



Chronic alcohol consumption, type 2 diabetes mellitus, insulin-like growth factor-I (IGF-I), and growth hormone (GH) in ethanol-treated diabetic rats

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ABSTRACT

Aims: Alcohol has deleterious influences on glucose metabolism which may contribute to the development of type 2 diabetes mellitus (T2DM). Insulin-like growth factor I (IGF-I) and growth hormone (GH), which interact with insulin to modulate metabolic control, have been shown to be related to impaired glucose tolerance. This study was conducted to assess the possibility that altered circulating IGF-I and GH levels contribute to the exacerbation of T2DM by alcohol use in type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats and non-diabetic Long-Evans Tokushima Otsuka (LETO) rats.

Main method: OLETF rats were pair-fed a Lieber-DeCarli Regular Ethanol diet and LETO rats were pair-fed a control diet for 6 weeks. At 6 weeks, an Intraperitoneal Glucose Tolerance Test (IP-GTT) was performed and IGF-I and GH levels were evaluated.

Key findings: Prior to an IP-GTT, OLETF-Ethanol (O-E) group had significantly a decrease in the mean glucose levels compared to OLETF-Control (O-C) group. At 120 min post IP-GTT, the O-E group had significantly an increase in the mean glucose levels compared to O-C group. The serum IGF-I levels were significantly lower and the serum GH levels were significantly higher in the O-E group than in L-C group.

Significance: These results suggest that IGF-I and GH are prominent in defining the risk and development of T2DM, and may be adversely affected by heavy alcohol use, possibly mediating its diabetogenic effects. Thus, the overall glucose intolerance in the setting of alcoholism may be attributable to inappropriate alteration of IGF-I and GH levels.

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Introduction

Impaired glucose tolerance, the major pathogenic feature of type 2 diabetes mellitus (T2DM), seems to be directly influenced by alcohol use (Hodge et al., 1993; Risinger and Cunningham, 1992). Several studies suggest that the mechanism behind this association may be a combination of the direct toxic effect of chronic alcohol exposure on the pancreatic islet cells and decreased suppression of hepatic glucose production as a result of impaired insulin action (Holbrook et al., 1990; Rosengren et al., 1988).

The free circulating insulin-like growth factor-1 (IGF-I) mediates several important effects of pituitary growth hormone (GH), which stimulates the synthesis of peripheral IGF-I in the predominant target,

the liver, as well as several other tissues including the lung, kidney, thymus, spleen, heart, muscle, and gonads. IGF-I shares 50% amino acid sequence homology with insulin, and as a result, elicits almost the same hypoglycemic response (Boulware et al., 1994). In addition to its insulin like effects, this growth-promoting peptide influences peripheral growth, differentiation, and survival in variety of cells and tissues (Daughaday and Rotwein, 1989). There is limited evidence that glucose intolerance in alcohol-dependent patients and insulin resistance in subjects with different degrees of glucose tolerance may be related to dysregulation in IGF-I (Sesti et al., 2005; Singh et al., 1988). GH acts by binding to its receptor (GHR) primarily at two sites, the liver and the growth plate. GH plays an important role in the growth process and has many metabolic functions, such as increasing muscle mass and bone mineral density, and stimulates islet cell growth and insulin secretion (Nielsen et al., 2001; Rhodes, 2000). Despite this, the observed effect of GH has been to counteract insulin's action and lead to insulin resistance in insulin target tissue (Coschigano et al., 2000).

Since IGF-1-GH axis is thought to play a prominent role in glucose tolerance and T2DM (Robertson et al., 2008; Vaessen et al., 2001), and chronic use of alcohol reduces circulating IGF-1 levels (Nguyen et al., 2012), we hypothesize that progression of T2DM might be influenced

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by IGF-I and its synthesis regulator, GH, after alcohol consumption, which may lead to aggravation of diabetes-related parameters in the setting of alcoholism. However, the pathophysiologic mechanisms affecting T2DM after alcohol ingestion are still largely unknown, and in particular the interaction between the glucose intolerance, IGF-I and GH remains unclear. This study was conducted to determine if chronic heavy alcohol exposure is potentially involved in glucose homeostasis and is mediated by alterations in IGF-I and GH, both of which have been previously linked to an increase risk for T2DM. In addition, extreme abnormalities of glucose tolerance in T2DM that occurred as a consequence of chronic use of alcohol were also investigated.

