Effects of smoking cessation or alcohol restriction on metabolic and fibrinolytic variables in Japanese men

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ABSTRACT

We investigated the effects of smoking cessation or alcohol restriction on metabolic and fibrinolytic variables in Japanese men. In the smoking study, 35 male subjects [32 ± 1 (S.E.M.) years] who habitually smoked cigarettes (29 ± 3 cigarettes/day) were told either to keep their usual smoking habits for 1 week, or to abstain from cigarette smoking, using a randomized crossover design. In the alcohol study, 33 male subjects (37 ± 1 years) who habitually drank alcohol (64 ± 6 ml of ethanol/day) were told either to keep their usual drinking habits for 3 weeks, or to reduce alcohol intake by at least up to a half of their usual drinking amount, using a randomized crossover design. In each study, venous blood samples were drawn after a 12-h overnight fast on the last day of each period, and metabolic and fibrinolytic variables were measured. One-week smoking cessation significantly increased serum high-density lipoprotein (HDL) cholesterol levels (P < 0.05), and significantly decreased serum lipoprotein (a) levels (P < 0.01) and plasma plasminogen activator inhibitor-1 levels (P < 0.05). In contrast, 3-week alcohol restriction significantly decreased serum HDL cholesterol levels (P < 0.05) and plasma tissue plasminogen activator levels (P < 0.05). These results suggest that smoking cessation has substantial and immediate benefits on lipid and fibrinolytic variables in habitual smokers, whereas alcohol restriction increases cardiovascular risks, in some respects, in habitual drinkers.

INTRODUCTION

Cigarette smoking is one of the strongest contributors to the risks of cardiovascular diseases, including coronary heart disease, stroke, sudden death, peripheral artery disease, and aortic aneurysm [1]. Considerable reductions in the risk of cardiovascular diseases occur immediately after the discontinuation of cigarette smoking [2], although the mechanisms responsible for this have not been fully elucidated. In contrast, it has been suggested that light-to-moderate alcohol consumption may protect against coronary heart disease [3] and ischaemic stroke [4] through its effects on high-density lipoprotein (HDL) cholesterol, coagulation factors, and other mechanisms.

So far, very few studies have been performed to investigate the effects of smoking cessation or alcohol restriction on fibrinolytic variables, as well as metabolic variables in the manner of interventional trials. Especially in studies on smoking cessation, some clinical problems often occur. Weight usually increases 2–5 kg after
quitting [5]. Weight gain is intimately associated with
harmful metabolic and fibrinolytic disturbances [6,7].
Weight gain after quitting may blunt or conceal the
benefit of smoking cessation. Therefore, it is necessary to
assess the effects of smoking cessation on cardiovascular
risk factors for a relatively short-term period during
which body weight does not show a significant change.
Accordingly, we investigated the effects on metabolic
and fibrinolytic variables of 1-week smoking cessation in
male habitual smokers, and of 3-week alcohol restriction
in male habitual drinkers respectively, using an inter-
vention and a randomized trial.

METHODS

Smoking study
In the smoking study, we studied 38 Japanese male
volunteers who were all habitual cigarette smokers (≥10
cigarettes daily; range 10–80) and who stated their desire
to stop smoking. Subjects were told either to keep their
usual smoking habits for 1 week (smoking period), or
to cease smoking (non-smoking period), using a random-
ed crossover design. Nineteen subjects were
assigned first to keep their usual smoking habits and the
other 19 subjects were assigned first to cease smoking.
During the first 3 days of the non-smoking periods, all
subjects were strongly encouraged, via telephone conver-
sations, to maintain the cessation of smoking.

