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Mesenteric Lymphatic–Perilymphatic Adipose Crosstalk: Role in Alcohol-Induced Perilymphatic Adipose Tissue Inflammation

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Abstract

Background—The digestive tract lymphatics transport approximately two-thirds of all lymph produced in the body and have a key role in mucosal immunity through their contribution to antigen transport and immune cell trafficking. Mesenteric lymphatic pumping function integrity is critical for maintaining homeostasis and lipid transport. We previously demonstrated that acute alcohol intoxication (AAI) increases mesenteric lymphatic amplitude of contraction and ejection fraction, enhancing the ability of the lymphatic vessels to pump lymph. AAI has been shown to disrupt intestinal barrier integrity, which would be expected to increase the endotoxin content of mesenteric lymph. In this study, we tested the prediction that AAI increases lymphatic permeability directly affecting perilymphatic adipose tissue (PLAT) milieu.

Methods—Male Sprague Dawley rats received an intragastric infusion of 2.5 g/kg of alcohol. Isovolumic administration of water (vehicle) served as control. PLAT was isolated for the determination of Evans Blue extravasation (permeability), cytokine content, and immunohistochemistry for inflammatory cell infiltration at 30 minutes and 24 hours after alcohol administration.

Results—PLAT isolated from AAI animals had greater Evans Blue concentrations and cytokine expression (24 hours post-AAI) and mast cell and neutrophil density than that isolated from controls. AAI resulted in significantly higher plasma lipopolysaccharide (endotoxin) levels, lower plasma adiponectin levels (at 30 minutes), and unchanged plasma visfatin levels.

Conclusions—The data indicate that AAI induces mesenteric lymphatic hyperpermeability, promotes PLAT inflammatory milieu and disrupts the systemic adipokine profile. These findings suggest an association between alcohol-induced lymphatic hyperpermeability and early manifestations of metabolic dysfunction as a result of alcohol abuse. We propose that crosstalk between lymph and PLAT results in adipose inflammation and adipokine dysregulation during AAI.

Keywords

Ethanol; Lipopolysaccharide; Endotoxin; Adipokine; Lymphatic Permeability

Human and Rodent studies indicate that acute and chronic alcohol consumption disrupts intestinal permeability, which increases leakage of various macromolecules including endotoxins such as lipopolysaccharide (LPS) (Lambert et al., 2003; Mathurin et al., 2000; Parlesak et al., 2000) and thus promotes subclinical inflammation. Subclinical chronic inflammation has been identified as a significant contributor to insulin resistance (Adachi et al., 1995; Lambert et al., 2003; Lindtner et al., 2013), and more specifically, local inflammation of adipose tissue may be the sentinel event that causes systemic insulin resistance and inflammation (Smith, 2002; Wisse, 2004). Adipose tissue inflammation is also associated with decreased levels of adiponectin, an adipokine with insulin-sensitizing and anti-inflammatory properties, and increased levels of visfatin, a visceral fat-derived adipokine that has been shown to positively correlate with type II diabetes (Adeghate, 2008; Li et al., 2010). Recent rodent studies demonstrated that binge alcohol drinking induces insulin resistance and impairs insulin-dependent responses in adipose tissue (Kang et al., 2007; Lindtner et al., 2013). Moreover, alcohol binge drinking significantly increases the risk of metabolic syndrome (Carlsson et al., 2003; Fan et al., 2008; Lee, 2012). However, the mechanisms underlying adipose-induced metabolic dysregulation associated with alcohol binge drinking are unknown.

In the gastrointestinal (GI) tract, where more than half of daily lymph is formed (Alexander et al., 2010), collecting lymphatic vessels play a fundamental role in intestinal lipid uptake (Breslin, 2014; Unthank and Bohlen, 1988). Lymphatics that originate in the intestinal layers (lacteals in the villi, submucosal lymphatic network, and lymphatic network in the smooth muscle layer) coalesce into the common collecting lymphatics near the mesenteric border of the intestine (Miller et al., 2010). A link between lymphatic function and adipose biology was described. Mice possessing functional inactivation of a single allele of the homeobox gene *Prox1* developed adult-onset obesity due to abnormal lymph leakage from ruptured lymphatic vessels. Notably, animals with leaky lymphatics had increased mesenteric adipose tissue prior to presenting an increase in total body weight. These findings suggest an important role for the lymphatic system in fat deposition and inflammation (Harvey, 2008; Harvey et al., 2005).

