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□ ORIGINAL ARTICLE □

Examination of Adhesion Molecules, Homocysteine and hs-CRP in Patients with Polygenic Hypercholesterolemia and Isolated Hypertriglyceridemia

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Abstract

Background Increased levels of selectins, adhesion molecules, hs-CRP and homocysteine are considered important as indicators of atherosclerosis. There is a significant amount of evidence that high LDL-C levels are a risk factor for coronary artery disease, whereas the relevance of isolated triglycerides is controversial. The present study aims to compare the levels of homocysteine, hs-CRP, E-selectin, sP-selectin, VCAM-1, ICAM-1 in patients with isolated hypertriglyceridemia and polygenic hypercholesterolemia.

Methods The following three groups were formed: polygenic hypercholesterolemia group (n=30), isolated hypertriglyceridemia group (n=30) and control group (n=30). These three groups were matched in terms of BMI, waist circumference and gender. Plasma high sensitive CRP, homocysteine, sVCAM-1, sICAM-1, sP-selectin, sE-Selectin levels of patients in these three groups were measured.

Results In the present study, mean values for sE-selectin, sVCAM-1 and sICAM-1 in the polygenic hypercholesterolemia group were significantly higher than in the other two groups ($p<0.001$). Homocysteine and hs-CRP levels were higher in the polygenic hypercholesterolemia group, compared to the isolated hypertriglyceridemia group ($p=0.019$, $p<0.001$; respectively) and the control group ($p<0.001$, $p<0.001$; respectively). Comparison of patients with hypertriglyceridemia to individuals in the control group did not yield a significant difference in terms of sE-selectin, sP-selectin, sVCAM-1, sICAM and homocysteine ($p>0.05$), where as the hs-CRP value was significantly higher in patients with isolated hypertriglyceridemia compared to the control group ($p=0.001$).

Conclusion The increase of adhesion molecules, homocysteine and hs-CRP in polygenic hypercholesterolemia subjects compared to the isolated hypertriglyceridemia group reflects their high cardiovascular risk.

Key words: polygenic hypercholesterolemia, isolated hypertriglyceridemia, adhesion molecules, selectins, homocysteine, hs-CRP

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Introduction

One of the key initial events in the development of atherosclerosis is the adhesion of monocytes to endothelial cells, with subsequent transmigration into the vascular intima. Leukocyte and vascular adhesion molecules (CAMs) such as selectins, integrins, sVCAM-1, and sICAM-1 play critical roles in the adhesion of monocytes to endothelial cells. *In vivo* animal studies and immunohistochemical studies of hu-

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man tissues have shown that these CAMs are expressed at increased levels in atherosclerotic plaques (1-3). sVCAM-1 concentration is directly correlated with LDL-C levels in isolated hypercholesterolemia (4).

It is generally accepted that an elevation in levels of total cholesterol and LDL-C cholesterol and a reduction in the level of HDL-C cholesterol place patients at a higher risk of initial and subsequent cardiac events. Cholesterol may increase adhesion of monocytes to endothelium (5). LDL-C mediated monocyte adhesion molecule overexpression may be blocked by lipid lowering drugs (6).

There is also no definitive data currently available on the behavior of endothelial adhesion molecules in polygenic hypercholesterolemia patients. Disorders in triglyceride metabolism may promote atherogenesis by increasing the expression of vascular CAMs. Patients with markedly elevated triglyceride levels also have decreased HDL-C levels and abnormalities in fatty acid metabolism. In vitro studies have shown that low HDL-C and oxidized fatty acids increase the endothelial expression of CAMs in response to cytokines (7). The importance of an isolated triglyceride elevation is much less clear. Recommendations for screening or treating isolated hypertriglyceridemia are limited. Hyperhomocysteinemia (HHCY) and hypercholesterolemia are linked to the development of atherothrombotic diseases (8). Hyperhomocysteinemia promotes inflammation by increasing expression of vascular cell adhesion molecule 1 (sVCAM-1) and increases oxidative modification of low-density lipoproteins, thereby promoting uptake of low-density lipoprotein cholesterol by macrophages (9). Although hyperlipidemia affects and increases the risk of cardiovascular events, little is known about the effect of isolated triglyceride elevation on homocysteine and adhesion molecules.

