# Circulating Granulocyte-Macrophage Colony-Stimulating Factor and Serum Fatty Acid Composition in Men and Women

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**Atherosclerosis is increasingly recognized as an inflammatory disease. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a proinflammatory cytokine, recently implicated as a prominent component of the regulatory network involved in atherogenesis. We aimed to study the relationship between circulating GM-CSF levels and serum fatty acid (FA) composition in 78 healthy subjects. The latter was analyzed by gas-liquid chromatography and GM-CSF by a high-sensitivity commercial enzyme-linked immunosorbent assay (ELISA). Among women (n = 40), serum GM-CSF levels were found to be positively associated with the proportion of palmitic acid (C16:0) and negatively with linoleic acid (C18:2$\omega$-6), docosahexaenoic acid (DHA, C22:6$\omega$-3), and the proportion of total essential FA. After excluding smoking women (n = 6), the associations among GM-CSF and serum linoleic acid concentration ($r = -0.49$, $P = .003$), arachidonic acid ($r = -0.52$, $P = .001$), and DHA ($r = -0.34$, $P = .04$) were strengthened. The ratio of palmitic to linoleic and DHA acids was the single best predictor of serum GM-CSF in all subjects. Together with arachidonic acid, it contributed to 22% of the GM-CSF variance in women, after taking into account the effects of age, body mass index (BMI), blood pressure, and smoking status. None of these associations were observed among men. In conclusion, serum FA composition is associated with circulating GM-CSF specifically in women. As human arterial and venous smooth muscle cells release GM-CSF, and treatment of endothelial cells with oxidized low-density lipoproteins results in a rapid expression of GM-CSF, the mechanisms involved in these associations and the sex-linked differences should be further explored.**


Atherosclerosis can be viewed as a chronic inflammatory disease in which the ensuing accumulation of monocytes/macrophages in the tissue constitutes the hallmark of the inflammatory response.[1] Different cytokines and chemical messengers constitute the main regulators of this inflammatory process, achieving biological effects individually as well as in association with each other. Plasma lipid composition is altered in parallel to this chronic inflammatory process and cytokine production.[2] Increased activity of the tumor necrosis factor-alfa (TNF-$\alpha$) system is observed in proportion to total and non–very-low-density lipoprotein (VLDL)/non–high-density lipoprotein (HDL)-cholesterol levels in healthy volunteers and in experimental models.[3-5] Fasting triglycerides, VLDL-triglycerides, and basal and postload free fatty acids (FA) are also associated with serum interleukin-6 concentrations in healthy subjects.[6] Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been increasingly implicated as a critical player in atherogenesis and as a prominent component of the regulatory network.[7,8] Damage to the vessel wall causes endothelial cell disruption, resulting in exposure of the underlying vascular smooth muscle cells. Human arterial and venous smooth muscle cells can be induced to release GM-CSF.[9,10]

The FA composition of serum and tissue lipids is believed to reflect the quality of dietary fat.[11] The study of serum FA composition is important in human atherosclerosis because significantly lower levels of polyunsaturated FA (linoleic and arachidonic acids) have been recorded in patients with myocardial infarction.[12] Kingsbury et al showed that plasma cholesteryl esters contained less linoleic acid in subjects with acute infarct or a vascular death than in 40 healthy patients.[13] Low tissue concentrations of linoleic acid are also associated with an increased risk of coronary heart disease (CHD) in Europeans.[14] This evidence suggests that a deficiency of essential FA jeopardizes cellular integrity. In fact, within populations, a higher linoleic acid concentration is regarded as cardioprotective.[15] However, the high proportions of linoleic acid in erythrocyte membranes found in Asians do not seem to protect this group against CHD,[16] indicating that other factors should be implicated. Dietary polyunsaturated FA appear to modulate the release of different cytokines.[17] The production of interleukin-1$\beta$, interleukin-6, TNF-$\alpha$, and GM-CSF by peripheral mononuclear cells has been found to be decreased by dietary polyunsaturated FA supplementation in women.[18,19]

We aimed to study the relationship between the proatherogenic lipid profile, measured as the ratio of saturated to polyunsaturated FA, and circulating GM-CSF levels in 78 subjects who were otherwise healthy.

## MATERIALS AND METHODS

### *Subjects*

Seventy-eight healthy subjects (40 women) were evaluated as a part of an ongoing epidemiological study of nonclassical cardiovascular risk factors. None of the subjects were taking any medication or had any evidence of metabolic disease other than obesity. The study was approved by the hospital ethics committee and informed consent was obtained from each subject.

