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Ethanol exerts anti-inflammatory effects in human adipose tissue *in vitro*.

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Running title: Anti-inflammatory effects of ethanol in human adipose tissue

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Abstract

Moderate alcohol consumption is suggested to be associated with reduced inflammation and morbidity. Human adipose tissue (AT) and obesity is characterised by low-grade inflammation, why the present study wanted to investigate the effects of ethanol on inflammation in human AT *in vitro*. Subcutaneous human AT was incubated with ethanol [11mM - 88mM] under non- or LPS-stimulated [50mg/mL] conditions. Protein and mRNA levels of adiponectin, IL-6, IL-8, TNF- α , MCP-1, and CD68 were assessed using ELISA and Real-Time RT-PCR, respectively.

Non-stimulated, ethanol incubations up to 24h increased adiponectin release and mRNA expression ($p < 0.01$) and decreased IL-6 release in both short term [1.5h] ($p < 0.05$) and long term [24h] ($p < 0.01$) incubations. Ethanol decreased LPS-stimulated IL-6, IL-8, TNF- α , and MCP-1 dose-dependently (all $p < 0.01$). Ethanol decreased CD68 mRNA ($p < 0.001$), which correlated with the investigated adipokines ($p < 0.05$) but not adiponectin ($p > 0.05$).

In conclusion, ethanol exerts anti-inflammatory effects in human AT, suggesting that ethanol may attenuate whole-body inflammation.

1. Introduction

Human adipose tissue (AT) produces and releases a number of bio-active proteins, collectively referred to as *adipokines* (Trayhurn and Wood, 2004), of which several are reported to be increased in overweight and obese subjects [*e.g.* interleukin (IL)-6 (Bastard et al., 2000), IL-8 (Bruun et al., 2003b), Tumour Necrosis Factor (TNF)- α (Hotamisligil et al., 1995), and Monocyte Chemoattractant Protein (MCP)-1 (Bruun et al., 2005)]. Interestingly, obesity is associated with a state of low-grade inflammation reported to precede metabolic diseases such as insulin resistance, type 2 diabetes, atherosclerosis and cardiovascular disease (Despres and Lemieux, 2006; Dandona et al., 2004). The actual link between obesity and low-grade inflammation is still unknown but may involve an increase in the production and release of pro-inflammatory adipokines [*e.g.* IL-6, IL-8, TNF- α , MCP-1] combined with a decrease in the anti-inflammatory adipokine, adiponectin (Arita et al., 1999), which may be related to the recently demonstrated increased infiltration of macrophages in AT from obese patients (Weisberg et al., 2003; Bruun et al., 2005; Canello et al., 2005).

Alcohol is known to exert numerous effects in humans of which the deleterious ones caused by excess consumption are well-known and strongly associated with excess morbidity and mortality [reviewed in (Klatsky, 2007)]. During the last decades several epidemiological studies have reported associations between low to moderate alcohol consumption and reduced cardiovascular morbidity and mortality (Gronbaek, 2002; Klatsky and Udaltsova, 2007). Even though causality remains to be elucidated, several *in vivo* studies have reported beneficial effects of various combinations of ethanol [*e.g.* wine, beer, liquor] on especially circulating levels of High Density Lipoprotein cholesterol (HDL) (Sacanella et al., 2002; Wannamethee et al., 2003; Badia et al., 2004; Sierksma et al., 2004). Another possible link between moderate alcohol consumption and the reduced risk of developing metabolic disease is suggested to be mediated through alcohol induced

anti-inflammatory effects via changes in classical [*e.g.* hs-CRP, fibrinogen] (Sierksma et al., 2002; Estruch et al., 2004) as well as newer risk markers [*e.g.* adiponectin, MCP-1, VCAM-1, ICAM-1] (Sierksma et al., 2004; Estruch et al., 2004). Supporting this hypothesis, several *in vitro* studies is demonstrating anti-inflammatory effects of ethanol in various cell-types such as spleen cell-cultures and macrophages (Friedman, 1998; Szabo et al., 1999; Saeed et al., 2004), blood monocytes (Szabo et al., 1999), and endothelial cells (Saeed et al., 2004).

Since human AT have an inflammatory potential and whole body (low-grade) inflammation in obese individuals seems to precede the metabolic diseases outlined above (Dandona et al., 2004), the aim of the present study was to investigate the effects of ethanol on the expression and secretion of various adipokines [*e.g.* adiponectin, IL-6, IL-8, TNF- α , and MCP-1] and the macrophage specific marker CD68 in human AT *in vitro*.

2. Materials and methods

2.1. Whole adipose tissue cultures

Subcutaneous AT from the abdominal and gluteal AT-depots was obtained from 34 normal to overweight but otherwise healthy individuals [29 females and 5 males; mean age: 38 ± 12 yrs.] undergoing liposuction for cosmetic reasons [mean body mass index (BMI): 25.4 ± 4.9 kg/m²]. None of the subjects received any medication known to influence AT metabolism.

