Mitochondrial Uncoupling Protein-2 Mediates Steatotic Liver Injury following Ischemia/Reperfusion*

Received for publication, August 14, 2007, and in revised form, November 21, 2007. Published, JBC Papers in Press, December 17, 2007, DOI 10.1074/jbc.M706784200

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Steatotic livers are not used for transplantation because they have a reduced tolerance for ischemic events with reduced ATP levels and greater levels of cellular necrosis, which ultimately result in total organ failure. Mitochondrial uncoupling protein-2 (UCP2) is highly expressed in steatotic livers and may be responsible for liver sensitivity to ischemia through mitochondrial and ATP regulation. To test this hypothesis, experiments were conducted in lean and steatotic (ob/ob), wild-type, and UCP2 knock-out mice subjected to total warm hepatic ischemia/reperfusion. Although ob/ob UCP2 knock-out mice and ob/ob mice have a similar initial phenotype, ob/ob UCP2 knock-out animal survival was 83% when compared with 30% in ob/ob mice 24 h after reperfusion. Serum alanine aminotransferase concentrations from lean livers. Lipid peroxidation (thiobarbituric acid-reactive substances) increased after reperfusion most significantly in the steatotic groups, but the increase was not observed in the ob/ob UCP2 knock-out mice subjected to ischemia. Liver ATP levels were increased in the ob/ob UCP2 knock-out animals after reperfusion when compared with the ob/ob mice but remained below the concentrations from lean livers. Lipid peroxidation (thiobarbituric acid-reactive substances) increased after reperfusion most significantly in the steatotic groups, but the increase was not affected by UCP2 deficiency. These results reveal that UCP2 expression is a critical factor, which sensitizes steatotic livers to ischemic injury, regulating liver ATP levels after ischemia and reperfusion.

Complications from liver steatosis represent a significant clinical concern, especially for liver surgeries including resection and transplantation. This is of escalating importance as nonalcoholic fatty liver disease is independently correlated to obesity and insulin resistance, which are both epidemic in the United States (1, 2). Steatotic livers are considerably more sensitive to acute stressors including ischemia/reperfusion (I/R) as experienced in transplantation, and organs meeting this criterion are routinely turned down for donation (3–6). Under I/R conditions, steatotic livers are ATP-depleted, and the predominant hepatocellular fate is shifted from apoptosis to oncostic necrosis, strongly implicating inappropriate energy homeostasis as the primary cause of liver sensitivity (7–10).

In a state of energy substrate abundance and forward shift in cellular redox potential, hepatocytes are thought to combat mitochondrial electron transport chain-derived reactive oxygen species (ROS) production through mitochondrial uncoupling. In normal lean livers, mitochondrial uncoupling protein-2 (UCP2) is confined to Kupffer cells; however, hepatocellular concentrations of UCP2 greatly increase with steatosis (7, 11, 12). Although the mechanism is not known, UCP2 facilitates passive proton conductance across the mitochondrial inner membrane into the matrix during respiration (13, 14). Maximum employment of electrochemical potential at ATP synthase is sacrificed by UCP2, and heat, rather than ATP, is produced. Proton conductance is thought to require post-translational activation by mitochondrial-derived ROS such as superoxide, specifically in the matrix (13, 15). Studies indicate that resting mitochondrial proton conductance is typically not changed regardless of differences in UCP2 expression but that ROS-stimulated proton conductance is lost when the gene responsible for UCP2 is knocked out (16, 17). Steatotic livers have chronically elevated levels of ROS, and physiological changes in UCP2 expression correlate directly with proton conductance (7, 18).