*Anthropometric measurements.* All subjects were evaluated through the body mass index (BMI), calculated as weight (in kilograms) divided by height (in meters squared), and the waist-to-hip ratio (WHR). Waist measurements were obtained with a soft tape midway between the lowest rib and the iliac crest. Hip circumference was measured at the widest part of the gluteal region.

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### Serum Samples

*Analysis of serum FA.* Following the method of Lepage and Roy,[20] 100 μL of serum was precisely weighted in glass tubes, and diluted with methanol-benzene 4:1 (vol/vol). Acetyl chloride was slowly added over a period of 1 minute. After transesterification, the pooled solvent extracts were dried under a gentle stream of nitrogen at room temperature. Residues were dissolved in 500 μL of hexane and an aliquot was injected into the chromatograph.

*Gas liquid chromatography.* FA were chromatographed as methyl esters on a 30-m fused silica column with an internal diameter of 0.25 mm. Analysis was performed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector. The column temperature was held at 80°C for 3 minutes, and in a stepwise fashion reached a plateau of 220°C. The injection port temperature was 250°C and the detector temperature was 270°C. Helium was used as carrier gas. An internal standard consisting of 50 μg of pentadecanoic acid (C15:0) was precisely weighed and added to the serum. GM-CSF was measured using a high-sensitivity commercial enzyme-linked immunosorbent assay (ELISA; R & D Systems, Abingdon, UK). Intra-assay and interassay coefficients of variation were 6.7% and 9.5%.

The serum glucose concentration was measured in duplicate by the glucose oxidase method. Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. Total serum triglycerides were measured by the reaction of glycerol-phosphate-oxidase and peroxidase.

### Statistical Analysis

Descriptive results of continuous variables are expressed as the mean ± SD. Before statistical analysis, normal distribution and homogeneity of the variances were tested. Parameters that did not fulfill these tests (individual FA and ratios, GM-CSF) were log-transformed. The relations between variables were analyzed by simple correlation, partial correlation, and multiple regression in a stepwise manner with forward selection. BMI was entered as a continous variable and smoking as nonsmoking/smoking. Only those FA with statistically significant associations on univariant analysis were considered. The regression coefficient generated by this analysis indicates the slope of the association between the dependent variable and the specified independent variable, after adjusting for other independent variables in the model. The standard error represents the variability in this association, and the significance is reflected by the $P$ value. The model $R^2$ indicates the percent of the variance in the dependent variable that is accounted for by the independent variables included in the model. Levels of statistical significance were set at $P < .05$.

## RESULTS

The main characteristics of the study subjects are listed in Table 1. The serum FA composition is shown in Table 2. Seven nonsmoking men and six nonsmoking women were clinically obese (BMI > 30 kg/m$^2$). Age, BMI, WHR, blood pressure, and smoking status did not significantly affect the proportion of circulating FA or GM-CSF.

Serum GM-CSF levels were found to be positively associated with the proportion of palmitic acid (C16:0) and negatively with linoleic acid (C18:2ω-6) (Table 2 and Fig 1) in all subjects. In addition, the proportion of serum docosahexaenoic acid (DHA, C22:6ω-3) was negatively associated with GM-CSF in women (Table 2). The proportion of total essential FA in serum was negatively associated with GM-CSF ($r = -0.26$, $P = .022$) (Fig 2). The ratio of palmitic to linoleic acid and the ratio of palmitic to linoleic and DHA acids correlated with serum GM-CSF in all subjects (Table 2 and Fig 3). All of these correlations persisted after adjusting for the effects of age, BMI, blood pressure, and smoking status in a multivariant regression analysis. However, when the study was performed separately in men and women, these associations remained significant only in women. After excluding smoking subjects (21 men and 6 women), GM-CSF was significantly associated with the concentrations of linoleic acid ($r = -0.49$, $P = .003$), arachidonic acid ($r = -0.52$, $P = .001$), and DHA ($r = -0.34$, $P = 0.047$) only in women. We performed a multiple linear regression analysis in a stepwise manner to predict circulating GM-CSF levels. Among all subjects, the ratio of palmitic to linoleic acids and DHA independently contributed to 10.4% of the variance in serum GM-CSF levels ($P = .0042$). In men, no independent associations were found. In women, the ratio of palmitic to linoleic acids and DHA ($P = .0048$) and the concentration of arachidonic acid ($P = .011$) independently contributed to 22% of the variance in serum GM-CSF levels. When the ratio was excluded from the analysis, the individual FA also contributed to 22% of the GM-CSF variance (Table 3).

