Lipoprotein(a) in Alcohol-Dependent Male Patients During a Six-Month Abstinence Period

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(Received 14 June 2002; first review notified 4 September 2002; in revised form 2 October 2002; accepted 24 October 2002)

Abstract — Aims: The best known and probably most important mechanism of health-protective moderate alcohol drinking is beneficial changes in plasma lipid levels. We determined changes in main plasma lipid levels in alcohol-dependent patients over a 6-month abstinence period. Methods: Fifty-four alcohol-dependent male patients, who were abstinent for less than 14 days, and 20 non-alcoholic males, who had not drunk alcohol for the last month, were studied. In all patients at the study start and after 4 weeks and 6 months observation, lipoprotein(a) [Lp(a)], total cholesterol (TC), HDL cholesterol (HDL), LDL cholesterol (LDL) and triglyceride concentrations, both fasting and 5 h after a fatty meal, were determined. Results: Alcohol-dependent patients had similar mean fasting and post-prandial plasma lipid levels as the control group, both at the study start and after 4 weeks of abstinence. Whereas, in alcohol-dependent patients after 4 weeks of abstinence, a significant decrease in Lp(a) and fasting HDL levels, as well as a significant increase in fasting LDL level and pro-atherogenic indices of plasma lipids [TC/HDL, (TC-HDL)/HDL, LDL/HDL, Lp(a)/HDL] were observed. Post-prandial levels of studied plasma lipids, except HDL, did not change over the 6-month observation period. In patients who did not remain abstinent for the whole observation period (n = 9), in comparison to abstinent patients, significantly higher HDL levels and a tendency to higher values of LDL, LDL/HDL, Lp(a) and Lp(a)/HDL were found. Conclusions: (1) Higher Lp(a) levels soon after alcohol withdrawal may be a factor potentially responsible for the increase of acute cardiac syndromes prevalent in the drinking and early abstinence period, in spite of high HDL concentration; (2) in alcohol-dependent male patients, after a 6-month abstinence period, pro-atherogenic plasma cholesterol fraction changes occurred, expressed by a decrease in HDL level and an increase in LDL concentration.

Introduction

The results of many epidemiological, experimental and interventional studies show that LDL cholesterol (LDL) is the main risk factor of coronary artery disease (Wood et al., 1998). Moreover, all measures that lead to LDL plasma concentration decrease (dietary and pharmacological), result in a decrease in cardiovascular events frequency (Scandinavian Simvastatin Survival Study Group, 1994). The Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (NCEP, 2001) considered this cholesterol fraction as a main therapy target both in primary, as well as in secondary, coronary artery disease prevention. This report, among the known principal atherosclerosis risk factors, enumerated low HDL cholesterol (HDL) level (<40 mg/dl), increased triglyceride (TGL) concentration (>150 mg/dl), with an increased lipoprotein(a) [Lp(a)] concentration as a newly recognized risk factors. Lp(a) is the main congenital lipid atherosclerosis risk factor. An Lp(a) level above 30 mg/dl increases cardiovascular event risk twice, independently of other lipid levels and five times, when LDL level is simultaneously increased (Fulcher, 1992). About 80% of plasma Lp(a) concentration is genetically determined, with the remaining 20% being related to environmental factors and cytokine concentrations (which function as acute phase proteins). Lp(a) acts as a pro-atherogenic factor in many ways: as LDL-lipoprotein (which, after oxidation, accumulates in foam cells and activates inflammatory processes), and as a false plasminogen (fibrinolysis inhibitor). Lp(a) also increases endothelial plasminogen activator inhibitor type 1 (PAI-1) synthesis and secretion, inhibits plasminogen activation by tissue and urokinase type plasminogen activators, facilitates plasmin binding with alpha2-antiplasmin (Edelberg and Pizzo, 1994) and also activates platelets adhesion and aggregation, and vascular smooth myocyte migration and proliferation (Barts and Wanner, 1994).