After removal the AT was kept under sterile conditions and transported to the laboratory within 60 min. At the laboratory, all subsequent procedures were carried out under a laminar airflow hood. The AT was washed several times in sterile saline. As previously described, the AT was minced into fragments of less than 10 mg and a total of 500mg of was placed in 50 mL plastic tubes containing 5mL medium 199 supplemented with Penicillin/Streptomycin (100 IU/mL),

antipain/leupeptin (20 $\mu\text{L}/\text{mL}$) and 1 nM insulin (1 $\mu\text{L}/100\text{ mL}$ medium) (Bruun et al., 2001). The AT fragments were pre-incubated in a humidified incubator (37 $^{\circ}\text{C}$) at an atmosphere of 5 % CO_2 . The pre-incubation time was adjusted in order to make the total incubation time comparable between the various experiments. After pre-incubation, AT fragments were incubated for 1.5, 6, 12, or 24h in medium 199 with different concentrations of ethanol [11 mM, 22 mM, 44 mM, or 88 mM]. Incubations were performed with or without lipopolysaccharide [LPS; up to 50 mg/L] in order to activate an inflammatory response in the AT. All experiments are carried out as duplicates in renewed medium 199. The culture medium obtained from the different incubations was kept at -20 $^{\circ}\text{C}$ until analyses were performed. The AT was snap frozen in liquid nitrogen and kept at -80 $^{\circ}\text{C}$ for later RNA extractions.

2.2. Determination of adipokine secretion and mRNA levels

Protein secretion to the culture medium was assessed using human enzyme-linked immunosorbent assays (ELISA; adiponectin; B-Bridge, AH diagnostics AS, Denmark and IL-6, IL-8, TNF- α , and MCP-1; DuoSet, R&D Systems Europe Ltd., UK). Samples were diluted to be within the assay range. The IL-6 assay had an intra-assay coefficient of variation of 5.5 %. The IL-8 assay had an intra-assay coefficient of variation of 7.9 %. The TNF- α assay had an intra-assay coefficient of variation of 6.7 %. The MCP-1 assay had an intra-assay coefficient of variation of 8.1 %.

For mRNA determination the following oligonucleotide primer pairs were used. adiponectin:

5'CATGACCAGGAAACCACGACT3'; 5'TGAATGCTGAGCGGTAT3', IL-6:

5'AAATGCCAGCCTGCTGACGAAG3'; 5'AACAACAATCTGAGGTGCCCATGCTAC3', IL-8:

5'TTGGCAGCCTTCCTGATTTTC3'; 5'AACTTCTCCACAACCCTCTG3', TNF- α :

5'CGAGTGACAAGCCTGTAGC3'; 5'GGTGTGGGTGAGGAGCACAT3', MCP-1:

5'CAATCAATGCCCCAGTCAC3'; 5'GATTCTTGGGTTGTGGGAGTG3', CD68:

5'CTACATGGCGGTGGAGTACAA3'; 5'ATGATGAGAGGCAGCAAGATGG3', and β -actin: 5'ACGGGGTCACCCACACTGTGC 3'; 5'CTAGAAGCATTGCGGTGGACGATG3'. RNA was isolated from ~250 mg of AT using the TriZol Reagent (Invitrogen, Carlsbad, CA). RNA was quantified by measuring absorbancy at 260 and 280 nm. The integrity of the RNA was checked by visual inspection of the two ribosomal RNAs, 18S and 28S, on an agarose gel. RNA was reversely transcribed with RT and random hexamer primers at 23 °C for 10 minutes, 42 °C for 60 minutes, and 95 °C for 10 minutes according to the manufacturer's instructions (GeneAmp RNA PCR kit, PerkinElmer Life and Analytical Sciences, Boston, MA). Then 2 μ l of each RT reaction was amplified in PCR-masternix containing the specific primers, Hot Star Taq DNA polymerase, and SYBR-Green PCR buffer. All samples were determined as duplicates. Real-time quantification of target gene [adiponectin, IL-6, IL-8, TNF- α , MCP-1, CD68] relative to housekeeping gene [β -actin] was performed using a SYBR-Green real-time PCR assay and an iCycler PCR machine (Bio-Rad, Hercules, CA). In brief, samples were incubated in separate tubes for initial denaturation at 95 °C for 10 minutes, followed by 40 PCR amplification cycles. Each cycle consisted of 30 seconds at 95 °C, 30 seconds at 57 °C and extension for 60 seconds at 74 °C. During final extension increasing fluorescence was measured in real time. Data was obtained as threshold cycle values (Ct = the cycle number at which fluorescence reached 10 times the SD of the background fluorescence). Relative gene expression of target gene to β -actin was calculated as described in the User Bulletin No. 2, 1997 from Perkin Elmer Life and Analytical Sciences (Perkin Elmer Cetus, Norwalk CT, USA).