## DISCUSSION

Damage to the vessel wall causes endothelial cell disruption, resulting in exposure of the underlying vascular smooth muscle cells. It has recently been demonstrated that human arterial and venous smooth muscle cells release GM-CSF.[9,10] Treatment of

**Table 1. Subjects Characteristics**

| | Men | Women | *P* |
|---|---|---|---|
| N | 38 | 40 | — |
| Age (yr) | 40.1 ± 13.3 | 38.1 ± 9.3 | NS |
| BMI (kg/m$^2$) | 25.4 ± 6 | 23.4 ± 3.9 | NS |
| WHR | 0.96 ± 0.05 | 0.86 ± 0.04 | <.0001 |
| Smokers (yes/no) | 21/17 | 6/34 | .003 |
| Systolic blood pressure (mm Hg) | 124.4 ± 17.3 | 117 ± 16 | NS |
| Diastolic blood pressure (mm Hg) | 71.3 ± 10.9 | 67.5 ± 9.8 | NS |
| Fasting glucose (mmol/L) | 5.0 ± 0.7 | 4.7 ± 0.6 | NS |
| Total cholesterol (mg/dL) | 211 ± 41 | 201 ± 38 | NS |
| Triglycerides (mg/dL) | 92.1 ± 43.6 | 77.1 ± 30 | NS |
| GM-CSF (pg/mL) | 2.34 ± 1.05 | 2.45 ± 1.6 | NS |

Abbreviation: NS, not significant.

**Table 2. Profile of Fatty Acids in Serum and Simple Correlations With Circulating GM-CSF**

| Variable | % of Total FA | | | Correlation With GM-CSF | | |
|---|---|---|---|---|---|---|
| | Men | Women | All | Men | Women | All |
| Fatty acid* | | | | | | |
| Saturated | | | | | | |
| Palmitic (C16:0) | 19.8 ± 2.2 | 18.9 ± 2.9 | 19.4 ± 2.6 | 0.20 | 0.33† | 0.28† |
| Stearic (C18:0) | 7.8 ± 0.8 | 7.6 ± 0.8 | 7.7 ± 0.8 | 0.03 | −0.15 | −0.05 |
| Monounsaturated | | | | | | |
| Palmitoleic (C16:1) | 0.33 ± 0.09 | 0.36 ± 0.09 | 0.35 ± 0.09 | 0.12 | 0.05 | 0.07 |
| Oleic (C18:1) | 20.4 ± 3.3 | 20.7 ± 3.6 | 20.6 ± 3.4 | −0.02 | 0.13 | 0.06 |
| $\omega$-6 polyunsaturated | | | | | | |
| Linoleic (C18:2$\omega$-6) | 31.9 ± 4.2 | 32.6 ± 3.9 | 32.2 ± 4.1 | −0.17 | −0.329† | −0.25† |
| Dihomo-$\gamma$-linoleic (C20:3$\omega$-6) | 1.65 ± 0.43 | 1.56 ± 0.35 | 1.60 ± 0.39 | −0.10 | 0.14 | 0.03 |
| Arachidonic (C20:4$\omega$-6) | 7.3 ± 1.4 | 7.2 ± 1.1 | 7.2 ± 1.29 | 0.00 | −0.03 | −0.01 |
| $\omega$-3 polyunsaturated | | | | | | |
| Eicosapentaenoic (C20:5$\omega$-3) | 0.46 ± 0.24 | 0.56 ± 0.35 | 0.51 ± 0.30 | 0.00 | −0.11 | 0.02 |
| DHA (C22:6$\omega$-3) | 2.03 ± 0.4 | 1.95 ± 0.7 | 1.99 ± 0.63 | 0.16 | −0.32† | −0.15 |
| Derived indexes | | | | | | |
| Ratio of C16:0 to C18:2 | 0.64 ± 0.14 | 0.60 ± 0.14 | 0.62 ± 0.14 | 0.21 | 0.39† | 0.31‡ |
| Ratio of C16:0 to C18:2+C22:6 | 0.60 ± 0.13 | 0.56 ± 0.13 | 0.58 ± 0.13 | 0.20 | 0.41‡ | 0.32‡ |

*Mean percentage (SD) of total FA content in serum; trace amounts of other FA omitted.
†$P < .05$; ‡$P < .01$.

endothelial cells with modified low-density lipoproteins also results in a rapid and large induction of the expression of GM-CSF.[21] In this study, we found that circulating GM-CSF is linked to the serum FA composition in apparently healthy women. The mechanism of this association should be further explored in future studies.