Moreover, epidemiological studies show a ‘J’ shaped relationship between the quantity of daily alcohol intake and coronary artery disease prevalence (Goldberg et al., 1995; Keil, 1997; Renaud et al., 1998; De Lorimier, 2000). This suggests that, in regularly drinking people, cardiovascular event risk may increase during abstinence. We assume that this also concerns alcoholics. Because the cardioprotective effect of regular alcohol drinking is attributed to the favourable influence on lipid metabolism, homeostasis, antioxidative balance (flavinoids), nitric oxide secretion, vascular tone and reological blood properties, alcohol drinking cessation could result in the disappearance of these beneficial changes. The main changes in fasting lipids level during the acute abstinence period in alcoholics are well recognized, but changes in Lp(a) and post-prandial lipid levels in abstinent alcoholics are not clear. Because of this, we have undertaken the present study.

Patients and Methods

This investigation was performed in 54 alcohol-dependent male patients, diagnosed according to ICD-10 criteria (World Health Organization, 1992), hospitalized in an Addiction Treatment Unit, Department of Psychiatry, The Ludwik Rydygier Medical University in Bydgoszcz (Poland) in 1999...
and 2000. Twenty non-alcoholic males acted as a control group, who did not drink alcohol for at least 1 month before the study. The inclusion criteria of the patients group were: male sex, age between 30 and 50 years, ICD-10 criteria of alcohol dependence performance, abstinence, continued motivation and end of misuse period not longer than 14 days before the study start. The exclusion criteria were: concomitant presence of diseases, which could have an influence on lipid metabolism (for example liver failure, nephrotic syndrome), psychotic disorders, dementia, addiction to substances other than alcohol (except smoking), and any drug taking. The demographic and clinical data of the alcohol-dependent patients studied are presented in Table 1.

In all patients, blood samples for biochemical determinations were taken after 14 h of fasting at the start of the study, and after 4 weeks and 6 months of abstinence, whereas in the control group, because of ethical reasons, blood samples were taken only at the beginning of the study and 4 weeks later. After blood sampling, all subjects received a standard breakfast, which consisted of two bread pieces with 82.5% fat-containing butter (0.5 g per kilogram of body mass). After 5 h, a second blood sample was taken to determine post-prandial plasma lipid levels. This time was chosen on the basis of the results of the study by Nikkila et al. (1994), which showed that the TGL levels 5 h after meal ingestion was a better cardiovascular event predictor than HDL concentration. Our standard breakfast composition was based on the butter loading test.

We determined the concentrations of the following plasma lipids: Lp(a), total cholesterol (TC), HDL and TGL. An LDL value, using the Friedewald pattern, was calculated only for patients with a TGL level below 400 mg/dl. We also performed (to exclude secondary causes of lipid metabolic disturbances) a glucose tolerance test, peripheral blood morphology, creatinine and thyrotropin concentration, as well as biochemical markers of alcohol misuse, namely: γ-glutamyltranspeptidase, aspartate aminotransferase, alanine amino transferase and mean corpuscular volume. Moreover, we calculated the plasma lipids pro-atherogenic indices, namely: TC to HDL concentration ratio (TC/HDL), TC minus HDL to HDL concentration ratio ([TC−HDL]/HDL], LDL to HDL concentration ratio (LDL/HDL) and Lp(a) to HDL concentration ratio [Lp(a)/HDL]. We considered as critical values of the first three indices 5, 4 and 4, respectively. We could not find in the available literature the predictive value of the Lp(a)/HDL ratio. Lp(a) concentration (only fasting) was assayed by an ELISA method, using the set manufactured by Cormay-La Roche. The other determinations were made using routine laboratory methods.

For the first 8 weeks of the study, alcohol-dependent patients were hospitalized in the Addiction Therapy Unit. They received a similar hypolipaemic diet, according to European Atherosclerosis Society (1992) recommendations (Pyörälä et al., 1994). Energy consumption was on average 2000 kcal per day, but in patients with body mass index (BMI) above 25 kg/m² the reduced diet (to 20 kcal/kg body mass) was recommended. A daily diet consisted of cereal products in one third, vegetables in one quarter, 15% of milk products and the rest in meat, fish or legumes. In this way, daily cholesterol consumption was lower than 300 mg and daily fat-energy consumption was lower than 30% (saturated fatty acids below 10% energy, mono-unsaturated fatty acids 10–15% energy and poly-unsaturated fatty acids 7–10% energy). In patients with a BMI above 25 kg/m² and in patients with hyper-triglyceridaemia (TGL > 200 mg/dl), no sugar consumption was recommended. During the study period, patients did not take any drugs.