In models where the consequences of UCP2 expression were measured, it was found that the effects are tissue- and model-specific. UCP2 expression protected brain, cardiomyocytes, and HepG2 cells from oxidative insults (19–21). In contrast, in cortical and mesencephalic cells, induced expression of UCP2 causes a switch from apoptotic to necrotic death pathways in response to cyanide toxicity (22, 23). More recently, it was shown that UCP2 overexpression in primary cardiomyocytes led to ATP depletion and loss of cellular viability following a model of hypoxia/reoxygenation (24). Because some of the effects of UCP2 in these models were attributed to the reduction of oxidative injury, a deficiency in the expression of UCP2 could exacerbate the inevitable oxidative stress associated with liver I/R. However, in steatotic livers subjected to ischemia, UCP2 expression was shown to be directly proportional to injury, including necrosis (7, 25). Because UCP2 is highly expressed in steatotic livers and is capable of causing partial mitochondrial depolarization, we considered that UCP2 promotes hepatocellular death during periods of limited ATP availability as seen in liver I/R, rather than protecting from oxi-
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dative injury. To test this hypothesis, we studied the effects of UCP2 deficiency in lean and steatotic (ob/ob) mice subjected to I/R on hepatic injury, energy production, and oxidative damage.

EXPERIMENTAL PROCEDURES

Animals—Colonial founders of UCP2 heterozygous mice cross-bred with ob/ob heterozygous mice on the C57BL6 background were a generous gift from Dr. Bradford Lowell (Beth Israel Deaconess, Harvard University). The genotypes of animals were determined as described previously (26), and leptin+/−/UCP2+/− animals were mated, generating lean and ob/ob (leptin−/−) mice that were either wild-type or UCP2 knock-out (UCP2−/−). 6–7-week-old male mice of each genotype were either sacrificed (baseline) or subjected to total hepatic ischemia and then sacrificed. For baseline and I/R studies, six animals per group were used, with the exception of the 24-h ob/ob wild-type (WT) group. A number of 10 was statistically determined for this group, prior to experimentation, which compensated for the low survival rate we historically experience with our model. Peripheral blood samples were collected under isoflurane inhalation anesthesia at the indicated time points by sterile cardiac puncture, and livers were excised. Median, right, and caudate lobes were snap frozen in liquid nitrogen and stored at −80 °C until analysis. The hepatic left lobe was fixed in 10% buffered formalin for histology. Breeding, housing, surgery, and euthanasia all complied with the protocols approved by the Medical University of South Carolina (MUSC) Institutional Animal Care and Use Committee.

Warm I/R—Prior to surgery, mice were anesthetized with a single intraperitoneal injection of pentobarbital at 50 mg/kg of body weight. The abdomen was shaved and prepped, and a small vertical incision was made, slightly to the right of midline through the skin and peritoneum. The porta hepatis was exposed, and a pediatric vessel loop was drawn around and tightened to induce total hepatic ischemia. Ischemia was performed for 15 min, the vessel loop was removed, and the portal vein, hepatic artery, and liver were observed for restoration of blood flow (reperfusion). Both layers of the incision were closed with running 5/0 prolene suture. Each animal was immediately placed in a temperature-controlled recovery cage and allowed free access to food and water upon waking. Surviving animals were monitored hourly following surgery and were sacrificed at 1 or 24 h following reperfusion for all groups.

Histological Evaluation—Hematoxylin and eosin staining and Oil Red O staining were performed by MUSC university pathological services. Slides were blinded and then graded for centrilobular necrosis in hematoxylin and eosin-stained slides on a 0–3 scale as described by Neil and Hubscher (27). Ten central veins, each under high powered magnification, were graded, and the average was used as the grade for each animal. Representative photomicrographs were captured digitally, and image brightness and size were adjusted using Adobe Photoshop® CS Version 8.

Serum Transaminase Measurement—Whole blood was allowed to clot at room temperature for 10 min followed by centrifugation at 3,500 × g for 5 min at room temperature, and serum was separated. Serum alanine aminotransferase (ALT) concentrations were measured with a Synchron LX20 system (Beckman Coulter) and expressed as IU/liter (Clinical Laboratory Services, Medical University of South Carolina).

UCP2 Immunoblotting—Lung (positive control) and liver mitochondria were isolated using a mitochondrial isolation kit from Sigma (MITO-ISO1), as described by the manufacturer. Fresh liver samples (50 mg) were minced and then homogenized using a polytetrafluoroethylene pestle under ice-cold conditions. To ensure a purified “heavy” mitochondrial fraction, free of lysosomal and peroxisomal contamination, the low and high speed centrifugation steps were run at 1,000 and 3,500 × g, respectively. Mitochondrial samples were diluted in a 50 mm HEPES buffer, and protein concentrations were measured by BCA assay (Pierce). Standardized samples were solubilized in lithium dodecyl sulfate (NuPage) sample buffer, heat-denatured, and run on an SDS-PAGE gel. Anti-mouse UCP2 primary antibody was purchased from Alpha Diagnostic International (San Antonio, TX), and cyclooxygenase IV primary antibody was purchased from Cell Signaling (Danvers, MA). Primary antibodies were incubated at concentrations of 1:1,000 and 1:10,000, respectively. Anti-rabbit horseradish peroxidase-linked secondary antibody was purchased from Cell Signaling.

