The relationship between circulating neutrophil gelatinase-associated lipocalin and early alteration of metabolic parameters is associated with dietary saturated fat intake in non-diabetic Korean women

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Abstract. Circulating neutrophil gelatinase-associated lipocalin (NGAL) is associated with obesity-related metabolic disorders. This study investigated the relationship between serum NGAL and early alteration of metabolic parameters in non-diabetic Korean women, particularly with respect to saturated fat (SFA) intake. Anthropometric parameters, fasting glycemic status, and levels of lipids, oxidative stress/inflammatory markers, and NGAL were measured in 82 non-diabetic Korean women [Super-healthy group (n=57) with 0 metabolic syndrome risk factor (MetS RF) and MetS-risk group (n=25) with MetS RF≥1]. Age, weight, waist circumference, blood pressure, fasting glucose, HbA1C, triglyceride, LDL and total-cholesterol, and NGAL levels were higher, and HDL-cholesterol was lower in the MetS-risk group than in the Super-healthy group. Age-adjusted serum NGAL levels were higher in the MetS-risk group than in the Super-healthy group. NGAL increased proportionally with increase in MetS RFs (p=0.038) and correlated positively with BMI, triglycerides, LDL- and total-cholesterol, interleukin-6, white blood cell count, and neutrophil%, and negatively with HDL-cholesterol and superoxide dismutase activity. Serum NGAL levels positively correlated with SFA intake before and after adjustment (age and BMI). Serum NGAL levels were higher in high-SFA consumers [≥7g/day, ≥7% of total calorie intake (TCI)] than in low-SFA consumers (<7g/day, <7% of TCI). Serum NGAL levels were highest in the MetS-risk group consuming higher SFA and lowest in the Super-healthy group consuming lower SFA. However, serum NGAL did not significantly differ between the low-SFA consuming MetS-risk and Super-healthy groups. The relationship between circulating NGAL and early alteration of metabolic parameters is associated with dietary SFA intake in non-diabetic Korean women.

Key words: Neutrophil gelatinase-associated lipocalin, Metabolic syndrome, Saturated fat intake, Inflammation, Oxidative stress

THE PREVALENCE of obesity is increasing worldwide, and approximately 32.5% of adults aged 20 and above in Korea are considered obese [body mass index (BMI) ≥ 25 kg/m²] [1]. Obesity increases the risk of various metabolic disorders such as hypertension, dyslipidemia, metabolic syndrome (MetS), and type 2 diabetes [2, 3], which are highly correlated with inflammation, immune dysfunction and oxidative stress [4-10]. A cluster of metabolic abnormalities collectively known as a MetS contributes to the increased risk of cardiovascular disease (CVD) and related mortality [11, 12].

Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; CVD, cardiovascular disease; DBP, diastolic blood pressure; FBG, fasting blood glucose; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; NGAL, neutrophil gelatinase-associated lipocalin; PUFA, polyunsaturated fatty acid; SBP, systolic blood pressure; SFA, saturated fatty acid; TG, triglyceride; TC, total cholesterol.

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Recent studies have suggested that serum levels of neutrophil gelatinase-associated lipocalin (NGAL) are associated with obesity, MetS, diabetes, and CVD [13-16]. NGAL, also known as lipocalin-2 or growth factor-stimulated superinducible protein 24, is a 25 kDa secretory glycoprotein [17] that was first identified as a protein secreted from human neutrophils [18]. It belongs to the lipocalin superfamily and circulates as a monomer, homodimer, or a heterodimer complex with matrix metalloproteinase [17]. NGAL was reported to participate in various functions, including apoptosis and innate immunity, but primarily in the response to bacterial infection [19, 20]. NGAL is also expressed in multiple tissues such as kidney, liver, uterus, and bone marrow [21-23]. Several reports have suggested that NGAL is a sensitive biomarker for various forms of kidney injuries [24-26]. For example, when the renal tubules are damaged because of infection or nephrotoxicants, or in ischemic conditions, the expression of NGAL in the tubular cells rises about 1,000-fold, resulting in an increase in plasma NGAL levels [26].

