Decreased High-Density Lipoprotein Cholesterol Level is an Independent Correlate of Circulating Tumor Necrosis Factor-α in a General Population

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ABSTRACT

Background: Recent studies implicate a pathophysiological role of tumor necrosis factor-α (TNF-α) in atherosclerosis, thus suggesting that serum TNF-α levels may be one of the biomarkers for future cardiovascular events. However, which anthropometric, metabolic, and inflammatory variables could regulate circulating TNF-α levels in humans is not fully understood. In this study, we examined the independent determinants of serum TNF-α levels in a Japanese general population.

Hypothesis: Anthropometric, metabolic , and inflammatory variables could regulate TNF-α.

Methods: A total of 213 Japanese subjects underwent a complete history, physical examination, and determination of blood chemistries, including TNF-α levels. Univariate and multivariate analyses were applied for the determinants of TNF-α levels.

Results: The average TNF-α levels were 13.4±0.81 pg/ml in males and 13.9±4.5 pg/ml in females, respectively. Univariate analysis showed that TNF-α levels were associated with age ($P = 0.007$), body mass index ($P = 0.034$), waist circumference ($P < 0.001$), high-density lipoprotein cholesterol (HDL-C; inversely, $P<0.001$), triglycerides ($P<0.001$), creatinine ($P<0.001$), uric acids ($P<0.001$), insulin ($P = 0.008$), homeostasis model assessment of insulin resistance (HOMA-IR; $P = 0.015$), high sensitivity C-reactive protein (hs-CRP; $P<0.001$), and fibrinogen ($P = 0.009$). By the use of multiple stepwise regression analyses, HDL-C (inversely, $P<0.001$) and hs-CRP ($P<0.001$) remained significant and were independently related to TNF-α levels ($R_2 = 0.153$).

Conclusions: The present study is the first demonstration that besides hs-CRP, a decreased HDL-C level is an independent determinant of circulating TNF-α in the Japanese general population. Elevation of TNF-α may partly explain the increased risk of cardiovascular events in patients with low HDL-C levels.

Introduction

There is a growing body of evidence, ranging from in vitro experiments to pathologic analysis to epidemiologic studies, that atherosclerosis is intrinsically an inflammatory-proliferative disease.[1] Further, recent experimental and clinical studies implicate a pathophysiological role of tumor necrosis factor-α (TNF-α) in atherosclerosis.[2–7] Indeed, TNF-α not only induces apoptotic cell death of endothelial cells (EC), but also elicits vascular inflammation, thus being involved in atherosclerosis.[2] In addition, plasma TNF-α concentration is correlated with metabolic perturbations, EC injury, and degrees of early atherosclerosis, all of which are considered prognostic markers of future cardiovascular events.[6] Moreover, elevation of TNF-α has also been reported to be an increased risk for recurrent coronary events after myocardial infarction.[7] These observations suggest that circulating TNF-α levels may be one of the biomarkers for future cardiovascular events and also a molecular target to prevent the development of this devastating disorder. However, which anthropometric, metabolic, and inflammatory variables could regulate circulating TNF-α levels in humans is not fully understood. In this study, we examined the independent determinants of serum TNF-α levels in the Japanese general population.

Research Design and Methods

Subjects

In 2007, in a fishing community in southwestern Japan (Uku), a total of 213 people (80 males and 133 females) underwent a complete history, physical examination, and

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determination of blood chemistries, including serum levels of TNF-α. The mayor and the welfare section of Uku approved this study. The Ethical Committee of Kurume University also approved this study. All participants gave informed consent.

Data Collection

A medical history and use of cigarettes and alcohol were ascertained by a questionnaire. Smoking and alcohol were classified as current habitual use or not. Height and weight were measured, and body mass index (BMI: kilograms per meter squared) was calculated as an index of the presence or absence of obesity. Blood pressure (BP) was measured in the sitting position using an upright standard sphygmomanometer. Vigorous physical activity and smoking were avoided for at least 30 minutes before BP measurement.

Blood was drawn from the antecubital vein in the morning after a 12 hour fast for the determination of lipids (total cholesterol, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], and triglycerides), fasting plasma glucose (FPG), glycosylated hemoglobin (HbA_{1C}), insulin, blood urea nitrogen (BUN), creatinine, uric acid, TNF-α, high sensitivity C-reactive protein (hs-CRP), plasminogen activator inhibitor-1 (PAI-1), and fibrinogen levels. Blood chemistries, including serum TNF-α levels, were measured at a commercially available laboratory (The Kyodo Igaku Laboratory, Fukuoka, Japan), using an enzyme-linked immunosorbent assay or an enzymatic assay method as described previously.[8] The homeostasis model assessment of IR (HOMA-IR) index was calculated from the values of fasting plasma glucose (mg/dl) and insulin (μU/ml) using the following formula: ([glucose × insulin]/405).

