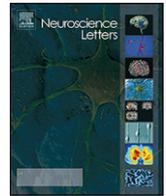




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Chronic ethanol ingestion, type 2 diabetes mellitus, and brain-derived neurotrophic factor (BDNF) in rats

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ABSTRACT

Chronic alcohol consumption contributes to the development of type 2 diabetes mellitus (T2DM) while decreasing the level of brain-derived neurotrophic factor (BDNF). BDNF may be an important regulator of glucose metabolism, so it may be associated with an increased risk for T2DM in alcoholism. We evaluated the association of chronic heavy alcohol exposure, T2DM and BDNF level. Ten week-old type 2 diabetic OLETF rats and non-diabetic LETO rats of similar weight were used. The rats were randomized by weight into four treatment groups: (1) OLETF-Ethanol (O-E, $n = 13$), (2) OLETF-Control (O-C, $n = 15$), (3) LETO-Ethanol (L-E, $n = 11$), and (4) LETO-Control (L-C, $n = 14$). The ethanol groups were fed an isocaloric liquid diet containing ethanol while the control groups were fed with the same diet containing maltose–dextran over a 6-week period using a pair-feeding control model in order to regulate different caloric ingestion. After 6 weeks of feeding, an Intraperitoneal Glucose Tolerance Test (IP-GTT) was performed and BDNF levels were analyzed. Prior to IP-GTT, the mean glucose levels in the O-E, O-C, L-E, and L-C groups were 90.38 ± 12.84 , 102.13 ± 5.04 , 95.18 ± 6.43 , and 102.36 ± 4.43 mg/dL, respectively. Thirty minutes after intraperitoneal injection, the mean glucose levels were 262.62 ± 63.77 , 229.07 ± 51.30 , 163.45 ± 26.63 , and 156.64 ± 34.42 mg/dL, respectively; the increased amount of the mean glucose level in the O-E group was significantly higher than that in the O-C group ($p < 0.05$). One hundred twenty minutes after intraperitoneal injection, the mean glucose levels were 167.38 ± 45.37 , 121.20 ± 18.54 , 106.73 ± 6.94 , and 104.57 ± 9.49 mg/dL, respectively; the increased amount of the mean glucose level in the O-E group was significantly higher than that in the O-C group ($p < 0.01$). The difference in mean glucose levels between the O-E group and O-C group was still significant even after adjusting for time ($p < 0.05$). Mean BDNF levels were 405.95 ± 326.16 , 618.23 ± 462.15 , 749.18 ± 599.93 , and 1172.00 ± 839.17 pg/mL, respectively; mean BDNF level in the O-E group was significantly lower than the L-C group ($p < 0.05$). In conclusion, the results of the present study suggest that chronic heavy alcohol ingestion may aggravate T2DM and may possibly lower BDNF level.

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Chronic alcohol consumption is a major health problem around the world [6] and one of the main causes of mortality in developed countries. Moreover, chronic alcohol abuse has been associated

with multiple health problems such as cancer, psychosocial and cognitive disorders as well as diabetes [9,22]. The goal of this study was to investigate the role of chronic heavy drinking in the development of type 2 diabetes mellitus (T2DM).

Recent studies have reported an association between the amount of ingested alcohol and susceptibility to T2DM. Previous studies have suggested that low to moderate amounts of alcohol ingestion reduced the risk of T2DM [1,20,23,31], whereas chronic heavy drinking increased the risk [4,10,31,32].

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Some experiments have demonstrated the effects of brain-derived neurotrophic factor (BDNF) on T2DM and its association with cognitive functions such as learning, or memory [2,27]. Both animal experiments and clinical research have shown that BDNF plays a major role in insulin resistance, a pathogenic feature of T2DM [18,19,30]. BDNF, a member of the neurotrophin family, is a regulator of general development. It modulates appetite, and is a potential therapeutic target for neurodegenerative diseases and addiction [5,13,21,26].

Interestingly, BDNF level has been shown to be significantly decreased after chronic ethanol exposure, which supports the concept that ethanol-induced cell damage might be affected by BDNF [11,16,24]. Both animal and human studies have demonstrated that BDNF level decreases after chronic alcohol ingestion, but increases after withdrawal of alcohol [11,12,15,24,28]. BDNF is associated with both T2DM and alcoholism, and may mediate the effect of alcoholism on T2DM. However, there are few published data on BDNF level in T2DM following chronic alcohol exposure. The goal of this study was to evaluate the effects of chronic heavy alcohol exposure on T2DM and its association with BDNF level.