Recently, NGAL was found to be abundantly expressed and secreted from adipocytes [27, 28]. Wang et al. reported that serum NGAL levels in obese individuals, particularly those with diabetes, were higher than those in lean non-diabetic individuals, even when adjusted for age and sex, although the significance disappeared when further adjusted for BMI [13]. A cross-sectional study conducted by Huang et al. in 2,519 Chinese subjects showed that serum NGAL levels in subjects with impaired glucose tolerance, impaired fasting glucose, and newly diagnosed type 2 diabetes were significantly higher than those in subjects with normal glucose regulation [14]. Moreover, NGAL levels showed a positive correlation with insulin resistance (IR), measured with the homeostasis model assessment-estimated IR (HOMA-IR) [14]. Ni et al. reported that serum NGAL levels in patients with coronary artery disease (CAD) were higher than those in non-CAD subjects. Similarly, NGAL levels in men with MetS were significantly higher than those in individuals without MetS [15]. Moreover, the number of MetS components positively correlated with serum NGAL levels [15]. In addition, Moreno-Navarrete et al. reported an interesting result for the relationship between NGAL and dietary intake showing that the change in the total intake of carbohydrates, total fat, or protein was not associated with changes in NGAL levels [29]. In contrast, the percentage change in the SFA intake relative to the total caloric intake was specifically associated with NGAL variations after weight reduction [29].

Thus far, no study has been conducted investigating the relationship between serum NGAL levels and early alteration of metabolic status in non-diabetic healthy Korean women, particularly with respect to dietary saturated fat intake. Therefore, in this study, we aimed to determine if serum NGAL levels reflect early alteration of metabolic parameters in non-diabetic Korean women, and if this relationship is associated with dietary saturated fat intake.

**Participants and Methods**

**Study participants and design**

The participants in this study were women recruited from the health promotion center at Dong-A University Hospital between January 2014 and March 2014. Exclusion criteria were diagnosis of vascular, renal, liver, or thyroid disease, acute or chronic inflammatory diseases, diabetes, cancer (diagnosed clinically or by anamnesis), orthopedic limitations, and weight loss/gain over the previous 6 months. None of the participants was taking antihypertensives, antidyslipidemics, antithrombotics, or antidiabetic medications. The participants were interviewed about their smoking and drinking behavior, and dietary patterns. They were subdivided into two groups according to their number of MetS risk factors (MetS RF): a “Super-healthy group” (MetS RF = 0, n = 57) and a “MetS-risk group” (MetS RF ≥ 1, n = 25). MetS RFs, which are based on a combination and modification of the NCEP-ATPIII guidelines, Asian-Pacific guidelines, and American Diabetes Association guidelines, include the following: 1) waist ≥ 85 cm; 2) systolic blood pressure (BP) ≥ 130 mmHg or diastolic BP ≥ 85 mmHg; 3) fasting blood glucose ≥ 100 mg/dL; 4) serum HDL cholesterol < 50 mg/dL; and 5) serum triglycerides ≥ 150 mg/dL. The final 82 individuals who met the criteria agreed to participate and signed the participation consent form. The written informed consent was obtained from all the participants, and the study was approved by the Institutional Review Board of Dong-A University.

**Anthropometric parameters, blood pressure, and blood collection**

Body weight and height were measured in the morning, with participants lightly clothed and without
shoes. Body weight was measured using a TBF-105 body fat analyzer (Tanita Corp. Tokyo, Japan), and standing height was measured with a wall stadiometer. Body mass index (BMI; kg/m^2) was calculated as body weight divided by height in square meters. Waist circumference was measured at the umbilical level in standing participants after normal expiration. BP was measured at seated patients’ arms after a rest for 20 minutes, using an automatic blood pressure monitor (HEM-7220, Omron, Matsusaka, Japan). After overnight fasting (12 hours), venous blood specimens were collected in tubes with or without EDTA treatment, centrifuged for the collection of serum or plasma, and then stored at -80°C until analysis.