Since the production and several biological functions of cytokines are under the control of products of arachidonic acid metabolism, it has been proposed that dietary effects on eicosanoid production could modify cytokine production. Meydani et al studied the effect of eicosapentaenoic acid (EPA) and DHA as dietary supplements in 12 women.[18] This supplementation significantly decreased GM-CSF production by peripheral blood mononuclear cells. Endres et al gave supplements of 18 g/d of fish oil containing 2.7 g of EPA and 1.85 g of DHA to 9 volunteers, and found a significant decrease (43%) in the production of interleukin-1$\beta$ by peripheral blood mononuclear cells.[19] Thus, essential FA supplementation appears to reduce the production of GM-CSF and other cytokines. From the latter reports it could be inferred that relatively low essential FA

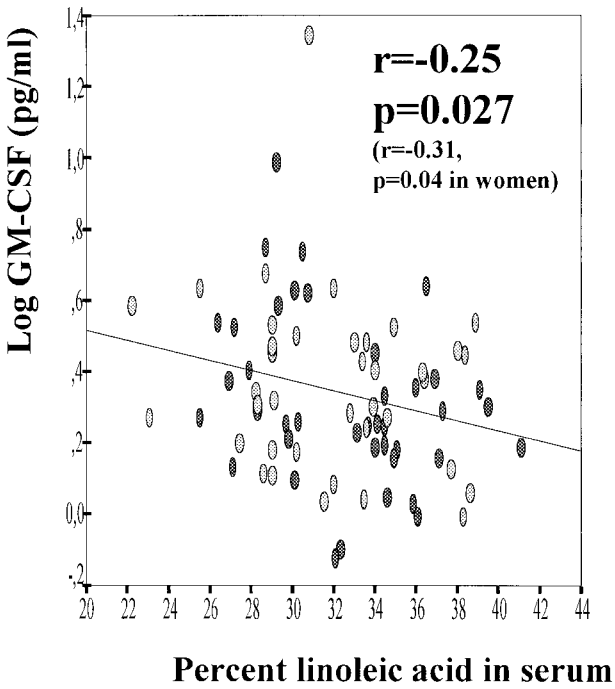


**Fig 1. Relationship between circulating GM-CSF and percent linoleic acid in serum (bold-filled circles, women).**

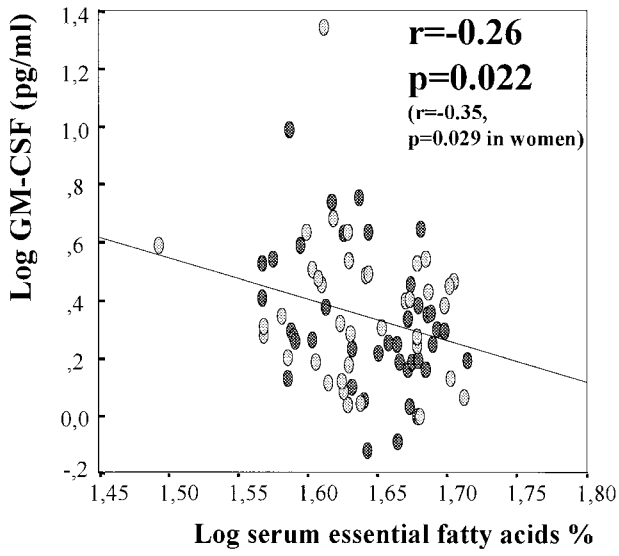


**Fig 2. Relationship between circulating GM-CSF and percent essential FA in serum (bold-filled circles, women).**

levels lead to increased GM-CSF production, as found in this study. However, it is possible that increased GM-CSF results in enhanced de novo synthesis of palmitic acid, or decreased synthesis of linoleic acid, DHA, and arachidonic acid in women. Finally, it is also possible that the associations between GM-CSF and serum FA composition are a mere byproduct of the inflammatory cascade. For example, cytokine stimulation of smooth muscle cell GM-CSF production is modified by indomethacin,[9] which suggests that arachidonic acid metabolism is closely linked to GM-CSF production.