Tokushima Institute, Otsuka Pharmaceutical (Tokushima, Japan) kindly provided 4-week-old male Otsuka Long–Evans Tokushima Fatty (OLETF) rats and non-diabetic male Long–Evans Tokushima Otsuka (LETO) rats of similar weight (Table 1). The animals were fed standard laboratory chow (25 g/day) until 10 weeks of age and their cages were kept at a controlled temperature ($23 \pm 2^\circ\text{C}$), humidity ($55 \pm 5\%$) and lighting (08:00–20:00) in our animal facility. The animals were monitored daily. They showed no sign of pain or distress during the feeding and experimental procedures. The rats were randomized by weight into four treatment groups: (1) OLETF-Ethanol (O-E, $n = 13$), (2) OLETF-Control (O-C, $n = 15$), (3) LETO-Ethanol (L-E, $n = 11$), and (4) LETO-Control (L-C, $n = 14$). The experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996.

The rats in the alcohol-fed group were fed with Lieber–DeCarli Regular EtOH (Cat. No. 710260, Dyets Inc., USA) over a 6-week period, as recommended by the manufacturer. The liquid diet contained 34% fat, 11% carbohydrate, 18% protein, and 36% ethanol. The control rats consumed Lieber–DeCarli Control diet (Cat. No. 710027, Dyets Inc., USA) with the calories from ethanol replaced by maltose–dextran. The liquid diet contained 35% fat, 47% carbohydrate, and 18% protein. Feeding was performed at 10:00 a.m. The O-E group did not eat all of the food provided, while the others finished all of their food so that caloric restriction was required. Pair-feeding controls were used with a synchronized pellet pair-feeding apparatus (Cat. No. 900006, Dyets Inc., USA) in order to regulate the different caloric ingestion; the animals were fed the same amount as the average of the O-E group members. One hundred mLs of an alcohol diet was given to the O-E and L-E groups. Body weight was measured every week to match it to the same level in all groups.

Table 1
The body weights of four groups (O-E, O-C, L-E, and L-C) before and after 6 weeks of feeding Lieber–DeCarli diet. Data are expressed as mean \pm S.D. and were analyzed by one-way ANOVA. There is no significant difference among the groups. O-E: OLETF-Ethanol; O-C: OLETF-Control; L-E: LETO-Ethanol; L-C: LETO-Control.

Group	n	Weight (g)	
		Baseline	After 6 weeks
O-E	13	291.00 \pm 25.19	312.69 \pm 22.96
O-C	15	294.53 \pm 21.28	327.47 \pm 22.78
L-E	11	272.18 \pm 16.50	316.82 \pm 13.11
L-C	14	282.64 \pm 15.75	331.50 \pm 17.85

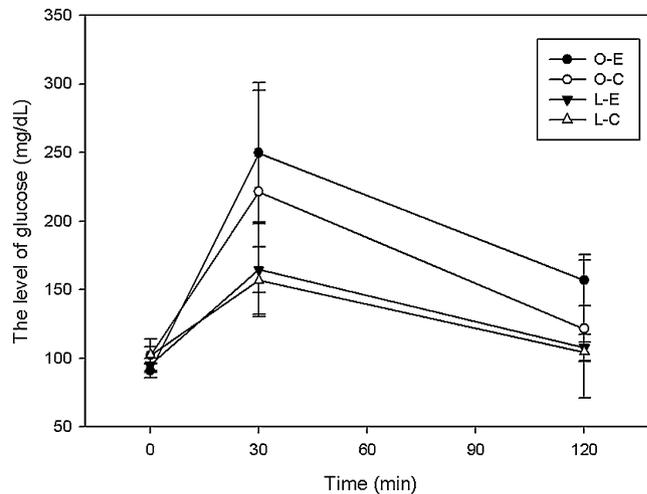


Fig. 1. Comparison of IP-GTT in the four groups (O-E, O-C, L-E, and L-C). Blood glucose levels were measured at baseline, and at 30 and 120 min after intraperitoneal injection. Data were analyzed by one-way ANOVA and repeated measures ANOVA. The increased amount of the glucose levels from baseline to 30 min in the O-E group is significantly higher than that in the O-C group ($p < 0.05$), and the same difference is observed at 120 min after intraperitoneal injection ($p < 0.01$). O-E: OLETF-Ethanol; O-C: OLETF-Control; L-E: LETO-Ethanol; L-C: LETO-Control.

