Alcohol Withdrawal–Induced Change in Lipoprotein(a) : Association With the Growth Hormone/Insulin-like Growth Factor-I (IGF-I)/IGF-Binding Protein-1 (IGFBP-1) Axis
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Alcohol Withdrawal–Induced Change in Lipoprotein(a) Association With the Growth Hormone/Insulin-like Growth Factor-I (IGF-I)/IGF-Binding Protein-1 (IGFBP-1) Axis

Marita Paassilta, Kari Kervinen, Markku Linnaluoto, Y. Antero Kesäniemi

Abstract—Lipoprotein(a) [Lp(a)] is an important risk factor for cardiovascular disease. Alcohol is one of the few nongenetic factors that lower Lp(a) levels, but the metabolic mechanisms of this action are unknown. Alcohol inhibits the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis. Alcohol might also affect IGF-binding protein-1 (IGFBP-1), which is an acute inhibitor of IGF-I. We studied how alcohol withdrawal affects Lp(a) levels and the GH/IGF-I/IGFBP-1 axis. Male alcohol abusers (n = 27; 20 to 64 years old) were monitored immediately after alcohol withdrawal for 4 days. Twenty-six healthy men, mainly moderate drinkers, served as control subjects. Fasting blood samples were drawn to determine Lp(a), IGF-I, and IGFBP-1 (by ELISA, RIA, and immunoenzymometric assay, respectively). Nocturnal (12 hours) urine collection was performed in 9 alcoholics and 11 control subjects for GH analyses (RIA). The groups were similar in age and body mass index. Lp(a), GH, and IGF-I tended to be lower and IGFBP-1 higher in the alcoholics immediately after alcohol withdrawal than in the control subjects. During the 4-day observation in alcoholics, Lp(a) levels increased by 64% and IGF-I levels by 41%, whereas IGFBP-1 levels decreased by 59% (P < .001 after ANOVA for all comparisons). Urinary GH levels tended to decline. The increase in Lp(a) correlated inversely with the changes in IGFBP-1 (r = −.63, P < .001, n = 27) and GH (r = −.70, P < .05, n = 9), but not with IGF-I. In multiple regression analysis, the main predictors for the increase in Lp(a) were IGFBP-1 and urinary GH. In conclusion, alcohol withdrawal induces interrelated and potentially atherogenic changes in Lp(a) and IGFBP-1 levels. (Arterioscler Thromb Vasc Biol. 1998;18:650-654.)

Key Words: lipoprotein(a) • alcohol • insulin-like growth factor-I • insulin-like growth factor binding protein-1 • lipoprotein

Accumulating data suggest that Lp(a) is an important risk factor for both clinical and preclinical atherosclerotic vascular disease.1–3 The Lp(a) particle is a normal LDL with apolipoprotein(a) glycoprotein attached to it by a disulfide bond.1,4 Interestingly, Lp(a) has also been implicated as a stimulus for smooth muscle cell proliferation,5 and this might provide an additional role for Lp(a) in the pathogenesis of atherosclerosis.

Plasma Lp(a) levels are largely genetically determined.6 Dietary and pharmacological attempts to reduce Lp(a) have been mainly unsuccessful.7 Nicotinic acid,8,9 estrogen,10,11 and alcohol12–15 are among the few factors that appear to lower Lp(a) levels. Lowered Lp(a) levels have also been observed during IGF-I administration.16,17 GH therapy has increased Lp(a) levels in normal subjects18 and in patients with GH deficiency19 and idiopathic osteoporosis.20 However, GH therapy with near physiological doses in GH deficiency21 has no effect on Lp(a) levels.