Inflammation, manifested by elevated serum levels of C-reactive protein (CRP) is associated with an increased risk of cardiovascular events. CRP can induce endothelial Lectin-like oxidized low-density lipoprotein (ox-LDL) receptor-1 (LOX-1), which is the primary endothelial receptor for oxLDL, may lead to the activation of proinflammatory genes including IL-8, sICAM-1, and sVCAM-1 (10). Little is known, however, about serum CRP levels in patients with polygenic hypercholesterolemia and isolated hypertriglyceridemia and the effect on adhesion molecules.

In the present study, homocysteine, hs-CRP, sE-selectin, sP-selectin, sVCAM-1 and sICAM-1 levels of 30 patients with polygenic hypercholesterolemia and 30 patients with isolated hypertriglyceridemia, all with no additional diseases, were evaluated in comparison with 30 healthy controls.

Materials and Methods

Study subject population and inclusion criteria

Study groups included individuals who presented to the Internal Diseases Clinic between April 2008 and June 2010 and who met the eligibility criteria. Written informed consent was obtained from each subject and research protocols were approved by the Ethical Committee of our institution. The patients were divided into study groups. The first group included patients with polygenic hypercholesterolemia, whose low-density lipoprotein cholesterol elevations were moderate (160-300 mg/dL) and whose serum triglyceride concentrations were within the reference range (50-150 mg/dL), while the second group included patients with isolated hypertriglyceridemia, where isolated hypertriglyceridemia was defined as serum triglyceride levels over 200 mg/dL and normal LDL-C levels (<130 mg/dL) with normal total serum cholesterol levels (< or=200 mg/dL). Thirty age- and gender-matched healthy volunteers were studied as the control group. History, physical examination, and routine chemical analysis showed that the control subjects had no evidence of any disease or any medications.

Eligibility criteria

Inclusion criteria were: All subjects were attendants of our outpatient clinic for routine check up. Inclusion criteria were: 1) adults 27 to 52 years, 2) non-obese individuals with BMI <25 kg/m^2 and waist circumference of <102 cm in males and <88 cm in females, 3) non-diabetic and non-hypertensive individuals, 4) non-smokers, 5) individuals with normal folate and vitamin B12 levels, and 6) non-menopausal women, 7) without a family history of premature onset of symptomatic CAD and physical findings such as tendinous xanthomas and xanthelasmas or arcus cornealis to rule out heterozygous familial hypercholesterolemia, and 8) cerebral or peripheral vascular disease.

Individuals in all three groups were evaluated according to the International Diabetes Federation, 2005 metabolic syndrome criteria and those without the metabolic syndrome were included in the study.

All study subjects were free of clinically manifest vascular disease and had not taken any lipid-lowering medication or any supplemental vitamins for at least 3 months. Patients with secondary hyperlipidemia, thyroid diseases, alcohol abuse, renal insufficiency (creatinine >1.5 mg/dL), liver cirrhosis, febrile disorders, acute or chronic inflammatory disease, malignancy, history of myocardial infarction, malnutrition or endocrine disorders, those using antiinflammatory medication, acetylsalicylic acid, antihistamines and corticosteroids, receiving hormone replacement therapy were excluded.

Body weight (kg) and height (cm) were measured. The body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference (WC) was measured as the minimum between the costal margin and iliac crest.