Statistical Methods

Because of skewed distributions, the natural logarithmic (ln) transformations were performed for triglycerides, FPG, insulin, HOMA-IR, hs-CRP, and PAI-1. Mean values with upper and lower 95% confidence intervals (CI) were exponentiated and presented as geometric mean±standard deviation (SD). The medications for hypertension, diabetes mellitus, and hyperlipidemia were coded as dummy variables. Univariate analysis was performed for determinants of TNF-α levels. To determine independent determinants of TNF-α levels, multiple stepwise regression analysis was performed. Statistical significance was defined as $P<.05$. All statistical analyses were performed with the use of the SAS system (SAS Institute, Cary, NC).

Results

Backgrounds of the subjects are presented in Table 1. Serum TNF-α levels were 13.4±0.81 pg/ml in males and 13.9±4.5 pg/ml in females, respectively. Table 2 shows results of univariate analysis for determinants of TNF-α

Table 1. Characteristics of Subjects

Parameters	Male (n = 80)	Female (n = 133)	Total (n = 213)
Age (years)	68.0±8.2	63.9±11.0	65.4±10.2
BMI (kg/m²)	23.9±2.8	23.4±3.3	23.6±3.1
Waist (cm)	85.0±8.0	77.4±8.6	80.3±9.1
Systolic BP (mm Hg)	130.0±17.4	129.2±21.3	129.5±19.9
Diastolic BP (mm Hg)	72.6±8.0	70.9±10.1	71.5±9.4
Total cholesterol (mg/dl)	197.2±34.8	204.8±37.6	201.9±36.6
HDL-C (mg/dl)	52.0±13.7	58.7±14.1	56.2±14.3
Triglycerides (mg/dl)[a]	92.3±15.6	78.3±15.3	83.1±15.5
BUN (mg/dl)	21.4±5.5	18.4±5.7	19.5±5.8
Creatinine (mg/dl)	0.82±0.17	0.61±0.14	0.69±0.18
Uric acid (mg/dl)	6.2±1.2	4.7±1.3	5.3±1.5
FPG (mg/dl)[a]	101.9±11.6	91.9±25.1	95.6±11.4
HbA_{1c} (%)	5.4±0.8	5.2±0.4	5.3±0.6
Insulin (μU/ml)[a]	3.8±2.1	3.5±1.7	3.6±1.8
HOMA-IR[a]	0.95±0.81	0.79±0.56	0.85±0.66
TNF-α (pg/ml)	13.4±0.81	13.9±4.5	13.7±4.3
hs-CRP (mg/dl)[a]	0.062±0.033	0.037±0.038	0.045±0.036
PAI-1 (ng/ml)[a]	27.1±1.8	17.8±1.7	20.9±1.8
Fibrinogen (mg/dl)	291.9±64.2	298.2±62.7	295.8±63.2
Alcohol (n)	50	10	55
Smoking (n)	14	3	17
Medication			
Hypertension (n)	33	63	96
Diabetes mellitus (n)	8	7	15
Dyslipidemia (n)	8	33	41

Unless otherwise indicated, data are means±SD.
[a] Log-transformed values were used in analyses.
Abbreviations: BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; n, number; PAI-1, plasminogen activator inhibitor-1; TNF-α, tumor necrosis factor-α ; hs-CRP, high sensitivity C-reactive protein. n, number; SD, Standard Deviation.

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Table 2. Correlates of TNF-α by Univariate Analysis

Parameters	β	SE	*P* Value
Age (years)	0.185	0.029	0.007
Sex	0.054	0.161	0.430
BMI (kg/m²)	0.145	0.094	0.034
Waist (cm)	0.217	0.032	< 0.001
Systolic BP (mm Hg)	−0.038	0.015	0.581
Diastolic BP (mm Hg)	−0.038	0.032	0.584
Total cholesterol (mg/dl)	−0.115	0.008	0.093
HDL-cholesterol (mg/dl)	−0.338	0.020	< 0.001
Triglycerides (mg/dl)[a]	0.240	0.658	<0.001
BUN (mg/dl)	0.070	0.052	0.308
Creatinine (mg/dl)	0.247	1.569	< 0.001
Uric acid (mg/dl)	0.016	0.010	<0.001
FPG (mg/dl)[a]	0.033	2.531	0.633
HbA_{1c} (%)	0.116	0.512	0.092
Insulin (μU/ml)[a]	0.182	0.473	0.008
HOMA-IR[a]	0.167	0.441	0.015
hs-CRP (mg/dl)[a]	0.298	0.220	< 0.001
PAI-1 (ng/ml)[a]	0.026	0.504	0.701
Fibrinogen (mg/dl)	0.178	0.005	0.009
Alcohol	−0.132	0.769	0.054
Smoking	−.0049	1.1013	0.477
Medication			
Hypertension	0.707	0.601	0.312
Diabetes mellitus	0.118	1.159	0.086
Dyslipidemia	0.024	0.758	0.732