Serum FA concentration is largely determined by dietary intake, and is a good indicator of habitual dietary fat intake in middle-aged adults, especially for the essential FA, linoleic acid (18:2$\omega$-6), $\alpha$-linolenic acid (18:3$\omega$-3), and DHA (22:6$\omega$-3). The correlations between dietary intake and tissue composition of these fatty acids are usually highly significant.[22,23]

The relationship between essential FA and GM-CSF seems to be restricted to women. Of note is that decreased GM-CSF production by peripheral blood mononuclear cells after polyunsaturated FA supplementation was also observed in women.[18] Interestingly, sex-based differences in early mortality after myocardial infarction have been well described.[24]

Smoking was a significant confounding factor. The associations among individual FAs and GM-CSF remained significant, and some of them were even strenghtened, among nonsmoking women. In case-control studies, the more cigarettes that were smoked, the lower the percentage of adipose linoleate.[25,26] In our study sample, the ratio of palmitic acid to linoleate was higher in those who smoked more than 20 cigarettes a day ($0.71 \pm 0.19$ *v* $0.63 \pm 0.15$, $P = .021$), indicating a lower percentage of linoleate in serum. After controlling for cigarette smoking, the relationship among individual FA and GM-CSF was not modified in men.

**Table 3. Multiple Linear Regression Analysis of the Association Between GM-CSF and Serum Individual Fatty Acids in Women**

| Dependent Variable | Independent Variables | Coefficient | Standard Error | *P* Value | $R^2$ |
|---|---|---|---|---|---|
| GM-CSF | C18:2 n-6 | −0.144 | 0.0622 | .026 | 0.22 |
| | C22:6 n-3 | −0.7803 | 0.321 | .0202 | |
| | C16:0 | 0.0011 | | .99 | |

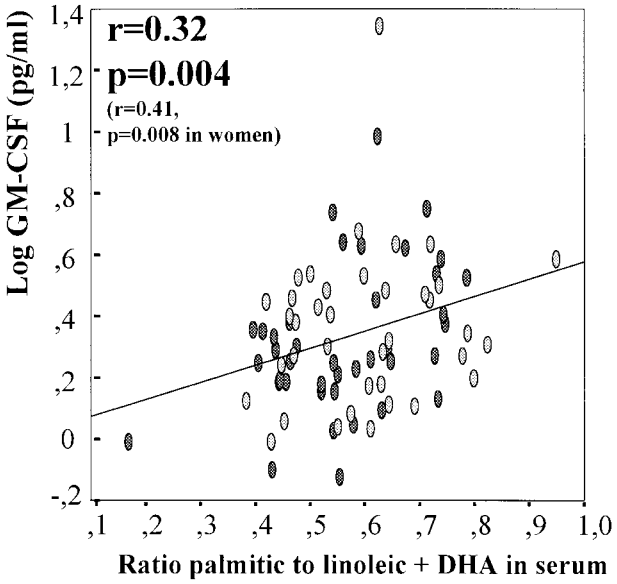


**Fig 3. Relationship between circulating GM-CSF and the ratio of palmitic to linoleic acid and DHA in serum (bold-filled circles, women).**

The association between serum essential FA and increased GM-CSF could be explained by other factors. Serum selenium, a well-known antioxidant factor, was directly associated with the percentage of essential FA and $\omega$-6 polyunsaturated FA, and inversely related to the percentage of saturated FA in phospholipids in one study.[27] A decreased antioxidant capacity in serum as a factor associated simultaneously with essential FA and raised serum GM-CSF levels cannot be excluded.

The associations described here are of potential interest in the understanding of the atherosclerotic process. Populations with a high mortality from CHD, such as the Scots and the Finns, have low concentrations in of linoleic acid.[28,29] The consumption of the $\omega$-3 polyunsaturated FA, EPA and DHA, is associated with a reduced risk of thrombotic brain infarction among American women.[30] These relationships are most likely to have a dietary explanation, because food is the source of these essential FA. EPA, in part converted from DHA, is transformed into a nonaggregatory agent, which increases the synthesis of a vasodilator, prostaglandin $I_3$, leading to further reductions in platelet aggregation and increased vasodilation.[31]

We have measured a cytokine—GM-CSF—with a highly sensitive method. The usual sandwich-format immunoassays recover free and some predictably bound cytokine, but miss other cytokines bound by unpredictable binding entities.[32] This caveat should be kept in mind when interpreting our results.

In summary, the proportions of linoleic acid, DHA, and palmitic acid in serum are associated with circulating GM-CSF. Because of the wide spectrum of biologic and pathologic events that can be affected by cytokines, the mechanisms of these associations should be further investigated.

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