Plasma Lp(a) levels are mainly determined by the apolipoprotein(a) production rate in the hepatocytes and the Lp(a) assembly.22 Liver is also the main synthesis site of IGF-I23 and IGFBP 1.24 In fact, low levels of IGF-I have been detected during both estrogen replacement therapy25,26 and niacin therapy,27 ie, in conditions with low Lp(a) levels. In addition, low levels of IGFBP-1, which is considered an acute regulator and inhibitor of IGF-I action,28 were recently shown to be associated with multiple factors predisposing to atherogenesis.29

Low Lp(a) levels have been observed in heavy alcohol consumers,12,13,15 but the mechanisms behind the alcohol-related reduction in Lp(a) are unknown. Since Lp(a) levels have been shown to rise rapidly after alcohol withdrawal,33 we decided to study the changes in the GH/IGF-I/IGFBP-1 axis after alcohol withdrawal to evaluate whether GH, IGF-I, and IGFBP-1 might be related to the change seen in Lp(a) after alcohol withdrawal.

Methods

Subjects
Twenty-eight consecutive male alcohol abusers admitted to the Alcoholism Treatment Unit in Oulu for withdrawal therapy were studied. Twenty-eight healthy nonalcoholic men volunteered as control subjects. One alcohol abuser with insulin-dependent diabetes...
Alcohol consumption, g/d 232

Other lipids and lipoproteins, IGF-I, and IGFBP-1 related to alcohol to the Alcoholism Treatment Unit and thereafter on three consecutive

TABLE 1. Baseline Characteristics of the Study Groups

Venous blood samples were drawn after an overnight fast (between

Study Design

All of the subjects had a good nutritional status and were clinically

TABLE 1. Baseline Characteristics of the Study Groups

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Alcohol Abusers</th>
<th>Healthy Control</th>
<th>P for the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>43.1±9.0</td>
<td>38.5±9.9</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.4±5.0</td>
<td>24.7±3.7</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol consumption, g/d</td>
<td>232±82</td>
<td>13±11</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.1±2.0</td>
<td>5.6±2.4</td>
<td>.01</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>2.4±0.74</td>
<td>2.0±0.71</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>1.97±0.90</td>
<td>2.27±0.82</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.62±0.21</td>
<td>0.58±0.43</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, median (range)</td>
<td>3.75–6.15</td>
<td>2.75–4.25</td>
<td>NS</td>
</tr>
<tr>
<td>GH, pg/mL*</td>
<td>2.56±3.14†</td>
<td>3.80±4.62‡</td>
<td>NS</td>
</tr>
<tr>
<td>IGF-I, nmol/L</td>
<td>8.8±2.9</td>
<td>10.3±4.3</td>
<td>NS</td>
</tr>
<tr>
<td>IGFBP-1, µg/L</td>
<td>4.8±3.6</td>
<td>4.2±3.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Results are presented as mean±SD, except for Lp(a) [median (range)].
† Nocturnal (12-hour) urinary GH secretion.
‡ n=9.
‡n=11.

Statistical Analysis

Results

The alcohol abusers did not differ from the control subjects in age or BMI (Table 1). In contrast, the alcohol abusers had 75% higher plasma HDL cholesterol (P<.001) and 28% lower LDL cholesterol (P<.01) levels at the end of the drinking period compared with the control subjects. Lp(a), GH, IGF-I, and IGFBP-1 levels did not differ significantly between the groups (Table 1), but Lp(a), GH, and IGF-I tended to be lower and IGFBP-1 higher in the alcoholics than in the control subjects.

Plasma lipids and lipoproteins, IGF-I, and IGFBP-1 in the alcoholics during the 4-day observation period after alcohol withdrawal are presented in Table 2. Lp(a) levels increased by 64% after alcohol withdrawal (P<.001). Total and HDL cholesterol concentrations showed a reduction of 8% and 21%, respectively, while VLDL cholesterol and plasma TG did not change during the same period. An increase of 41% in IGF-I and a decrease of 59% in IGFBP-1 (P<.001 for both withdrawal. An overnight (7 PM to 7 AM) urine collection for GH analyses was performed on 9 consecutive alcoholics on admission (before the first blood sampling day) and during the night before the fourth day of monitoring. Five control subjects were monitored for a 4-day period for Lp(a), IGF-I, IGFBP-1, and GH, while a single blood sample was obtained from the rest (n=21). Urine collection was performed among 11 control subjects. The plasma samples were stored at −70°C and the urine samples at −20°C.