Materials and methods

Animals

Fifty-four week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats and non-diabetic male Long-Evans Tokushima Otsuka (LETO) rats were purchased from Central Lab Animal Inc. (Seoul, Korea). The rats were divided into four groups: LETO-Control (O-C, $n = 9$), LETO-Ethanol (O-E, $n = 7$), OLETF-Control (L-C, $n = 9$), OLETF-Ethanol (L-E, $n = 8$). The rats were maintained in a controlled temperature (22–24 °C) with a 12 h light/dark cycle. All experiments were approved by the Catholic University Animal Care and Use Committee (CUMC-2012-0027-01). Animal care and experimental procedures conformed to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised in 1996.

Diet and treatment

The rats in the alcohol-fed group received 100 mL of Lieber-DeCarli Regular Ethanol diet (Cat. No. 710260, Dyers Inc., Bethlehem, PA) including 36% ethanol and control rats were fed a Lieber-DeCarli control diet (Cat. No. 710027, Dyers Inc., Bethlehem, PA) daily for six weeks, as recommended by the manufacturer. Animals were weighted on a weekly basis, with the goal of matching weights all groups, based on the previous reports (Jung et al., 2011).

Intraperitoneal glucose tolerance test

IP-GTT was performed before sacrificing the animals. The animals were fasted overnight (17 h) and given intraperitoneal glucose (2 g/kg, dextrose). Blood was then taken from the tail vein, and the glucose levels were measured using a portable glucose meter (One Touch Ultra, Johnson & Johnson Medical, USA). The glucose level was obtained at 0 min (fasting), 30 min, and 120 min after glucose administration.

Measurement of serum samples

After 6 weeks of feeding Lieber-DeCarli diet, following starvation for 12 h, the animals were anesthetized with isoflurane (1% to 2% with 0.8 L/min O₂ administrated with a facemask) and blood (3–5 mL) was drawn directly from the heart. Samples were centrifuged at 800 ×g at 25 °C for 20 min and stored at –80 °C until the analysis was performed. The biochemical parameters of serum were measured using a Selectra 2 auto-analyzer (Vital Scientific, Netherlands). Serum IGF-I concentrations were determined by the RAT/MOUSE IGF-I Single Plex kit (RMIGF-187 K, LINCO Research, St. Charles, MO, USA) and the GH levels were measured by the Brain Derived Neurotrophic Factor Sandwich ELISA kit (No. CYT306, Chemicon, USA). The serum insulin levels were measured using the Rat Metabolic Magnetic Bead Plate (No. RMHMAG-84K, Millipore, USA).

Statistical analysis

The data are expressed as the mean ± SEM for groups based on 7–9 rats in each group. Each experiment was repeated at least twice. Differences between groups were analyzed by analysis of variance (ANOVA) using SPSS (Version 15.0; SPSS Inc., Chicago, IL, USA). ANOVA results followed by Tukey's post hoc test with $p < 0.05$ were considered to be statistically significant.

Results

Changes in body weight

At baseline, the body weights in the different groups ranged between 275.78 g and 300.75 g, with no statistically significant differences among the groups (Table 1). After 6 weeks of feeding Lieber-DeCarli diet, the body weights in the L-C (from 275.78 ± 4.41 to 326.33 ± 4.48 g) and L-E (from 273.00 ± 9.50 to 320.00 ± 3.78 g) groups were both increased compared to baseline, but they were not significantly different from each other. The body weight increase in the O-C group (from 278.55 ± 8.22 to 320.00 ± 3.00 g) was similar to the O-E group (from 300.75 ± 8.73 to 319.25 ± 9.28 g). The average calculated amounts of alcohol based on liquid alcohol consumption in the L-E and O-E group were 9.35 and 9.86 g/kg/day, respectively; this constituted heavy alcohol consumption.