2.3. Statistical analysis

The SigmaStat 2.03 statistical packet (SPSS, Chicago, IL., USA) was used for the calculations. Normality was tested using the Kolmogorov-Smirnov test. A paired Students *t*-test or appropriate non-parametric statistics was applied when comparing mean differences between control/LPS and

ethanol/LPS+ethanol incubations. To determine the relationship between the mRNA expression of CD68 and the investigated adipokines bivariate correlations with a Pearson correlation coefficient (r_p) was used. Results are presented as mean \pm s.e.m. Threshold for significance was set at $p < 0.05$.

2.4. Ethics

Informed, written consent was obtained from all subjects and experiments were performed in accordance with the Helsinki II Declaration. The study was approved by the Ethical Committees of Aarhus.

3. Results

3.1. Effect of ethanol on adiponectin in human AT

In the non-stimulated experiments (without LPS), ethanol incubation for 1.5h [11mM] was found to increase adiponectin mRNA expression by up to 2.5-fold ($p < 0.05$, data not shown). After incubation for 24h, adiponectin release was significantly increased by all ethanol concentrations by up to 33% ($p < 0.001$, Fig. 1A) and adiponectin mRNA expression was increased by up to 2-fold at ethanol concentrations of 44mM ($p < 0.05$) and 88mM ($p < 0.01$), respectively (Fig. 1B).

Incubation with LPS significantly reduced adiponectin mRNA expression by 76 % ($p < 0.05$) but addition of ethanol at the same time was not able to counteract this LPS-induced inhibition of adiponectin mRNA expression (data not shown).

3.2. Effects of ethanol on IL-6 in human AT

In the non-stimulated (without LPS) time-course experiments, ethanol was found to induce a short lived decrease in IL-6 already after 1.5 h ($p < 0.05$, Fig. 2). Hereafter, the release of IL-6 returned to

initial levels the next 6-12h after which a sustained decrease in the release of IL-6 was observed ($p < 0.05$, Fig. 2). The inhibitory short term effects were found to be comparable for all four different ethanol concentrations, whereas the long term inhibitory effects on IL-6 release were found to be affected by ethanol in a dose-dependent manner (Fig. 2).

Incubation for 24h with LPS [50mg/L] resulted in a 4-fold increase ($p < 0.001$, Fig. 3) in the release of IL-6. Ethanol reduced the LPS-induced release of IL-6 in a dose-dependent manner with a maximal decrease of ~30 % at an ethanol concentration of 88mM ($p < 0.01$, Fig. 3). A similar trend was observed at the gene expression level, however, only an ethanol concentration of 88mM was found to reduce IL-6 mRNA expression by 36 % ($p < 0.05$, data not shown).

3.3. Effects of ethanol on IL-8, TNF α , and MCP-1 in human AT

In the non-stimulated (without LPS) incubations, ethanol had no short term effects on IL-8 but was after incubation for 24h found to decrease IL-8 release by 17 % at an ethanol concentration of 11mM ($p < 0.05$) and by 23 % at an alcohol concentration of 88mM ($p < 0.01$, data not shown).

LPS [50mg/L] was found to induce a 4-fold increase in the release of IL-8 ($p < 0.001$, Fig. 4A). Ethanol induced a dose-dependent decrease in the LPS-induced IL-8 release by ~30% at an ethanol concentration of 88mM ($p < 0.05$, Fig. 4A). Incubation with LPS significantly increased IL-8 mRNA expression by 9-fold ($p < 0.001$) but addition of ethanol was not able to counteract this LPS-induced increase in IL-8 mRNA expression (data not shown).

Ethanol was without effects on TNF- α release under non-stimulated (without LPS) conditions even after incubations up to 24h.

LPS [50mg/L] was found induce a robust 20-fold increase in TNF- α release ($p < 0.001$, Fig. 4B) after incubation for 24 h. Ethanol was found to decrease the release of TNF- α in a dose-dependent

manner reaching a maximal inhibition of ~28 % at an ethanol concentration of 88mM ($p<0.01$, Fig. 4B). Incubation with LPS significantly increased TNF- α mRNA expression by 8-fold ($p<0.001$) but addition of ethanol was not able to counteract this LPS-induced increase in TNF- α mRNA expression (data not shown).

Ethanol was without effects on MCP-1 release under non-stimulated (without LPS) conditions in incubation up to 24h. Incubation with LPS [50mg/L] for 24h was found to increase MCP-1 release by 4-fold ($p<0.001$, Fig. 4C). Ethanol was found to decrease the LPS-induced release of MCP-1 in a dose-dependent manner reaching a maximal inhibition of ~39 % at an ethanol concentration of 88mM ($p<0.01$, Fig. 4C). Incubation with LPS significantly increased IL-8 mRNA expression by 4-fold ($p<0.001$) but addition of ethanol was not able to counteract this LPS-induced increase in IL-8 mRNA expression (data not shown).