Biochemical analyses

All blood samples were obtained in the morning between 08 : 00 and 09 : 00 a.m. after overnight fasting. Fresh serum was used for determining concentrations of cholesterol and

Table 1. Lipid Profile of the Subjects

Parameters	Control group	Polygenic hypercholesterolemia group	Isolated hypertriglyceridemia	p
Total-C (mg/dL)	164.9±9.4	271.4±15.0	192.0±6.0	<0.001
TG (mg/dL)	116.4±12.3	122.0±13.4	234.0±22.2	<0.001
HDL-C (mg/dL)	49.0±6.4	49.0±6.6	49.2±6.5	0.8
LDL-C (mg/dL)	90.7±4.4	199.0±12.2	97.1±6.3	<0.001

Table 2. Comparison of Age, BMI, Waist Circumference and Gender among the Three Groups

Parameters	Control group	Polygenic hypercholesterolemia group	Isolated hypertriglyceridemia	p
Age (yr)	40.5±7.6	40.3±6.2	40.6 ±8.1	0.9
BMI (kg/m^2)	22.3 ±1.1	22.4 ±1.2	22.2 ±1.3	0.9
Wc (cm)	83.9±7.0	85.7±6.8	84.3±7.3	0.5
Sex	16F/14M	17F/13M	15F/15M	0.8

triglycerides in low-density lipoprotein and high-density lipoprotein fractions.

Aliquots of plasma for analyses of high-sensitivity C-reactive protein (CRP), cell adhesion molecules and homocysteine were centrifuged at 3,000 rpm for 10 minutes at room temperature within an hour. After centrifugation, the serum samples were frozen and stored at -80°until analysis.

Serum total cholesterol, triglyceride, LDL-C, HDL-C, high sensitive CRP, homocysteine, sVCAM-1, sICAM-1, sP-selectin and sE-Selectin levels of all subjects in the three groups were measured. Total cholesterol, triglyceride and HDL-C were measured using enzymatic colorimetric method. LDL-C was calculated using the, Friedewald formula: LDL-C: total cholesterol-(HDL-C+ Triglyceride/5). The TG levels were lower than 400 mg/dL in each subject.

sE-Selectin, sVCAM-1, sICAM-1 tests were carried out by ELISA kits from Ray Bio and sSP-selectin was measured using the ELISA method with ELISA kit from DIACLONE Research (France). Homocysteine test was performed by the enzyme immunoassay method by Homocysteine EIA kit from AXIS-SHIELD (United Kingdom) using high sensitive CRP nephelometric method and image immune chemistry (Beckman Coulter, Fullerton, CA, USA) device.

Statistical analyses

Results were expressed as mean value ± standard deviation. Statistical evaluation of the findings was performed by using SPSS 11.0 for Windows statistical software. One-way analysis of variance (ANOVA) in conjunction with Tukey's Highly Significant Difference (HSD) test was applied when comparing multiple groups. Comparison between groups of continuous variables was performed by using Student's t-test or Mann-Whitney U-test. The Chi-square test was used to compare the categorical parameters. Pearson's correlation coefficients were calculated for estimation of the level of association between variables in all groups. Results were expressed as mean ± SD. A p value of <0.05 was considered as statistically significant.

Results

Ninety subjects aged 27 to 52 were included in the study. Thirty subjects were enrolled into each of the polygenic hypercholesterolemia, isolated increased triglyceride and control groups. Of the subjects, 48 were females and 42 were males and their mean age was 40.4 and mean body mass index was 22.3. In the polygenic hypercholesterolemia group, mean total cholesterol was 271.4 mg/dL and mean LDL-C was 199 mg/dL, while, in the isolated hypertriglyceridemia group, subjects' mean triglyceride was 234 mg/dL (Table 1). There were no statistically significant differences between the three groups in terms of age, BMI, waist circumference, or gender (Table 2).

There were statistically significant differences among all three groups in terms of mean sVCAM-1, sE-selectin and sICAM-1 (Table 3; p<0,001). Mean sVCAM-1, sE-selectin and sICAM-1 levels of patients with polygenic hypercholesterolemia were statistically significantly higher than the control group (p<0.001) and the isolated hypertriglyceridemia group (p<0.001). Comparison of subjects with isolated hypertriglyceridemia with the subjects in the control group did not yield a significant difference in terms of sVCAM-1, sE-selectin and sICAM-1 (p>0.05). Mean sP-selectin values did not differ significantly among the three groups (Table 3; p= 0.09).