Biochemical Assays

After isolating VLDL (d<1.006 g/mL) by ultracentrifugation, the HDL cholesterol concentration in the VLDL-free fraction was determined by an enzymatic method after precipitation of LDL with heparin-manganese chloride. Plasma cholesterol and triglyceride concentrations were determined by enzymatic colorimetric methods (Boehringer, Mannheim GmbH) using a Kone Specific analyzer (Kone Specific, Selective Chemistry Analyzer, Kone Instruments).

Plasma Lp(a) concentrations were determined by an enzyme-linked immunosorbent assay method (Biopool Ltd), which has been shown to correlate well with the other Lp(a) assay methods. The CVs within and between Lp(a) assays were 5.0% and 6.6%, respectively. IGF-I was determined by a double antibody disequilibrium RIA (Incastar Stillwater) after an extraction procedure. The intra-assay CV was 5.9% and the interassay CV 11.8%. IGFBP-1 was measured by using an immunoenzymometric assay (IEMA TEST, Medix Biochemica), the intra-assay and interassay CVs being 4.1% and 8.6%, respectively. Urinary GH was determined by a sandwich RIA technique (31hIGH U Coartia, bioMérieux) with a detection limit of 0.5 pg/mL. The intra-assay CVs at 1.4 and 13.6 pg/mL were 9.3% and 8.1%, respectively.

Selected Abbreviations and Acronyms

BMI = body mass index
CV = coefficient of variation
GH = growth hormone
IGF-I = insulin-like growth factor-I
IGFBP-1 = IGF-binding protein-1
Lp(a) = lipoprotein(a)
RIAl = radioimmunoassay

mellitus and two control subjects with alcohol consumption of more than 40 g/d were excluded. The amount and quality of alcoholic beverages consumed during the previous 2 weeks were recorded using a questionnaire based on the method of Khavari and Farber. All alcoholics had been drinking daily for at least 1 week before admission. Two of the control subjects were teetotalers and the others were social drinkers. The mean daily alcohol consumption was 232 g among the alcohol abusers and 13 g among the control subjects (Table 1). All of the subjects had a good nutritional status and were clinically free from liver, kidney, or heart dysfunction. In one subject, albumin and Thrombotest Simplastin A (TT-SPA) values were lower than normal [39 g/L (34 to 46 g/L) mean (range)] and TT-SPA values. The mean values (reference value) for alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), and mean corpuscular volume of erythrocytes (MCV) in the alcohol abusers was 57 U/L (<50 U/L), 162 U/L (<80 U/L), and 98 fL (<96 fL), respectively. MCV and the activities of ALT and GGT were analyzed in the laboratory of the Oulu University Hospital using standard methods. Written informed consent was obtained from all the participants, and the Ethical Committee of the University of Oulu approved the study.

Study Venous blood samples were drawn after an overnight fast (between 7 and 8 AM) from the alcohol abusers on the first day after admission to the Alcoholism Treatment Unit and thereafter on three consecutive days during the abstinence period to investigate the changes in Lp(a), other lipids and lipoproteins, IGF-I, and IGFBP-1 related to alcohol withdrawal. An overnight (7 PM to 7 AM) urine collection for GH analyses was performed on 9 consecutive alcoholics on admission (before the first blood sampling day) and during the night before the fourth day of monitoring. Five control subjects were monitored for a 4-day period for Lp(a), IGF-I, IGFBP-1, and GH, while a single blood sample was obtained from the rest (n=21). Urine collection was performed among 11 control subjects. The plasma samples were stored at −70°C and the urine samples at −20°C.