Measurement of Whole Liver ATP—Samples (50 mg) from frozen livers were cut and homogenized in 500 μl of ice-cold cell lysis buffer (150 mm NaCl, 50 mm Tris, 1% Triton X-100, 0.1% SDS, and 1% deoxycholate, pH 7.5) and then acidified with 1.5% trichloroacetic acid (for ATPase inhibition). Homogenates were centrifuged at 20,000 × g, and supernatants were diluted 1:100 in Tris acetate buffer (0.1 mm, containing sodium acetate). Each diluted sample was mixed with reconstituted luciferin-luciferase solution (Enliten®, Promega), and the ATP concentration was immediately measured luminometrically. For normalization between samples, total cellular protein from each sample of liver homogenate was determined by BCA assay. Aliquots of sample homogenates were collected prior to the addition of trichloroacetic acid for the protein assay.

Measurement of Lipid Peroxidation—Lipid peroxidation in whole liver was monitored as the production of malondialdehyde with slight modifications (28). Liver samples (30 mg) were cut from the frozen tissue and immediately homogenized in 500 μl of ice-cold phosphate-buffered saline. Homogenates were treated with an equal volume of 10% trichloroacetic acid and centrifuged at 14,000 × g. The supernatant was removed, combined with 2-thiobarbituric acid (0.76%), boiled for 10 min, and cooled, and absorbance was measured at 532 nm with 1,1,3,3-tetraethoxypropane as a standard. Aliquots of homogenate were taken prior to the addition of trichloroacetic acid and measured for protein concentration by BCA assay, for the purpose of standardization.

Statistical Analysis—All values are expressed as mean ± S.D. An α value of 0.05 was established prior to experimentation as the limit for statistical significance. For a single pairwise comparison, a two-tailed t test was used. For multiple independent groups, one-way ANOVA was used, and to correct for unequal sample sizes post-I/R due to unequal animal death between groups, post-test correction was done with the Tukey-Kramer multiple comparison test using JMP® 4 statistical software.
RESULTS

Characterization of Lean and Steatotic UCP2<sup>−/−</sup> Mice—The functional comparison of WT and UCP2 knock-out (KO) livers was conducted using 6–7-week-old lean and ob/ob, UCP2<sup>−/−</sup>, and WT C57BL/6 mice. In ob/ob (leptin<sup>−/−</sup>) mice, the chosen age followed the development of significant liver steatosis but is prior to the onset of insulin resistance, which UCP2 is known to affect (26). ob/ob UCP2 KO mice were not phenotypically different from normal ob/ob mice at this age, as was similarly reported by Baffy et al. (29), who developed and provided this animal model. Additionally, no phenotypic differences were observed between the lean groups. Body weight, food intake, degree of liver steatosis, ratio of macro to micro steatosis, liver weight, and liver morphology were not different between the ob/ob groups or between the lean groups. Steatosis was markedly increased in the livers of both ob/ob groups when compared with their lean controls (Fig. 1a). Measurement of UCP2 protein from isolated mitochondria showed an approximate 1.5-fold greater level of UCP2 expression in the livers of ob/ob WT animals when compared with the WT lean controls (p < 0.05) (Fig. 1b).

UCP2 Deficiency Protects against I/R Injury in ob/ob Mice—We have previously shown that ob/ob mice are significantly more sensitive to total hepatic I/R than their lean littermates (3, 7, 30). To investigate the role of UCP2 expression on liver I/R injury, 15 min of total hepatic ischemia was performed followed by 1 or 24 h of reperfusion. Animal survival was measured at 24 h following reperfusion. Those animals that survived 24 h of reperfusion typically do not die of I/R-related injuries in our model. At 24 h, all lean animals were alive, regardless of the UCP2 phenotype. Survival in the ob/ob WT group was 30%, which was consistent with our previously published studies (30, 31). Animal survival of 83% in the UCP2 KO ob/ob group was greater than the ob/ob control group (Fig. 2a).