Abstinence was controlled during hospitalization on the basis of physical examination as well as alcohol presence in exhaled air and the above-mentioned biochemical markers of alcohol misuse. After discharge from the Unit, abstinence was monitored by interview, level of biochemical markers, objective familial interview, and medical documentation analysis (from out-patient department).

As mentioned above, the study included 54 alcohol-dependent male patients and 20 control males, who denied ever misusing alcohol or drinking for the previous month. For the examination after 4 weeks of abstinence, 47 (87%) patients and 18 (90%) persons from the control group turned up, and after 6 months of observation, 27 (50%) patients turned up, but only 18 (33%) remained abstinent till this period.

All subjects gave their informed consent to participate in this study, which was approved by the Local Ethics Committee of The Ludwik Rydygier Medical University in Bydgoszcz. The investigation was in compliance with the Declaration of Helsinki for medical research.

| Table 1. Demographic and clinical data of the study group |
|----------------------------------|------------------------|
| Feature                          | Alcohol-dependent males (n = 54) |
| Age (years)                      | 40.8 ± 8.0             |
| MAST (score)                     | 44.9 ± 21.5            |
| Age of alcohol dependence onset (years) | 22.2 ± 6.4            |
| Length of alcohol dependence (years) | 17.7 ± 7.0             |
| Number of drinking days during 90 days before the study start (days) | 50.9 ± 25.6 |
| Number of standard drinks drunk during 90 days before the study start (drinks) | 952.0 ± 670.5, 142.8 g of pure alcohol per day |
| Number of standard drinks drunk during 30 days before the study start (drinks) | 259.1 ± 176.6, 116.6 g of pure alcohol per day |
| Smoking [n (%)]                  | 45 (90)                |
| Mean daily nicotine dose (mg/day)—in smoking patients | 28.6 ± 13.1 |
| Mean daily tar dose (mg/day)—in smoking patients | 337.0 ± 144.0 |
| Systolic blood pressure (mmHg)   | 114.3 ± 14.0           |
| Diastolic blood pressure (mmHg)  | 75.3 ± 8.9             |
| Body mass index (kg/m²)          | 25.0 ± 3.0             |
| Waist–hip ratio                 | 0.97 ± 0.05            |

Values are means ± SD, unless otherwise indicated.

MAST, Polish version of the Michigan Alcoholism Screening Test (Faliciki et al., 1986).
Statistical significance was determined using, respectively, unpaired and paired Student’s t-tests, chi-square tests and two-factorial ANOVAs with three repetitions and Tukey post hoc tests in statistical software STATISTICA PL 5.0.

RESULTS

The studied alcohol-dependent male patients had similar mean plasma lipid levels as in the control group, both at the study start and after 4 weeks of abstinence (Table 2). However, in alcohol-dependent patients after just 4 weeks of abstinence, we observed the significant decrease of Lp(a) and fasting HDL concentration (Fig. 1) as well as the increase of fasting LDL concentration and the values of plasma lipids pro-atherogenic indices [TC/HDL, (TC–HDL)/HDL, LDL/HDL, Lp(a)/HDL]. The specificity of such changes for alcoholics after alcohol drinking cessation was confirmed using ANOVA for the first 4 weeks of the observation period. We found a significant interaction effect between the time of abstinence duration (4 weeks) and recent alcohol drinking withdrawal in changes in Lp(a) level ($F = 8.8, P = 0.047$), fasting ($F = 22.04$, $P = 0.0001$) and post-prandial ($F = 5.03, P = 0.029$) HDL concentrations, changes in TC/HDL values ($F = 6.52$, $P = 0.013$) and LDL/HDL values ($F = 9.89, P = 0.0041$). Simultaneously, after 4 weeks of abstinence, the percentage of patients with a prognostic unfavourable (TC–HDL)/HDL ratio value above 4 rose significantly (20 vs 40%; $P = 0.03$). In alcohol-dependent patients after the next 5 months of abstinence, small increases of fasting HDL (significant) and LDL (not significant) levels were observed (Fig. 1). As a result, in comparison with the initial values, in alcohol-dependent males after 6 months of abstinence, significantly lower fasting (13%) and post-prandial (16%) mean HDL values were observed (Fig. 1). In this patient group, increases in the plasma lipid pro-atherogenic indices were also found. The mean TC level increased in this period by 6% and LDL by 18%, although not significantly. Post-prandial levels of studied plasma lipids, except HDL, have also not changed significantly over the 6-month observation period.

