Published in final edited form as:

Alcohol Clin Exp Res. 2011 May ; 35(5): 939–945. doi:10.1111/j.1530-0277.2010.01424.x.

Effects of acetaldehyde on hepatocyte glycerol uptake and cell size: implication of Aquaporin 9

James J. Potter, B.S.¹, Ayman Koteish, M.D.¹, James Hamilton, M.D.¹, Xiaopu Liu, B.S.¹, Kun Liu, Ph.D.¹, Peter Agre, M.D.¹, and Esteban Mezey, M.D.¹

¹Department of Medicine, The Johns Hopkins University School of Medicine, Bloomberg School of Public Health, Baltimore, MD 21205-2195

²Malaria Research Institute, Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Baltimore, MD 21205-2195

Abstract

Background—The effects of ethanol and acetaldehyde on uptake of glycerol and on cell size of hepatocytes and a role Aquaporin 9 (AQP9), a glycerol transport channel, were evaluated.

Methods—The studies were done in primary rat and mouse hepatocytes. The uptake of [¹⁴C] glycerol was determined with hepatocytes in suspension. For determination of cell size, rat hepatocytes on coated dishes were incubated with a lipophilic fluorochrome that is incorporated into the cell membrane and examined by confocal microscopy. A three dimensional z scan of the cell was performed, and the middle slice of the z scan was used for area measurements.

Results—Acute exposure to acetaldehyde, but not to ethanol, causes a rapid increase in the uptake of glycerol and an increase in hepatocyte size, which was inhibited by HgCl₂, an inhibitor of aquaporins. This was not observed in hepatocytes from AQP9 knockout mice, nor observed by direct application of acetaldehyde to AQP9 expressed in *Xenopus Laevis* oocytes. Prolonged 24 hours exposure to either acetaldehyde or ethanol did not result in an increase in glycerol uptake by rat hepatocytes. Acetaldehyde decreased AQP9 mRNA and AQP9 protein, while ethanol decreased AQP9 mRNA but not AQP9 protein. Ethanol, but not acetaldehyde, increased the activities of glycerol kinase and phosphoenolpyruvate carboxykinase.

Conclusions—The acute effects of acetaldehyde, while mediated by AQP9, are probably influenced by binding of acetaldehyde to hepatocyte membranes and changes in cell permeability. The effects of ethanol in enhancing glucose kinase, and phosphoenolpyruvate carboxykinase leading to increased formation of glycerol-3-phosphate most likely contribute to alcoholic fatty liver.

Keywords

acetaldehyde; ethanol; glycerol; cell size; Aquaporin 9

Aquaporins are membrane proteins that serve as channels that facilitate movement of water across membranes. Aquaporins 7 and 9, which transport glycerol as well as water, are present in the adipocytes and the liver respectively (Maeda et al., 2008). The coordinated regulation of these two glycerol channels leads to release of glycerol from the adipocytes and its uptake by the liver (Maeda et al., 2008). Glycerol is a precursor of gluconeogenesis and a direct source of glycerol-3-phosphate for triglyceride synthesis (Reshef, et al., 2003).

Ballooning of hepatocytes is a common feature of alcoholic steatohepatitis (Lefkowitz, 2005). The ballooning is most likely due to a combination of protein and water retention by the hepatocytes. The purpose of this study was to determine the effects of ethanol and acetaldehyde on hepatocyte uptake of glycerol and on cell size and the role of aquaporin 9 in these changes.

MATERIALS AND METHODS

Materials

Male Sprague Dawley rats were obtained from Charles River laboratories (Wilmington, MA). Aquaporin 9 knockout mice (AQP9 KO) and wild-type mice were provided by K.L. and P.A. from our Institution (Rojek, et al., 2007). The animals received human care in compliance with the guidelines of the Animal Care and Use Committee of the Johns Hopkins University. Fetal bovine serum (FBS) was purchased from Life Technologies, Inc. (Gaithersburg, MD). Acetaldehyde was from Fisher Scientific (Pittsburgh, PA).