Hepatocyte injury was assessed by serum ALT measurement. UCP2 deficiency did not affect baseline ALT concentrations, whereas ob/ob levels were significantly elevated when compared with their respective lean controls in that a 4.5-fold increase was observed when comparing the WT ob/ob group with their lean controls (Fig. 2b).
against the WT lean controls, and a 3.8-fold increase was measured when comparing ob/ob KO mice with the KO lean controls (Fig. 2b). 1 h following I/R, ALT concentrations in the ob/ob WT mice were increased 11-fold over baseline values. ALT levels in the ob/ob UCP2 KO animals increased 4-fold over baseline and were 38% of that observed in ob/ob UCP2 KO livers (d). Scale bar for all images, 200 μm. e, grading of centrilobular necrosis for each animal was conducted on a 0–3 scale, scored per high powered field, and represented graphically. At 24 h, necrosis was significantly greater in the ob/ob control livers when compared with the ob/ob UCP2 KO livers, whereas both groups were greater than their lean controls. Mean values are expressed ± S.D. (n = 3–6/group); means with different text superscripts are significantly different, p < 0.05 (error bars); ANOVA.

In addition to measuring the extent of necrosis, TUNEL staining was performed. At 24 h following reperfusion, staining and morphology, including chromatin condensation and apoptotic body formation in zone 2, indicated apoptosis, but the incidence of staining in this zone was minor when compared with the necrosis in zone 3 (data not shown). Importantly, no differences were measured in the incidence of positive staining in zone 2 between WT and UCP2 KO ob/ob and lean groups at 24 h, and the incidence of staining was low in all groups. Also, no increase in TUNEL staining was observed 1 h following reperfusion when compared with baseline values.

Hepatic ATP Concentrations Are Sustained in UCP2−/− ob/ob Mice Following I/R, whereas Lipid Peroxidation Is Not Affected—Previous studies involving models of UCP2 deficiency or overexpression vary with respect to the effect of the expression of this protein on ATP levels. To determine the effect of UCP2 deficiency on ATP, ATP levels in the liver were measured before and after I/R. The concentrations of ATP found in the livers of WT and UCP2 KO lean mice were equivalent, but both were greater than those observed in the ob/ob groups at baseline and 1 or 24 h after I/R (Fig. 4a). UCP2 deficiency failed to alter the concentration of ATP in the ob/ob mice at baseline. After I/R, ATP content in WT and UCP2 KO lean livers at 1 and 24 h were decreased ~25% when compared with baseline levels but were not significantly different from one another. In contrast, ATP content in ob/ob WT mice decreased from 8.6 ± 1.1 nmol/mg of protein at baseline to 3.8 ± 1.1 nmol/mg of protein 1 h after I/R (56% decrease) and remained at this level at 24 h. The ob/ob KO mice exhibited a
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FIGURE 4. UCP2 deficiency increases liver ATP concentrations following ischemia/reperfusion but has no effect on lipid peroxidation. At baseline and following ischemia, the concentration of ATP from whole liver tissue was measured by chemiluminescent assay, and lipid peroxidation was monitored by TBARS measurement. a, differences were not observed at baseline relating to UCP2 expression, whereas the concentrations of ATP in ob/ob livers were significantly reduced. Following reperfusion, UCP2 deficiency resulted in the increased concentration of ATP in ob/ob livers. b, measurement of TBARS showed no difference based on UCP2 expression, whereas peroxidation was increased in ob/ob livers following reperfusion. For all groups, average values are expressed ± S.D. (n = 3–6/group). Means with different text superscripts are significantly different, p < 0.05 (error bars); ANOVA.

consistent with what we observed in the steatotic livers following I/R, the loss of ATP would lead to increases in cytosolic free Ca\(^{2+}\), increased plasma membrane permeability through reperfusion and further increased after 24 h, independent of the presence or absence of UCP2. Thus, the absence of UCP2 had no effect on I/R-induced hepatic lipid peroxidation, whereas the steatotic phenotype was linked directly to oxidative injury.