[a] Log-transformed values were used in analyses.
Abbreviations: BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high sensitivity C-reactive protein; PAI-1, plasminogen activator inhibitor-1; TNF, tumor necrosis factor-α .

levels. Parameters statistically and significantly related to TNF-α levels were age ($P = 0.007$), BMI ($P = 0.034$), waist circumference ($P<0.001$), HDL-C (inversely, $P<0.001$), triglycerides ($P<0.001$), creatinine ($P<0.001$), uric acids ($P<0.001$), insulin ($P = 0.008$), HOMA-IR ($P = 0.015$), hs-CRP ($P<0.001$), and fibrinogen ($P = 0.009$). Because these

Table 3. Correlates of TNF-α by Multiple Stepwise Regression Analysis

Parameters	β	SE	*P* Value
HDL-C (mg/dl)	−0.327	1.151	<0.001
hs-CRP (mg/dl)[a]	0.235	0.216	<0.001
Uric acid (mg/dl)	0.815		0.071
Age	0.111		0.085
Insulin (μU/ml)[a]	0.075		0.258
HOMA-IR[a]	0.056		0.401
Waist (cm)	0.031		0.431
Fibrinogen (mg/dl)	0.017		0.828
Triglycerides (mg/dl)[a]	0.014		0.863

$R^2 = 0.153$
[a] Log-transformed values were used in analyses.
Abbreviations: HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high sensitivity C-reactive protein.

significant parameters were closely correlated with each other, multiple stepwise regression analysis was performed. Finally, HDL-C (inversely, $P<0.001$) and hs-CRP ($P<0.001$) remained significant (Table 3) and were independently related to TNF-α levels ($R^2 = 0.153$). In addition, we found here that HDL-C levels were inversely correlated with hs-CRP levels in univariate analysis ($P<0.01$). In multiple linear regression analysis adjusted for age and gender, HDL-C levels were one of the independent determinants of hs-CRP levels (beta $= -0.139$, SE $= 0.743$, P value $= 0.041$).

Discussion

TNF-α has been shown to suppress the insulin signaling in both adipose tissues and skeletal muscles by inducing serine phosphorylation of insulin receptor substrate-1 and by subsequently inhibiting the activation of phosphoinositol-3-kinase.[9] In addition, overexpression of a soluble form of TNF-α receptor fragment has been reported to improve insulin resistance in obese rats.[10] Since circulating TNF-α level is elevated in patients with insulin resistance and/or the metabolic syndrome,[11–13] it is generally thought that TNF-α plays a central role in the pathogenesis of insulin-resistant metabolic derangements. Further, there is accumulating evidence that TNF-α also participates in the development of cardiovascular disease in at-risk patients with insulin resistance and/or the metabolic syndrome.[2,5,6] These observations suggest that circulating TNF-α level may be a molecular target to improve insulin resistance and subsequently prevent future cardiovascular events in high-risk patients. This is a reason why circulating TNF-α level was chosen in this study as the cytokine of interest.

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Adipose tissues are the source of a number of proinflammatory cytokines such as TNF-α.[14,15] However, adipose tissue TNF-α expression levels do not necessarily reflect circulating levels of TNF-α in obese subjects.[16] Therefore, which anthropometric, metabolic, and inflammatory variables could regulate circulating TNF-α levels in humans remains to be elucidated. In this study, we demonstrated for the first time that besides hs-CRP, the most powerful inflammatory biomarker of future cardiovascular events,[17] decreased HDL-C levels was an independent determinant of circulating levels of TNF-α in a general population. The present results suggest that elevation of TNF-α may partly explain the increased risk of future cardiovascular events in patients with low HDL-C levels.

In this study, we did not clarify how low HDL-C levels were independently correlated with serum levels of TNF-α in our subjects. However, HDL-C has been shown to reduce TNF-α production from monocyte-macrophages upon contact with stimulated T cells or an ischemia-reperfusion injured heart.[18,19] These observations suggest that HDL-C may be a negative regulator of circulating TNF-α levels through its anti-inflammatory properties. In addition, since HDL-C has also been reported to have antioxidative properties in vivo,[20,21] HDL-C could protect plasma lipids including LDL-C against oxidation, thus suppressing the proinflammatory signaling of oxidized lipids, which could lead to the reduction of serum levels of TNF-α. Further, in the present study, since waist circumference was not an independent determinant of circulating TNF-α in multiple stepwise regression analysis, subclinical inflammation rather than central obesity could be a main regulator of circulating levels of TNF-α in a general population.