Alcohol study
In the alcohol study, we studied 33 Japanese male
volunteers who drank alcoholic beverages daily. In-
formation about their drinking habits was obtained using
a questionnaire, with an inclusion criterion of ≥30 ml
daily ethanol consumption. Beer was the most common
alcoholic beverage, followed by sake. Some subjects
drank wine, whiskey or other spirits. All subjects con-
sumed alcohol in the evening and none drank in the
morning or afternoon. Subjects were told either to keep
their usual drinking habits for 3 weeks (usual alcohol
period), or to reduce their alcohol intake by at least up to
a half of their usual drinking amounts (reduced alcohol
period), using a randomized crossover design. Seventeen
subjects were assigned first to the usual alcohol period and
the other 16 subjects were assigned first to the
reduced alcohol period. Daily alcohol consumption was
recorded by each subject throughout the study. Daily
alcohol intake was calculated as the equivalent in ml of
absolute ethanol, according to actual ethanol concentra-
tion of the alcoholic beverages.

In each study, subjects were selected and recruited
from male volunteers who responded to advertisements
seeking male smokers or drinkers to assess the effects of
smoking cessation or alcohol restriction on metabolic
and fibrinolytic variables. In both studies, no subjects
had a history of diabetes mellitus or vascular disease and
were not taking regular medication. The subjects all
agreed to participate in the study after receiving a detailed
explanation of its nature and purpose, and each subject
gave written informed consent. The study protocol was
in accordance with the Declaration of Helsinki (1989) of
the World Medical Association and was approved by the
institutional review board of Dokkyo University School of
Medicine.

Blood sampling and analyses
In both studies, venous blood samples were drawn, after
a 12-h overnight fast and after 15 min of supine rest, from
subjects on the last day of each period. Blood samples for
fibrinolytic determinations were collected into dispos-
able siliconized vacuum glass tubes containing 0.1 vol. of
3.8% (w/v) trisodium citrate. Samples were centrifuged
at 3000 g for 15 min at 4 °C immediately after collection
to separate plasma, which was then stored in plastic tubes
at −80 °C until laboratory determinations were per-
formed. The samples for the determination of other
variables were collected into plain tubes, which were
centrifuged at 3000 g for 15 min at 4 °C. After separation,
the serum samples were stored at 4 °C and analysed
within a few days.

In the smoking study, serum nicotine and cotinine
concentrations were measured by gas chromatography as
described by Jacob et al. [8]. Cotinine, a metabolite of
nicotine, has a much longer half-life and fluctuates much
less throughout the day than does nicotine and is widely
used as a marker of daily nicotine intake [9]. We used
cotinine to assess compliance with the cessation protocol.
In the alcohol study, serum γ-glutamyl transpeptidase,
one of the biochemical markers of daily alcohol intake,
was determined using an autoanalyser, Hitachi 7170
(Hitachi, Ltd., Instruments, Tokyo, Japan).

In both studies, plasma glucose and serum lipids were
determined using an autoanalyser, Hitachi 7170. Low-
density lipoprotein (LDL) cholesterol was estimated by
the method of Friedewald et al. [10]. Serum lipoprotein
(a) was measured with an ELISA kit (Lp (a) Latex
Daiichi, Daiichi Pure Chemicals Co., Ltd., Tokyo,
Japan). Plasma glucose was estimated using an auto-
analysers, Hitachi 7170, following the glucose oxidase
method. Serum insulin was determined using radio-
immunoassay kits, Insulin Riabead II (Dinabot Co., Ltd.,
Chiba, Japan). The levels of plasma plasminogen activator
inhibitor-1 (PAI-1) antigen were also determined using ELISA kits (Biopool,
Umea, Sweden) [12]. Plasma fibrinogen was determined
using the one-stage clotting assay employing a fully
Statistical analysis

Values are expressed as means ± S.E.M. In both studies, comparisons between the two periods were made using Wilcoxon’s rank sum test. Statistical significance was accepted at the level of P < 0.05.

RESULTS

Smoking study

Of the 38 subjects, three resumed smoking during the non-smoking period. Therefore, we analysed the data of the remaining 35 subjects. The baseline characteristics of the subjects are shown in Table 1.