Changes in blood glucose concentrations

Glucose concentrations during the IP-GTT were elevated in the alcohol consumption groups at all time-points from 30 to 120 min (Fig. 1). Prior to glucose injection, the mean glucose levels in the L-C, L-E, O-C, and O-E groups were 101.22 ± 1.27, 94.28 ± 2.34, 104.44 ± 0.73, and 91.63 ± 3.13 mg/dL, respectively. The mean glucose levels in the O-E group were significantly lower than that of the L-C group at that time-point ($p < 0.05$). Thirty minutes after IP injection, the mean glucose levels in the L-C, L-E, O-C, and O-E groups were 139.00 ± 8.46, 181.47 ± 6.75, 255.89 ± 13.80, and 285.625 ± 23.11 mg/dL, respectively; all group had an increase in the mean glucose levels compared to the baseline. The increase in mean glucose levels in the O-E group was significantly greater than that of either the L-C group ($p < 0.001$) or the O-C group ($p < 0.001$). One hundred twenty minutes after IP injection, the mean glucose levels in the L-C, L-E, O-C, and O-E groups were 99.44 ± 2.52, 109.14 ± 1.72, 131.44 ± 4.92, and 192.13 ± 12.36 mg/dL, respectively; again, all group had an increase in the mean glucose levels compared to the baseline measurement. The increase in the mean glucose levels in the O-E group was still significantly higher than that of the L-C ($p < 0.001$) and the O-C groups ($p < 0.001$) at the later time-point. In contrast, the overall glucose response in the L-C and the L-E groups was not significantly different at 30 min and 120 min post-glucose load, but the increase in the mean glucose levels in the L-E group was significantly higher than that in the L-C group.

Table 1
Body weight by group (O-C, O-E, L-C, and L-E) at baseline and after six weeks of feeding Lieber-DeCarli diet.

Group	N	Weight (g)	
		Baseline	After 6 weeks
L-C	9	275.77 ± 4.42	326.33 ± 4.48
L-E	8	273.00 ± 9.50	320.00 ± 3.78
O-C	9	278.55 ± 8.22	320.00 ± 3.00
O-E	7	300.75 ± 8.73	319.25 ± 9.28

Data were analyzed by one-way ANOVA and repeated measures ANOVA. There were no significant differences among the groups. O-E: OLETF-Ethanol; O-C: OLETF-Control; L-E: LETO-Ethanol; L-C: LETO-Control.

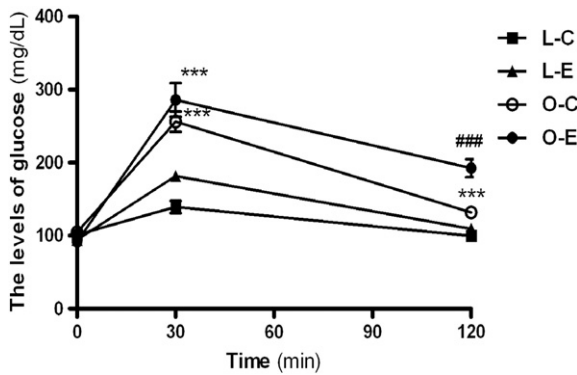


Fig. 1. Comparison of IP-GTT by group (O-E, O-C, L-E, and L-C). Blood glucose levels were measured at baseline and 30 min and 120 min after intraperitoneal glucose administration. Data were analyzed by one-way ANOVA and repeated measures ANOVA. $***p < 0.001$ vs. L-C group, $###p < 0.001$ vs. O-C group.

Changes in blood serum levels of IGF-I, GH, and insulin

Fig. 2A shows the mean IGF-I levels in the L-C, L-E, O-C, and O-E groups, which were $229,888.89 \pm 31,859.26$, $140,142.86 \pm 13,659.86$, $153,844.44 \pm 27,918.90$, and $115,414.29 \pm 9,847.93$ pg/mL, respectively; The mean IGF-I level in the O-E group was significantly lower than that in the L-C group ($p < 0.05$). IGF-I levels in the L-E and O-C groups were lower than those in the L-C group but these differences were not significant. **Fig. 2B** displays that the mean GH levels in the L-C, L-E, O-C, and O-E groups, which were 2341.25 ± 262.04 , 3321.43 ± 483.89 , 3037.78 ± 820.76 , and 7157.50 ± 2123.52 pg/mL, respectively; the mean GH level in the O-E group was significantly lower than that in the L-C group ($p < 0.05$). The GH levels in the L-E and O-C groups were lower than those in the L-C group but these differences were not statistically significant. **Fig. 2C** gives the mean insulin levels by group. In the L-C, L-E, O-C, and O-E groups, the mean insulin levels were 538.9 ± 130.0 , 535.7 ± 41.10 , 704.6 ± 71.50 , and 265.5 ± 29.56 pg/mL, respectively. The mean insulin levels in

O-E group were significantly lower than those in the L-C group and the O-C group ($p < 0.05$; $p < 0.01$, respectively).