There was a significant difference among the three groups

Table 3. Comparisons of Adhesion Molecules, hs-CRP and Homocysteine Levels in Three Groups (mean ± SD)

	Polygenic hypercholesterolemia	Isolated hypertriglyceridemia	Control	p value
sVCAM-1 (ng/mL)	533.7 ± 23.3	496.8 ± 30	483.7 ± 29,3	<0.001
sICAM-1 (ng/mL)	294.7 ± 41,9	204.5 ± 23.6	195.9 ± 22.6	<0,001
sP-selectin (ng/mL)	254.5 ± 52.6	244.5 ± 49.9	226.0 ± 46.7	0.09
sE-Selectin (ng/mL)	48.5 ± 6.5	36.7 ± 4.6	34.7 ± 3.5	<0.001
h-CRP (mg/L)	3.9 ± 1.8	2.01 ± 1.4	1.0 ± 0.5	<0.001
Homocysteine (µmol/L)	15.7 ± 4.5	13.1 ± 3.9	12.6 ± 3.9	0.004

Table 4. Pearson's Correlation Analysis for LDL-c, sE-selectin, sP-selectin Levels, sVCAM-1, sICAM-1 Levels, hs-CRP and Homocysteine Levels in the Whole Study Group (n=90)

	LDL-C	HDL-C	HHCY	sVCAM-1	sICAM-1	sE-selectin	hs-CRP	sP-selectin
LDL-C		NS	r=0.379 p<0.001	r=0.626 p<0.001	r=0.846 p<0.001	r=0.795 p<0.001	r=0.683 p<0.001	r=0.227 p<0.032
HDL-C	NS		r= - 0.275 p=0.009	r= - 0.260 p=0.010	r= - 0.232 p=0.028	r= - 0.225 p=0.033	NS	NS
Homocysteine	r=0.379 p<0.001	r= - 0.275 p=0.009		r=0.737 p<0.001	r=0.641 p<0.001	r=0.713 p<0.001	r=0.724 p<0.001	r=0.711 p<0.001
sVCAM-1	r=0.626 p<0.001	r= - 0.260 p=0.010	r=0.737 p<0.001		r=0.857 p<0.001	r=0.881 p<0.001	r=0.858 p<0.001	r=0.706 p<0.001
sICAM-1	r=0.846 p<0.001	r= - 0.232 p=0.028	r=0.641 p<0.001	r=0.857 p<0.001		r=0.967 p<0.001	r=0.875 p<0.001	r=0.550 p<0.001
sE-selectin	r=0.795 p<0.001	r= - 0.225 p=0.033	r=0.713 p<0.001	r=0.881 p<0.001	r=0.967 p<0.001		r=0.903 p<0.001	r=0.594 p<0.001
hs-CRP	r=0.683 p<0.001	NS	r=0.724 p<0.001	r=0.858 p<0.001	r=0.875 p<0.001	r=0.903 p<0.001		r=0.680 p<0.001
sP-selectin	r=0.227 p<0.032	NS	r=0.711 p<0.001	r=0.706 p<0.001	r=0.550 p<0.001	r=0.594 p<0.001	r=0.680 p<0.001	

in terms of mean hs-CRP and homocysteine (p<0.001, p= 0.004; respectively). Homocysteine and hs-CRP levels were higher in the polygenic hypercholesterolemia group, compared to the isolated hypertriglyceridemia group (p=0.019, p <0.001; respectively) and the control group (p<0.001, p< 0.001; respectively). Subjects with hypertriglyceridemia did not differ significantly from the control group regarding homocysteine (p>0.05), whereas hs-CRP value was higher in the subjects with isolated hypertriglyceridemia than in the control group (p=0.001).

Pearson's correlation analysis revealed that LDL-C levels were correlated with sE-selectin, sP-selectin levels, sVCAM-1, sICAM-1 levels, hs-CRP and homocysteine levels in the whole study group (Table 4). Pearson's correlation analysis also showed that TG levels were not correlated with the variables sE-selectin and sP-selectin levels, sVCAM-1 and sICAM-1 levels in the whole group. For HDL-C, there was a negative correlation between sE-selectin, sVCAM-1, sICAM-1 levels and homocysteine levels.