3.4. CD68 in relation to ethanol, LPS, and adipokines in human AT

No correlation was found between CD68 and the investigated adipokines under non-stimulated (without LPS) conditions in incubations up to 24h. Ethanol alone was without effects on CD68 mRNA expression under the same non-stimulated conditions in incubations up to 24h.

In the LPS-stimulated [50mg/L] incubations [24h] CD68 mRNA expression correlated with IL-6 ($p<0.001$), IL-8 ($p<0.05$), TNF- α ($p<0.01$), and MCP-1 ($p<0.05$) but not with adiponectin ($p>0.05$) (Table 1). In addition, LPS was found induce a 2-fold increase in CD68 mRNA expression ($p<0.05$) and ethanol [88mM] was found to decrease CD68 mRNA expression by ~50% ($p<0.001$, data not shown).

4. Discussion

The present paper is to our knowledge the first to report that ethanol exerts general and significant anti-inflammatory effects in whole human AT *in vitro*, by inducing an increase in adiponectin and a parallel decrease in IL-6, IL-8, TNF- α , and MCP-1. Interestingly, incubation with ethanol induced a time-dependent IL-6 response with a significant short-term [1.5h] decrease, a plateau phase for 6-12h where IL-6 release returned to initial control values, after which a sustained and significant long-term [>24 h] decrease was observed (Fig. 2). Previous studies have found IL-6 release and mRNA expression to display a biphasic response upon various stimuli in human visceral AT cultures (Fain et al., 2005) and 3T3-L1 adipocytes (Fasshauer et al., 2003) supporting the observed findings on IL-6. The molecular pathways involved is suggested to via the receptor complex nuclear factor- κ B (NF- κ B), since acute or short-term [1.5h] ethanol treatment is reported to decrease IL-6 release from human isolated and LPS-stimulated monocytes via transient decrease in NF- κ B-activity (Mandrekar et al., 1997) and similarly, short-term [2-4h] incubation with specific inhibitors of the NF- κ B pathway is reported to decrease IL-6 release from human visceral AT cultures (Fain et al., 2005).

Alcohol (ethanol) intake is in observational studies found to exert a J-shaped pattern in relation to mortality with an increase in mortality in abstainers, a decrease in mortality in light to moderate drinkers primarily due to a reduction in cardiovascular disease, followed by a dose-dependent increase in mortality in heavy drinkers [>40 g ethanol/day] (Klatsky and Udaltsova, 2007). The background for this pattern is still obscure, however, an increasing number of human studies report a similar J-shaped pattern in relation to various inflammatory markers (Sacanella et al., 2002; Wannamethee et al., 2003; Imhof et al., 2004), suggesting that inflammation may be involved in the observed J-shaped relationship between ethanol and metabolic disease. This is of particular interest, since low-grade inflammation is reported to precede metabolic diseases such as insulin resistance, type 2 diabetes, atherosclerosis and cardiovascular disease (Despres and Lemieux, 2006; Dandona

et al., 2004). These observational studies are supported by recent rodent studies in which treatment with chronic and high concentrations of ethanol induce insulin resistance (Onishi et al., 2003; Kang et al., 2007), paralleled by an increase in AT-macrophage infiltration and a subsequent increase in AT-inflammation [*e.g.* increase in IL-6, TNF- α , MCP-1 and a decrease in adiponectin] (Kang et al., 2007). In line with the abovementioned rodent studies, recent human intervention studies have shown that moderate alcohol intake is able to decrease inflammation via an increase plasma levels of adiponectin by 10-15 % (Sierksma et al., 2004; Beulens et al., 2007), which is in agreement with our *in vitro* findings. Moreover, moderate alcohol consumption is reported to improve insulin sensitivity in relatively insulin resistant subjects, an effect that may be mediated via the stimulatory effect of alcohol on adiponectin (Sierksma et al., 2004; Thamer et al., 2004). Thus, in addition to weight loss (Yang et al., 2001; Madsen et al., 2008) and treatment with the antidiabetic drugs, the thiazolidinediones (Maeda et al., 2001), ethanol seems to be able to up-regulate endogenous adiponectin production in humans.

Adiponectin is rather specifically expressed in mature adipocytes but whole AT cultures as used in the present study consists of several other cell-types including inflammatory cells [*e.g.* monocytes, macrophages], known to infiltrate the AT in relation to the size and topographical localisation of the AT (Weisberg et al., 2003; Bruun et al., 2005; Canello et al., 2005). Several studies have found inverse interactions between adiponectin and adipokines such as IL-6 and TNF- α (Yokota et al., 2000; Bruun et al., 2003a; Suganami et al., 2005). The short term [1.5h] ethanol-induced up-regulation of adiponectin may be mediated directly via effects on the adipocytes *per se*. However, the observed correlation between CD68 and the pro-inflammatory adipokines [IL-6, IL-8, TNF- α , MCP-1], but not the anti-inflammatory adiponectin, confirms previous reports that the SV-fraction of the AT is the primary source of the inflammatory adipokines (Fain et al., 2004; Bruun et al., 2005) and suggests that the observed long term [24h] increase in adiponectin release and mRNA

expression may be mediated indirectly via paracrine loops in the AT involving especially TNF- α and IL-6, since these pro-inflammatory proteins was found to be down-regulated in parallel.