Correlation between levels of blood glucose and IGF-I

There was a negative correlation between blood glucose and IGF-I levels ($r = -0.54$, $p = 0.0305$) (**Fig. 3**). However, serum GH levels did not significantly correlate with blood glucose and IGF-I levels ($r = 0.24$, $p = 0.2369$; $r = -0.22$, $p = 0.4224$, respectively).

Discussion

Several earlier studies have focused on the effect of chronic alcohol use on growth factors such as neurotrophins that are expressed only in the nervous system, as well as fibroblast growth factor (FGF), insulin, and IGF-1, which are ubiquitously expressed (Sonntag and Boyd, 1989; Yoon et al., 2006). Low circulating levels of brain-derived neurotrophic factor (BDNF) and increased severity of insulin resistance as measured by the homeostasis model assessment index, which is based on fasting glucose and insulin levels, have been observed in individuals with alcoholism (Krabbe et al., 2007). BDNF may also regulate glucose metabolism to reduce food intake and lower blood glucose in the obese diabetic mice (Nakagawa et al., 2000). Thus, the malfunction of some of the growth/trophic factor system has been of considerable interest given its potential contribution to the negative effects of T2DM in the setting of chronic alcohol consumption.

In this study, alcohol-related alterations that have previously been implicated in the pathophysiology of T2DM were observed with the IP-GTT. The slope of the line showing plasma glucose between 30 and 120 min of the GTT in **Fig. 1** shows a parallel between the diabetic and non-diabetic rats treated with alcohol, suggesting that the rate of glucose disposal might have been impaired in these animals. Ethanol treatment diminished fasting glucose and increased postprandial glucose levels. These effects were greater in the OLETF rats than in the LETO rats, implying that alcohol did aggravate pre-existing T2DM in the OLETF rats, but did not, by itself, result in the presentation of T2DM in the LETO rats. It is well known that the impaired response to insulin and β -cell failure in the setting of alcohol-related glucose intolerance is likely important

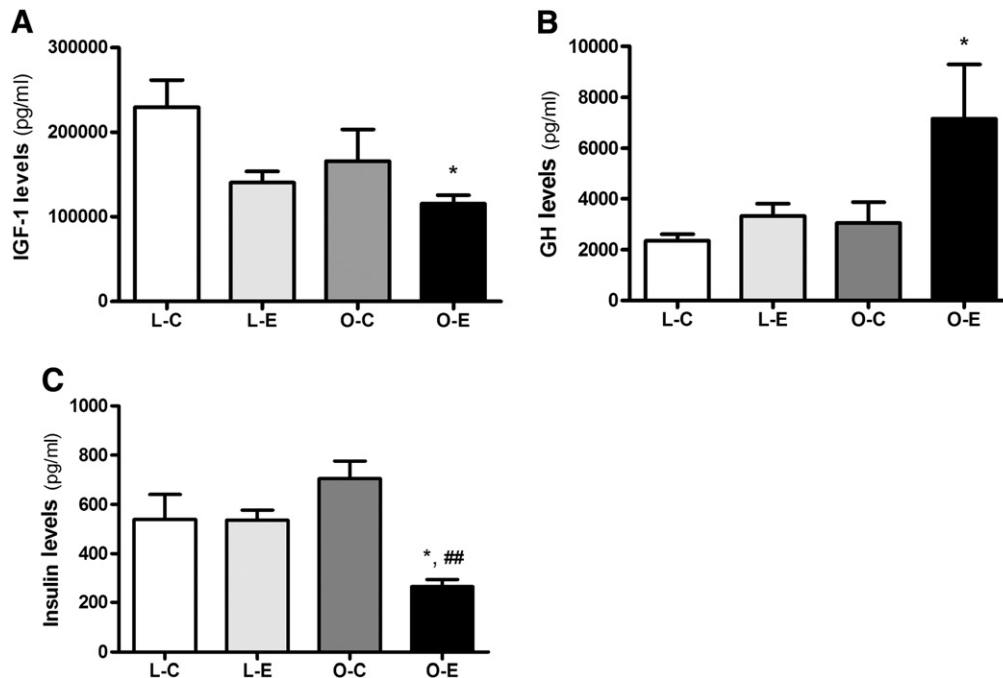


Fig. 2. Comparison of IGF-1 levels (A), GH levels (B), and insulin levels (C) by group (O-E, O-C, L-E, and L-C). Data were analyzed by one-way ANOVA. $*p < 0.05$ vs. L-C group, $##p < 0.01$ vs. O-C group. Vertical bars indicate the S.E.