Systemic LPS from the GI tract is primarily transported through the portal vein to the liver, where significant detoxification occurs, and through the lymphatic route directly into the systemic circulation, accounting for most of the systemic bioactive LPS (Azuma et al., 1983; Olofsson et al., 1986; Wang et al., 2010). Endotoxemia results in lymphatic vessel hyperpermeability (Brookes et al., 2009). The perilymphatic adipose tissue (PLAT) surrounding all collecting lymphatic vessels and lymph nodes is the proximal target for leaked macromolecules from lymphatic vessels. Thus, it is possible that PLAT has a critical role in alcohol-mediated immunomodulation (Pond, 2005) and metabolic dysregulation (Catalano et al., 2010; Rutkowski et al., 2009). Our previous studies focused on the impact of acute alcohol intoxication (AAI) on lymphatic contractile function (Souza-Smith et al., 2010, 2012, 2013). In these studies, we used isolated mesenteric collecting lymphatic vessels from rats that received an intragastric bolus of 2.5 g/kg of alcohol, mimicking an alcohol binge episode. Our results showed that AAI decreases contraction frequency and increases mesenteric lymphatic amplitude of contraction and ejection fraction, enhancing the ability of the lymphatic vessels to pump lymph (Souza-Smith et al., 2010). These results,

together with the reported alcohol-induced increase in gut LPS trans-location (Lambert et al., 2003; Parlesak et al., 2000), suggest greater LPS dissemination to the systemic circulation via lymphatic vessels during AAI. We hypothesize that AAI induces lymphatic hyperpermeability and increases LPS leak from lymphatic vessels, promoting PLAT inflammation and altered adipokine profile. We believe these derangements in mesenteric lymphatic function may be an initial pathophysiological mechanism contributing to alcohol-induced metabolic dysregulation. We propose that lymphatic-derived macromolecules modulate PLAT inflammatory and adipokine profiles, which over time, contributes to metabolic dysregulation culminating in insulin resistance.

MATERIALS AND METHODS

Animals

All animal studies were approved by the Institutional Animal Care and Use Committee at the Louisiana State University Health Sciences Center and were performed in accordance with the guidelines of the NIH Guide for the Care and Use of Laboratory Animals (8th edition). Male Sprague Dawley rats (270 to 350 g body wt) were housed in a controlled temperature (22°C) and controlled illumination (12:12 hour light–dark cycle) environment. After arrival, the rats were allowed a 1-week acclimation period and were provided standard rat chow (2018 Teklad Global 18% Protein Rodent Diet; Harlan, Indianapolis, IN) and water ad libitum.

Gastric Catheter Placement and Alcohol Administration Protocol

AAI was induced as previously described (Phelan et al., 2002; Souza-Smith et al., 2010, 2013). Rats were anesthetized with ketamine and xylazine (90 and 9 mg/kg, respectively). A sterile catheter was aseptically placed into the antrum of the stomach and exteriorized at the nape of the neck. Following a 2-day recovery period from the surgical procedure, conscious and unrestrained animals received an intragastric bolus of 30% ethanol (EtOH; 2.5 g/kg) via the gastric catheter, mimicking a binge-drinking episode. We have previously shown this intragastric administration of EtOH to produce blood alcohol levels of 200 to 300 mg/dl within 30 minutes of administration (Souza-Smith et al., 2010). A time-matched control group received isovolumic intragastric administration of vehicle (water). We have previously shown that there are no differences between water and dextrose administration in this experimental setting (Souza-Smith et al., 2010). The animals were sacrificed either 30 minutes or 24 hours after AAI. Two time points were used because it has been shown that when alcohol is present in the system, or not completely metabolized, there is an acute alcohol-induced suppression in pro-inflammatory cytokines (Bhatty et al., 2011).