Discussion

In the present study, mean values for plasma sE-selectin, sVCAM and sICAM were significantly higher in the group with polygenic hypercholesterolemia compared to the other two groups. Comparison of subjects with hypertriglyceridemia with those in the control roup did not reveal any statistically significant differences in terms of sE-selectin, sP-

selectin, sVCAM-1, sICAM or homocysteine, while hs-CRP was significantly higher in the isolated hypertriglyceridemia group compared to the control group. Mean plasma levels of sP-selectins in all three groups did not yield a significant difference.

The correlation analyses across all subjects demonstrated a positive, highly significant relationship between LDL-C cholesterol and sVCAM, sICAM, sE-Selectin, sP-selectin, homocysteine and hs-CRP levels. On the other hand, TG levels were not significantly correlated with these parameters. Recommendations for screening or treating isolated hypertriglyceridemia have been limited. It has been demonstrated that hypertriglyceridemia is associated with increased plasma concentrations of soluble adhesion molecules (11-13). However, unlike the present study, HDL-C levels differed between the patient and control groups in those studies. HDL-C levels were lower in patient groups than in the control group. In a study by Cockerill et al, HDL-C cholesterol was added to the endothelial cell culture and noted a reduction in the expression of VCAM-1, sE-selectin and ICAM-1 in the cell culture medium (7). Additionally, a negative association between tHcy and HDL-C cholesterol has been reported (14). It is believed that there were no differences between the subjects with isolated triglyceridemia and those in the control group in terms of adhesion molecules and homocysteine.

Triglyceride is a fatty substance that is composed of three fatty acids. Each of these acids is attached to a glycerol molecule. Before breakfast after an overnight fast, most of the triglyceride in the blood comes from the liver in the form of VLDL particles. Whether elevated triglyceride levels in the blood lead to atherosclerosis and heart attacks is controversial. It is difficult to conclusively prove that elevated triglyceride by itself can cause atherosclerosis. However, it is increasingly recognized that elevated triglyceride is often associated with other conditions that increase the risk of atherosclerosis, including obesity, low levels of HDL-C, insulin resistance and poorly controlled diabetes mellitus, and small, dense LDL-C particles (15).

A recent review of the literature concluded that treating isolated hypertriglyceridemia does not prevent coronary events (15). In PROCAM study, the cardiovascular risk of hypertriglyceridemic subjects was strongly influenced by the concurrent cholesterol and HDL-C cholesterol levels, whereas the cardiovascular event rate of the subjects with high triglycerides and normal cholesterol was the same as that of normolipidemic controls (16). The National Cholesterol Education Program Adult Treatment Panel does not recommend drug therapy for hypertriglyceridemia except in familial dyslipidemias (17). The American College of Physicians does not recommend screening for hypertriglyceridemia (18) and the US Preventive Services Task Force is not for or against screening for elevated triglyceride levels (19).

Together with previous reports, this emphasizes the importance of the fact that isolated hypertriglyceridemia may not show increased plasma sICAM-1, sVCAM-1, homocysteine and soluble E-selectin levels for atherosclerosis and coronary heart disease with the results of the present investigation in which there were no differences in between patients with hypertriglyceridemia and control subjects. However, isolated hypertriglyceridemic patients showed “isolated high CRP levels” in our study. Isolated hypertriglyceridemia could conceivably lead to a chronic inflammatory state, which can contribute further to endothelial dysfunction.

High serum lipid levels, especially high LDL-C levels have been shown to be related strongly to the development of atherosclerosis (20, 21). Results from both clinical and animal studies show that a high level of LDL-C in the blood is pivotal in the production of atherosclerosis. It is crucial to understand how elevated blood LDL-C or cholesterol levels can induce a proinflammatory, proatherogenic state. Conflicting results have been reported. LDL-C may promote adhesion molecules. LDL-C increases endothelial cell affinity for monocytes and it induces expression of vascular adhesion molecule (VCAM)-1 and sE-selectin in human vascular endothelial cells (22). However, other studies have not confirmed elevated levels of sICAM-1 and sVCAM-1 in hypercholesterolemia subjects (23, 24). Kowalski et al recorded elevated levels of sICAM-1 but not of sVCAM-1 in individuals with hyperlipidemia IIb (25).