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Statistical Analysis

Statistical analysis was carried out with the software package SPSS for Windows (Release 6.1, SPSS Inc.). The results are presented as mean±SD unless otherwise stated. Logarithmic transformation was used to normalize the distribution of the data for Lp(a), GH, IGF-I, plasma, and VLDL triglycerides. A standard t test of means was then used to compare the homogeneity of the study groups. The strength of the linear association between two variables was measured using Pearson correlation coefficients. For analyses of the time-dependent changes in the variables, repeated-measures ANOVA module of SPSS was used. Thereafter, paired t tests were performed to evaluate the difference in the variables between days. GH and the variables that gave statistical significance (P<.05) after ANOVA were analyzed in a stepwise multiple linear regression model. In this procedure, independent variables that best predicted the value of the dependent variable were estimated by means of R² (explanatory value of the model).

Results

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comparisons) levels were observed after the cessation of drinking. Urinary GH levels tended to decline during the monitoring period (from 2.56±3.14 to 1.10±1.00 pg/mL, P<.05; mean±SD). In the five control subjects who were monitored for 4 days, Lp(a), IGF-I, IGFBP-1, and GH levels did not change during a 4-day monitoring period (data not shown).

IGFBP-1, Lp(a), and Other Lipids
At the end of the drinking period, IGFBP-1 showed a correlation with LDL cholesterol (r=−.62, P=.001) and the other lipids (HDL and VLDL cholesterol and plasma and VLDL triglycerides, r=−.41, −.46, −.47, and −.41; P<.05 for all). The change in IGFBP-1 during the 4-day observation was correlated negatively with the changes in plasma and VLDL triglycerides (r=−.48, −.43, respectively, P<.05 for both). Multiple-regression analysis showed that the best predictor for the change in IGFBP-1 was Lp(a) (R²=.608, P=.013).

The change (from day 1 to day 4) in Lp(a) levels during the 4-day monitoring showed a negative association with the change in IGFBP-1 (r=−.63, P<.001) (Fig 1). In multiple regression analysis, the main predictors of the change in Lp(a) were IGFBP-1 alone or together with GH (R²=.650, P=.016 and R²=.828, P=.005, respectively). IGFBP-1 was also the main predictor of the change in plasma triglycerides (R²=.530, P=.026).

IGF-I
The change in IGF-I during the monitoring period did not show any correlation with the other variables. Multiple regression analysis revealed the best predictive parameters for the change in IGF-I to be plasma cholesterol and triglycerides (R²=.850, P=.003).

GH
The change in nocturnal (12 hours) urinary GH secretion correlated negatively with the change in plasma Lp(a) levels (r=−.70, P<.05) (Fig 2), but not with any other variables. The change in GH was best predicted by Lp(a) in the multiple regression analysis (R²=.492, P=.035).

Discussion
The three major findings in the present study were the following: (1) In agreement with a previous study, Lp(a) levels increased rapidly after alcohol withdrawal. (2) Plasma levels of IGFBP-1 showed a remarkable decrease after the cessation of drinking, and the change correlated with that in Lp(a). The change in Lp(a) was mainly predicted by the changes in IGFBP-1 and GH. Conversely, Lp(a) was the main predictor for the changes in IGFBP-1 and GH. (3) The change in IGF-I after alcohol withdrawal mirrors the change seen in Lp(a) similarly to the change seen during niacin and estrogen therapies. The increase in IGF-I related to alcohol withdrawal did not, however, correlate with the increase in Lp(a).
This finding suggests that IGF-I may not be involved in the regulation of Lp(a) during alcohol withdrawal. The decreasing trend seen in the GH levels during the monitoring is suggested to reflect the negative feedback by increased IGF-I.

Acute moderate alcohol intake has been shown to increase IGFBP-1 levels\(^43\) and reduce GH levels.\(^44\) Therefore, social alcohol consumption—shown to decrease Lp(a) levels\(^45\)—may also have affected the GH/IGF-I/IGFBP-1 axis in the control group of the present study. This may partly explain why the control group, which mainly consisted of social drinkers, did not differ from the alcohol abusers in GH, IGF-I, IGFBP-1, and Lp(a) levels.