IP-GTT was performed before sacrificing the rats. All rats were fasted for 17 h after transfer to a clean cage and then weighed. Fasting glucose level was obtained from the venous blood of a small tail clip for baseline levels. Next, intraperitoneal injection of D-glucose (2 g/kg) was performed and blood glucose levels were obtained at 30 and 120 min later. The blood glucose level was measured with an ACCU-CHECK Active (Roche Diagnostics, Korea) portable glucose meter. The blood was collected from the heart after 6 weeks of ethanol ingestion.

BDNF levels were measured using the rat pituitary LINCplex immunoassay kit (RPT86K-07, LINCO Research, St. Charles, MO, USA).

One-way analysis of variance (ANOVA) and repeated measures ANOVA were used to compare the data from IP-GTT. Body weights and BDNF levels were analyzed by one-way ANOVA. Planned comparisons were used for post hoc analysis. $p < 0.05$ was considered significant. Statistical calculations were performed using SPSS software for Windows (version 15.0, SPSS, Inc., Chicago, IL).

The average amounts of ingested ethanol in the L-E and O-E group were 9.94 and 9.74 g/kg/day, respectively; this corresponds to a heavy amount of ethanol ingestion. Table 1 shows that there was no significant difference in body weights among the groups. It shows that body weight was not a confounding factor.

Fig. 1 shows that prior to glucose injection, the mean glucose levels in the O-E, O-C, L-E, and L-C groups were 90.38 ± 12.84 , 102.13 ± 5.04 , 95.18 ± 6.43 , and 102.36 ± 4.43 mg/dL, respectively. The mean glucose level in the O-E group was lower than that of any other group ($p < 0.05$).

Thirty minutes after intraperitoneal injection, the mean glucose levels in the O-E, O-C, L-E, and L-C groups were 262.62 ± 63.77 , 229.07 ± 51.30 , 163.45 ± 26.63 , and 156.64 ± 34.42 mg/dL, respectively; all groups showed increases in the mean glucose levels compared to the baseline. The increased mean glucose level in the O-E group was significantly higher than that in the O-C group ($p < 0.05$). The increased mean glucose level in the L-E group was also higher than that in the L-C group but the difference was not significant.

One hundred twenty minutes after intraperitoneal injection, the mean glucose levels in the O-E, O-C, L-E, and L-C groups were 167.38 ± 45.37 , 121.20 ± 18.54 , 106.73 ± 6.94 , and

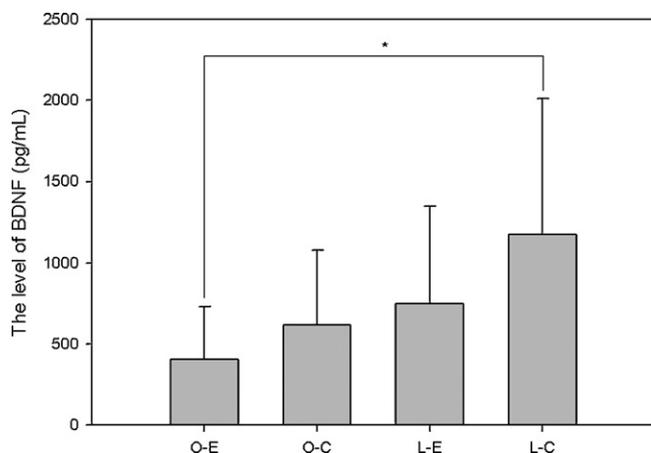


Fig. 2. Comparison of BDNF levels in the four groups (O-E, O-C, L-E, and L-C). Data were analyzed by one-way ANOVA. BDNF level in the O-E group is significantly lower than that in the L-C group ($p < 0.05$). * $p < 0.05$. O-E: OLETF-Ethanol; O-C: OLETF-Control; L-E: LETO-Ethanol; L-C: LETO-Control.

104.57 ± 9.49 mg/dL, respectively; all groups showed increases in the mean glucose levels compared to the baseline. The increased mean glucose level in the O-E group was still significantly higher than that in the O-C group ($p < 0.01$). The increased mean glucose level in the L-E group was also higher than that in the L-C group but the difference was not significant.

After adjusting for time, the difference in mean glucose levels between the O-E and O-C groups was still significant ($p < 0.05$), but not between the L-E and L-C groups.

Fig. 2 shows that mean BDNF levels in the O-E, O-C, L-E, and L-C groups were 405.95 ± 326.16, 618.23 ± 462.15, 749.18 ± 599.93, and 1172.00 ± 839.17 pg/mL, respectively. Mean BDNF level in the O-E group was significantly lower than the L-C group ($p < 0.05$). BDNF levels in the L-E and O-C groups were lower than that in the L-C group but the differences were not significant.