In response to the robust LPS-induced stimulation of IL-6, IL-8, TNF- α , and MCP-1 in the human AT incubations, ethanol exerted a significant and dose-dependent inhibition of all of the investigated adipokines. Although this finding is new in relation to AT incubations, it is essentially in line with several previous *in vitro* studies performed in human isolated monocytes (Szabo et al., 1995; Szabo et al., 1996; Szabo et al., 1999). LPS is a potent pro-inflammatory agent via an activation of the toll-like receptor4 (TLR4), which is reported to act as a central receptor probably mediating some of the anti-inflammatory effects induced by ethanol *in vitro* (Pruett and Pruett, 2006; Dai and Pruett, 2006). In addition to modulate TLR4 activity, ethanol is also reported to decrease the activity of NF- κ B *in vitro* (Szabo et al., 1999; Saeed et al., 2004; Klatsky and Udaltsova, 2007). The general decrease in inflammatory markers as found in the present study is probably caused by an alcohol-induced decrease in the activity in the NF- κ B system since activation of this system is known to induce the expression of adipokines in human AT as well as to induce preadipocyte proliferation (Lacasa et al., 2007). However, since adiponectin is reported to reduce the activity of the NF- κ B system (Ouchi et al., 2000), it remains to be elucidated whether the alcohol-induced decrease in IL-6, IL-8, TNF- α , and MCP-1 found in the present study is related directly to a decrease in NF- κ B activity or an adiponectin induced decrease in NF- κ B activity is also involved.

The concordant findings in human AT and human monocytes is probably related to the observed infiltration and activity of inflammatory cell-types such as monocytes and macrophages in human AT (Weisberg et al., 2003; Fain et al., 2004; Bruun et al., 2005). The increase in AT-resident macrophages is associated with adiposity as well as a state of AT-inflammation, potentially reversible through weight loss (Cancello et al., 2005; Bruun et al., 2006). In line with these

findings, we found robust correlations between CD68 and IL-6, IL-8, TNF- α , and MCP-1 but not adiponectin in the LPS-incubations. Even though the number of macrophages in the present incubations must be considered as constant ethanol significantly reduced mRNA expression of the macrophage surrogate marker CD68, suggesting that ethanol may reduce the inflammatory potential of the macrophages already present in the AT incubations and thereby supporting the overall anti-inflammatory findings in the present manuscript. The ethanol-concentrations used in the present incubations range from 11 mM to 88 mM, which is comparable to the concentrations used in previous *in vitro* studies (Szabo et al., 1995; Szabo et al., 1999) and known to be associated with a cell viability of ~90% *in vitro* (Friedman, 1998). *In vivo*, in humans, an ethanol concentration of 88mM may be lethal. The intake of ~24g (females) and ~36g (males) of ethanol will within 30min result in a peak circulating ethanol-concentration of ~11mM and up to 44mM will result in various degrees of intoxication, however, since ethanol consumption in humans is associated with a prompt degradation in the liver, it is difficult to extrapolate from the *in vitro* concentrations used in the present study to the actual ethanol concentration in the AT *in vivo*. In the present study, already low concentrations of ethanol [11mM] was found to decrease LPS-stimulated IL-6, IL-8, TNF- α , and MCP-1, and increase the anti-inflammatory adipokine adiponectin. The observed relationship between low concentrations of ethanol and a general decrease in inflammation is supported by other studies in which the anti-inflammatory adipokine, IL-10, was increased using similar ethanol concentration (Mandrekar et al., 1996; Szabo et al., 1996), high concentrations of ethanol oppositely reduced adiponectin mRNA expression in (rodent) epididymal AT (Kang et al., 2007), and recently human *in vivo* studies found low to moderate alcohol consumption to increase circulating adiponectin levels (Sierksma et al., 2004; Englund et al., 2006).

In conclusion, ethanol was found to exert significant anti-inflammatory effects in a time- and dose-dependent manner in human AT fragments *in vitro*. Even though excess ethanol consumption *in*

vivo by far exceeds the beneficial effects of low to moderate consumption as reported in several epidemiological studies, the findings presented may have clinical implications since they support previous *in vivo* findings that ethanol (in low to moderate concentrations) may be beneficial attenuating whole body low-grade inflammation which is a characteristic feature in patients with obesity.

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Conflicts of interest: the authors have nothing to declare.

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Legends to figures.