How could cessation of alcohol intake affect IGFBP-1 and Lp(a)? Alcohol alters the redox state of the liver by increasing the NADH/NAD ratio and thus promoting reduced intracellular state.\(^35\) High NADH/NAD ratio is also characteristic for metabolic acidosis, a condition shown to increase plasma levels of IGFBP-1 and the IGFBP-1 content in the rat liver.\(^36\) Theoretically, normalization of the redox state after alcohol withdrawal might have contributed to the observed reduction in IGFBP-1 levels. It is also possible that the removal of the hepatotoxic agent, ie, alcohol, allows the liver to resume its normal synthetic function and thus normalize IGFBP-1 and Lp(a) production. Since the rise in Lp(a) occurs rapidly, the mechanisms behind it may also be related to changes in the catabolism of Lp(a).

Nutritional factors are important in the regulation of IGF-I and IGFBP-1. Alcohol abusers may often present with hepatic damage and nutrient deprivation.\(^39,40\) However, a careful clinical examination of the subjects ruled out severe liver damage, and the normal BMI and serum albumin levels revealed that the subjects were in a good nutritional condition.

Previous data on the effects of alcohol on stress hormones, such as catecholamines and cortisol, are inconsistent. Increased\(^41\) or unchanged\(^42\) plasma cortisol levels have been observed after alcohol intake. IGF-I levels may be reduced\(^43\) or unaffected\(^44\) by corticosteroid excess. IGFBP-1 levels have been shown to decrease\(^45\) or increase\(^46\) during cortisol administration. In addition, dexamethasone treatment after renal transplantation has been shown to induce a dose-dependent reduction in Lp(a).\(^47\) Therefore, the changes in Lp(a), IGF-I, and IGFBP-1 and the observed association between Lp(a) and IGFBP-1 during alcohol withdrawal are possibly not related to cortisol. Unfortunately, neither plasma nor urinary catecholamines and cortisol were measured in the present study.

Enhanced expression of IGF-I has been observed in human atherosclerotic plaques\(^48\) and in rat allograft arteriosclerosis.\(^49\) In addition, low levels of IGFBP-1 have recently been associated with low HDL cholesterol, increased insulin, proinsulin, and BMI—factors known to increase the risk for cardiovascular disease.\(^28\) IGF-I was not shown to be associated with cardiovascular risk factors in that study. The present data provide new evidence that IGFBP-1 may also be associated with Lp(a), a risk factor for CHD. In the present study, alcohol withdrawal may have enhanced the bioavailability of IGF-I due to a concomitant increase in IGF-I and a decrease in IGFBP-1 levels. The role of GH may also be important in the modulation of Lp(a) levels, but this requires further studies, because the number of subjects analyzed for GH in the present study was limited.

In conclusion, alcohol withdrawal induces a rapid rise in plasma Lp(a) levels. The increase in Lp(a) is associated with the decrease in IGFBP-1. After cessation of drinking, IGF-I levels increase simultaneously with Lp(a), but they do not correlate with each other. Alcohol-induced changes in the liver, such as reduced redox state, could partly explain the observed changes in Lp(a) and IGFBP-1 levels seen after alcohol withdrawal. These changes, low IGFBP-1 together with high Lp(a), may be important factors predisposing to atherosclerosis. The possible interrelationship between Lp(a) and IGFBP-1 in a normal population awaits further studies.

Acknowledgments

This study was supported by grants from the Finnish Foundation for Alcohol Studies and the Medical Council of the Academy of Finland. We are indebted to Saja Kortetjarvi, Anna-Riitta Malinen, and Eila Saarikoski for their outstanding technical assistance. Thanks are also due to the staff of the Kivihiarju Alcoholism Treatment Unit for their kind cooperation during the sample collection.

References