As shown in Table 2, body weight did not differ significantly between the smoking and the non-smoking periods. Body weight did not differ significantly between the smoking and the non-smoking period. Therefore, we analysed the data of the remaining 35 subjects. The baseline characteristics of the subjects are shown in Table 1.

Table 1 Characteristics of the subjects of the smoking study (n = 35)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Smoking period</th>
<th>Non-smoking period</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.9 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>74.5 ± 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.6 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>119 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74.3 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette consumption (number/day)</td>
<td>28.6 ± 2.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Body weight, serum nicotine and cotinine, metabolic variables and fibrinolytic variables in the smoking and non-smoking periods

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Smoking period</th>
<th>Non-smoking period</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>74.4 ± 2.0</td>
<td>74.6 ± 2.0</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum nicotine (µg/l)</td>
<td>10.5 ± 1.6</td>
<td>0.98 ± 0.79</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Serum cotinine (µg/l)</td>
<td>190 ± 21</td>
<td>42.3 ± 10.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/l)</td>
<td>4.68 ± 0.15</td>
<td>4.70 ± 0.15</td>
<td>0.72</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mmol/l)</td>
<td>2.77 ± 0.13</td>
<td>2.67 ± 0.11</td>
<td>0.20</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mmol/l)</td>
<td>1.34 ± 0.04</td>
<td>1.40 ± 0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum triacylglycerols (mmol/l)</td>
<td>1.26 ± 0.13</td>
<td>1.35 ± 0.19</td>
<td>0.63</td>
</tr>
<tr>
<td>Serum lipoprotein (a) (mg/l)</td>
<td>112 ± 19</td>
<td>99.1 ± 16.7</td>
<td>0.003</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.46 ± 0.07</td>
<td>5.44 ± 0.08</td>
<td>0.77</td>
</tr>
<tr>
<td>Serum insulin (m-units/l)</td>
<td>8.14 ± 1.10</td>
<td>8.00 ± 1.43</td>
<td>0.36</td>
</tr>
<tr>
<td>Plasma PAI-1 antigen (µg/l)</td>
<td>66.6 ± 8.4</td>
<td>52.3 ± 6.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Plasma tPA antigen (µg/l)</td>
<td>7.09 ± 0.39</td>
<td>6.89 ± 0.33</td>
<td>0.54</td>
</tr>
<tr>
<td>Plasma fibrinogen (g/l)</td>
<td>2.16 ± 0.07</td>
<td>2.14 ± 0.08</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Table 3 Characteristics of the subjects of the alcohol study (n = 33)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.9 ± 1.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172 ± 1</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>73.9 ± 1.8</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.0 ± 0.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>128 ± 2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81.2 ± 1.5</td>
</tr>
<tr>
<td>Alcohol consumption (ml of ethanol/day)</td>
<td>63.6 ± 6.3</td>
</tr>
</tbody>
</table>

Table 4 Alcohol intake, body weight, serum γ-glutamyl transpeptidase, metabolic variables and fibrinolytic variables in the usual alcohol and reduced alcohol periods

