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Low ethanol consumption induces enhancement of insulin sensitivity in liver of normal rats

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Abstract

Moderate amounts of alcohol intake have been reported to have a protective effect on the cardiovascular system and this may involve enhanced insulin sensitivity. We established an animal model of increased insulin sensitivity by low ethanol consumption and here we investigated metabolic parameters and molecular mechanisms potentially involved in this phenomenon. For that, Wistar rats have received drinking water either without (control) or with 3% ethanol for four weeks. The effect of ethanol intake on insulin sensitivity was analyzed by insulin resistance index (HOMA-IR), intravenous insulin tolerance test (IVITT) and lipid profile. The role of liver was investigated by the analysis of insulin signaling pathway, GLUT2 gene expression and tissue glycogen content. Rats consuming 3% ethanol showed lower values of HOMA-IR and plasma free fatty acids (FFA) levels and higher hepatic glycogen content and glucose disappearance constant during the IVITT. Neither the phosphorylation of insulin receptor (IR) and insulin receptor substrate-1 (IRS-1), nor its association with phosphatidylinositol-3-kinase (PI3-kinase), was affected by ethanol. However, ethanol consumption enhanced liver IRS-2 and protein kinase B (Akt) phosphorylation (3 times, $P<0.05$), which can be involved in the 2-fold increased ($P<0.05$) hepatic glycogen content. The GLUT2 protein content was unchanged. Our findings point out that liver plays a role in enhanced insulin sensitivity induced by low ethanol consumption.

Keywords: Low-ethanol intake; Insulin sensitivity; Insulin receptor substrate-2; Akt/PKB; Hepatic glycogen

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Introduction

There are some evidences that moderate consumption of any type of alcoholic beverage is associated with the reduction of overall morbidity and mortality, mainly due to the reduction of cardiovascular disease (CVD) risk factors (Doll, 1997; Klatsky et al., 1992; Rimm et al., 1991; Valmadrid et al., 1999). Low-to-moderate ethanol (EtOH) consumption has been reported to decrease blood pressure, platelet aggregation, fibrinogen, LDL-cholesterol and triglycerides, and to increase HDL cholesterol (Bell et al., 2000; Brunner et al., 1996; Doll et al., 1994; Hojnacki et al., 1988; Meade et al., 1979; Mukamal et al., 2001; Nanchahal et al., 2000; Pellegrini et al., 1996; Soleas et al., 1997; Vliegenthart et al., 2002). Interestingly, these parameters affected by ethanol overlap with several components involved in Insulin Resistance Syndrome (Bjorntorp, 1992; Reaven, 1988, 1993).

One decade has passed since the first report suggested that insulin would play a major role in ethanol protective effects (Razay et al., 1992). Even though other studies have outlined that low-to-moderate alcohol consumption (12–24 g EtOH or 1–2 drinks a day) is associated with enhanced insulin sensitivity (Bell et al., 2000; Facchini et al., 1994; Kiechl et al., 1996; Lazarus et al., 1997; Mayer et al., 1993), some could not report this relationship (Cordain et al., 2000; Todoroki et al., 1994) and others could not completely confirm it after adjustment of other risk factors such as body mass index (BMI) and central adiposity (Bell et al., 2000). These findings also leave the field open to doubts because these epidemiological studies have used imprecise methods to evaluate insulin resistance and large range of alcohol intake.

To clarify this phenomenon, an animal model for the study of the effect of low ethanol intake was previously characterized (Furuya et al., 2003). The effect of various ethanol concentrations intake (0.5%, 1.5%, 3%, 4.5%, and 7% *v*/*v* EtOH in the drinking water) for 4 weeks was investigated in normal Wistar rats and the results showed an inverted U-shaped relationship between alcohol intake and insulin sensitivity (Furuya et al., 2003). In that study, animals consuming 3% EtOH showed the highest insulin sensitivity, based on the increase of plasma glucose disappearance rate during the insulin tolerance test and based on the reduction of insulin secretion during the glucose tolerance test.

The cellular mechanisms behind the enhanced insulin sensitivity effect of alcohol are, so far, unknown. To address this issue, we have investigated the effect of low ethanol consumption on in vivo insulin sensitivity and on liver insulin signaling pathway, GLUT2 gene expression and glycogen content.

Materials and methods

Materials

Ethanol (EtOH 99.5% pure) was obtained from Labsynth (Diadema, SP, Brazil). Glycogen type II from oyster was obtained from Sigma (St. Louis, MO, USA). Palmitic acid was from Matheson Coleman and Bell Manufacturing Chemists (Norwood, OH, USA). Anti-IR, anti-IRS-1, and anti-IRS-2 were purchased from Santa Cruz Technology, Inc (Santa Cruz, CA, USA). Anti-PI 3-kinase was from Upstate Biotechnology, Inc (Lake Placid, NY, USA). Anti-Akt was obtained from New England Biolabs Inc. (Beverly, MA, USA). Enhanced chemiluminescence (ECL) detection system, [α-^{32}P]-dCTP and [^{125}I]-Protein A were from Amersham Pharmacia Biotech Buckinghamshire, England. Regular insulin (Iolin®) was from Biobras, Montes Claros, MG, Brazil. ^{32}P-probes were prepared with the "Random Primers DNA Labeling System Kit" from Gibco-BRL (NY, USA).