Figure 1

Effects of ethanol on adiponectin in human adipose tissue

Adiponectin release (Fig. 1A) and mRNA expression (Fig. 1B) was assessed after 24h in non-stimulated adipose tissue incubations with increasing ethanol concentrations [11mM - 88mM].

Data represent mean values \pm s.e.m. (N=7), with protein concentrations in [μ g/L] and mRNA control values set at 100. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ as compared to control values.

Figure 2

Effects of ethanol on IL-6 in non-stimulated human adipose tissue.

The effects of increasing ethanol concentrations [closed circle 11mM, open circle 22mM, closed triangle 44mM, open triangle 88mM] on IL-6 release was investigated in non-stimulated adipose tissue incubated up to 24h.

Data represent mean values \pm s.e.m., with control values set at 100 (N=8). * $p < 0.05$ and ** $p < 0.01$ as compared to control values.

Figure 3

Effects of ethanol on IL-6 in LPS-stimulated human adipose tissue.

The effects of increasing ethanol concentrations [11 mM - 88 mM] on IL-6 release was investigated in LPS-stimulated [50mg/L] adipose tissue incubated for 24h.

Data represent mean values \pm s.e.m. (N=9). * $p < 0.05$ and ** $p < 0.01$ as compared to control values.

Figure 4

Effects of ethanol on IL-8, TNF- α , and MCP-1 in LPS-stimulated human adipose tissue.

The effects of increasing ethanol concentrations [11 mM - 88 mM] on (A) IL-8, (B) TNF- α , and (C) MCP-1 release was investigated in LPS-stimulated [50mg/L] adipose tissue incubated for 24h.

Data represent mean values \pm s.e.m. (N=9). * $p < 0.05$ and ** $p < 0.01$ as compared to control values.

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Table 1

Correlation between CD68 and adipokines in LPS-stimulated AT-incubations *in vitro* (N=15).

| | CD68 mRNA |
|--------------------|--------------------------|
| Adiponectin mRNA | $r_p=0.09$ ($p>0.05$) |
| IL-6 mRNA | $r_p=0.81$ ($p<0.001$) |
| IL-8 mRNA | $r_p=0.52$ ($p<0.05$) |
| TNF- α mRNA | $r_p=0.76$ ($p<0.01$) |
| MCP-1 mRNA | $r_p=0.64$ ($p=0.01$) |