<table>
<thead>
<tr>
<th></th>
<th>Usual alcohol period</th>
<th>Reduced alcohol period</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol intake (ml of ethanol/day)</td>
<td>70.1 ± 4.6</td>
<td>19.1 ± 2.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>73.9 ± 1.8</td>
<td>73.8 ± 1.8</td>
<td>0.40</td>
</tr>
<tr>
<td>Serum γ-GTP (units/l)</td>
<td>62.7 ± 6.0</td>
<td>54.2 ± 4.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/l)</td>
<td>4.85 ± 0.13</td>
<td>4.89 ± 0.11</td>
<td>0.84</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mmol/l)</td>
<td>2.78 ± 0.11</td>
<td>2.61 ± 0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mmol/l)</td>
<td>1.36 ± 0.06</td>
<td>1.27 ± 0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum triacylglycerols (mmol/l)</td>
<td>1.88 ± 0.33</td>
<td>1.93 ± 0.35</td>
<td>0.40</td>
</tr>
<tr>
<td>Serum lipoprotein (a) (mg/l)</td>
<td>107 ± 22</td>
<td>114 ± 24</td>
<td>0.15</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.78 ± 0.10</td>
<td>5.68 ± 0.08</td>
<td>0.35</td>
</tr>
<tr>
<td>Serum insulin (m-units/l)</td>
<td>11.2 ± 1.5</td>
<td>10.4 ± 1.4</td>
<td>0.15</td>
</tr>
<tr>
<td>Plasma PAI-1 antigen (µg/l)</td>
<td>44.8 ± 3.9</td>
<td>40.8 ± 6.8</td>
<td>0.54</td>
</tr>
<tr>
<td>Plasma tPA antigen (µg/l)</td>
<td>9.70 ± 0.49</td>
<td>8.92 ± 0.43</td>
<td>0.04</td>
</tr>
<tr>
<td>Plasma fibrinogen (g/l)</td>
<td>2.34 ± 0.12</td>
<td>2.39 ± 0.12</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Both serum nicotine and cotinine levels were significantly lower during the non-smoking period than during the smoking period. Although serum total cholesterol, LDL cholesterol, and triacylglycerol levels did not differ significantly between the two periods, serum HDL cholesterol levels were significantly higher and serum lipoprotein (a) levels were significantly lower during the non-smoking period than during the smoking period. Fasting plasma glucose and serum insulin levels did not differ significantly between the two periods. Plasma PAI-1 levels were significantly lower during the non-smoking period than during the smoking period, while plasma tPA and fibrinogen levels did not differ significantly between the two periods.

Alcohol study

All subjects completed the study protocol. The baseline characteristics of the subjects are shown in Table 3.

As shown in Table 4, body weight did not differ significantly between the usual alcohol period and the
reduced alcohol period. Daily alcohol intake was significantly lower during the reduced alcohol period than during the usual alcohol period. Serum γ-glutamyl transpeptidase levels were significantly lower during the reduced alcohol period than during the usual alcohol period. Although serum total cholesterol, LDL cholesterol, triacylglycerol, and lipoprotein (a) levels did not differ significantly between the two periods, serum HDL cholesterol levels were significantly higher during the usual alcohol period than during the reduced alcohol period. Fasting plasma glucose and serum insulin levels did not differ significantly between the two periods. Plasma tPA levels were significantly lower during the reduced alcohol period than during the usual alcohol period, while plasma PAI-1 and fibrinogen levels did not differ significantly between the two periods.

**DISCUSSION**

The findings obtained in the present study demonstrated that 1-week smoking cessation produced a significant increase in serum HDL cholesterol levels and significant decreases in serum lipoprotein (a) and plasma PAI-1 levels in Japanese male smokers, and 3-week alcohol restriction produced significant decreases in serum HDL cholesterol and plasma tPA levels in Japanese male drinkers. In the present study, neither 1-week smoking cessation nor 3-week alcohol restriction changed body weight significantly.

HDL cholesterol is well established as a major protective factor against coronary heart disease [13]. In the present study, serum HDL cholesterol levels were significantly lower during the smoking period than during the non-smoking period, verifying that smoking cessation is effective at increasing serum HDL cholesterol levels. The result is in agreement with a number of earlier observations. For example, findings from the Framingham Offspring Study [14] showed that cigarette smoking was associated with a significant reduction in serum HDL cholesterol levels in both genders. In addition, passive smoking has also been shown to decrease HDL cholesterol levels [15]. Regarding the mechanism responsible for these, the smoke component was suggested to influence enzymes that regulate lipoprotein metabolism such as lecithin: cholesterol acyltransferase (LCAT) or hepatic lipase [16]. In contrast, a number of observational studies have reported that mild-to-moderate alcohol intake reduces the risk of coronary artery disease, and the major mechanism was suggested to be the ability of alcohol to raise HDL cholesterol concentrations [17,18]. De Oliveira e Silva et al. [19] recently reported that ethanol increased HDL cholesterol by raising transport rates of the major HDL apolipoproteins, apo A-I and apo A-II. In the present study, serum HDL cholesterol levels were significantly higher during the usual alcohol period than during the reduced alcohol period, confirming earlier observations.