Determination of Lymphatic Permeability

Alcohol and vehicle were diluted in a solution of 1% Evans Blue dye and administered via an intragastric catheter. Thirty minutes after intragastric administration of alcohol or vehicle mixed in Evans Blue, and the animals were sacrificed as previously described (Souza-Smith et al., 2010). Briefly, rats were anesthetized with ketamine and xylazine (90 and 9 mg/kg, respectively), a midline laparotomy was performed, and the gut with associated mesentery was excised and pinned in a dissection chamber containing 4°C albumin physiological salt

solution. PLAT was carefully excised from the mesentery, free of connective tissue, large blood vessels, and lymphatic vessels and was stored at -80°C until Evans Blue concentration was measured (Sawane et al., 2013). PLAT was homogenized (40 mg of PLAT/100 μl of formamide) and incubated for 24 hours at 37°C . Tissues were centrifuged for 10 minutes at $23,447 \times g$ at room temperature, and the aqueous infranatant lying beneath the fat was collected. An Evans Blue standard curve was generated from serial dilutions of a 1 mg/ml stock (10, 1, 0.1, 0.01, and 0.001 $\mu\text{g}/\text{ml}$). Evans Blue concentration from PLAT digest was spectrophotometrically assessed (620 nm) based on the standard curve and normalized by PLAT weight (Sawane et al., 2013).

Cytokine and Adipokine Measurements

PLAT and blood samples were obtained at 30 minutes or 24 hours after alcohol or vehicle administration. PLAT (150 mg) was isolated per animal, and homogenized in 1 ml/100 mg of tissue of tissue protein extraction reagent (T-PER; ThermoScientific, Rockford, IL) buffer containing protease inhibitors. Homogenates were used in an ELISArray kit (Rat Inflammatory Cytokines Multi-Analyte ELISArray Kit; Qiagen, Valencia, CA) to measure 12 different cytokines (interleukin [IL]-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, interferon [IFN]- γ , tumor necrosis factor [TNF]- α , granulocyte-macrophage colony-stimulating factor [GM-CSF], and RANTES) from control and AAI groups 30 minutes and 24 hours postalcohol administration. Circulating adiponectin (EDM Millipore, Billerica, MA) and visfatin (EIA Raybiotech, Norcross, GA) were measured from blood plasma samples using commercially available rat ELISAs.

PLAT Mast Cell and Neutrophil Content

In a separate group of animals treated as described above, mesenteric branches containing adipose tissue, blood, and lymphatic vessels were isolated and fixed in 4% paraformaldehyde, processed for paraffin embedding, and sectioned at a thickness of 5 μm . Toluidine blue (Sigma-Aldrich, St. Louis, MO) was used for histological staining of mast cells (Poglio et al., 2010). Antineutrophil antibody (NIMP-R14; Abcam, Cambridge, MA) was used for immunohistochemical detection of neutrophils. Immunolocalization of neutrophils was performed as described (Elgazar-Carmon et al., 2008), and by cell size and nuclear morphology, control experiments were performed by omitting the primary antibody. Representative color micrographs were obtained from tissue sections (7 to 13/group) using $10\times$ objectives for mast cell analyses and $60\times$ for neutrophil analyses. Slides were imaged in a blinded manner using an Olympus DP72 Digital Camera System mounted to an Olympus BX51 TRF Microscope (Olympus, Center Valley, PA).

LPS Measurement

Animals were treated as described above, and plasma was isolated and stored at -80°C until analysis. A commercial LPS ELISA kit (Biomatik, Wilmington, DE) was used to measure plasma levels of circulating LPS. Plasma (50 μl) was used undiluted and diluted 1:5 in the assay according to the manufacturer's protocol. Colorimetric measurements (450 nm) were performed on an xMark Spectrophotometer (Bio-Rad Laboratories, Hercules, CA). Values were compared to a standard curve of LPS (1:3 serial dilutions) ranging from 12.35 to 1,000 ng/ml.