**Serum fasting glucose, glycosylated hemoglobin (HbA1C), lipid profiles, albumin levels, and blood cell counts**

Fasting glucose levels were measured using the glucose oxidase method with a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, USA). HbA1C was measured using the VARIANT II TURBO HbA1C Kit-2.0 (Bio-Rad, Hercules, CA, USA) with a Variant analyzer (Variant II TURBO, Bio-Rad, Hercules, CA, USA). Fasting levels of total cholesterol, triglyceride, and low-density lipoprotein (LDL) cholesterol were measured using commercially available kits with a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). After precipitating serum chylomicrons, very low-density lipoprotein (VLDL), and LDL with dextran sulfate magnesium, high-density lipoprotein (HDL) cholesterol levels in the supernatants were enzymatically measured. Fasting serum albumin levels were measured using commercially available kits with a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). White blood cell (WBC) and neutrophil counts were determined using the HORIBA ABX diagnostics system (HORIBA ABX SAS, Parc Euromedicine, France).

**Plasma interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels**

Plasma IL-6 and TNF-α levels were measured using the Quantikine® HS ELISA Kit (R&D systems, Inc., Minneapolis, MN, USA), according to the manufacturer’s instructions. Resultant color reactions were measured using an iMark™ microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA, USA). The wavelength correction was set at 490 and 560 nm.

**Serum superoxide dismutase (SOD) activity and NGAL levels**

Serum SOD activity was assessed using a kit based on the generation of superoxide radicals by xanthine and hypoxanthine (BioVision, Inc., Milpitas, California, USA). Absorbance was read at 450 nm with the iMark™ microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA, USA). Serum NGAL levels were measured using a Human LCN2 Immunoassay kit (Antibody and Immunoassay Services, University of Hong Kong, HK, China). The absorbances of the resulting reaction mixtures were read at 450 nm with the iMark™ microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA, USA). The intra- and inter-assay coefficients of variation were 2.68% and 8.36%, respectively.

**Markers of liver and kidney function**

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using a modified method from the International Federation of Clinical Chemistry. Serum creatinine levels were measured with a kinetic colorimetric (Jaffe) assay. Serum urea nitrogen (BUN) levels were measured using a kinetic UV assay.

**The assessment of dietary intake and physical activity level**

Information on each participant’s usual diet was obtained using a 24-hour recall method. All participants were given written and verbal instructions by a registered dietitian on completion of a recent 3-day (2 weekdays and 1 weekend) dietary intake. Dietary energy values and nutrient content were calculated using the Computer Aided Nutritional Analysis Program (CAN-pro 4.0, Korean Nutrition Society, Seoul, Korea). Total energy expenditure (kcal/day) was calculated from activity patterns including basal metabolic rate, physical activity for 24 hours [30], and specific dynamic actions of food. Basal metabolic rate for each participant was calculated with the Harris-Benedict equation [31].

**Statistical analysis**

Statistical analyses were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Student’s *t*-test was used to compare parameters between the two groups. The correlation between basic and biochemical parameters, and NGAL levels...
was tested by Pearson or partial correlation analyses. Parameters were compared between the two groups using one-way analysis of variance (one-way ANOVA) with the Bonferroni correction. A general linear model analysis (post-hoc multiple comparison tests) followed by Bonferroni correction was also performed to evaluate the differences in the parameters among the subgroups after adjustment for the confounding factors of age and BMI. Skewed variables were log-transformed for statistical analysis. For descriptive purposes, mean values are presented using untransformed values. The results are expressed as mean ± standard error (SE) or percentages. If the two-tailed p value was less than 0.05, data were considered statistically significant.