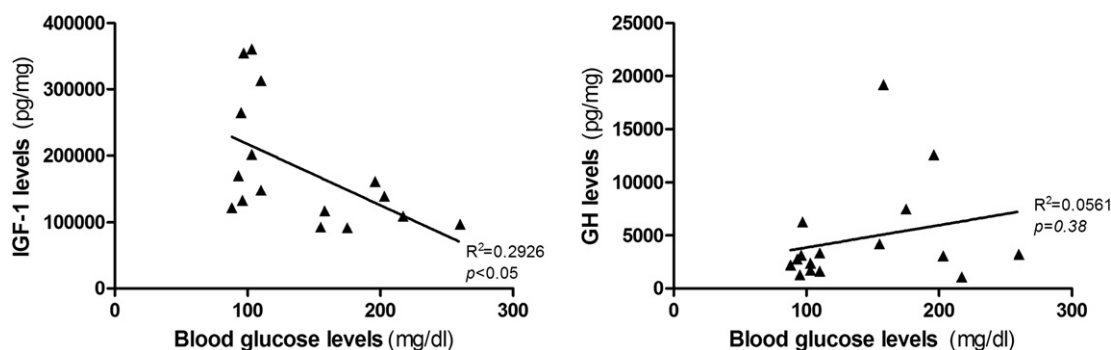


Fig. 3. Correlation between levels of blood glucose and IGF-1 and GH. Circulating concentrations of glucose, IGF-1, and GH detected in serum samples taken from L-C and O-E group. Representative correlations in blood glucose are shown as a scatter-plot with IGF-1 or GH (closed triangles = IGF-1 or GH) plotted. Data were analyzed using Pearson's correlation coefficients. There was significant correlation at level of 0.05 between blood glucose and changes of IGF-1 ($r = -0.54$).

risk factors for the development and progression of T2DM (Patto et al., 1993). As a result of this β -cell dysfunction and inadequate insulin release, postprandial glucose levels increase due to incomplete suppression of hepatic glucose production and decreased efficiency of liver and muscle glucose uptake (Kahn, 2001; Wannamethee et al., 2002). In addition to this, continued alcohol metabolism interferes with hepatic gluconeogenesis, which leads to lower blood glucose levels, as well as insulin secretion that is also reduced as the level of blood glucose falls.

The most notable findings of the present study are that ethanol treated OLETF rats presented significantly lower serum IGF-1 levels, higher serum GH levels, and lower serum insulin levels than compared to those in the non-ethanol-treated LETO rats, which suggest that the comorbidity of alcohol dependence and T2DM may create a synergic influence in IGF-1 and GH levels. Studies have consistently reported that IGF-1 deficiency is associated with decreased insulin sensitivity and is ultimately linked to T2DM (Friedrich et al., 2012; McDonald et al., 2007; Sesti et al., 2005). IGF-1 is also a prominent signaling molecule in the central nervous system (CNS), suggesting that it may have a reduced neurotrophic influence in the brain in the setting of diabetes (Porte et al., 2005). It has recently been suggested that IGF-1 levels may indicate a positive prognostic indicator in metabolic syndrome, and increases in log-transformed IGF-1 concentrations have been shown to markedly reduce metabolic syndrome (Sesti et al., 2005). Plasma IGF-1 levels have also been shown to be decreased in diabetes (Asplin et al., 1989). Our findings suggest that the lower IGF-1 levels in T2DM associated with alcohol use in this study might be a reflection of the severity of the metabolic disturbance. Additionally, one could speculate that oxidative stress, which is considered to have a substantial effect on apoptosis through the formation of lipid hydroperoxides, may result in an overall decrease in circulating IGF-1 levels. Chronic heavy alcohol use also increases mitochondrial reactive oxygen species (ROS) production, which through mitochondrial damage is one of the earliest events in glucose intolerance. The deleterious effects of ethanol on β -cells also include an exquisite sensitivity to oxidative stress (Dembele et al., 2009). Moreover, ROS-dependent β -cells dysfunction is thought to be involved in glucolipotoxicity and may be associated with the progression from metabolic syndrome to T2DM (Guichard et al., 2008).

