In addition to the altered fasting metabolic profile, comparison of postprandial lipid and glucose metabolism revealed that men characterized by high levels of visceral adipose tissue presented increased responses of TG, insulin, and glucose after the mixed-meal test. Moreover, glucose intolerance has been linked to oxidation status or lipid peroxidation (28). Consistent with this notion, our data show that higher levels of plasma MDA and urinary excretion of 8-epi-$PGF_{2\alpha}$ in subjects with HVFA compared with those with LVFA. In addition, we have shown that urinary excretion of 8-epi-$PGF_{2\alpha}$ and plasma MDA correlate with other more commonly used adiposity indexes, such as BMI and WHR. Nevertheless, we found some specific relations between body fat measures and these two measures of lipid peroxidation that may result from differences in specificity and sensitivity in assessing lipid peroxidation (29, 30). MDA is a general indicator of oxidative stress rather than a specific marker of lipid peroxidation and it is known to vary in response to the diet (23, 24). Dietary factors such as the amounts of fat intakes and alcohol even in nonobese subjects, especially when calorie intake is in excess of body needs, may induce the accumulation of visceral fat (13), which is characterized by its high lipogenic activity as well as its accelerated lipolytic activity (24).

Oxidative stress, including lipid peroxidation, can negatively influence the antioxidant system (28, 29) and consequently cause greater DNA damage (31). Evaluation of DNA damage in lymphocytes has been used as a biological marker in the detection, monitoring, and prognosis of chronic degenerative diseases such as atherosclerosis (31). Differences in the extent of DNA damage in the normal population have been reported to not only depend on aging but also depend on eating and smoking habits (9, 32). In this study, the HVFA fat group had increased cigarette smoking, higher lipid peroxidation, and consequently with higher tail moment of DNA compared with subjects included in the LVFA group. However, most interestingly, even after adjusting for cigarette smoking, DNA damage and lipid peroxides between the two groups were still significantly different. This suggests an independent association between visceral fat accumulation and oxidation. Increased oxidative stress is also known to cause endothelial dysfunction by increased vascular superoxide anion production and subsequent decrease in nitric oxide (NO) bioavailability through superoxide-induced NO degradation (12). Endothelial dysfunction has been also associated with a range of CVD risk factors including smoking (33), visceral fat accumulation independent of BMI (34) and subcutaneous fat area (35), and fasting or postprandial hypertriglyceridemia (11). All of these risk factors were observed in our nonobese men with HVFA.

There is substantial evidence supporting a correlation between visceral fat accumulation and endocrine abnormalities including low concentrations of testosterone and IGF-1 (4, 36, 37). Generally, serum concentrations of testosterone have a negative correlation with visceral fat accumulation in men (38–40). Because testosterone decreases the lipoprotein lipase activity of visceral fat cells, it can limit the fat accumulation in visceral region (38–40). On the other hand, it increases the number of β-adrenergic receptors and the activity of hormone sensitive lipase, accelerating the lypolysis from visceral fat (38–40). IGF-1 also plays an important role in body fat quantity and distribution by reducing abdominal VFA, and building up muscles and bones (34, 35). Serum concentrations and the activities of testosterone and IGF-1 are inversely correlated with age, smoking, and visceral fat (37, 39–42). Accordingly, our study shows that subjects within the HVFA group had lower levels of testosterone and IGF-1 compared with LVFA group. From this experimental design, we cannot conclude whether these reduced levels might cause visceral fat accumulation or, alternatively, visceral fat accumulation itself might bring a reduction in testosterone and IGF-1 levels. Serum leptin levels were not significantly different between the two groups, in agreement with reports showing that leptin levels were related with total or subcutaneous fat accumulations rather than with visceral fat accumulation (43, 44).

Finally, these nonobese men with HVFA showed positive energy balance and lower levels of muscle area at midthigh compared with their low visceral fat counterparts, consistent with previous studies showing that body fat and muscle distribution relate to behavioral habits including diet, physical activity, and smoking (36, 45).

In conclusion, we found significant correlations between abdominal fat distribution, postprandial lipemia, and lipid peroxidation in nonobese apparently healthy men. Specifically, our data show that visceral fat accumulation at the L4 level is the most significant correlate of impaired postprandial lipid response, lipid peroxidation, DNA damage of lymphocytes, and endothelial dysfunction, all of them implicated in cardiovascular morbidity and mortality risk. The mechanisms behind the relationship between visceral obesity, lipid peroxidation, DNA damage, and endothelial dysfunction need to be elucidated in order to implement successful therapeutic approaches. It has been previously shown that visceral adipose tissue accumulation at L4 and L5 is positively associated with C-reactive protein levels, suggesting that the expanded abdominal fat depot may induce a low-grade inflammatory state (46, 47), which in turn will stimulate lipid peroxidation (48). This increased lipid peroxidation could then promote DNA damage, which has been shown to contribute to vascular dysfunction (49). Moreover, vascular dysfunction may also result directly from visceral obesity independently of other conventional risk factors (50).

A most important finding is the fact that all these associations were observed among subjects who would have been considered normal by the commonly used anthropometrical risk factors. This is consistent with the dramatic increases in CVD rates observed in Korea in the absence of significant BMI increases, supporting the greater relevance of fat distribution. Therefore, the major goal of intervention studies should be the reduction of visceral fat through physical exercise and heart-healthy nutrition guidelines even for those subjects who are considered at low risk based on their BMI values. Moreover, similar studies are needed on White subjects to investigate the generality of our findings.

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