On the other hand, homocysteine may promote lipid peroxidation of LDL-C (26). Elevation of plasma tHcy also has been shown to accelerate the development of atherosclerosis in the hypercholesterolemic Apoe-deficient (Apoe -/-) mouse (27). HcyH is proinflammatory and upregulates the expression of human aortic endothelial cell adhesion molecules (28). As with homocysteine, CRP is an important risk factor for atherosclerosis and coronary artery disease. Vincenzo et al proposed that CRP directly stimulates the inflammatory reaction of arteriosclerosis by inducing the expression of adhesion molecule in vascular endothelial cells (10). Hs-CRP levels were found to be significantly correlated with Hcy levels in patients with diabetes mellitus (29).

In the present study, mean plasma levels of sE-selectin, sVCAM-1 and sICAM-1 in the group with polygenic hypercholesterolemia were significantly higher than in the other two groups. Indeed, hyperlipidemic individuals showed a significant correlation between LDL-C, sE-selectin, sP-selectin levels, sVCAM-1, sICAM-1 levels, hs-CRP and homocysteine levels, which is in accordance of other authors confirming an evident and independent correlation between LDL-C cholesterol levels and coronary heart disease.

There are two main limitations in this study. First, we did not examine IDL, Lpa levels, which are important parameters in atherosclerosis. Because beta-quantification is cumbersome and requires sophisticated equipment, we use the Friedewald formula, which also includes LDL-C, IDL-cholesterol, and Lp(a)-cholesterol in the estimation of LDL-C.

This formula is valid when triglycerides are below 400 mg/dL. Published evaluations of current direct LDL tests in-

dicate that the correlation between a direct LDL method to the reference method was no better than the correlation observed using the Friedewald equation, when triglycerides are between 200 mg/dL and 400 mg/dL (30-33). There was a good correlation between the results of LDL-C assayed by the proposed method and the beta-quantification reference method involving these reports (r=0.990, r=0.994, r=0.994, respectively). The Working Group on Lipoprotein Measurement of the NCEP recommends that the most prudent course at present is to measure LDL-C by methods similar to those used to establish the epidemiological database on which the relations between cardiovascular risk and LDL-C have been established. These methods are limited to ultracentrifugation and Friedewald formula calculation. Hypertriglyceridemia is often associated with the elevation of plasma LDL-C and IDL. Isolated hypertriglyceridemia is defined in subjects as elevated triglycerides but LDL-C is normal (34). We selected isolated hypertriglyceridemia patients who had higher than 200 mg/dL with normal LDL cholesterol levels. On the other hand, plasma Lp (a) levels are inversely related to TG levels in hypertriglyceridemic patients (34, 35). The other limitation is the small sample size in each group. The results, although could be considered as preliminary due to the relatively small sample size could improve the understanding on the development of coronary arterial disease in these two metabolic situations especially in similar HDL-C levels.

Comparison of plasma concentration of these molecules between patients with hypertriglyceridemia and subjects in the control group did not yield a significant difference. Plasma concentrations of sP-selectin were not significantly different among the three groups. The increase of adhesion molecules, homocysteine and hs-CRP in polygenic hypercholesterolemia subjects compared to the isolated hypertriglyceridemia group reflects their high cardiovascular risk. However, it is controversial how much isolated hypertriglyceridemia correlates directly with coronary artery disease and further studies are needed to clarify whether treatment for this condition leads to meaningful clinical outcomes.

In conclusion, we determined that plasma concentrations of the adhesion molecules sVCAM, sICAM, sE-selectin, homocysteine and hs-CRP were statistically significantly increased compared to the hypertriglyceridemia and control groups.

The authors state that they have no Conflict of Interest (COI).