Statistical Data Analysis

Summarized data are presented as mean $\pm$ SEM and the *N* indicated. Student's *t*-test was used to detect significant differences between 2 groups (control vs. alcohol at 30 minutes or at 24 hours post-AAI). GraphPad Prism 4.0 Software (GraphPad, La Jolla, CA) was used, and data were considered statistically significant at $p < 0.05$.

RESULTS

AAI Increases Mesenteric Collecting Lymphatic Permeability

Lymphatic permeability was determined by Evans Blue extravasation into PLAT. Evans Blue concentration was significantly increased in PLAT from AAI animals in comparison with PLAT from control animals 30 minutes post-AAI or vehicle administration, suggesting increased lymphatic leak (Fig. 1A). Figure 1B illustrates Evans Blue in mesenteric lymphatic vessel.

AAI Promotes PLAT Inflammatory Milieu

Adipose inflammatory profiles were measured in PLAT isolated 30 minutes and 24 hours post-AAI. To dissect the acute direct effects of alcohol from those that may be more long lasting, PLAT cytokines were also measured 24 hours after AAI. Our results did not detect significant differences in PLAT cytokine content between vehicle- and alcohol-administered animals at 30 minutes post-AAI (data not shown). However, the expression of anti-inflammatory cytokines (IL-4, IL-10, and IL-13), pro-inflammatory cytokines (IL-6, TNF- α , and GM-CSF), and chemokine (RANTES) was greater in PLAT from AAI animals compared to control animals at 24 hours post alcohol administration (Fig. 2). Table 1 shows the average of PLAT cytokine raw absorbance values for the 4 groups studied.

AAI Promotes Immune Cell Recruitment to PLAT

Perilymphatic adipose tissue mast cells and neutrophils were quantified and averaged per field. Representative micrographs of PLAT stained with Toluidine blue for mast cells and immunohistochemistry for neutrophils are shown in Figs 3 and 4, respectively. An increase in the average number of mast cells (Fig. 3) and neutrophils (Fig. 4) per field was observed in PLAT of AAI animals at 30 minutes and 24 hours post-AAI compared to PLAT from vehicle-administered animals.

AAI Disrupts Adipokine Expression

To evaluate the role of AAI on adipokine profiles, we measured circulating levels of adiponectin and visfatin. Both adipokines are related to adipose tissue inflammation, whereby in the presence of inflammation adiponectin is decreased and visfatin is increased (Adeghate, 2008; Li et al., 2010). AAI resulted in significantly lower plasma adiponectin levels (~23%) at 30 minutes. Although not significant, adiponectin levels were still reduced by approximately 14% 24 hours post-AAI (Fig. 5A). No significant changes in circulating visfatin were noted between the groups at 30 minutes or 24 hours after AAI or vehicle administration (Fig. 5B).

AAI Increases Circulating LPS Levels

To our knowledge, the contribution of GI-derived LPS to AAI-mediated lymphatic permeability, PLAT inflammatory milieu, and adipokine profile has never been investigated. To confirm that AAI disrupts intestinal barrier integrity, plasma LPS levels were measured. Circulating LPS was significantly increased at 30 minutes and 24 hours after alcohol administration in comparison with vehicle-administered controls (Fig. 6).