Cell Culture

Hepatocytes were isolated by in situ perfusion through the portal vein as described previously (Mezey, et al., 1986). Rat or mouse hepatocytes were cultured in 25-cm² tissue culture flasks precoated with bovine type I collagen in DMEM containing 10% FBS, fungizone (2.5 µg/ml), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 °C with a humidified atmosphere of 5 % CO₂ and 95 % air.

For experiments with acetaldehyde, the media was first changed to serum-free low glucose (1 gm/L) DMEM containing the following six supplemental growth factors (SGF6): epidermal growth factor (10 µg/L), transferrin (0.5 mg/L), selenous acid (5 µg/L), linoleic acid (0.5 mg/L), bovine serum albumin (0.5 mg/L), and fetuin (0.5 mg/L). After 18 h acetaldehyde was added to a final concentration of 200 µM together with 4-methylpyrazole (0.1 mM) and cyanamide (50 µM) and the flasks were tightly capped. The cells were spiked with acetaldehyde at 12 h for total treatment time of 24 h and harvested at 24h.

Glycerol uptake

The effects of ethanol and acetaldehyde on glycerol uptake were determined in isolated rat hepatocytes in suspension in DMEM in capped flasks. Acetaldehyde (200 µM), ethanol (100 mM), or isovolumetric buffer was added to the hepatocyte suspension and the uptake of glycerol determined immediately or after 24 hour incubation which in the later case included spiking with acetaldehyde at 12 hours. Thereafter, the determination of glycerol uptake was started by the addition of 1 mM unlabelled glycerol and [¹⁴C] glycerol 0.3 µCi/ml (1 Ci (= 37 GBq/mmol) (Amersham Biosciences, Piscataway, NJ). To determine the effects of HgCl₂, an inhibitor of aquaporins, hepatocyte suspensions were preincubated for 15 min with 0.1 mM HgCl₂ prior to the addition of the [¹⁴C] glycerol. Four separate flasks were used for each of the variables at each harvest time. The cell suspension was gently rotated on a roller until harvesting of the cells. At harvesting, the cell suspensions were removed and centrifuged at 10,000g for 1 min through dibutyl phthalate to separate the cells from the media (Fariss et al., 1985) and to stop the uptake of glycerol. The cells are collected in a 10% perchloric acid layer which is beneath the dibutyl phthalate and the radioactivity in the cell lysate determined in a scintillation counter (Promeneur et al., 2007).

Glycerol permeability coefficient in AQP9 oocytes

The acute effect of acetaldehyde (200 µM) on the glycerol permeability coefficient was determined in *Xenopus Laevis* oocytes which had been injected with 5 ng of rat AQP9 cRNA and expressed AQP9 (AQP9 oocytes) and in empty control oocytes. The

acetaldehyde was added immediately after the addition of the glycerol and the swelling of the cells was monitored by video microscopy as described previously (Carbrey et al. 2003).

Cell size measurement

The effects of acetaldehyde were determined on cell areas. Primary rat or mouse hepatocytes were grown on 35mm glass bottom poly-D-lysine coated dishes (MatTek Corporation, Ashland MA). The cells were incubated for 15 min with 10 μ g of the lipophilic fluorochrome 1,1'-dioctadecyl-3-3'-3'-tetramethylindo-carbocyanine perchlorate (Invitrogen, Eugene OR), dissolved in 10 μ L of dimethyl sulfoxide (DMSO) that is incorporated into the cell membrane. The cells were then washed with PBS and examined by laser confocal microscopy at 561 nm, the emission maximum of the fluorochrome. Serial images were captured every 30 seconds for 5 min and exported from the Zeiss LSM Image software for analysis by MetaMorph Image Analysis software. The change in area over time for individual cells was determined for both control and acetaldehyde treated cells. A three dimensional z scan of the cell was performed, and the middle slice of the z scan was used for the area measurements. The areas are expressed as a percentage of the zero time measurement.