DISCUSSION

This experimental series addresses a longstanding issue of the role of UCP2 in increasing the sensitivity of steatotic livers to I/R. Previous studies have only illustrated a correlation between steatotic liver UCP2 expression and intolerance to I/R. Here, we established that UCP2 expression in the steatotic liver is primarily responsible for the increased hepatic injury observed as a result of I/R. The loss of the UCP2 protein in the steatotic liver resulted in greater animal survival, less hepatocellular injury measured histopathologically by necrotic index and by ALT, and greater ATP content after I/R. In the steatotic animals, ALT levels increased significantly at 1 h and trended toward recovery by 24 h. Lean livers had a greater tolerance for I/R, regardless of UCP2 phenotype, and injury to this group of animals was minimal, suggesting that UCP2 has a minor or no role in I/R injury in the absence of steatosis. However, whether or not UCP2 is expressed in lean hepatocytes or whether UCP2 deficiency may affect lean liver tolerance to more severe ischemic stress (longer ischemia) is not known.

Liver ATP concentrations were not different between the steatotic groups (WT versus UCP2 KO) at baseline despite the absence of UCP2 expression in the KO group. The livers of both ob/ob groups contained significantly less ATP than their lean counterparts, implicating systems other than UCP2 for the differences in the concentrations of ATP observed between lean and steatotic livers at baseline. It is important to note that no compensatory uncoupling has been observed by an up-regulation of other uncoupling proteins or systems in the fatty UCP2 and steatotic livers at baseline. It is known whether the basal level of ROS production is great enough to stimulate full UCP2 activation (35, 36). Studies in models of physiologically relevant levels of UCP2 expression suggest that under normal conditions, UCP2 activation is minimal in the absence of a significant oxidative stimulant (13, 37). Likewise, in isolated mitochondria, UCP2 does not affect mitochondrial proton conductance unless activated by superoxide production (13, 38). Although no differences in ATP content were observed at baseline, ATP content in the ob/ob UCP2 KO livers (6.7 nmol/mg of protein, 1 h) were greater than those in ob/ob WT livers (3.8 nmol/mg of protein) after I/R. It is possible that a dramatic increase in ROS production associated with reperfusion may induce mitochondrial depolarization through full activation of UCP2, thus accounting for the differences in ATP content observed in the presence and absence of UCP2 in the steatotic groups. Alternatively, UCP2 could contribute to early mitochondrial failure through an indirect induction of the mitochondrial permeability transition, which is sensitive to changes in mitochondrial polarization.

Lesser degree of liver ATP loss (29%) 1 h after I/R, and ATP content remained at this level at 24 h. After I/R, ATP content in ob/ob mice remained less than in lean mice, and ATP content in ob/ob mice remained less than in ob/ob KO mice. Thus, hepatic ATP content markedly decreases in ob/ob mice, whereas ob/ob mice lacking UCP2 exhibit less ATP loss following I/R.

UCP2 has been linked directly to cellular tolerance to ROS production and oxidative stress, providing the possibility for increased oxidative injury when using this model of UCP2 deficiency in the liver. The predominant oxidative damage to cells resulting from ischemia to the liver is lipid peroxidation (32–34). To address the consequences of deleting UCP2 on oxidative stress following liver ischemia, whole liver lipid peroxidation was measured by TBARS. No differences in the levels of lipid peroxidation were observed between any of the groups at baseline (Fig. 4b). Furthermore, the level of TBARS did not increase following 1 or 24 h of reperfusion in lean mice. In contrast, lipid peroxidation increased in ob/ob mice after 1 h of reperfusion and further increased after 24 h, independent of the presence or absence of UCP2. Thus, the absence of UCP2 had no effect on I/R-induced hepatic lipid peroxidation, whereas the steatotic phenotype was linked directly to oxidative injury.
nonspecific anion channel formation, and subsequent oncotic necrosis (9). The incidence of apoptosis (TUNEL staining) was low and not different among all groups 24 h after I/R, supporting the idea that necrosis is the primary cell fate in steatotic livers following I/R.