DISCUSSION

We have investigated the impact of a single bolus of binge alcohol administration on gut lymphatic permeability, PLAT cytokine and adipokine expression, and circulating LPS levels. Our results show that PLAT from alcohol-administered animals had greater concentration of Evans Blue compared to controls, indicating greater lymphatic permeability. PLAT pro-inflammatory cytokine production was greater in PLAT from alcohol compared to vehicle-administered animals at 24 hours postalcohol administration. PLAT mast cell and neutrophil densities were significantly greater in alcohol-administered animals than in controls. AAI was associated with decreased circulating levels of adiponectin, but no change in visfatin was observed at 30 minutes or 24 hours after alcohol administration. Finally, AAI animals had significantly increased circulating levels of LPS compared to controls. These results suggest that AAI-induced mesenteric lymphatic hyperpermeability promotes a PLAT inflammatory milieu and impairs adipokine profiles in PLAT. The gut-derived endotoxins, specifically LPS, could be directly mediating these early alterations in the PLAT milieu and reflecting the lymphatic/PLAT crosstalk that we predict disrupts metabolic regulation and contributes to increased risk for systemic alcohol-induced insulin resistance.

Our previous studies show that AAI alters mesenteric collecting lymphatic pump function, decreasing mesenteric lymphatic tone, and increasing lymphatic ejection fraction (Souza-Smith et al., 2010). These alterations enhance the ability of lymphatics to transport lymph during the alcohol-intoxicated state, which we predict favors LPS lymphatic dissemination. Binge drinking causes a rapid rise in circulating LPS (Abdelmegeed et al., 2013), but the contribution of LPS dissemination via the lymphatic route during binge alcohol consumption has not been well characterized. Although lymphatic vessels display unidirectional and inward uptake of large molecules, collecting lymphatics also allow the leakage of large molecules into interstitial spaces during endotoxemia, reflecting the function of collecting lymphatics as exchange vessels (Cromer et al., 2014). Our studies demonstrate an increase in mesenteric lymphatic permeability, and we expect this lymphatic hyperpermeability to result in lymph leak, potentially impacting surrounding tissues, especially PLAT (Sawane et al., 2013). Lymph leakage to PLAT will stimulate a PLAT inflammatory milieu and promote pre-adipocyte differentiation and accumulation of adipocytes (Harvey et al., 2005). Our working hypothesis is that alcohol-induced increase in gut-derived endotoxins, such as LPS, promotes lymphatic leak leading to lymphatic-PLAT crosstalk, promoting PLAT inflammation.

Dysfunctional adipose tissue is a key mediator of insulin resistance (Smith, 2002). Adipose tissue-derived inflammatory cytokines have been implicated in the induction of insulin

resistance via inhibition of adipose tissue insulin signaling (Ruan and Lodish, 2003). Additionally, endotoxemia and bacterial translocation initiate an inflammatory cascade in adipose tissue that is characterized by pro-inflammatory cytokine secretion (Creely et al., 2007). It is also worth noting that very recent findings show acute binge drinking to increase serum endotoxin and bacterial DNA levels in humans (Bala et al., 2014). Furthermore, 3-day binge-like alcohol intoxication induces whole-body insulin resistance, suggesting that binge drinking could set the stage for metabolic syndrome and type II diabetes (Lindtner et al., 2013). Our results show that a single episode of AAI is sufficient to produce marked increases in PLAT inflammatory cytokines, promote immune cell recruitment, decrease circulating adiponectin levels, and increase circulating LPS levels, implying a potential role of alcohol in metabolic dysregulation. The increased inflammatory response in PLAT could be a consequence of alcohol-induced lymphatic vessel hyperpermeability, allowing lymph contents to leak into PLAT. As previously noted, the lymphatic system may be the primary means of dissemination of LPS, rather than dissemination through portal circulation (Azuma et al., 1983; Konrad and Wueest, 2014). Although we did not measure lymph LPS due to technical constraints of the rat model, we observed an increase in circulating LPS levels, which suggests that lymph LPS levels may also have been increased. Therefore, it is plausible and likely that lymph-derived LPS contributes to PLAT inflammation. This hypothesis is supported by studies showing that high-fat-diet-induced increases in circulating LPS is an early event preceding metabolic dysregulation (Cani et al., 2007). For example, a short exposure to high-fat diet leading to a “prediabetic” state increases bacterial translocation to mesenteric adipose tissue, suggesting the development of diet-induced metabolic dysregulation (Amar et al., 2011).