Determination of messenger RNA by real time quantitative polymerase chain reaction RT-qPCR

A 7900 HT (Applied Biosystems, Foster City, CA) and SDS 2.2.1 software was used to perform RT-qPCR at the The Johns Hopkins DNA Analysis Facility. Total cellular RNA from a portion of liver was isolated and purified using RNA STAT 60 reagent, and following their protocol. The concentration of the isolated RNA was determined from the optical density at 260nm and its purity from the 260nm/280nm OD ratio. The isolated RNA was stored at -80° C. RT-qPCR for Aquaporin-9 (AQP9), peroxisome proliferator-activated receptor α (PPAR α), glycerol kinase and phosphoenol pyruvate carboxykinase mRNA were performed using sequence-specific probes from TaqMan gene expression assays of Applied Biosystems. Probes for mouse AQP9, PPAR α glycerol kinase, cytosolic phosphoenolpyruvate carboxykinase and β -actin (as endogenous control) were obtained from Applied Biosystems. Superscript III first strand synthesis from Invitrogen (Carlsbad, CA) was used to synthesize first strand cDNA from the purified RNA. Gene-transcript levels of the above probes were compared to β -actin, the housekeeping endogenous control. Variation in the amount of the transcripts was corrected by the level of expression of the β -actin gene in each individual sample.

Western Blot Analysis—The cells were lysed in NP-40 lysis buffer containing 50 mM Tris-HCl buffer, pH 8.0, 400 mM NaCl, 5 mM EDTA, 1% NP-40, 1 mM PMSF, protease inhibitor cocktail (Roche Diagnostics, GMBH, Mannheim, Germany) for 1 h at 4° C and then centrifuged at 3,000g for 10 min at 4° C. The cytosol protein in the supernatant was initially stored at -80° C. The proteins were separated on mini-SDS gels at 100 V for 1 h and electrotransferred to nitrocellulose transblot membranes (BioRad, Hercules, CA). The membranes were washed in PBS, pH 7.6, containing 0.1% Tween 20 (PBS-T), blocked with 5% (W/V) dry nonfat milk in PBS-T for 1 h, rinsed with PBS-T and then incubated with rabbit anti-rat antibodies to AQP9, PPAR α , and β actin, obtained from Santa Cruz Biotechnology, Inc, Santa Cruz, CA. After repeated washing, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000 dilution; Amersham Biosciences) at room temperature for 1 h. The membranes were then washed again and visualized by enhanced chemiluminescence reaction (ECL Plus; Amersham Biosciences). Densitometry was determined using Image J v 1.30 obtained from NIH.

Glycerol kinase activity

Glycerol kinase activity was determined in the hepatocyte homogenates with [¹⁴C] glycerol as described by Lamb et al., 1977. The radioactivity in glycerol phosphate was collected on DEAE cellulose disks and counted. Glycerol kinase activity was expressed per mg of protein. Protein was determined by the method of Lowry et al., 1951.

Phosphoenolpyruvate carboxykinase activity

Cytosolic phosphoenolpyruvate carboxykinase activity was assayed in hepatocyte homogenates with deoxyguanosine 5'diphosphate as a substrate as described by Petrescu et al., 1979. The enzyme activity was expressed per mg of protein.

Statistical Analysis

In most measurements, the mean and the standard error of the mean were calculated. The data was analyzed with the Student's *t* test or by two way analysis of variance (ANOVA) when comparing means of more than two groups.

RESULTS

Acetaldehyde acutely increases glycerol uptake

Acute acetaldehyde exposure increased the uptake of glycerol by rat hepatocytes (Fig. 1). The uptake of glycerol in the presence of acetaldehyde (200 μ M) was maximal at 3 minutes ($p < 0.05$ as compared to control) and then decreased. By contrast, ethanol (100 mM) did not result in a significant increase in glycerol uptake as compared to control. The enhanced uptake of glycerol in the presence of acetaldehyde was markedly inhibited by HgCl₂ (0.1 mM), an inhibitor of aquaporins ($p < 0.05$). HgCl₂ also inhibited glycerol uptake in the presence of ethanol (not shown).

Acute acetaldehyde exposure (100 μ M) increased glycerol uptake from wild-type mice ($p < 0.05$) (Fig. 2), but did not increase glycerol uptake by hepatocytes isolated from AQP9 KO mice. Acetaldehyde concentration of 200 μ M resulted in membrane damage in mouse but not in rat hepatocytes.