In patients who failed to remain abstinent for the whole observation period ($n = 9$), compared with abstinent alcoholics, we found, at 6 months, significantly higher fasting (62.2 ± 28.9 vs 44.6 ± 11.8 mg/dl, $P = 0.03$) and post-prandial (71.4 ± 37.8 vs 42.8 ± 14.9 mg/dl, $P = 0.01$) HDL levels (Fig. 2), as well as a tendency to lower values of LDL level (128.7 ± 30.8 vs 162.1 ± 64.1 mg/dl, $P = 0.15$), LDL/HDL ratio (2.7 ± 1.7 vs 3.9 ± 1.6, $P = 0.08$), Lp(a) concentration (12.4 ± 13.1 vs 26.2 ± 22.5 mg/dl, $P = 0.16$) and Lp(a)/HDL ratio (0.21 ± 0.2 vs 0.60 ± 0.48, $P = 0.055$).

DISCUSSION

In our study, we estimated the changes in fasting and post-prandial plasma lipid levels in alcohol-dependent male patients after a 6-month abstinence period. To determine that the observed changes were related to alcohol withdrawal, we included only patients who abstained less than 2 weeks before the study start. Moreover, we have compared the results obtained with the lipid values in non-alcoholic males, who have not drunk alcohol for the last month. The studied and control group had similar clinical and demographic data (other than alcohol drinking). Plasma lipid levels in the first 4 weeks also did not differ significantly (Table 2). In alcohol-dependent males during the observation period, significant decreases in fasting and post-prandial HDL levels as well as increases in fasting LDL concentration and plasma lipids pro-atherogenic indices were found. Similar changes in plasma cholesterol fractions after alcohol drinking cessation were observed in other studies (Kervinen et al., 1991; Langer et al., 1992; Lamiss et al., 1994; Goldberg et al., 1995) and in our previous paper (Budzyński et al., 2000). These observations can be accounted for by the disappearance of metabolic ethyl

Table 2. The mean fasting and post-prandial plasma lipid levels in alcohol-dependent patients and in the control group at the study start and after 4 weeks of abstinence

<table>
<thead>
<tr>
<th>Parameter (mg/dl)</th>
<th>ADP (n = 54)</th>
<th>CG (n = 20)</th>
<th>ADP (n = 47)</th>
<th>CG (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a)</td>
<td>26.0 ± 26.3</td>
<td>17.5 ± 19.1</td>
<td>23.9 ± 26.0</td>
<td>17.2 ± 5.8</td>
</tr>
<tr>
<td>TC</td>
<td>226.6 ± 46.2</td>
<td>216.0 ± 37.3</td>
<td>220.7 ± 50.6</td>
<td>208.9 ± 33.7</td>
</tr>
<tr>
<td>HDL</td>
<td>50.3 ± 13.5</td>
<td>47.2 ± 13.2</td>
<td>46.8 ± 11.6</td>
<td>46.8 ± 11.6</td>
</tr>
<tr>
<td>LDL</td>
<td>138.4 ± 36.9</td>
<td>138.7 ± 32.1</td>
<td>149.4 ± 36.9</td>
<td>134.3 ± 27.3</td>
</tr>
<tr>
<td>TGL</td>
<td>168.7 ± 97.0</td>
<td>152.1 ± 105.5</td>
<td>165.2 ± 83.5</td>
<td>132.8 ± 116.3</td>
</tr>
<tr>
<td>(TC–HDL)/HDL</td>
<td>4.7 ± 1.4</td>
<td>5.2 ± 2.6</td>
<td>5.6 ± 2.1</td>
<td>4.9 ± 2.2</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>3.0 ± 1.1</td>
<td>3.2 ± 1.7</td>
<td>3.9 ± 1.6</td>
<td>3.0 ± 1.5</td>
</tr>
<tr>
<td>Lp(a)/HDL</td>
<td>0.54 ± 0.56</td>
<td>0.39 ± 0.47</td>
<td>0.58 ± 0.64</td>
<td>0.34 ± 0.15</td>
</tr>
<tr>
<td>TCp</td>
<td>227.7 ± 45.9*</td>
<td>212.0 ± 33.7</td>
<td>234.5 ± 53.8*</td>
<td>202.8 ± 31.0*</td>
</tr>
<tr>
<td>HDLp</td>
<td>48.8 ± 13.1*</td>
<td>45.8 ± 12.8*</td>
<td>41.8 ± 13.2</td>
<td>43.4 ± 13.0**</td>
</tr>
<tr>
<td>LDLp</td>
<td>132.3 ± 37.7</td>
<td>122.6 ± 29.9</td>
<td>146.2 ± 54.9</td>
<td>117.8 ± 27.3</td>
</tr>
<tr>
<td>TGLp</td>
<td>233.0 ± 135.2</td>
<td>214.8 ± 158.1</td>
<td>245.3 ± 146.2**</td>
<td>222.3 ± 170.8**</td>
</tr>
</tbody>
</table>