Fig. 1A

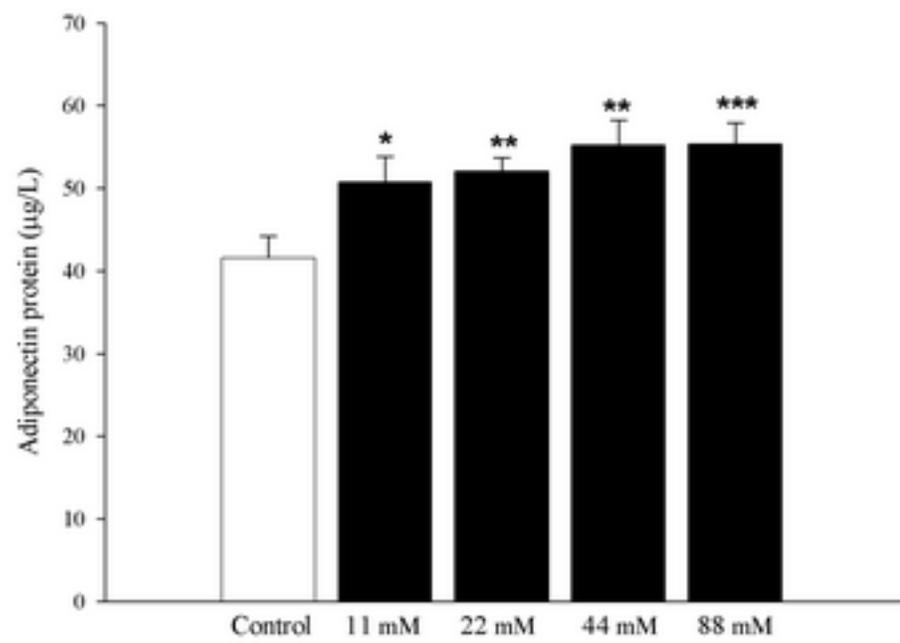


Fig. 1B

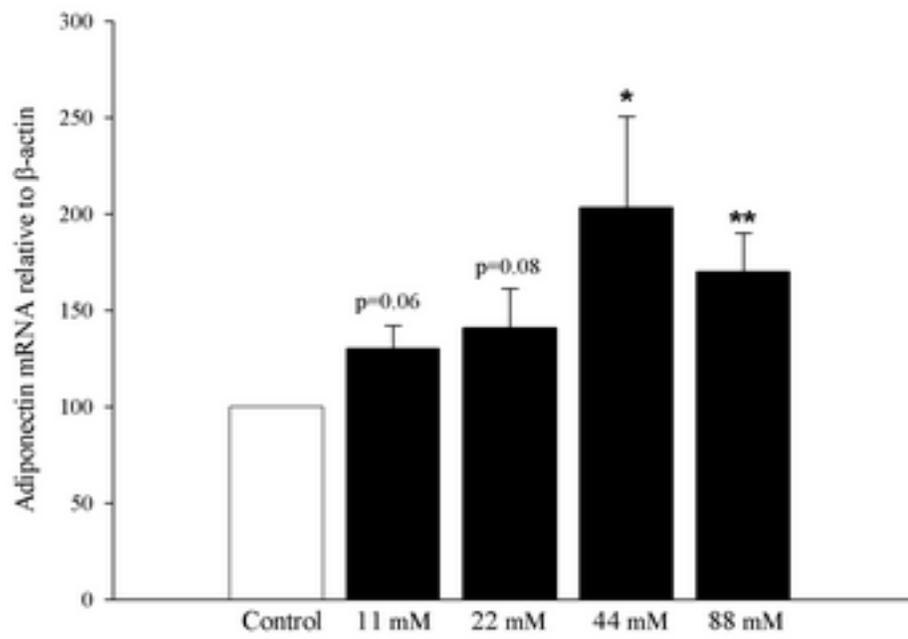


Fig. 2

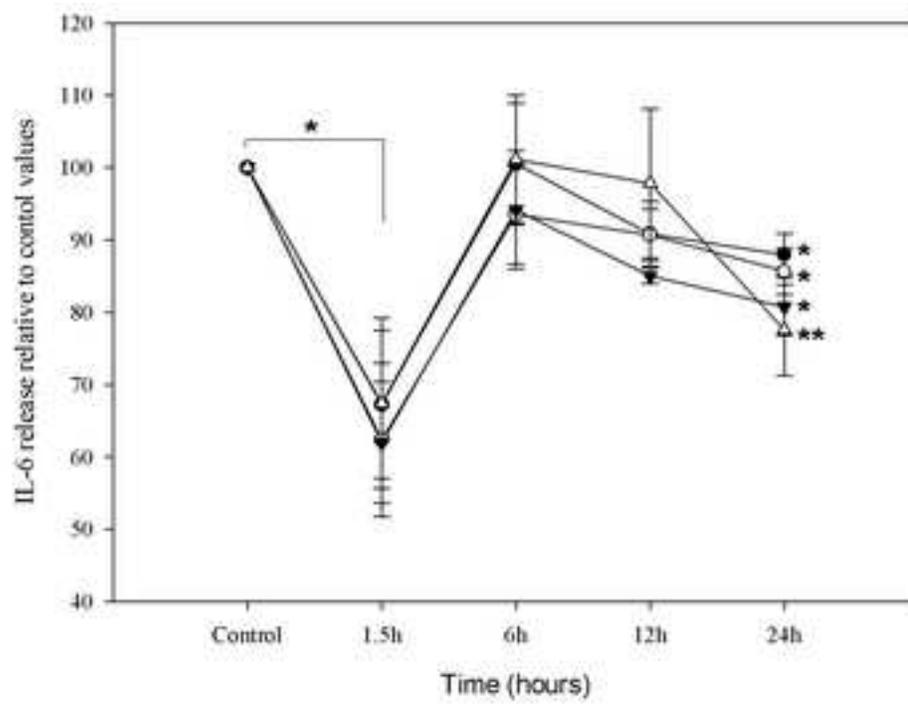


Fig. 3