Acetaldehyde (200 μ M), however, did not result in significant changes in the glycerol permeability coefficient in AQP9 oocytes. The glycerol permeability coefficients were 1.35 ± 0.25 and $1.21 \pm 0.37 \times 10^{-6}$ /sec in empty control oocytes as compared to 21.0 ± 0.18 and $18.5 \pm 1.72 \times 10^{-6}$ /sec in AQP9 oocytes in the absence and presence of acetaldehyde respectively. Glycerol uptake was not increased significantly after chronic exposure of rat hepatocytes to acetaldehyde (200 μ M) or ethanol (100 mM) for 24 hours (Fig. 3). The initial rapid uptake of glycerol after 24 hour exposure to acetaldehyde, ethanol or no additions (control) was maximal at 0.5 min with a subsequent fall in glycerol content. In the case of acetaldehyde, however, there was further uptake at 5 min. HgCl₂ (0.1 mM) inhibited the initial 0.5 glycerol uptake in the presence of acetaldehyde ($p < 0.05$).

Acetaldehyde increases cell size

Exposure of rat hepatocytes to acetaldehyde (200 μ M) resulted in a rapid increase in cell area (Fig. 4A), not observed in the absence of acetaldehyde (not shown). Also the enhancing effect of acetaldehyde was abrogated in the presence of 0.1 mM HgCl₂. Ethanol (100 mM) had no significant effect on cell area (not shown).

The effect of acetaldehyde on cell areas was also determined in hepatocytes isolated from wild-type and AQP9 KO mice. Acetaldehyde (100 μ M) resulted in a greater increase in cell size in wild-type mouse hepatocytes than in AQP9 KO hepatocytes ($p < 0.05$) (Fig. 4B). The

enhancing effect of acetaldehyde on hepatocyte area was inhibited by 0.1 mM HgCl₂ to values comparable to those observed with the AQP9 KO hepatocytes.

Prolonged exposure to acetaldehyde decreases AQP9

Exposure of mouse hepatocytes to acetaldehyde (200 μ M) for 24 h resulted in decreased AQP9 mRNA (Fig. 5A) and AQP9 protein (Fig. 5B). Exposure to ethanol (100 mM) decreased AQP9 mRNA (Fig. 5A) but had no significant effect on AQP9 protein (Fig. 5B).

Ethanol increases the activities of glycerol kinase and phosphoenolpyruvate carboxykinase

Glycerol kinase and phosphoenolpyruvate carboxykinase are important in the formation of glycerol-3 phosphate which can serve as a precursor of gluconeogenesis. Glycerol kinase catalyzes the direct conversion of glycerol to glycerol-3-phosphate, while phosphoenolpyruvate carboxykinase, which catalyzes the conversion of oxaloacetate to phosphoenol pyruvate, is important in the formation of glycerol-3-phosphate from precursors other than glucose and glycerol. Twenty-four hour exposure of mouse hepatocytes to acetaldehyde and ethanol increased glycerol kinase mRNA (Fig. 6A), while ethanol, but not acetaldehyde, increased glycerol kinase activity (Fig. 6B). Also acetaldehyde and ethanol increased phosphoenolpyruvate carboxykinase mRNA (Fig. 6C), while only ethanol increased phosphoenolpyruvate carboxykinase activity (Fig. 6D).

PPAR α is known to up regulate genes involved in hepatic gluconeogenesis such as glycerol kinase. Twenty-four hour exposure to acetaldehyde (200 μ M) increased mouse hepatocyte PPAR α mRNA, while ethanol had no significant effect on PPAR α mRNA (Fig. 7). However, neither acetaldehyde nor ethanol had any significant effects on PPAR α protein (data not shown)

DISCUSSION

This study shows that acetaldehyde causes a rapid increase in the uptake of glycerol and an increase in cell size in hepatocytes. These effects are inhibited by HgCl₂, an inhibitor of aquaporins (Kozono et al., 2002) suggesting that they are mediated by AQP9, which is the principal aquaporin responsible for the transport of glycerol and water into the liver (Maeda et al., 2008). Also, the acute effect of acetaldehyde in increasing glycerol uptake and cell size was absent in hepatocytes isolated from AQP9 KO mice.