### Results

**General characteristics and biochemical parameters of study participants**

General characteristics, biochemical parameters, and dietary intake of study participants are presented in Table 1. Age, BMI, waist circumference, systolic/diastolic BPs, fasting glucose, HbA1C%, and triglycerides and LDL cholesterol in the MetS-risk group were higher and HDL cholesterol levels were lower than those in the Super-healthy group (all p-values were less than 0.05). Total cholesterol in the MetS-risk group tended to be higher than that in the Super-healthy group, but this difference was not statistically significant. Other parameters such as serum levels of

| Table 1 General characteristics, biochemical parameters an dietary intake of study women |
|---------------------------------------------|--------------------------|---------------------|---------------------|
| Age (year)                                  | 44.6 ± 1.19             | 51.9 ± 2.08         | 0.002              |
| BMI (kg/m²)                                 | 21.8 ± 0.31             | 25.1 ± 0.75         | <0.001             |
| Waist (cm)                                  | 71.8 ± 0.82             | 80.2 ± 1.85         | <0.001             |
| Glucose (mg/dL) *                           | 85.9 ± 0.88             | 98.6 ± 2.94         | <0.001             |
| HgbA1c (%)                                  | 5.18 ± 0.04             | 5.46 ± 0.08         | 0.001              |
| Systolic BP (mm Hg)                         | 108.7 ± 1.01            | 119.4 ± 2.84        | <0.001             |
| Diastolic BP (mmHg)                         | 69.6 ± 0.75             | 73.7 ± 1.60         | 0.009              |
| Current smoker (%)                          | 1.80                    | 0.00                | 0.695              |
| Current drinker (%)                         | 42.9                    | 48.0                | 0.155              |
| Triglyceride (mg/dL) *                      | 65.9 ± 4.05             | 124.5 ± 16.7        | 0.002              |
| HDL-cholesterol (mg/dL)                     | 71.1 ± 1.63             | 57.0 ± 3.35         | <0.001             |
| LDL-cholesterol (mg/dL)                     | 114.2 ± 3.28            | 135.9 ± 7.47        | 0.012              |
| Total cholesterol (mg/dL)                   | 188.0 ± 3.15            | 206.3 ± 8.93        | 0.062              |
| Albumin (g/dL)                              | 4.41 ± 0.02             | 4.40 ± 0.05         | 0.914              |
| AST (U/L)                                   | 25.6 ± 1.05             | 25.1 ± 1.33         | 0.792              |
| ALT (U/L)                                   | 20.4 ± 1.63             | 24.7 ± 2.53         | 0.159              |
| Creatinine (mg/dL)                          | 0.76 ± 0.01             | 0.71 ± 0.02         | 0.053              |
| BUN (mg/dL)                                 | 13.2 ± 0.43             | 12.8 ± 0.76         | 0.675              |
| Uric acid (mg/dL)                           | 3.97 ± 0.10             | 4.18 ± 0.19         | 0.270              |
| Daily dietary intake                        |                         |                     |                    |
| TEE/TCI                                     | 0.94 ± 0.04             | 0.95 ± 0.05         | 0.587              |
| Protein (%)                                 | 17.0 ± 0.47             | 17.5 ± 0.61         | 0.602              |
| Fat (%)                                     | 25.2 ± 0.94             | 21.9 ± 1.55         | 0.065              |
| Carbohydrate (%)                            | 57.8 ± 1.12             | 60.6 ± 1.84         | 0.176              |
| Fiber (g/day)                               | 22.7 ± 1.06             | 22.9 ± 1.83         | 0.927              |
| Cholesterol (mg/day)                        | 300.4 ± 24.4            | 253.6 ± 27.8        | 0.259              |
| SFA (g/day)                                 | 7.89 ± 0.66             | 8.05 ± 1.18         | 0.980              |
| MUFA (g/day)                                | 9.62 ± 0.76             | 8.85 ± 1.34         | 0.601              |
| PUFA (g/day)                                | 9.00 ± 0.55             | 7.77 ± 0.90         | 0.234              |

Data are means ± S.E. or percentage (%); * tested by log transformed; tested by independent t-test (student t-test); BMI, body mass index; HgbA1c, glycated hemoglobin; BP, blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; MUFA, monounsaturated fats; PUFA, polyunsaturated fats; SFA, saturated fats; TEE, total energy expenditure; TCI, total calorie intake.
albumin, AST, ALT, creatinine, BUN, and uric acid, and consumption of alcohol and cigarettes were not significantly different between the two groups. Regarding information on dietary intake of study participants, no significant differences were observed between the two groups.

