Association Between Plasma Lipids Profile and Lipids Oxidizability in Healthy Men

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Abstract
Introduction: The aim of the present study was to determine the susceptibility of lipids to Cu-induced peroxidation in diluted plasma and its relation with plasma lipids and lipoproteins in a group of healthy men.
Materials and Methods: In 100 healthy men volunteers (age range 20-55 years with a mean of 36.8±10.3 years), fasting plasma levels of lipoprotein (a) [Lp (a)], total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and triglycerides (TG) were assayed. The Cu2+-induced lipid peroxidation was evaluated. Lipid oxidation was estimated by monitoring the change of conjugated dienes in the diluted plasma following the addition of Cu^{2+}. The kinetic curves of the accumulation of lipid peroxide products were prepared, and a number of quantitative parameters including lag time, time of maximal oxidation rate (T-max), and maximal accumulation of absorbing products (OD-max) were evaluated.
Results: The TG concentrations were positively correlated with lag time and T-max (r=0.33, $P < 0.01$ and r=0.24, $P < 0.05$) respectively. Also, TC and LDL-C were positively correlated with OD-max (r=0.28, $P < 0.01$ and r=0.26, $P < 0.05$ respectively), and HDL-C was negatively correlated (r=-0.23, $P < 0.05$) with T-max. No significant correlation was observed between other variables and lipid oxidizability parameters.
Conclusions: Results of this research indicate that TG increased the resistance of LDL and VLDL against initiation of lipid oxidation. In addition, HDL-C induced the susceptibility of lipid oxidizability.
Keywords: Diluted Plasma, Copper, Lipid Oxidizability, Healthy Men
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Introduction

The oxidation of lipids and lipoproteins is involved in the pathogenesis of atherosclerosis.[1] The modification of plasma lipoproteins, particularly low-density lipoproteins (LDLs), is responsible for the excessive internalization of oxidized LDL via the less tightly controlled scavenger receptor' pathway, which results in the formation of foam cells and atherosclerotic plaques.[2] Lipoprotein(a) [Lp(a)] is one of the plasma lipoproteins that is considered as an independent risk factor for cardiovascular disease.[3] It has been suggested that elevated levels of proinflammatory oxidized phospholipids primarily carried by LP(a) represents a genetic susceptibility to increased oxidative stress, which this claim is controversial.[4,5] Much effort has been devoted to the development of in vitro assays for assessing the susceptibility of lipoproteins to various oxidants as well as to a possible correlation between the 'oxidizability' of LDL and lipid-related pathologies.[6] Copper-induced oxidation of isolated LDL is frequently used as an in vitro model for the resistance of LDL lipids to oxidation.[7,8] The lengthy procedure involved in LDL fractionation and the possible modification of the lipids during isolation of LDL, limit the clinical usefulness of kinetic data on LDL 'oxidizability' and justify further efforts aimed at the evaluation of the susceptibility of lipids to oxidation in no fractionated plasma or serum.[9] Several studies have used plasma or serum as a biological model in this context.[10,11] It seems that oxidation of lipids in the unfractionated plasma is a better, reliable, and flexible biological model to evaluate the lipid oxidizability.[12-14] These studies have used whole serum or plasma diluted from 2-fold to 150-fold and copper concentrations ranging from 50 mmol/L to 3.75 mmol/L.[12] Together, these studies have established that the measured oxidation in serum or plasma is a consequence of lipoprotein oxidation that correlates well with the oxidation of LDL.[14-16] Copper-induced oxidation of lipids in non-fractionated serum is correlated with the complex nature of the interrelated effects of the various factors

that influence the oxidation, including those of water-soluble antioxidants, anticoagulants used for plasma preparation and ions present in the diluting media.[17,18] Considering that finding the best and most accurate method for measuring the oxidizability of LDL is still challenging, the present study has aimed to investigate the relationship between serum lipids level, especially LP (a), and lipid oxidizability. In the present study, serum plasma was used due to the lower cost in used method.

Materials and Methods

This descriptive and cross-sectional study was conducted on 100 randomized healthy men referred to Yazd Central Laboratory. The mean age was 36.8±10.3 (ranged between 20 to 55 years). The inclusion criteria for enrolling the study included having a negative history for cardiovascular, hepatic, renal, genetic and endocrine disorders and also no positive history for smoking and hypertension. Blood samples were taken from the patients after 12 hours fasting and some were mixed with heparin for preparing plasma, and a part was incubated at room temperature for serum isolation for one hour.

In order to study lipid oxidizability, plasma was stored at - 70°C for a maximum 2 months period. The serum concentration of triglyceride and cholesterol were measured using "MAN". The high-density lipoprotein-cholesterol (HDL-C) was also determined by "Pars Azmoon" laboratory kits and TECHNICON-RA-1000 autoanalyzer.