The lack of effect of acetaldehyde on glycerol permeability coefficient in AQP9 oocytes is most likely due to the rapid influx of glycerol after its addition which is already close to maximal in the absence of acetaldehyde. Ethanol had no significant effect on either glycerol uptake or hepatocyte size. The mechanism by which acetaldehyde modifies AQP9 and/or alters membrane permeability to enhance glycerol and water transport is unknown. Most likely it is related to effects of acetaldehyde on binding to hepatocyte membrane proteins which was previously described to occur without changes in membrane integrity and function (Barry et al., 1984). Acetaldehyde was also shown to increase paracellular permeability in Caco-2 cells (Rao, 1998) and cellular permeability in human hepatoma cells (Henzel et al., 2004).

Continuous exposure of hepatocytes to acetaldehyde for 24 hours resulted in a decrease in AQP9 mRNA and AQP9 protein without a significant change in the uptake of glycerol. Ballooning of hepatocytes is a common feature of alcoholic steatohepatitis (Lefkowitz, 2005) while it is much less common in nonalcoholic steatohepatitis (Matsuda et al., 1985). The ballooning is most likely due to a combination of protein and water retention by the hepatocytes. Acetaldehyde causes protein retention by decreasing protein secretion due to

formation of acetaldehyde-tubulin adducts (Tuma et al., 1991) resulting in microtubule dysfunction (Lieber, 1983). As regards water retention, ethanol (70 mM) added to mouse liver slices was shown to increase hepatocyte water volume measured with ion sensitive microelectrodes to monitor changes in the intracellular activity of tetramethylammonium ion (Wondergem and Davis, 1994).

Ethanol in this study increased the activities of glycerol kinase and phosphoenolpyruvate carboxykinase. Both of these enzymes are important in the formation of glycerol-3-phosphate which can serve as a precursor of gluconeogenesis or lead to the esterification of fatty acids with the formation of triglycerides. On the other hand, cytosolic phosphoenolpyruvate carboxykinase which catalyzes the conversion of oxaloacetate to phosphoenol pyruvate is important in the formation of glycerol-3-phosphate from precursors other than glucose and glycerol by the process of glyceroneogenesis (Reshef et al., 2003; Nye et al., 2008). As regards gluconeogenesis, ethanol and acetaldehyde are known to inhibit gluconeogenesis due principally to an increase in NADH/NAD⁺ ratio during their metabolism (Cederbaum and Dicker, 1981). Furthermore acetaldehyde inhibits gluconeogenesis from glycerol (Cederbaum and Dicker, 1979). Hence the effects of ethanol in enhancing the above enzymes with the formation of glycerol-3-phosphate are not expected to have a positive effect on gluconeogenesis.

As regards triglyceride formation, alcoholic fatty liver is due principally to increased availability of fatty acids because of increased synthesis and decreased degradation of fatty acids as a consequence of the increased NADH/ NAD⁺ ratio occurring during ethanol metabolism (Lieber, 2004). Also contributing to the decreased fatty acid oxidation is the interference by ethanol of the DNA transcription activation properties of PPAR α on genes that are involved in free fatty acid oxidation (You and Crabb, 2004). The effects of ethanol in enhancing glucose kinase, and phosphoenolpyruvate carboxykinase with increased formation of glycerol-3-phosphate leading to the esterification of fatty acids most likely contributes to alcoholic fatty liver.

PPAR α induces the expression of genes involved in the conversion of glycerol to glucose such as glycerol kinase, but does not induce AQP9 (Patsouris, et al., 2004). Acetaldehyde in this study increased PPAR α mRNA, but neither ethanol nor acetaldehyde had a significant effect on PPAR α protein. In previous studies, ethanol was shown to increase PPAR α mRNA in MCF-7 breast cancer cell lines in culture, while acetaldehyde has no effect (Venkata et al., 2008). On the other hand, chronic ethanol administration reduced PPAR α mRNA in rat and mice livers and furthermore ethanol (200 mM) and acetaldehyde (50 μ M) were shown to reduce PPAR α transcriptional activity of a PPAR response element luciferase reporter in transfected biliary epithelial cells (Lee et al., 2008).