In contrast, the effect of chronic alcohol consumption on was to significantly increase the serum GH levels. Our result is supported by the earlier finding that liver-specific IGF-1 gene depleted mice exhibited elevated GH levels and impaired activation of early signaling events in response to insulin, aligned with low plasma IGF-1. These were associated with susceptibility to severe insulin resistance and diabetes (Usui et al., 2002; Yakar et al., 2001). Another potential mechanism that has been described is that where insufficient negative feedback between the hypothalamus and pituitary leads to low plasma IGF-1, causing GH hypersecretion and reduced insulin sensitivity, especially in the skeletal muscle. The priming effect of the GH excess generates a state of insulin resistance by impairing the ability of insulin to inhibit glucose production and promote glucose

utilization. Furthermore, continued GH excess causes fasting hyperglycemia, and subjects may develop diminished fasting insulin and an extended decline in their insulin response to a given glucose load (Colao et al., 2004; Yakar et al., 2004). Thus, alterations in GH affect the ability to meet metabolic demand, influencing the capacity to maintain islet β -cell mass and adequate insulin secretion, and could present an intriguing biological mechanism linking chronic alcohol consumption and T2DM.

In parallel with these observed changes in IGF-1 and GH, serum insulin levels in ethanol treated OLETF rats were significantly lower than in the non-ethanol treated LETO rats. Insulin is required for GH-stimulated secretion of IGF-1 from the liver. High concentrations of alcohol are considered to reduce insulin binding and suppress insulin intracellular signaling, inducing a state of reversible insulin resistance. Thus, a role for insulin has been demonstrated in several studies concerning the involvement of chronic alcohol use in glucose metabolism (Singh et al., 1988). Previous studies have described the complicated relationship between insulin and IGF-1, in that the insulin resistance associated with chronic hyperinsulinemia may be linked to low IGF-1 and GH receptor resistance, while hypoinsulinemia may actually induce low IGF-1 (Bereket et al., 1995; Brugts et al., 2010). In concordance with previous reports, impaired glucose tolerance was characterized not only by changes in low IGF-1 and high GH, but also by a fall in fasting insulin (Sesti et al., 2005). The results of the present study suggest that impaired insulin secretion, as indicated by dysregulation of the IGF-1 and GH system, may contribute to alcohol-induced diabetes.

Most importantly, we found a strong negative correlation between the concentrations of postprandial blood glucose and IGF-1. At the same time, blood glucose concentrations did not significantly correlate with GH levels. In contrast to the primary action of GH, which lessens glucose uptake, increases hepatic glucose production, decreases the conversion of glucose to fat and reduces the responsiveness of target tissue to insulin, several studies have addressed controversial results with respect to GH and glucose homeostasis (Liu et al., 2004). Thus, IGF-1 and GH seem to represent the overall diabetogenic effects of chronic alcohol consumption, but are being further investigated.

There are possible limitations in this study. One is that the impact of chronic excessive alcohol use on hepatic gluconeogenesis in diabetic rats, which might help to distinguish the combined effects of alcohol and T2DM from that of alcohol or T2DM alone, was not tested and thus needs further study. Although observations of the histological effects of chronic heavy alcohol consumption in animals with diabetes did not figure into our study, several findings have demonstrated that alcohol administration to diabetic rats results in more severe hepatic damage than that observed in non-diabetic rats (Shanmugam et al., 2011). Additionally, although the evidence suggested that IGF-1 represents a viable therapeutic target in modulating glucose dysfunction related to alcoholism, addition of more animals experimented with strategies to increase serum IGF-1 levels could strengthen our results. Therefore, further studies are necessary to confirm these effects.

In summary, we have demonstrated that in ethanol treated OLETF rats, the serum IGF-I and insulin levels decrease, the serum GH levels increase, and these changes are implicated in changes in glucose intolerance. The results of the present study suggest that IGF-I and GH may be important candidates as mediators between alcohol consumption and T2DM, and play a broader role in the deteriorating glucose homeostasis seen with alcohol use. The present study was not sufficient to fully understand the abnormalities of glucose metabolism in alcohol-related T2DM, yet therapeutic strategies that increase in serum IGF-I levels may be promising to prevent diabetogenic sequelae of alcohol dependence.

Conclusions

In conclusion, fasting serum IGF-I and GH measured may have predictive values for not only the development of glucose intolerance but also the progression of T2DM, which are influenced by chronic alcohol consumption. This study further clarifies the role of IGF-I and GH, and provides insight into the effects of chronic alcohol use in the pathophysiology of T2DM.

Conflict of interest statement

None of the original material contained in the manuscript has been submitted for consideration or will any of it be published elsewhere. All of authors in the manuscript participated sufficiently in the process of this study. All authors agreed with the copyright transfer. The authors declare no conflict of interest.

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