Limitations

Unfortunately, we did not have any more detailed information about the medications for hypertension, diabetes mellitus or dyslipidemia in our subjects. Therefore, although univariate analysis revealed no significant correlation between serum TNF-α levels and medications for hypertension, diabetes mellitus or dyslipidemia (Table 2) and that the medications did not affect the association of HDL-C levels with TNF-α levels in multiple linear regression analysis, we cannot totally exclude the possibility that some anti-inflammatory agents (eg, blockers of the renin-angiotensin system, glitazones, statins, niacin) could affect serum levels of TNF-α and its correlation with HDL-C levels in our cases. Further study with relatively large numbers of patients could clarify this issue. In addition, our study was a cross-sectional one and, therefore, did not elucidate the causal relationship between low HDL-C and high TNF-α levels. Longitudinal and/or interventional studies are needed to clarify whether elevation of HDL-C levels could decrease circulating levels of TNF-α and subsequently reduce the risk of future cardiovascular events in a general population.

References

1. Manitovani A, Bussolino F, Dejana E. Cytokine regulation of endothelial cell function. *FASEB J*. 1992;6:2591–2599.
2. Bruunsgaard H, Skinhoj P, Pedersen AN, Schroll M, Pedersen BK. Ageing, tumor necrosis factor-α (TNF-α) and atherosclerosis. *Clin Exp Immunol*. 2000;121:255–260.
3. Yamagishi S, Inagaki Y, Abe R, et al. Nifedipine inhibits apoptotic cell death of cultured endothelial cells induced by tumor necrosis factor-α. *Drugs Exp Clin Res*. 2003;29:141–145.
4. Yamagishi S, Inagaki Y, Kikuchi S. Nifedipine inhibits tumor necrosis factor-α -induced monocyte chemoattractant protein-1 overexpression by blocking NADPH oxidase-mediated reactive oxygen species generation. *Drugs Exp Clin Res*. 2003;29:147–152.
5. Yamagishi S, Nakamura K, Matsui T. Role of oxidative stress in the development of vascular injury and its therapeutic intervention by nifedipine. *Curr Med Chem*. 2008;15:172–177.
6. Skoog T, Dichtl W, Boquist S, et al. Plasma tumor necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. *Eur Heart J*. 2002;23:376–383.
7. Ridker PM, Rifai N, Pfeffer M, et al. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation*. 2000;101:2149–2153.
8. Enomoto M, Adachi H, Yamagishi S, et al. Positive association of serum levels of advanced glycation end products with thrombogenic markers in humans. *Metabolism*. 2006;55:912–917.
9. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor-α inhibits signaling from the insulin receptor. *Proc Natl Acad Sci USA*. 1994;91:4854–4858.
10. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science*. 1993;259:87–91.
11. Matsushita K, Yatsuya H, Tamakoshi K, et al. Comparison of circulating adiponectin and proinflammatory markers regarding their association with metabolic syndrome in Japanese men. *Arterioscler Thromb Vasc Biol*. 2006;26:871–876.
12. Moon YS, Kim DH, Song DK. Serum tumor necrosis factor-α levels and components of the metabolic syndrome in obese adolescents. *Metabolism*. 2004;53:863–867.
13. Nilsson J, Jovinge S, Niemann A, Reneland R, Lithell H. Relation between plasma tumor necrosis factor-α and insulin sensitivity in elderly men with non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 1998;18:11991–1201.
14. Borst SE. The role of TNF-α in insulin resistance. *Endocrine*. 2004;23:177–182.
15. Wisse BE. The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. *J Am Soc Nephrol*. 2004;15:2792–2800.
16. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. *J Clin Invest*. 1995;95:2409–2415.
17. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res*. 2001;89:763–771.
18. Burger D, Dayer JM. High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and chronic inflammation? *Autoimmun Rev*. 2002;1:111–117.
19. Calabresi L, Rossoni G, Gomaraschi M, et al. High-density lipoproteins protect isolated rat hearts from ischemia-reperfusion injury by reducing cardiac tumor necrosis factor-α content and enhancing prostaglandin release. *Circ Res*. 2003;92:330–337.
20. Klimov AN, Gurevich VS, Nikiforova AA, et al. Antioxidative activity of high density lipoproteins in vivo. *Atherosclerosis*. 1993;100:13–18.
21. Kontush A, de Faria EC, Chantepie S, Chapman MJ. A normotriglyceridemic, low HDL-cholesterol phenotype is characterised by elevated oxidative stress and HDL particles with attenuated antioxidative activity. *Atherosclerosis*. 2005;182:277–285.

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