Evidence suggests that adipocytes and recruited immune cells participate in the pathogenesis of inflammation-induced insulin resistance (Shoelson et al., 2006). Thus, immune cell recruitment may also contribute to PLAT inflammation. Mast cells express an array of receptor systems to recognize and remove pathogens. Once activated, mast cells release mediators with proinflammatory, enzymatic, vasoactive, antimicrobial, and anticoagulant properties (Altintas et al., 2011). Mast cells are abundant in visceral fat of obese mice (Altintas et al., 2011). Neutrophils are the largest fraction of white blood cells, and adipose tissue neutrophils produce cytokines and chemokines, thus potentially contributing to macrophage infiltration and adipose inflammation (Talukdar et al., 2012). Increased visceral fat mast cells, neutrophil, and macrophage infiltration has been shown to be associated with insulin resistance in rodents (Altintas et al., 2011; Elgazar-Carmon et al., 2008). Our results show an increase in mast cells and neutrophils in the PLAT from alcohol-administered animals compared to controls, suggesting that alcohol-induced immune cell recruitment to PLAT could also be contributing to PLAT inflammation. Whether these cells are recruited by the systemic vasculature or from lymphatic vessels remains to be explored.

Adipose tissue-derived inflammatory cytokines have been implicated in the induction of insulin resistance via inhibition of adipose tissue insulin signaling (Ruan and Lodish, 2003). Adiponectin and visfatin, adipokines expressed in visceral adipose tissue, have contrasting roles in insulin sensitivity (Adeghate, 2008; Li et al., 2010). Low daily doses of alcohol have been reported to lead to ~12.5% enhancement of circulating adiponectin levels. In contrast, higher doses of alcohol result in about ~20 to 25% decrease in adiponectin levels (Beulens et

al., 2006; Xu et al., 2003), which is speculated to be the result of either direct alcohol effects on adipocytes or indirect effects through modulation of cytokine production levels (Xu et al., 2003). Adiponectin also has potent immuno suppressive properties, with critical relevance to cytokine regulation by stimulating anti-inflammatory mediators and suppressing pro-inflammatory cytokines (Wolf et al., 2004). The latter might be the case in the present study. Here, we observed that a single episode of alcohol intoxication reduced circulating adiponectin levels by approximately 23% (30 minutes post-AAI) before an increase in PLAT cytokine expression (24 hours post-AAI) was detected. A possible speculation is that the preceding decrease in adiponectin levels contributes to the PLAT inflammatory milieu seen 24 hours post-AAI. Visfatin is an adipokine that is increased in obesity and diabetes (Pravdova and Fickova, 2006). Although we did not find significant differences of visfatin levels in between AAI and controls at the time points studied, there is insufficient evidence to rule out the importance of this adipokine in alcohol-induced insulin resistance, especially in multiple binge or chronic exposure. The importance of visfatin in alcohol-induced insulin resistance warrants further investigation.

A link between visceral adipose tissue phenotype and insulin resistance has been proposed (Bergman et al., 2006; Cruz et al., 2002; Frayn, 2000), but the underlying mechanisms remain to be elucidated. Moreover, the influence of PLAT inflammatory milieu, induced by binge alcohol administration, on metabolic dysregulation is unknown. It is important to note that different abdominal fat depots exhibit unique features, such as different metabolic properties and venous drainage (Golan et al., 2012). Mesenteric adipose tissue or PLAT and omental fat (negligible amount in mice and rats) (Murano et al., 2008) are drained by the portal vein (Item and Konrad, 2012). As a result, free fatty acids, cytokines, and adipokines released from PLAT directly to the liver may impact metabolism.

In summary, this study provides evidence that 1 episode of AAI induces lymphatic vessel hyperpermeability, promoting PLAT inflammation, and alters adipokine profile. To our knowledge, there are no previous reports on the role of lymphatic-PLAT crosstalk as a mechanism underlying pathophysiological responses to binge alcohol administration. Our study uses a novel approach that links lymphatic function and PLAT and the results suggest potential mechanisms of alcohol-induced metabolic dysregulation. Insights into how AAI disrupts the visceral adipose tissue milieu, particularly how constituents leaked from lymphatic vessels contribute to PLAT-induced insulin signaling impairment leading to the development of systemic insulin resistance are the focus of our future studies.