Furthermore, LP (a) levels were measured by electro immunodiffusion techniques in the serum. Lipid oxidizability was studied by peroxidation products (Conjugated dynes) and the absorbance rate was measured at 245 nm.[12] Copper-induced plasma lipid peroxidation was estimated in 60-fold diluted plasma in 20 mM 60 times by phosphate-buffered saline (PBS) containing 0.16 M NaCl and 720 μM sodium citrate (pH = 7.4). The lipid oxidation procedure was performed in a water bath at 37°C and initiated by adding 30 μL of 4.5 mM CuSO$_4$ solution, to give final Cu^{2+} concentration of 70 μM. The kinetic of conjugated dienes formation was monitored by spectrophotometer (Dual wavelength spectrophotometer, Milton Ray) at 245 nm, every 10 minutes for at least 300 minutes.

Excel Microsoft software program was used for plotting the kinetic curves of lipid peroxidation product (absorbance rate changes at 245 nm versus time in minute). A number of quantitative oxidation parameters including latency to onset of oxidation (Lag time), the maximum rate of oxidation (V-max), the maximum concentration of lipid peroxide products accumulation (OD max) and the time needed to gain the maximum rate of oxidation (T-max) were considered to evaluate the relationship between serum lipids level and lipid oxidizability.

Statistical Analyses

All quantitative data are presented as the mean ± SD. Statistical analysis was performed using SPSS version 20. The student *t* test, Pearson rank correlation coefficient were used to analyze the results according to the normal distribution of variables.

Results

The mean age of participants was 36.8±10.3 years. Lipid levels and mean oxidizability parameters of plasma lipids are shown in Table 1 and Table 2, respectively. Mean of plasma Lp (a) level was 18.3±18.7 mg/dL. When heparin plasma was diluted 60-fold with PBS containing 0.15 M NaCl and incubated with 70 μM Cu^{2+}, its absorbance at 245 nm was found to increase (Figure 1). The oxidizability of the plasma was characterized using the parameters which are typically used to characterize the oxidizability of plasma, namely lag-phase and propagation phase duration and maximal oxidation rate (maximal oxidation rate measured within the propagation phase).

There was no significant correlation observed between serum Lp (a) levels and oxidizability parameters, but triglyceride concentration was significantly correlated with Lag time and T max ($P = 0.001$ and $r = 0.33$; $P = 0.01$ and $r = 0.28$, respectively). A negative, but not significant correlation was found between lag time and plasma cholesterol levels. The mean total cholesterol concentration was significantly

Table 1. Studied Variables in Population Study

Variables	Mean ± SD
Age	36.8 ± 10.3
Total cholesterol (mg/dL)	200.9 ± 40
Triglycerides (mg/dL)	179.4 ± 96.7
HDL-C (mg/dL)	38 ± 12.4
LDL-C (mg/dL)	128.3 ± 45.7
LP(a) (mg/dL)	18.3 ± 18.7

Table 2. Mean of Quantitative Oxidation Parameters of Plasma Lipids

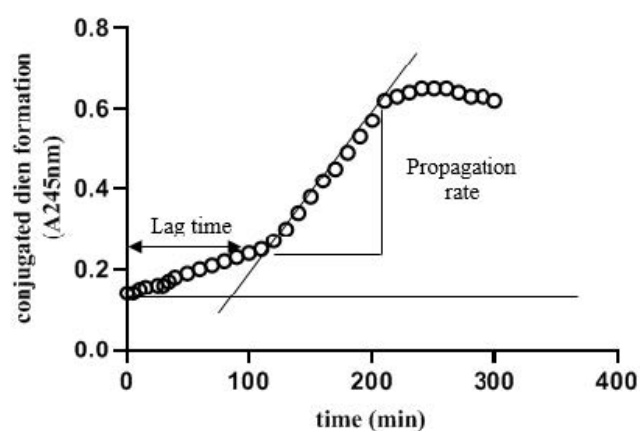| Variable	Mean ± SD
Lag Time (min)	103.8 ± 18.1
T max (min)	187.6 ± 25.1
OD max	0.508 ± 0.123
V max (OD/min)	3.1 ± 0.6

Figure 1. Conjugated Diene Monitoring During Copper-Induced Plasma Oxidation. Conjugated diene formation during copper-mediated plasma oxidation was monitored for 300 min by the changes in 245 nm wavelength absorbance.