Lipoprotein (a) is a cholesteryl ester- and apolipoprotein B-containing particle, which differs from LDL by the additional presence of a glycoprotein termed apolipoprotein (a), which is homologous to plasminogen [20,21]. Recently, several lines of studies have suggested that increased concentrations of plasma lipoprotein (a) are associated with a higher prevalence and risk of cardiovascular disease, independent of associations of other lipids/lipoproteins and risk factors [22–24]. Very few investigations have been performed to study the effect of smoking on lipoprotein (a) concentrations. Hughes et al. [25] reported that smoking was not related to serum lipoprotein (a) levels as well as serum total cholesterol and LDL cholesterol levels in a cross-sectional comparison of smokers and non-smokers. To the best of our knowledge, this is the first study to investigate the effect of smoking cessation on serum lipoprotein (a) levels using an intervention and a randomized trial. The present finding highlights a further benefit of smoking cessation on cardiovascular risks.

PAI-1, the primary endogenous inhibitor of tPA and urokinase, may play an important role in inhibiting arterial clot lysis [26]. Increased plasma PAI-1 is found in patients with ischaemic stroke [27], in survivors of myocardial infarction [28,29], and in other thrombotic disorders [30–33]. Only a few studies have been carried out to investigate the effects of smoking on fibrinolysis. For example, Simpson et al. [34] reported that plasma PAI-1 antigen was significantly higher in smokers than in non-smokers, and with intermediate levels in former smokers. They observed similar trends for plasma PAI-1 activity, although this did not reach statistical significance. In the present study, plasma PAI-1 levels were significantly higher in the smoking study subjects than those in the alcohol study subjects (P < 0.05, by Student’s unpaired t test). This finding appears to be due to the fact that all participants were habitual smokers in the smoking study, while about a half of the participants were non-smokers in the alcohol study. To the best of our knowledge, no studies have been carried out to investigate the effects of smoking or smoking cessation on plasma PAI-1 levels using an intervention and a randomized trial. The present results suggest that stopping smoking returns impaired fibrinolysis towards normal.

Epidemiological studies have suggested that moderate alcohol consumption reduces the risk of cardiovascular morbidity and mortality [3,4]. This cardioprotective benefit may be mediated, in part, by promoting fibrinolysis via changes in fibrinolytic components and/or activity, resulting in the decreased risk for thrombosis, coronary artery disease, and eventual myocardial infarction. However, estimates of benefits of alcohol owing to fibrinolytic factors are less robust than in the case of...
HDL cholesterol because fewer clinical studies have assessed these markers. In the present study, plasma tPA levels were significantly lower during the reduced alcohol period than during the usual alcohol period. The finding is in agreement with a previous experimental study by Grenett et al. [35]. They have reported that low ethanol levels transcriptionally up-regulated tPA gene expression in cultured human umbilical vein endothelial cells.

Smoking cessation is often associated with an increase in food intake and weight gain [5]. Alcohol restriction may also change food intake in drinkers. In the present study, neither 1-week smoking cessation nor 3-week alcohol restriction changed body weight significantly, although food intake was not assessed. It is obvious that efforts should be made not to increase body weight after quitting smoking over the long term.

In conclusion, the present results suggest that smoking cessation has substantial and immediate benefits on lipid and fibrinolytic variables in habitual smokers, whereas quitting smoking over the long term may also change food intake in drinkers. In the present study, plasma tPA levels were significantly lower during the reduced alcohol period than during the usual alcohol period. The finding is in agreement with a previous experimental study by Grenett et al. [35]. They have reported that low ethanol levels transcriptionally up-regulated tPA gene expression in cultured human umbilical vein endothelial cells.

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