In conclusion this study shows that acetaldehyde acutely results in an increase in glycerol uptake and hepatocyte cell size that is possibly mediated by AQP9. On the other hand prolonged exposure to acetaldehyde reduces AQP9 and does not enhance cell size or glycerol uptake. The effects of ethanol in enhancing glucose kinase, and phosphoenolpyruvate carboxykinase without changes in AQP9 most likely lead to increased formation of glycerol-3-phosphate contributing to alcoholic fatty liver.

Acknowledgments

The authors acknowledge assistance from the Hopkins Digestive Disease Basic Research Development Center (National Institutes of Health grant 2462388) in the performance of this study.

Supported by grants AA000626 and HL48268 from The United States Public Health Service.

REFERENCES

- Barry RE, McGivan JD, Hayes M. Acetaldehyde binds to liver cell membranes without affecting membrane function. *Gut*. 1984; 25:412–416. [PubMed: 6706221]
- Carbrey JM, Gorelick-Feldman DA, Kozono D, Praetorius J, Nielsen S, Agre P. Aquaglyceroporin AQP9: solute permeation and metabolic control of expression in liver. *Proc Natl Acad Sci. USA*. 2003; 100:2945–2950. [PubMed: 12594337]
- Cederbaum AI, Dicker E. A comparison of the effects of ethanol and acetaldehyde on glucose production from various precursors by isolated rat liver cells. *Curr Alcohol*. 1979; 7:71–81. [PubMed: 552351]
- Cederbaum AI, Dicker E. Effect of cyanamide on the metabolism of ethanol and acetaldehyde and on gluconeogenesis by isolated rat hepatocytes. *Biochem Pharmacol*. 1981; 30:3079–3088. [PubMed: 7337724]
- Fariss MW, Brown MK, Schmitz JA, Reed DJ. Mechanism of chemical-induced toxicity. I. Use of a rapid centrifugation technique for the separation of viable and nonviable hepatocytes. *Toxicol Appl Pharmacol*. 1985; 79:283–295. [PubMed: 4002230]
- Kosono D, Yasui M, King LS, Agre P. Aquaporin water channels: atomic structure and molecular dynamics meet clinical medicine. *J Clin Invest*. 2002; 109:1395–1399. [PubMed: 12045251]
- Henzel K, Thorborg C, Hofmann M, Zimmer G, Leuschner U. Toxicity of ethanol and acetaldehyde in hepatocytes treated with ursodeoxycholic or tauroursodeoxycholic acid. *Biochim Biophys Acta*. 2004; 1644:37–45. [PubMed: 14741743]
- Lamb RG, Wood CK, Landa BM, Guzelian PS, Fallon HJ. Studies of the formation and release of glycerolipids by primary monolayer cultures of adult hepatocytes. *Biochim Biophys Acta*. 1977; 489:318–329. [PubMed: 922033]
- Lee JH, Banerjee A, Ueno Y, Ramaiah SK. Potential relationship between hepatobiliary osteopontin and peroxisome proliferator-activated receptor α expression following ethanol-associated hepatic injury *in vivo* and *in vitro*. *Toxicol Sci*. 2008; 106:290–299. [PubMed: 18703563]
- Lefkowitz JH. Morphology of alcoholic liver disease. *Clin Liver Dis*. 2005; 9:37–53. [PubMed: 15763228]
- Lieber CS. Alcohol, protein nutrition, and liver injury. *Curr Concepts Nutr*. 1983; 12:49–71. [PubMed: 6342974]
- Lieber CS. Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. *Alcohol*. 2004; 34:9–19. [PubMed: 15670660]
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem*. 1951; 193:265–275. [PubMed: 14907713]
- Maeda N, Funahashi T, Shimomura I. Metabolic impact of adipose and hepatic glycerol channels aquaporin7 and aquaporin 9. *Nat Clin Pract Endocrinol Metab*. 2008; 4:627–634. [PubMed: 18852723]
- Matsuda Y, Takada A, Sato H, Yasuhara M, Takase S. Comparison between ballooned hepatocytes occurring in human alcoholic liver disease and nonalcoholic liver diseases. *Alcohol Clin Exp Res*. 1985; 9:366–370. [PubMed: 3901809]
- Mezey E, Potter JJ, Rodes DL. Effect of glucagon on alcohol dehydrogenase activity in rat hepatocyte culture. *Gastroenterology*. 1986; 91:1271–1277. [PubMed: 3758618]
- Nye CK, Hanson RW, Kalhan SC. Glyceroneogenesis is the dominant pathway of triglyceride glycerol synthesis *in vivo* in the rat. *J. Biol Chem*. 2008; 283:27565–27574. [PubMed: 18662986]
- Patsopuris D, Mandard S, Voshol PJ, Escher P, Tan NS, Havekes LM, Koenig W, Marz W, Tafuri S, Wahli W, Muller M, Kersten S. PPAR α governs glycerol metabolism. *J Clin Invest*. 2004; 114:94–103. [PubMed: 15232616]
- Petrescu I, Bojan O, Saied M, Barzu O, Schmidt F, Kuhnle HF. Determination of phosphoenolpyruvate carboxykinase activity with deoxyguanosine 5'-diphosphate as a nucleotide substrate. *Anal Biochem*. 1979; 96:279–281. [PubMed: 474956]
- Promeneur D, Liu Y, Maciel J, Agre P, King LS, Kumar N. Aquaglyceroporin PbAQP during intraerythrocytic development of the malaria parasite *Plasmodium berghei*. *Proc Natl Acad Sci USA*. 2007; 104:2211–2116. [PubMed: 17284593]