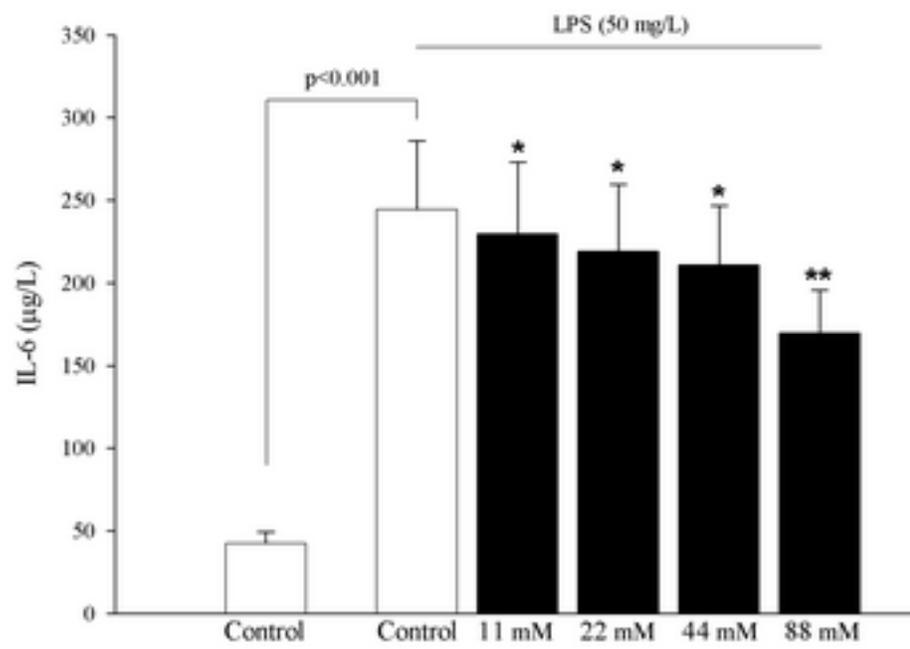


Fig. 4A

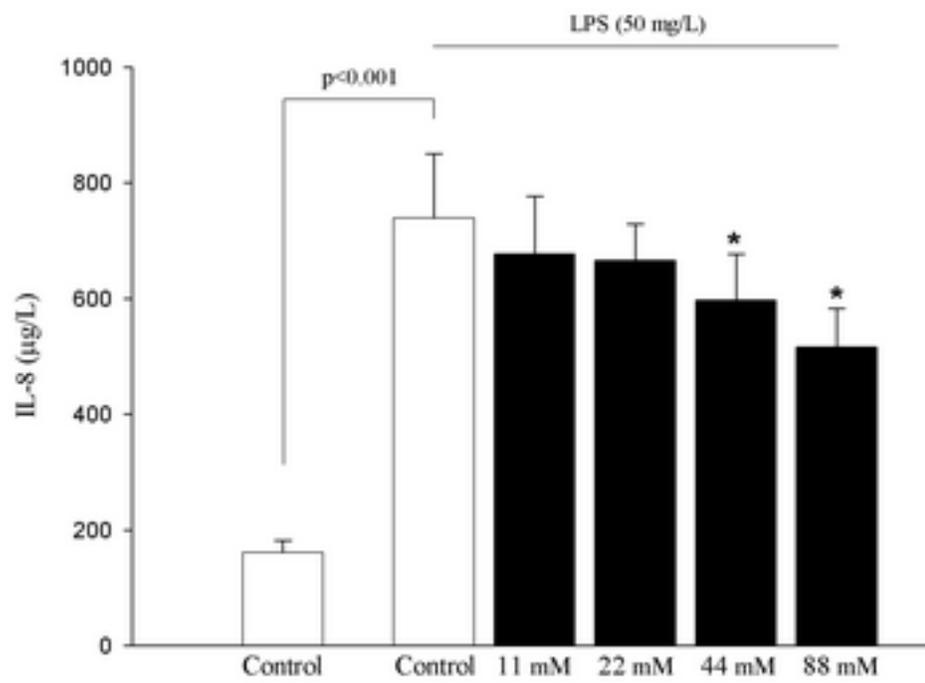


Fig. 4B

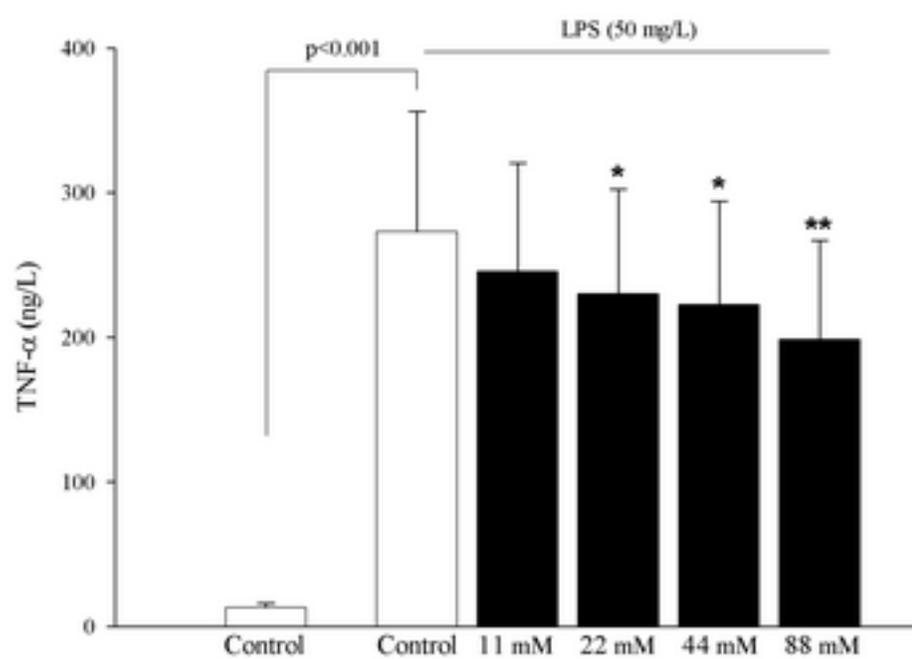


Fig. 4C