Comparison of inflammatory, immune, and antioxidant markers between the Super-healthy group and the MetS-risk group

As shown in Table 2, no significant differences were found between the two groups in inflammatory (IL-6 and TNF-α levels), immune (WBC count and neutrophil percentage), and antioxidant (SOD activity) markers. The WBC count in the MetS-risk group tended to be higher than that in the Super-healthy group, but the average values in both groups were within the normal range.

Serum NGAL levels according to the number of MetS RFs in non-diabetic women

Fig. 1(a) shows the serum NGAL levels of the two participant groups. The average serum NGAL level in the Super-healthy group was significantly lower than that in the MetS-risk group, after adjustment for age (Super-healthy group: 118.60 ± 5.31 ng/mL, MetS-risk group: 140.79 ± 8.06 ng/mL; p = 0.028). As shown in Fig. 1(b), serum NGAL levels increased proportionally with the increasing number of MetS RFs (MetS RF 0: 118.60 ± 5.31 ng/mL, MetS RF 1-2: 135.9 ± 8.5 ng/mL, and MetS RF ≥3: 168.2 ± 19.5 ng/mL; p = 0.038), after adjustment for age.

Table 2  Inflammatory, immune and antioxidant biomarkers in study women

<table>
<thead>
<tr>
<th></th>
<th>Super-healthy (n=57)</th>
<th>MetS-risk (n=25)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/mL)</td>
<td>1.08 ± 0.16</td>
<td>0.96 ± 0.17</td>
<td>0.650</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.04 ± 0.09</td>
<td>1.38 ± 0.41</td>
<td>0.276</td>
</tr>
<tr>
<td>White blood cell (×10⁹/L)</td>
<td>4.85 ± 0.18</td>
<td>5.35 ± 0.26</td>
<td>0.086</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>53.74 ± 1.37</td>
<td>52.64 ± 1.96</td>
<td>0.652</td>
</tr>
<tr>
<td>SOD activity (%)</td>
<td>69.36 ± 0.99</td>
<td>70.27 ± 1.25</td>
<td>0.599</td>
</tr>
</tbody>
</table>

Data are means ± S.E. or percentage (%), * tested by log transformed; tested by independent t-test (student t-test). There were no significant differences in the values between the two groups. IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; SOD, superoxide dismutase.

Fig. 1  Serum NGAL levels according to MetS risk

(a) Serum NGAL levels in the Super-healthy group and the MetS-risk group (MetS RF ≥ 1). (b) Serum NGAL levels according to MetS RF. The columns and error bars in both graphs indicate the means ± standard error. Results were obtained by general linear model analysis followed by Bonferroni correction with adjustment for age; * p < 0.05 compared with the MetS-risk group. Sharing the same alphabet indicates no significant difference in each variable in the same row. MetS RF, metabolic syndrome risk factor number; NGAL, neutrophil gelatinase-associated lipocalin.
Relationships between serum NGAL levels and BMI, fasting lipid profiles, and inflammatory, immune, and antioxidant related biomarkers

As shown in Fig. 2, serum NGAL levels were positively correlated with BMI ($r = 0.308, p = 0.005$); fasting levels of lipids such as triglycerides ($r = 0.306, p = 0.006$), LDL cholesterol ($r = 0.269, p = 0.016$) and total cholesterol ($r = 0.313, p = 0.005$); WBC count ($r = 0.324, p = 0.003$); neutrophil percentage ($r = 0.408, p < 0.001$); and IL-6 levels ($r = 0.222, p = 0.047$). In contrast, serum NGAL levels were negatively correlated with SOD activity ($r = -0.252, p = 0.024$).

Correlation between serum NGAL levels and saturated fat intake

We performed correlation analyses to investigate the relationship between serum NGAL levels and daily dietary nutrient intake. Among the nutrients examined, saturated fat (SFA) intake was significantly correlated with serum NGAL levels. As shown in Fig. 3, serum NGAL levels were positively correlated with SFA intake before and after adjustment for age and BMI (SFA intake in g/day: $r_0 = 0.237, p_0 = 0.038$; $r_1 = 0.234, p_1 = 0.043$; SFA as a percentage of total calorie intake (TCI)/day: $r_0 = 0.251, p_0 = 0.023$; $r_1 = 0.279, p_1 = 0.012$).