- Rao RK. Acetaldehyde- induced increases in paracellular permeability in Caco-2 cell monolayer. *Alcohol Clin Exp Res*. 1998; 22:1724–1730. [PubMed: 9835287]
- Reshef L, Olswang Y, Cassuto H, Blum B, Croniger CM, Kalhan SC, Tilghman SM, Hanson RW. Glyceroneogenesis and the triglyceride/fatty acid cycle. *J Biol Chem*. 2003; 278:30413–30416. [PubMed: 12788931]
- Rojek AM, Skowronski MT, Fuchtbauer EM, Fuchtbauer AC, Fenton RA, Agre P, Frokiaer J, Nielsen S. Defective glycerol metabolism in aquaporin 9 (AQP9) knockout mice. *Proc Nat Acad Sci USA*. 2007; 104:3609–3614. [PubMed: 17360690]
- Tuma DJ, Smith SL, Sorrell MF. Acetaldehyde and microtubules. *Ann NY Acad Sci*. 1991; 625:786–692. [PubMed: 2058934]
- Venkata NG, Aung CS, Cabot PJ, Monteith GR, Roberts-Thomson SJ. PPAR α and PPAR β are differentially affected by ethanol and the ethanol metabolite acetaldehyde in the MCF-7 breast cancer cell line. *Toxicol Sci*. 2008; 102:120–128. [PubMed: 18003597]
- Wondergem R, Davis J. Ethanol increases hepatocyte water volume. *Alcohol Clin Exp Res*. 1994; 18:1230–1236. [PubMed: 7531405]
- You M, Crabb DW. Recent advances in alcoholic liver disease II. Minireview: molecular mechanisms of alcoholic fatty liver. *Am J Physiol Gastrointest Liver Physiol*. 2004; 287:G1–G6. [PubMed: 15194557]

Fig. 1.

Acute effect of acetaldehyde and ethanol on uptake of glycerol by rat hepatocytes. Acetaldehyde (200 μ M), ethanol (100mM), or isovolumetric buffer (control) was added to hepatocyte suspensions (2×10^6 cells/ml) followed immediately by addition of [14 C] glycerol. The uptake of glycerol was measured at short time intervals for acetaldehyde (●), ethanol (□), control (○) and acetaldehyde + 0.1 mM HgCl₂ (▲). The data is presented as means of 4 determinations per time intervals. The vertical bars indicate standard errors.

