Effects of Alcohol Consumption on Hepatocellular Injury in Japanese Men

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To clarify the effects of alcohol consumption on hepatocellular injury, we examined aspartate and alanine aminotransferases (AST and ALT), and γ-glutamyltransferase (GGT), together with weekly alcohol consumption calculated from a self-rating questionnaire, in 1113 Japanese salesmen. The thresholds of associations between alcohol consumption and liver markers were estimated by the benchmark dose (BMD) method. The AST, ALT and GGT were positively correlated with alcohol intake ($p<0.001$), as well as age and body mass index (BMI); the relations to alcohol were statistically significant even when controlling for age, BMI and smoking habit. Although the AST and GGT were associated with four types of alcoholic beverage ($p<0.01$), it was only whisky that had close relation to the ALT ($p<0.05$). The thresholds of alcohol consumption (ethanol g/week), i.e., 95% lower confidence limits of the BMD, were 362 for AST, 660 for ALT, and 252 for GGT. The thresholds for GGT and AST in Japanese men seem to be somewhat higher than those reported in Western countries. It is suggested that hepatocellular injury (i.e., AST elevation) in Japanese men may emerge at the ethanol level of more than 50 g/day.

In Japan, it has been said “sake (Japanese alcoholic beverage of fermented rice) is the best of all medicines” since the ancient; likewise, most ecological studies in Western countries have suggested that wine is more effective in reducing risk of mortality from a certain disease (e.g., coronary heart disease), than beer or spirits (Grønbæk 2002). On the contrary, it is well known that alcohol causes health issues such as liver cirrhosis, overweight, hypertension, and stroke (Macdonald 1999). Therefore, a certain threshold of alcohol consumption, at which such health issues begin to take place, should be between the sensible drinking and overdrinking. The upper
limit of moderate intake has been reported to range from 24 to 80 g/day of 100% ethanol (Kalant and Poikolainen 1999), but the value may differ in human race or alcohol culture (Steffensen et al. 1997; Hoffmeister et al. 1999; Stewart 2002).

The critical dose at which adverse effects of alcohol emerge differs in the target organ. A couple of markers such as $\gamma$-glutamyltransferase (GGT) or carbohydrate deficient transferrin (CDT) may be the most appropriate for the early detection of nondependent hazardous or harmful drinking (Sillanaukee et al. 2000; Conigrave et al. 2002), inasmuch as the elevated GGT or CDT is not always indicative of a clinically diagnosed disease. Aspartate aminotransferase (AST) seems to be a primary marker for hepatocellular injury (Stewart 2002), because it is more specific than other liver enzymes for detecting alcohol-induced hepatocyte necrosis (Cohen and Kaplan 1979). Although some information has been developed as to the relationships between alcohol consumption and either AST or alanine aminotransferase (ALT) (Steffensen et al. 1997; Arndt et al. 1998; Nagaya et al. 1999; Honjo et al. 2001; Lee et al. 2001; Stewart 2002), the threshold of alcohol-associated AST (or ALT) elevation remains controversial.

In this study, AST, ALT and GGT, together with weekly alcohol consumption calculated from a self-rating questionnaire, were examined in Japanese men to clarify the effects of alcohol consumption on the hepatocellular injury, by using the benchmark dose (BMD) approach formulated by Budtz-Jørgensen et al. (2001).

**MATERIALS AND METHODS**

A self-reported questionnaire with detailed explanations of the study purpose was distributed to approximately 3400 salesmen of motor vehicle stores resided in Akita Prefecture, Japan in June 2002, and 1244 of them consented to our proposal and returned it to the occupational health nurse of the health insurance union (response rate, 37%). Of the respondents, 131 were excluded according to the following exclusion criteria: (1) Subjects did not undergo the mandatory health checkup, conducted under the Industrial Safety and Health Law in Japan, in April-May 2002, (2) they suffered from ischemic heart disease, chronic renal failure, alcoholic dependency diagnosed by DSM-IV (American Psychiatric Association 1994), liver cirrhosis or liver cancer, previously, and (3) the reported questionnaire had some imperfect information on drinking and smoking habits. The study population, accordingly, consisted of 1113 men (respondents) aged 18-29 years (34.1%), 30-39 years (22.8%), 40-49 years (24.4%), 50-59 years (17.0%), and 60-68 years (1.7%); and, of 1731 non-respondents (i.e., remaining salesmen) who underwent the same health checkup. The study protocol was approved by the ethical review committee of the Akita Health Insurance Union of Motor Vehicle Stores.