Table 3. Correlation Between Plasma Lipid and Lipoproteins Concentration and Quantitative Parameters of Oxidizability

Variable	Lipids				
	LP(a)	HDL-C	LDL-C	Triglyceride	Total Cholesterol
Lag-time (min)	r = -0.009, P = 0.92	r= -0.205, P = 0.042	r= -0.06, P = 0.56	r= 0.337, P = 0.001	r= 0.006, P = 0.95
V-max (OD/min)	r = -0.103, P = 0.316	r= -0.024, P = 0.811	r=0.142, P = 0.166	r= -0.065, P = 0.526	r= 0.072, P = 0.479
T-max	r = -0.031, P = 0.765	r= -0.203, P = 0.045	r= 0.010, P = 0.926	r= 0.24, P = 0.018	r= 0.014, P = 0.892
OD-max	r = -0.045, P = 0.659	r= -0.066, P = 0.516	r= 0.253, P = 0.013	r= 0.145, P = 0.156	r= 0.283, P = 0.005

correlated with OD max (P = 0.005, r = 0.28), while the mean plasma concentration of LDL-C was not correlated with V max and lag time, but there was a significant correlation with OD max (P = 0.013, r = 0.25) (Table 3).

Discussion

In this study, a significant relationship was found between the concentration of triglycerides levels with Lag time and T max, which was in accordance with the results from de Man et al's study.[19] It seems that normal ranges of triglycerides, increases LDL resistance to oxidation in *in vitro* samples and alternatively, triglyceride-rich samples would be more resistant to oxidation. It is likely that the VLDL contains more antioxidants than LDL, and since the bulk of triglycerides are carried by VLDL, increasing the oxidation time will result in a triglycerides rise. As cholesterol is one of the oxidized substrates, increasing its concentration could be due to a rise in the maximum oxidation products. High cholesterol is also associated with increases in LDL/VLDL ration, which contain fewer antioxidants and is, therefore, more susceptible to oxidation.[16] Another finding observed in this study was an inverse relationship between HDL concentration and the lag time, which indicates that HDL reduces resistance to oxidizability process.[16,20] The most epidemiological studies indicate that plasma HDL is considered as a protective factor against coronary heart disease. The prevalence of atherosclerosis has been reported to be lower in the patients with high HDL levels.[21] Actually, HDL can bind to cholesterol and lipid peroxidation products which inhibits LDL oxidation process. Moreover, HDL influences the release of endothelial relaxing factor and prostacyclin stability that plays a protective role against oxidation.[22] On the contrary, the results from previous studies have shown that HDL ratio is more sensitive to oxidation induction process.[23,24] Accordingly, HDL contains the main part of plasma lipid peroxides which is oxidized faster than LDL *in vitro*. Similar to oxidized LDL, oxidized HDL can convert to a toxic particle and start atherosclerosis. It is worth mentioning that HDL has beneficial properties such as stimulating the cholesterol flow from foam cells and antioxidant activity. These properties will be reduced following HDL oxidation.[25] Besides, HDL oxidation and paraoxonase activity also lead to the loss of antioxidant activity.[26] The HDL oxidation rate depends on several factors, such as HDL composition, particle size and concentration of the solution, which is used for oxidation as well as copper ion concentrations.[27,28] As mentioned before, HDL is more susceptible to oxidation induction and also accelerates LDL oxidation progression.[23] Hydroperoxides immigration from

oxidized HDL to LDL (which is partially oxidized), followed by increased production of free radicals in LDL, is responsible for the LDL oxidation rise in the high concentrations of copper ion.[29] In the present study, an inverse relation was detected between serum HDL and T max, which is in contrast to the findings of Lin et al.[29] Also, LP(a) levels were not significantly associated with oxidizability parameters but a significant effect of LP(a) was not found on the oxidation rate and oxidizability, at least at low concentrations.[30,31] Results may change if the oxidizability parameters get examined in subjects with high concentrations of Lp (a) (over 50 mg/dL). Lipid and lipoprotein fractions (especially LDL) and total plasma can be used to study the oxidizability of lipoproteins and lipids. The main advantage of oxidizability examination on LDL is to remove the interference of other materials and components. It should be noted that LDL isolation is difficult and time-consuming and LDL damage would be likely to occur during the separation process. The disadvantage of oxidation induction on whole plasma is interfering components. Alternatively, this method is simpler and less expensive and does not require any special devices. Furthermore, this method gives a better image of oxidation conditions *in vivo* due to its biological environment. The major limitation of this study was that only male gender and healthy subjects were evaluated.

Conclusions

The results of this study showed that at least at low concentrations, serum Lp (a) has no significant effect on the rate of lipid oxidation and the lipids oxidizability properties in healthy subjects. Extremely LDLs and triglycerides show more resistance to oxidation compared to cholesterol and LDLs.

Conflict of Interest Disclosures

No potential conflicts of interest were disclosed.

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