*P<0.05 vs control. [†]P <0.05 vs acetaldehyde + HgCl₂.

Fig. 2.

Acute effect of acetaldehyde on uptake of glycerol by Aquaporin 9 knockout (AQP9 KO) mouse hepatocytes. Acetaldehyde (100 μ M), or isovolumetric buffer (control) was added to hepatocyte suspensions (1×10^6 cells/ml) obtained from wild-type and AQP9 KO mice followed immediately by addition of [14 C] glycerol. The uptake of glycerol was measured at short time intervals for wild-type mouse hepatocytes: control (○) acetaldehyde (●) and AQP9 KO mouse hepatocytes: control (□) acetaldehyde (■). The data is presented as means of 6 determinations per time intervals. The vertical bars indicate standard errors. * $P < 0.05$ vs wild-type hepatocyte control.

Fig. 3. Effects of prolonged (24 hour) exposure to acetaldehyde (200 μ M), ethanol (100mM), or isovolumetric buffer (control) on [14 C] glycerol uptake by rat hepatocyte suspensions (2×10^6 cells/ml). The uptake of glycerol was measured at short time intervals for acetaldehyde (●), ethanol (□), control (○) and acetaldehyde + 0.1 mM HgCl₂ (▲). The data is presented as means of 4 determinations per time intervals. [†]P < 0.05 vs acetaldehyde + HgCl₂.

Fig. 4.

Effects of acetaldehyde on cell area of hepatocytes. A. Rat hepatocytes exposed to 200 μ M acetaldehyde in the absence (●) and presence 0.1 mM HgCl₂ (▲). B. Wild-type (●) and AQP9 KO mouse hepatocytes (○) exposed to 100 μ M acetaldehyde. Effects of 0.1 mM HgCl₂ on wild-type (▲) and AQP9 KO mouse hepatocytes (△) exposed to 100 μ M acetaldehyde. Cell areas were determined from a three dimensional z scans of the cells using the middle slice of the z scan for the area measurements. The vertical bars indicate standard errors of the mean of 6 determinations. * $P < 0.05$ vs AQP9 KO mouse hepatocytes.

Aquaporin 9 Relative Densitometry (% of Control)

Fig. 5. Effects of prolonged (24 hour) exposure to acetaldehyde (200 μ M) (A) and ethanol (100 mM) (E) as compared to control (C) on: (A) Aquaporin 9 mRNA and (B) Aquaporin 9 protein in mouse hepatocytes. The mRNA was determined by real-time polymerase chain

reaction. The relative expressions of the cDNAs were normalized against β -actin DNA in the same samples. Aquaporin protein was determined by Western blot. The values were expressed as means $\pm$ SE of 6 samples each. * $P < 0.05$ vs control.

Glycerol Kinase mRNA (% of Control)

Glycerol Kinase Activity (nmoles/mg protein/min)

Fig. 6.

Effects of prolonged exposure to acetaldehyde (A), and ethanol (E) as compared to control (C) on: (A) glucose kinase mRNA, (B) glucose kinase activity, (C) phosphoenolpyruvate carboxykinase mRNA and (D) phosphoenolpyruvate carboxykinase activity. Mouse hepatocytes were exposed to 200 μ M acetaldehyde (A) or 100 mM ethanol (E) for 24 hours. The mRNAs were determined by real-time polymerase chain reaction. The relative expressions of the cDNAs were normalized against β -actin DNA in the same samples. All values were expressed as means $\pm$ of 6 samples each. * $P < 0.05$ vs control. ** $P < 0.01$ vs control.

Fig. 7.

Effects of prolonged (24 hour) exposure to acetaldehyde (200 μ M) (A) and ethanol (100mM) (E) as compared to control (C) on PPAR α mRNA in mouse hepatocytes. The mRNA was determined by real-time polymerase chain reaction. The relative expressions of the cDNAs were normalized against β -actin DNA in the same samples. The values were expressed as means $\pm$ SE of 6 samples each. *P<0.05 vs control.