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Fig. 1. (A) Perilymphatic adipose tissue (PLAT) Evans Blue concentration expressed as percent of control animals. (B) Representative image of mesenteric lymphatic vessels containing Evans Blue (arrows) surrounded by PLAT. Red indicates mesenteric blood vessels. Values are mean $\pm$ SE, $N = 4$ /group. * $p < 0.05$ versus control.

Fig. 2. Perilymphatic adipose tissue (PLAT) cytokine and chemokine expression at 24 hours postalcohol or vehicle administration. Values are mean $\pm$ SE, $N = 3$ /group. * $p < 0.05$ versus control.

Fig. 3.

Distribution of perilymphatic adipose tissue (PLAT) mast cells as seen with Toluidine blue staining. Representative image of PLAT field analyzed (10×) showing PLAT surrounding lymphatics and blood vessels (**far left**). Mast cells (shown by arrow) in PLAT from control (11 fields—**left**) and alcohol animals (13 fields—**right**) at 30 minutes in **top panels** and control (10 fields—**left**), and alcohol animals (12 fields—**right**) at 24 hours in **bottom panels**. The average number of mast cells per field is greater in acute alcohol intoxication (AAI) animals when compared to control groups (**far right**). Values are mean $\pm$ SE, $N = 4$. * $p < 0.05$ versus time-matched control.

Fig. 4.

Immunolocalization of neutrophils in perilymphatic adipose tissue (PLAT) using anti-NIIMP-R14 antibody. Representative image of negative control (**far left**).

Immunolocalization of neutrophils (shown by arrow) per field (60×) in PLAT from groups: control 30 minutes (7 fields—**top left**), alcohol 30 minutes (7 fields—**top right**), control 24 hours (6 fields—**bottom left**), and alcohol 24 hours (6 fields—**bottom right**).

Immunolocalization was performed in an average of 3 areas per field. High average number of neutrophils per field was observed in alcohol treated animals at 30 minutes and 24 hours postintoxication when compared with control groups (**far right**). Values are mean $\pm$ SE, $N=4$. * $p < 0.05$ versus time-matched control.

Fig. 5. Plasma levels of adiponectin (**A**) and visfatin (**B**) in acute alcohol intoxication (AAI) and control animals at 30 minutes and 24 hours post-AAI. Values are mean $\pm$ SE, $N = 6$ to 7 /group. * $p < 0.05$ versus time-matched controls.

Fig. 6.

Plasma levels of lipopolysaccharide (LPS) in acute alcohol intoxication (AAI) and control animals at 30 minutes and 24 hours post-AAI. Values are mean $\pm$ SE, $N = 4$ to 7/group. * $p < 0.05$ versus time-matched controls.

Table 1

Average of Perilymphatic Adipose Tissue (PLAT) Cytokine Raw Absorbance Values Normalized by PLAT Weight (g) of all 4 Groups

Absorbance/ PLAT (g)	Control 30 minutes	AAI 30 minutes	Control 24 hours	AAI 24 hours
IL-1 α	6.79	5.64	5.00	9.38
IL-1 β	10.53	7.61	6.86	14.60
IL-2	12.90	8.83	11.25	20.53
IL-4	5.90	4.72	5.09	8.99
IL-6	13.30	10.23	11.20	22.62
IL-10	11.86	8.67	9.77	18.48
IL-12	10.62	8.12	8.96	18.06
IL-13	6.54	4.95	4.88	8.50
IFN- γ	9.01	6.98	7.14	13.78
TNF- α	12.49	10.27	11.11	18.62
GM-CSF	6.39	5.23	5.37	9.82
RANTES	6.75	4.98	5.60	9.39

AAI, acute alcohol intoxication.