ADP, alcohol-dependent patients; CG, control group; TC, total cholesterol; HDL, HDL cholesterol; LDL, LDL cholesterol; TGL, triglycerides; Lp(a), lipopr otein(a); TCp, post-prandial total cholesterol; HDLp, post-prandial HDL cholesterol; LDLp, post-prandial LDL cholesterol; TGLp, post-prandial triglycerides. Statistical significance: differences between the ADP and CG groups were not significant, both in the fasting and post-prandial state, as well as both at the study start and after 4 weeks of abstinence; after comparison before and after the meal (unpaired Student’s t-test): $^aP < 0.05$, $^{**}P < 0.01$; after comparison of the levels at the study start and after 4 weeks, in respective groups (paired Student’s t-test): $^bP < 0.001$, $^{*}P < 0.01$, $^{*}P < 0.05$. 

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alcohol activity. Ethanol induces some changes in enzymes involved in lipid metabolism, especially increases in lecithin-cholesterol acyltransferase (LCAT), post-heparin lipoprotein lipase (LPL), hepatic lipase (HL) and cholesterol ester transfer protein activities and decreases in hydroxymethyl-glutaryl-CoA reductase activity (Valimaki et al., 1986; Savolainen et al., 1990; Steinberg et al., 1991; Hagiage et al., 1992; Valimaki et al., 1993; Nishiwaki et al., 1994; Pownall, 1994; Goldberg, 1996). That the changes in HDL concentration and TC/HDL, LDL/HDL ratios observed were due to discontinuation of alcohol intake is supported by the ANOVA analysis and by the significant increase in HDL level observed in relapsing patients (Fig. 2).

These results indicate that, in alcohol-dependent males during abstinence, pro-atherogenic changes proceed in fasting plasma lipid levels. These changes may lead to an increase of cardiovascular events risk. Epidemiological studies show that an increase in TC level by 1% increases the cardiovascular event risk by 2% (Law et al., 1994), and a decrease of HDL by 1 mg/dl increases cardiovascular event risk by 3% (Gordon et al., 1989). In this way, the cardiovascular event risk in our alcohol-dependent patients increased after 6 months of abstinence by about 12–18% for TC and 21% for HDL (total increase by about 40%). On the basis of the same data, we may also conclude that patients with alcohol drinking relapse had a 52% reduced cardiovascular event risk than patients, who have kept abstinence for the whole 6-month period (Fig. 2). Additional greater cardiovascular risk was related to the increase of plasma lipid pro-atherogenic indices. For example, a value of TC/HDL ratio above 5 is related to four times greater cardiovascular event risk in patients with a TGL level below 200 mg/dl, and increased seven times when TGL level is above 200 mg/dl (Assmann et al., 1992).