Serum NGAL levels according to MetS risk and saturated fat intake

Study participants were subdivided into two groups according to SFA intake level, and into four groups according to SFA intake level and the presence of MetS RF (Fig. 4). Fig. 4(a) shows that serum NGAL levels in participants consuming $\geq 7$ g of SFA/day were significantly higher than those in participants consuming $<7$ g of SFA/day. An intake of 7 g of SFA/day represents the 50th percentile of SFA intake in participants in this study. Fig. 4(b) shows that serum NGAL levels in participants with MetS RF who consumed $\geq 7$ g of SFA/day were significantly higher than the levels in the other three groups. However, when the SFA intake was $<7$ g/day, serum NGAL levels were not significantly different between the Super-healthy and MetS-risk groups.

In addition, the guidelines of Korean dietary reference intakes recommend that the SFA intake be $<7$% of TCI. Therefore, we subdivided study participants into two groups: $<7$% of TCI from SFA intake and $\geq 7$% of TCI from SFA intake. Consuming $\geq 7$% of TCI from SFA represents the upper 25th percentile of participants in this study. The graph in Fig. 4(c) shows that serum NGAL levels in participants consuming $\geq 7$% of TCI from SFA were significantly higher than NGAL levels in those consuming $<7$% of TCI from SFA. The graph in Fig. 4(d) shows that serum NGAL levels in participants with MetS RF who were consuming $\geq 7$% of TCI from SFA were the highest among the four subgroups. In addition, among Super-healthy participants, serum NGAL levels in higher SFA consumers ($\geq 7$% of TCI) were significantly higher than those in lower SFA consumers ($<7$% of TCI). However, when the SFA intake was $<7$% of TCI, serum NGAL levels were not significantly different between the Super-healthy and the MetS-risk group.

Correlation between serum NGAL levels and inflammation related parameters according to saturated fat intake

The relationship between serum NGAL levels and inflammation related parameters were differently observed according to SFA intake (Supplementary Table 1). Serum NGAL levels significantly correlated with inflammation related parameters in subjects consuming $\geq 7$ g of SFA/day. On the other hand, the significant correlations were not observed in those consuming $<7$ g of SFA/day. Similar patterns were also observed when SFA intake was categorized by % of TCI, but some of significances turned to tendency or disappeared, which may be due to small number of subjects consuming SFA intake $\geq 7$% of TCI/day.

Discussion

This present study shows for the first time that circulating NGAL levels may reflect early alteration of metabolic parameters in non-diabetic Korean women, and that this relationship is associated with dietary SFA intake. Among the high SFA consumers, serum NGAL levels in the MetS-risk group were significantly higher than those in the Super-healthy group. However, among low SFA consumers, serum NGAL levels were not significantly different between the MetS-risk and Super-healthy groups. Thus, this study suggests that the relationship between circulating NGAL levels and early alteration of metabolic parameters was associated with dietary SFA intake in non-diabetic Korean women.

NGAL is produced in small amounts by the epithelial cells in various kinds of tissues and organs such
Fig. 2 Relationships between serum NGAL levels and body mass index; serum fasting lipid profiles; and inflammatory, immune and antioxidant related biomarkers

Correlations between serum NGAL and (a) BMI, (b) serum triglyceride, (c) serum LDL cholesterol, (d) serum total cholesterol, (e) WBC count, (f) neutrophil percentage, (g) IL-6 levels, and (h) SOD activity. Correlations were analyzed using Pearson correlation analysis; * tested after log-transformation. BMI, body mass index; IL-6, interleukin-6; NGAL, neutrophil gelatinase-associated lipocalin; SOD, superoxide dismutase; WBC, white blood cells.
Correlations between serum NGAL and (a) SFA intake (g/day) and (b) SFA % of TCI/day; tested by Pearson ($r_p$) and partial ($r_1$) correlation analysis. $p_0$, $p$-value from Pearson correlation analysis (unadjusted), $p_1$, $p$-value from partial correlation analysis (adjusted for age and body mass index). NGAL, neutrophil gelatinase-associated lipocalin; SFA, saturated fat; TCI, total calorie intake.