The weekly amount of each type of alcoholic beverage consumed was asked in the above questionnaire, including questions such as "how many 180 ml-cups (or 1800 ml-bottles) of sake do you drink in a week?" or "how many 350 ml-cans (500 ml-cans, or 633 ml-bottles) of beer do you drink in a week?" Types of alcoholic beverage listed were sake, beer, shochu (Japanese distilled alcoholic beverage primarily made from wheat or sweet potatoes), whisky, wine, and others (e.g., plum wine, brandy, gin, or vodka). A total of 100% ethanol equivalent dose (g/week) was calculated for each subject on the assumption that sake, beer, shochu, whisky, and wine contain 15%, 5%, 20 (or 25)% 40%, and 12% of ethanol, respectively. About 5% of 894 drinkers (45 men), who consumed 100% ethanol equivalent dose of more than 800 g/week, were directly asked about recent alcohol consumption by the occupational health nurse five months after the questionnaire collection, in order to validate the reported alcohol consumption. Smoking habit was also examined in the questionnaire, and the response was scored as “nonsmoker”=0 and “smoker”=1.

Data of liver markers, i.e., serum AST, ALT and GGT, together with HDL-cholesterol (HDL-CH) and body mass index (BMI), were obtained
from each record of the annual health checkup. AST, ALT, and GGT were measured at 37°C according to the principles recommended by the Japan Society of Clinical Chemistry (reference intervals were 12 to 36 IU/liter for AST, 8 to 36 IU/liter for ALT, and 6 to 85 IU/liter for GGT) (Kawai 1993); and, the normal range of HDL-CH was between 41 and 70 mg/100 ml.

The difference between weekly alcohol consumptions answered in the questionnaire and interview was analyzed by the paired-sample t-test. The significance levels of the associations between alcohol consumption and liver markers (logarithmically transformed AST, ALT, and GGT) in the 1113 respondents were tested by the Pearson’s product moment correlation coefficient (r). Also, multiple regression analysis was performed to examine the relations of alcohol consumption and possible confounders (age, BMI, and smoking) to the liver markers.

The BMD was defined as the weekly dose of ethanol that resulted in an increased probability of abnormal liver marker by a benchmark response (BMR), i.e., from $P_0$ to $P_0+BMR$. The cutoff value (i.e., 37 IU/liter) was specified from the equation (2) of the Methods section.

Fig. 1. A dose-effect relationship between alcohol consumption and aspartate aminotransferase (AST) in 1113 salesmen for benchmark dose (BMD) calculation, after controlling for age, body mass index and smoking. $P_0$ and benchmark response (BMR) indicate an abnormal rate (5%) in the non-drinkers and an excess risk (5%) above $P_0$ in the drinkers, respectively. The BMD is the dose of alcohol consumption that results in the increased probability of abnormal AST, i.e., from $P_0$ to $P_0+BMR$. The cutoff value (i.e., 37 IU/liter) was specified from the equation (2) of the Methods section.
The normalized value for each confounder was employed in the above regression model. A lower confidence limit for BMD (BMDL) was then calculated as the statistical 95% lower bound of the BMD (Budtz-Jørgensen et al. 2001). The power parameter $K$ has been restricted to values equal to or above 1, thus allowing the dose effect curve to be nonlinear. Since previous applications of this method have used a $P_0$ of 5% and a BMR of 5% (Budtz-Jørgensen et al. 2001; Murata et al. 2002, 2003), we also applied the linear ($K=1$) and $K$-power dose effect curves, set at the $P_0$ of 5% and the BMR of 5% (and 2%). All analyses were performed by using the Statistical Package for the Biosciences (SPBS V9.5), including the BMD program (Murata and Yano 2002).