OLETF rats provide a model for the pathophysiology of human T2DM. Prior studies have shown that ethanol ingestion of over 4-weeks in rodents is equivalent to chronic ingestion in humans [3,25]. So ethanol ingestion for 6-weeks in this study is assumed to be a chronic exposure to alcohol. The amount of ethanol in the alcohol-fed groups was excessive.

The major findings of the present study are that chronic heavy drinking aggravates T2DM and may possibly lower BDNF level. Alcohol-related changes associated with the pathophysiology of T2DM were observed during IP-GTT. The fasting glucose level in the O-E group was lower than that of any other group. It may be due to chronic ingested ethanol causing liver damage. Recently one pilot study suggested that hypoglycemia is common in chronic alcoholics with liver damage [8], but we did not evaluate the liver damage. The ethanol-induced changes in blood glucose levels during IP-GTT were different in the diabetic versus non-diabetic groups. The glucose levels in the ethanol-fed diabetic group showed significantly larger changes than the control-fed diabetic group 30 min after glucose administration, and this was maintained at the 120 min evaluation point. On the other hand, the glucose level of the ethanol-fed non-diabetic group changed at a level similar to the control-fed non-diabetic group. Therefore, ethanol appeared to have little effect on T2DM in the non-diabetic groups. The relationship between the amount of alcohol ingestion and risk of T2DM has been examined in relatively few studies. Several studies have suggested that low to moderate alcohol ingestion increased insulin sensitivity, which might provide protecting against the development of T2DM [1,20,26]. On the other hand, chronic heavy drinking was thought to be a risk factor for T2DM [4,32]. The results of the

present study confirm previous findings; T2DM was made worse by chronic heavy ethanol ingestion. The diabetic rats had a low-to-normal fasting glucose level and an extremely high postprandial glucose level that was difficult to return to baseline levels. They were further aggravated by chronic heavy ethanol ingestion. However, this effect of ethanol on glucose levels was not observed in non-diabetic rats. Therefore, the diabetic state appears to be more susceptible to heavy alcohol ingestion than the non-diabetic state.

The exact mechanism of the effects of alcohol on the development of T2DM has not been determined. A number of recent studies have reported on the relationship between BDNF and T2DM [14,17,18,34]. The major pathogenic features of T2DM are insulin resistance and subsequent insufficient insulin secretion [7]. Animal-based researches have suggested that BDNF plays a role in insulin resistance [18,19,30]. BDNF has been suggested as a regulator of glucose metabolism by directly acting on the hypothalamus [18], and is thought to have a protective effect on pancreatic islets [33].

There have been several studies suggesting that alcohol ingestion affects BDNF level. One study reported that ethanol altered cellular pathways related to BDNF signaling [29]. Human studies have shown that BDNF levels in alcoholic patients were lower than normal controls [12], but increased one-week after alcohol withdrawal [11]. Animal studies have shown a similar pattern; BDNF mRNA expression was decreased following chronic alcohol exposure, but increased after its withdrawal [15,28]. In addition, one *in vitro* study showed that BDNF mRNA expression was increased with acute alcohol exposure, but was decreased after continuous exposure [16]. It seems alcohol affects BDNF level differently according to exposure duration. Such findings suggest that BDNF may be linked to the pathophysiology of T2DM after alcohol ingestion.

A possible limitation in this study is that there is no data on blood alcohol concentration to confirm the effect of Lieber–Decarli diet on blood alcohol level, because this requires a large amount of blood sample. Additionally, we have already confirmed that this feeding method significantly increases the blood alcohol concentration in OLETF and LETO rats.

Our study shows that BDNF level in the ethanol-fed diabetic group was significantly lower than that in the control-fed non-diabetic group. However the difference in BDNF level between the ethanol-fed non-diabetic group or the control-fed diabetic group and the control-fed non-diabetic group was not significant. So the present study cannot evaluate that BDNF level is an important mediator or a marker for the effect of alcohol on T2DM. But it appears that chronic heavy alcohol ingestion and T2DM may create a synergetic effect on the reduction of BDNF level.

The present study cannot explain why the effects of chronic heavy ethanol exposure on the glucose and BDNF levels are more severe in T2DM. Nevertheless, this is the first study to report the effects of chronic heavy ethanol exposure on both T2DM and BDNF in an animal model.

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