References

1. Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apostprandialarently healthy men. Lancet **351**: 88-92, 1998.
2. Hwang S-J, Ballantyne CM, Sharret AR, et al. Circulating adhesion molecules VCAM-1 ICAM-1 and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation **96**: 4219-4225, 1997.
3. Blann AD, Amiral J, Mc Collum CN. Circulating endothelial cell/leukocyte adhesion molecules in ischaemic heart disease. British J Haem **95**: 263-265, 1996.
4. Desideri G, Marinucci MC, Tomassoni G, Masci PG, Santucci A, Ferri C. Vitamin E supplementation reduces plasma VCAM-1 and von Willebrand factor levels and increases nitric oxide concentrations in hypercholesterolemic patients. J Clin Endocrinol Metab **87**: 2940-2945, 2002.
5. Fu C, He J, Li C, Shyy JY, Zhu Y. Cholesterol increases adhesion of monocytes to endothelium by moving adhesion molecules out of caveolae. Biochim Biophys Acta **1801**: 702-710, 2010.
6. Serrano CV Jr, Pesaro AE, de Lemos JA, et al. Native LDL-C-cholesterol mediated monocyte adhesion molecule overexpression is blocked by simvastatin. Cardiovasc Drugs Ther **23**: 215-220, 2009.
7. Cockerill GW, Rye K-A, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol **15**: 1987-1994, 1995.
8. Olszewski AJ, Szostak WB, Bialkowska M, Rudnicki S, McCully KS. Reduction of plasma lipid and homocysteine levels by pyridoxine, folate, cobalamin, choline, riboflavin and troxerutinin in atherosclerosis. Atherosclerosis **75**: 1-6, 1989.
9. Lentz SR. Does homocysteine promote atherosclerosis? Arterioscler Thromb Vasc Biol **21**: 1385-1386, 2001.
10. Romano G, Corrado E, Muratori I, et al. Carotid and peripheral atherosclerosis in patients who underwent primary percutaneous coronary intervention and outcome associated with multifocal atherosclerosis. Int Angiol **25**: 389-394, 2006.
11. Lundman P, Eriksson MJ, Silveira A, et al. Relation of hypertriglyceridemia to plasma concentrations of biochemical markers of inflammation and endothelial activation (C-Reactive Protein, Interleukin-6, Soluble Adhesion Molecules, von Willebrand Factor, and Endothelin-1). Am J Cardiol **91**: 1128-1130, 2003.
12. Lupattelli G, Lombardini R, Schillaci G, et al. Flow-mediated vasoactivity and circulating adhesion molecules in hypertriglyceridemia: association with small, dense LDL-C cholesterol particles. Am Heart J **140**: 521-526, 2000.
13. Abe Y, El-Masri B, Kimball KT, et al. Soluble cell adhesion molecules in hypertriglyceridemia and potential significance on monocyte adhesion. Arterioscler Thromb Vasc Biol **18**: 723-731, 1998.
14. Liao D, Tan H, Hui R, et al. Hyperhomocysteinemia decreases circulating high-density lipoprotein by inhibiting apolipoprotein A-I Protein synthesis and enhancing HDL-C cholesterol clearance. Circ Res **99**: 598-606, 2006.
15. Cucuzzela M, Smith P, Nashelsky J. When should we treat isolated high triglycerides? J Fam Pract **53**: 142-144, 2004.
16. Assmann G, Schulte H. Results and conclusions of the Prospective Cardiovascular Münster (PROCAM) Study. In: Lipid Metabolism Disorders and Coronary Heart Disease. 21. Assmann G, Ed. MMV Medizin Verlag, 1993: 67.
17. Rubinstein A. National Cholesterol Education Program Second Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Pannel II). Circulation **89**: 1333-1445, 1994.
18. LaRosa JC. Cholesterol agonistics. Ann Intern Med **124**: 505-508, 1996.
19. Atkins D, DiGuiseppi C. Screening for high blood cholesterol and other lipid abnormalities. In: Guide to Clinical Preventive Services. 2nd ed. Williams & Wilkins, Baltimore, MD, 1996: 15-39.
20. Anderson KM, Castelli WP, Levy D. Cholesterol and mortality. 30 years of follow-up from the Framingham study. JAMA **257**: 2176-2180, 1987.
21. Martin MJ, Hulley SB, Browner WS, Kuller LH, Wentworth D. Serum cholesterol, blood pressure and mortality: Implications

from a cohort of 361,662 men. Lancet **2**: 933-936, 1986.