Serum NGAL levels according to MetS risk and saturated fat intake

(a) Serum NGAL levels according to SFA intake (g/day), (b) serum NGAL levels according to SFA intake (g/day) and MetS risk, (c) serum NGAL levels according to SFA intake (% of TCI), and (d) serum NGAL levels according to SFA intake (% of TCI) and MetS risk. Data are presented as means ± standard error. Data were analyzed using the general linear model followed by Bonferroni correction with adjustment for age and BMI. * $p < 0.05$; sharing the same alphabet indicates no significant difference in each variable in the same row. Korean dietary reference intakes recommend saturated fat intake of less than 7% of TCI. SFA intake ≥ 7g/day in this study represents the 50th percentile of study participants. SFA intake ≥ 7% of TCI in this study represents the 25th percentile of study participants. BMI, body mass index; NGAL, neutrophil gelatinase-associated lipocalin; SFA, saturated fat; TCI, total calorie intake.
as kidney, lung, digestive tract, and prostate [21-25]. NGAL is easily detected in the urine since it passes through the kidney because of its low molecular weight [24, 25, 32], and therefore, it has often been measured in renal studies [24, 25]. It also accumulates in the renal cortical tubules and blood vessels [32]. As mentioned above, recent studies have shown positive correlations between serum NGAL levels and the risk of obesity, MetS, diabetes, and CVD [13-16]. According to Wang et al., NGAL levels are positively correlated with adiposity indexes such as BMI, waist circumference, and body fat percentage [13]. Consistent with these findings, our present study shows a positive correlation between serum NGAL levels and BMI. The physiological relationship between circulating NGAL levels and obesity remains unclear. However, the results of a few studies have suggested that NGAL is abundantly expressed and secreted from adipocytes [27, 28], which supports the association between circulating NGAL levels and obesity. In addition, circulating NGAL levels positively correlated with parameters of obesity-related metabolic disorders, including abnormal lipid profiles, hyperinsulinemia, fasting glucose, and HOMA-IR [13]. These findings are consistent with the findings of our study. Law et al. identified that NGAL deficiency attenuates the development of aging- and obesity-associated hyperglycemia, hyperinsulinemia, and IR using their NGAL knockout mouse model [33]. They reported that NGAL activates the arachidonate 12-lypoxygenase metabolic pathway, and stimulates the expression of TNF-α, an inflammatory cytokine in adipose tissue, which may in turn magnify the local inflammation and cause impaired energy homeostasis and systemic IR [33]. While the study of Wang et al. [13] did not show a significant correlation between serum NGAL levels and LDL cholesterol, our study does show a significant positive correlation between serum NGAL levels and LDL and total cholesterol.

Abnormalities of lipid metabolism are often accompanied by chronic inflammatory conditions [34]. Consequently, there is an increase in the production of adipokines such as NGAL, TNF-α, and IL-6, which in turn regulate insulin sensitivity, lipid metabolism, and cardiovascular homeostasis, potentially resulting in dyslipidemia [34]. Recently, NGAL has been proposed to be involved in the inflammatory response because the proinflammatory transcription factor, NF-κB transactivates NGAL expression by binding to a consensus motif in the promoter region of the NGAL gene [35]. In the present study, the number of white blood cells, proportion of neutrophils, and plasma IL-6 levels were significantly positively correlated with circulating NGAL levels. Ni et al. found a significant positive correlation between serum NGAL levels and the proportion of neutrophils in both men and women [15]. This finding, considered together with the significant correlation found between IL-6 and NGAL in this study, suggests that the expression of NGAL is transactivated through proinflammatory transcription factors such as NF-κB [35]. In addition, the present study also shows a significant negative correlation between circulating NGAL and SOD activity, an antioxidant biomarker. Roudkenar et al. reported that NGAL expression induced and upregulated by reactive oxygen species can be attenuated by anti-oxidants [36].