**RESULTS**

Main characteristics of the salesmen in Akita are shown in Table 1. No significant differences between the 1113 respondents and 1731 non-respondents were seen in BMI, AST, ALT, or HDL-CH ($p>0.1$, Mann Whitney’s U-test) except age and GGT ($p<0.05$). The weekly alcohol consumption answered in the questionnaire did not significantly differ from that in the interview ($p>0.2$, data not shown). The alcohol consumption was significantly correlated with the HDL-CH in the respondents (Spearman rank correlation coefficient $r_s=0.152$, $p<0.001$). Also, proportions of mainly beer and shochu consumers in the drinkers were 60.6% and 24.2%, respectively.

Weekly alcohol consumption was significantly correlated with log-transformed AST and ALT (Table 2).

<table>
<thead>
<tr>
<th>Table 1. Main characteristics of car salesmen in Akita, Japan</th>
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<tbody>
<tr>
<td>Respondents ($n=1113$)</td>
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<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>mean ± s.d. (range)</strong></td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>Body mass index (kg/m$^2$)</td>
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<tr>
<td>Drinkers (100% ethanol g/week)</td>
</tr>
<tr>
<td>Smokers</td>
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<tr>
<td>Liver enzyme activities (IU/liter):</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
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<tr>
<td>$\gamma$-glutamyltransferase</td>
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<tr>
<td>HDL-cholesterol (mg/100 ml)</td>
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<tr>
<td><strong>Median.</strong></td>
</tr>
</tbody>
</table>

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<tr>
<th>Table 2. Relations of alcohol consumption, age, body mass index (BMI), and smoking to log-transformed aspartate and alanine aminotransferases (AST and ALT) and $\gamma$-glutamyltransferase (GGT) in 1113 salesmen: results of multiple regression analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple correlation coefficient Standardized regression coefficients</td>
</tr>
<tr>
<td>Alcohol</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>AST</td>
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<tr>
<td>ALT</td>
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<tr>
<td>GGT</td>
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</tbody>
</table>

* $p<0.05$, ** $p<0.01$, *** $p<0.001$. 
Effects of Alcohol on Liver Enzymes

The relationships between alcohol consumption and liver markers were statistically significant when controlling for age, BMI, and smoking (Table 2). Also, only whisky in four types of alcoholic beverage was significantly related to ALT, while all types of alcoholic beverage had significant relations to AST and GGT (Table 3).

To test the goodness of fit of the dose-effect model between alcohol consumption (d) and liver markers, we performed the multiple regression analysis using alcohol consumption, age, BMI and smoking as independent variables, and AST as a dependent variable. When changing the curve, the log-likelihood ratios of the regression analysis were 203.4 for linear model (d), 149.7 for K-power model (d^K), 149.6 for square root model (√d), and 138.7 for logarithmic model (log[d+1]); and, all of the significance levels (p) were less than 0.001 (χ^2 test). Accordingly, we applied the linear dose effect curve (i.e., K=1) to the BMD calculation. The BMDLs (100% ethanol g/week),

\( r = 0.248, p < 0.001 \), ALT \( r = 0.139, p < 0.001 \), and GGT \( r = 0.387, p < 0.001 \). Similarly, age and BMI were positively correlated with all the markers \( r = 0.160 \sim 0.423 \) for age and \( r = 0.267 \sim 0.503 \) for BMI, \( p < 0.001 \). The relationships between alcohol consumption and liver markers were statistically significant when controlling for age, BMI, and smoking (Table 2). Also, only whisky in four types of alcoholic beverage was significantly related to ALT, while all types of alcoholic beverage had significant relations to AST and GGT (Table 3).