22. Allen S, Khan S, Al-Mohanna F, Batten P, Yacoub M. Native low-density lipoprotein-induced calcium transients trigger VCAM-1 and E-selectin expression in cultured human vascular endothelial cells. J Clin Invest **101**: 1064-1075, 1998.

23. Morisaki N, Saito I, Tamura K, et al. New indices of ischemic heart disease and aginig: studies on the serum levels of soluble adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) in patients with hypercholesterolemia and ischemic heart disease. Arteriosclerosis **131**: 43-48, 1997.

24. Blann AD, Seigneur M, Steiner M, Miller JP, McCollum CN. Circulating ICAM-1 and VCAM-1 in peripheral artery disease and hypercholesterolaemia: relationship to the location of atherosclerotic disease, smoking, and in the prediction of adverse events. Thromb Haemostas **79**: 1080-1085, 1998.

25. Kowalski J, Okopien B, Madej A, et al. Levels of sICAM-1, sVCAM-1and MCP-1 in patients with hyperlipoproteinemia IIa and IIb. Int J Clin Pharmacol Ther **39**: 48-52, 2001.

26. Hirano K, Ogihara T, Miki M, et al. Homocysteine induces iron-catalyzed lipid peroxidation of low density lipoprotein that is prevented by alpha-tocopherol. Free Rad Res **21**: 267-276, 1994.

27. Zhou J, Werstuck GH, Lhotak S, et al. Association of multiple cellular stress pathways with accelerated atherosclerosis in hyperhomocysteinemic apolipoprotein E-deficient mice. Circulation **110**: 207-213, 2004.

28. Silverman MD, Tumuluri RJ, Davis M, Lopez G, Rosenbaum JT, Lelkes PI. Homocysteine upregulates vascular cell adhesion molecule-1 expression in cultured human aortic endothelial cells and enhances monocyte adhesion. Arterioscler Thromb Vasc Biol **22**: 587, 2002.

29. Araki A, Hosoi T, Orimo H, Ito H. Association of plasma homocysteine with serum interleukin-6 and C-peptide levels in patients with type 2 diabetes. Metabolism **54**: 809-814, 2005.

30. Suguichi H, Irie T, Uji Y, et al. Homogenous assay for measuring low-density lipoprotein cholesterol in serum with triblock copolymer and α-cyclodextrin sulfate. Clin Chem **44**: 522-531, 1998.

31. Naito HK. Coronary artery disease and disorders of lipid metabolism. In: Clinical Chemistry. 4th ed. Kaplan L, Pesce AJ, Kazmierczak SC, Eds. Mosby, 2003: 603-638.

32. Lindsey CC, Graham MK, Johnston TP, Kiroff CG, Freshley A. A clinical comparison of calculated versus direct measurement of low-density lipoprotein cholesterol level. Pharmacotherapy **24**: 167-172, 2004.

33. Rıfai N, Ianotti E, DeAngelis K, Law T. Analytical and clinical performance of a homogeneous enzymatic LDL-cholesterol assay compared with ultracentrifugation-dextran sulfate-Mg method. Clin Chem **44**: 1242-1250, 1998.

34. Dunbar RL, Rader DJ. Demystifying triglycerides: A practical approach for the clinician. Cleve Clin J Med **72**: 661-680, 2005.

35. Bartens W, Rader DJ, Talley G, Brewer HB Jr. Decreased plasma levels of lipoprotein(a) in patients with hypertriglyceridemia. Atherosclerosis **108**: 149-147, 1994.

36. Werba JP, Safa O, Gianfranceschi G, Michelagnoli S, Sirtori CR, Franceschini G. Plasma triglycerides and lipoprotein(a): inverse relationship in a hyperlipidemic Italian population. Atherosclerosis **101**: 203-211, 1993.

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