The present study found a significant relationship between serum NGAL levels and SFA intake among dietary nutrients. Serum NGAL levels in the high SFA consumers were significantly higher than those in the low SFA consumers, even after adjusting for confounding factors. Among the high SFA consumers, serum NGAL levels in MetS-risk participants were significantly higher than those in Super-healthy ones. Interestingly, however, among low SFA consumers, serum NGAL levels were not significantly different between the MetS-risk and Super-healthy groups. Our results may be partly supported by several previous studies. Moreno-Navarrete et al. [29] reported that fat overload (particularly with SFA) acutely increased circulating NGAL in patients with IR and the highest postprandial triglyceride concentration. According to Cani et al. [37], high-fat feeding alters intestinal permeability and gene expression coding for proteins involved in the gut tight junctions, which allows intestinal lipopolysaccharides (LPS) to easily access in bloodstream. Erridge et al. [38] also identified that fat intake leads to increase of LPS levels in human bloodstream. In addition, intra-lipid infusion significantly pronounced systemic inflammatory response to LPS [39]. In fact, immune system homeostasis can be challenged by continuous external insults, such as SFA-rich diets, pathogen-associated molecular patterns (i.e. LPS), infection and oxidative stress [40-43]. It was also well reported that both SFA and LPS affect the immune system through similar pathways. For example, oxidative and inflammatory stress by circulating immune cells could be induced by SFA challenge.
after oral consumption or intravenous administration, which increasing nuclear factor-κB-binding activity in mononuclear cells [44, 45]. Thus, we assumed that circulating NGAL which has antibacterial effects could be highly secreted responding to increased circulating LPS in high SFA consumers, particularly in those with MetS risk (i.e. high TG concentration, impaired glycemic control etc.).

This study has some limitations. 1) This was a cross-sectional study that did not assess time-sequential associations because the exposure and outcomes were collected at one point in time. 2) Given the restricted study population, the results may not be applicable to men or other ethnic groups with different clinical and biochemical characteristics. Despite these limitations, the study results suggest that the serum NGAL level may be a useful marker for reflecting early metabolic changes in non-diabetic healthy women, even with a low risk of MetS. It also suggests that the serum NGAL level is highly correlated with dietary SFA intake. The results of this study indicate that further studies are needed to investigate whether NGAL acts as a sensitive prognostic indicator of early metabolic changes, by examining the variability of NGAL during dietary intervention in the MetS risk group.

Acknowledgement

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Disclosure

The authors have nothing to disclose.

Supplementary Table 1 Correlation between serum NGAL levels and inflammation related parameters according to SFA intake

<table>
<thead>
<tr>
<th></th>
<th>SFA intake &lt; 7g/day (n=40)</th>
<th>SFA intake ≥ 7g/day (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r0</td>
<td>p0</td>
</tr>
<tr>
<td>IL-6 (pg/mL) a</td>
<td>-0.196</td>
<td>0.260</td>
</tr>
<tr>
<td>WBC count</td>
<td>0.147</td>
<td>0.399</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>0.241</td>
<td>0.164</td>
</tr>
<tr>
<td>SOD activity %</td>
<td>-0.034</td>
<td>0.846</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>SFA intake &lt; 7% of TCI/day (n=61)</th>
<th>SFA intake ≥ 7% of TCI/day (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r0</td>
<td>p0</td>
</tr>
<tr>
<td>IL-6 (pg/mL) a</td>
<td>0.116</td>
<td>0.391</td>
</tr>
<tr>
<td>WBC count</td>
<td>0.230</td>
<td>0.086</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>0.364</td>
<td>0.005</td>
</tr>
<tr>
<td>SOD activity %</td>
<td>-0.107</td>
<td>0.427</td>
</tr>
</tbody>
</table>

Correlations were analyzed using Pearson (r0) and partial (r1) correlation analysis. a tested by log transformed; p0, p-value from Pearson correlation analysis (unadjusted); p1, p-value from partial correlation analysis (adjusted for age and body mass index). IL-6, interleukin-6; NGAL, neutrophil gelatinase-associated lipocalin; SFA, saturated fat; SOD, superoxide dismutase; TCI, total calorie intake; WBC, white blood cells.

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