To test the goodness of fit of the dose-effect

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**Table 3.** Relations of four types of alcoholic beverage (sake, beer, shochu and whisky; g/week of ethanol), age, body mass index, and smoking to log-transformed aspartate and alanine aminotransferases (AST and ALT) and γ-glutamyltransferase (GGT) in 1113 salesmen: results of multiple regression analysis with p-values in parentheses

<table>
<thead>
<tr>
<th>Liver marker</th>
<th>K-power model</th>
<th>Linear model^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMD</td>
<td>BMDL</td>
</tr>
<tr>
<td>AST</td>
<td>254</td>
<td>196</td>
</tr>
<tr>
<td>ALT</td>
<td>760</td>
<td>413</td>
</tr>
<tr>
<td>GGT</td>
<td>135^b</td>
<td>117^b</td>
</tr>
</tbody>
</table>

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**Table 4.** Benchmark dose (BMD) of alcohol consumption (g/week of ethanol) and its lower 95% confidence limit (BMDL) at a benchmark response (BMR) level of 2% and 5% for aspartate and alanine aminotransferases (AST and ALT) and γ-glutamyltransferase (GGT) in 1113 salesmen, after controlling for age, body mass index and smoking

<table>
<thead>
<tr>
<th>Liver marker</th>
<th>K-power model</th>
<th>Linear model^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMD</td>
<td>BMDL</td>
</tr>
<tr>
<td>AST</td>
<td>543</td>
<td>418</td>
</tr>
<tr>
<td>ALT</td>
<td>1621</td>
<td>880</td>
</tr>
<tr>
<td>GGT</td>
<td>290^b</td>
<td>252^b</td>
</tr>
</tbody>
</table>

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^a Cutoff values specified were 37 for AST, 60 for ALT and 114 for GGT, when K=1.
^b K=1.
set at the $P_0$ of 5% and BMR of 5%, were 362 for AST, 660 for ALT, and 252 for GGT (Table 4).

**DISCUSSION**

In the self-reported questionnaire of this study, the subjects were asked about the number of cans (or bottles) of each alcoholic beverage per week, although the questionnaire utilized in many health check centers of Japan includes a cognitively difficult task to summarize drinking behavior (de Vries et al. 1999), e.g., subjects must calculate the equivalent dose of sake per day, if they drink beer or whisky (Nagaya et al. 1999). Then, the significant association was observed between the estimate of alcohol use and HDL-CH in the 1113 salesmen; by using this correlation, qualitative evidence is provided that alcohol use is being measured with at least some degree of validity (Giovannucci et al. 1991; de Vries et al. 1999). In addition, the alcohol consumption was confirmed in about 5% of the drinkers by the interview method, against inaccuracy due to underreporting of alcohol consumption (Steffensen et al. 1997). Therefore, our questionnaire is suggested to provide a useful approach for estimating weekly alcohol consumption.

The respondents and non-respondents of our study population did hardly differ (Table 1), although the response rate was low (37%); certainly, since the non-respondents were somewhat younger than the respondents, the GGT in the former was thought to be relatively lower. Also, proportions of 31 180 000 male employees in Japan in 2001 were 23.4% at 18-29 years of age, 23.7% at 30-39 years, 21.3% at 40-49 years, 23.1% at 50-59 years, and 8.4% at 60-69 years (Ministry of Public Management, Home Affairs, Posts and Telecommunications 2002a), and similar to our study population regarding the age distribution. Thus, our respondents would be almost representative of Japanese male workers.

The AST, ALT and GGT in the present study were significantly correlated with weekly alcohol consumption, as well as age and BMI; the relations to alcohol were statistically significant even when controlling for age, BMI and smoking habit. Also, the GGT and AST were associated with all types of alcoholic beverage, while the ALT had weak relation to only whisky consumption; this may have been due to the different drinking pattern (e.g., with or without food) of each alcoholic beverage (Bellentani et al. 1997). It is suggested that the elevated level in ALT may be affected by other causes such as hepatitis B or C virus (HBV or HCV) infection (Anderson et al. 2000) and/or high BMI (Burns et al. 1996; Poikolainen and Vartianine 1997), rather than by a type of alcoholic beverage. In fact, since it has been reported that about 2% of Japanese adults in the general population have HBV or HCV, and that most of them without any symptoms did not always undergo medical treatment (Tanaka 1997), such HBV/HCV carriers may have been included in the present study.