In our study, we have observed a significant decrease in Lp(a) levels during the succeeding months of abstinence (Fig. 1). These results show some differences in comparison with the majority of other studies. Only Willeit et al. (1995) showed that alcohol drinking was associated with a tendency towards higher Lp(a) levels, but Valimaki et al. (1993), Iso et al. (1996) and Paassilta et al. (1998) observed a negative correlation between quantity of alcohol intake and Lp(a) plasma concentration, whereas in our study Lp(a) level was highest soon after alcohol drinking cessation and decreased in succeeding months. A simultaneous significant increase in the Lp(a)/HDL ratio was observed. The changes in Lp(a) and the Lp(a)/HDL ratio during 6 months of abstinence observed in the present work are in accordance with epidemiological studies, which show ‘J’ shaped relationships between quantity of alcohol drunk and cardiovascular event prevalence (Goldberg et al., 1995; Keil, 1997; Renaud et al., 1998; De Lorimier, 2000). By reason of the fact that an Lp(a) level above 30 mg/dl is the independent cardiovascular event risk factor, in our patients Lp(a)-related cardiovascular risk was the highest at the study start. This suggests that the increase of Lp(a) plasma concentration may be a potential factor in the pro-atherogenic alcohol effect (right arm of ‘J’ shaped curve). On the other hand, the increase in the Lp(a)/HDL ratio after 4 weeks of abstinence may be the next mechanism explaining the left arm course of this ‘J’ shaped curve, in addition to known changes in HDL and LDL levels. The results of The Familial Atherosclerosis Treatment Study suggested that Lp(a) level and the Lp(a)/HDL ratio are better related to coronary artery narrowing before the hypolipaemic therapy start, than other lipid concentration values (Maher et al., 1995). The observed higher Lp(a) level at the study start may be explained by the influence of inflammatory cytokines, which regulate about 20% of Lp(a) plasma concentration [the same level as the decreased mean Lp(a) levels in our patients during abstinence]. The increased level of inflammatory cytokines due to alcohol drinking has previously been reported (Naveau et al., 2001; Poullis and Mendall, 2001; Uesugi et al., 2001, 2002). The higher level of Lp(a) observed just after alcohol drinking cessation suggests a potentially pro-atherogenic alcohol misuse effect connected with the activity of this lipoprotein.

In our study, we also investigated post-prandial plasma lipid changes during abstinence. We studied these parameters, because for most of the day, people are in a post-prandial state. The Nikkila et al. (1994) study confirmed this suggestion. Our results showed that post-prandial TGL level is probably a better predictor of pro-atherogenic lipid activity than fasting HDL level. Alcohol drinking may influence post-prandial lipid levels via: changes in lipid intestinal absorption (intestinal barrier injury, bile and pancreatic juice excretion), lipid turnover (changes in pituitary–thyroid and pituitary–adrenal axes function) and metabolism (changes in LPL, HL and LCAT activities). These changes disappear after alcohol withdrawal, which may be of importance in post-prandial lipaemia. But, in our study, we did not observe an effect of...
abstinence on post-prandial lipid levels. Only post-prandial HDL level decreased with abstinence, as was the case for fasting HDL. We have not found any investigations in the literature in which changes in post-prandial lipaemia during abstinence in alcohol-dependent patients were studied. But previous results showed that drinking 60 g of vodka (24 g of pure ethanol) or 370 ml of white wine (44.4 g of pure ethanol) with meals leads to a significant increase of post-prandial TGL level, independently of abstinence or regular alcohol drinking in the past (Superko, 1992; Pownall, 1994).

In conclusion, we suggest that: (1) higher Lp(a) levels soon after alcohol withdrawal may be a potential factor responsible for the increase in acute coronary syndromes prevalent in the drinking and early abstinence period, in spite of high HDL concentration; (2) in alcohol-dependent male patients, abstinence for 6 months, pro-atherogenic plasma cholesterol main fraction changes occur, which, on the basis of epidemiological studies, could increase the cardiovascular event risk by about 40%; (3) the increase in HDL concentration is the main lipid metabolic parameter mediating the anti-atherogenic effect of alcohol.

ACKNOWLEDGEMENTS

The study was supported by the State Committee for Scientific Research grant no. 4 POSD 071 18 awarded for project realization in 2000–2001.

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