Concerning the estimation of the critical dose, the no-observed-adverse-effect level (NO-AEL) is the highest dose at which no statistically or biologically significant adverse effects are identified (National Research Council 2000). However, the BMD approach tends to be used in the field of environmental health in place of the NOAEL, inasmuch as a number of shortcomings of the NOAEL have been identified, including not adequately reflecting the shape of the dose response and not appropriately accounting for study size (National Research Council 2000; Crump 2002). The BMDL has been applied as an alternative to the NOAEL to provide a point of departure for low-dose extrapolation (National Research Council 2000).

The implication of our outcome is that, if Japanese men have continued to consume ethanol of 41 g/day (BMD for GGT), the proportion of drinkers with abnormal GGT increases by 5%, as compared to the proportion of non-drinkers with it. The threshold in Japanese men (BMDL for GGT, 36 g/day) is higher than two previous ones (i.e., 10.5 and 26.3 g/day) calculated from 3974 Finnish men aged 25-74 years (Sillanaukee et al. 2000) and from 7677 German men aged 20-75
years (Hoffmeister et al. 1999). The discrepancy may have been attributable to the different method of calculations. Otherwise, the sensitivity of GGT to alcohol consumption may differ in the distribution of alcohol/aldehyde dehydrogenase (ALD/ALDH) genotypes; because, there exists a highly prevalent polymorphism in the low Km ALDH (ALDH2) gene in Japanese when compared with the Caucasoid (Goedde et al. 1992), and Takeshita et al. (2000) demonstrated significantly lower activities of liver markers in drinkers with the inactive ALDH2 than in those with the active ALDH2. Further research is required to examine the race difference in liver function, with consideration for ADH/ALDH genotypes.

In this study, the threshold (BMDL) of alcohol consumption for abnormal AST was estimated to be approximately 50 g/day. On the other hand, the risk for developing liver damage steadily increased when ethanol consumption exceeded 30 g/day in the Dionysos study (Bellantani et al. 1997; Bellentani and Tiribelli 2001). Despite the discrepancy in these values, excess prevalence of liver damage in the cohort Dionysos population, compared to teetotaler, was 2.7% for alcohol intake of 31-60 g/day, and 6.9% for 61-90 g/day, and our finding indicated that the BMD for AST, set at the excess risk (BMR) of 5%, was 65 g/day. Therefore, it is suggested that hepatocellular injury (i.e., AST elevation) may emerge at the ethanol level of more than 50 g/day in the light of the excess prevalence of 5%.

All observational studies have weaknesses, because all important determinants (e.g., ADH/ALDH genotypes) cannot be controlled a priori. In the present study, some confounders such as age, BMI and smoking habit, were considered in the process of data analysis, although ADH/ALDH genotypes were not examined. There was of course no selection bias because comparisons were not made. Also, annual alcohol consumption per household in Akita has been reported to be more than those in other Japanese prefectures (Ministry of Public Management, Home Affairs, Posts and Telecommunications 2002b). Because of a wide range of alcohol consumption, the statistical power would be potent, despite the presence of the limitations that the sample size of ours was smaller than those of other studies (Hoffmeister et al. 1999; Sillanaukee et al. 2000), and that “healthy men” were not defined in the same manner as the Dionysos study with ultrasonography of the liver, and HBV-antigen and HCV-antibody tests (Bellantani et al. 1997).

In epidemiological studies, the odds ratio and relative risk are frequently used. Since these values depend on the used unit/category, they do not always imply a benchmark of preventive goals. By contrast, the BMD method appears to provide a promising approach for estimating the threshold point (not a “range”) of dose-effect relationships of hazardous factors in clinical medicine (Murata et al. 2003). And, current evidence on alcohol effects can directly indicate that an early hepatocellular injury is more detectable by AST (i.e., BMDL of 50 g/day) than by ALT (i.e., BMDL of 94 g/day). For the evidence based prevention of alcohol-induced diseases, it is important to scrutinize the threshold, as described above, of alcohol consumption for other target organs, possibly by using the same method.

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