The extent of lipid peroxidation was significantly higher in ob/ob animals when compared with lean controls after I/R. The lean animals did not exhibit an increase in lipid peroxidation following I/R, whereas a time-dependent increase in TBARS concentrations at 1 and 24 h was observed in the ob/ob animals. The absence of UCP2 failed to alter the extent of lipid peroxidation. We suggest that UCP2 in steatotic mice does not play a role in protecting against lipid peroxidation following I/R. Nevertheless, oxidative stress may contribute to the liver injury that was observed in the absence of UCP2 and may reduce mitochondrial and cellular viability. Previous studies suggested that UCP2 has the capacity to inhibit ROS production, allowing the possibility for increased oxidative stress in the knock-out groups (19–21). Likewise, the reduced levels of ATP observed in the ob/ob WT groups could be a consequence or cause of increased oxidative stress or reduced cellular capacity for repair. Therefore, despite the lack of differences in lipid peroxidation between the ob/ob groups after I/R, oxidative stress may still play a significant role.

In addition to oxidative stress, numerous other mechanisms of injury have been linked to liver I/R, which may contribute in this model, including sinusoidal disruption, endothelial cell injury, endotoxin sensitivity, and resultant inflammatory responses (31, 39–41). It is unlikely that UCP2 expression affects any of these injury pathways, and therefore, it was not surprising that the deletion of UCP2 failed to completely protect the steatotic liver from the consequences of I/R injury.

Although our findings compellingly establish the protective effect conferred by deleting UCP2 in steatotic livers exposed to I/R, other studies report conflicting roles of UCP2 with respect to oxidative and ischemic stressors in different tissues. For example, in brain, cardiomyocytes, and HepG2 cells, UCP2 overexpression was associated with protection from oxidative insult (stroke, peroxide, and hypoxia, respectively) (19–21). In the brain and in cardiomyocytes, protection from apoptosis was attributed to a decrease in mitochondrial electrochemical potential, decrease in ROS production, decrease in mitochondrial Ca\(^{2+}\) uptake, and subsequent decrease in proapoptotic signaling. Protection against hypoxia in HepG2 cells was explained through a general reduction of cellular oxidative damage and resultant prevention of apoptosis (19). In complete contrast, UCP2 deficiency protected against brain ischemia in one model of cerebral occlusion, presumably through the preservation of microglial GSH stores (42). In an alternative study of primary cardiomyocytes, the induced overexpression of UCP2 decreased ATP levels as well as cellular survival following hypoxia and reoxygenation (24). Overexpression of UCP2 leads to the decrease of cellular ATP and induction of oncotic cell death in HeLa cells, even in the absence of stress, but has no effect on normal diploid fibroblasts (43). In addition, UCP2 negatively regulates insulin secretion in pancreatic β cells and regulates antimicrobial ROS production in macrophages (26, 44).

The divergent responses observed above may be the result of the different models and stressors, the degree of UCP2 overexpression, and/or compensatory responses of the cells to the loss or overexpression of UCP2. The balance between the availability of ATP and the capacity to protect from oxidative damage is different in steatotic livers subjected to I/R than in the other tissues noted above. In an alternative study using ob/ob UCP2 KO mice, it was found that ATP levels and cellular viability (apoptosis) were improved following Fas-mediated injury, suggesting a role of UCP2 similar to our study (45). In this, and our fatty liver model, it appears that the physiological levels of hepatocyte UCP2 expression are inadequate to combat the oxidative burden, as the deletion of UCP2 failed to have any consequence on oxidant formation in the liver. However, as stated earlier, it is unknown how much ATP depletion affects oxidation repair or how much UCP2 directly affects ROS production in the liver.

We observed the protection of ATP stores in the ob/ob UCP2 knock-out fatty livers following I/R along with a general reduction of injury. The differences with respect to UCP2 expression between different organs and mechanisms of injury may represent variance in the capacity of UCP2 to protect against oxidative stress, overall differences in the sensitivity to oxidative stress, or different metabolic strategies and requirements between tissues. Therefore, in some settings, UCP2 can have positive effects, whereas it has negative effects in others. Given these differences, it is possible that through common mechanisms relating to mitochondrial uncoupling, UCP2 can have variable effects. In the steatotic liver, it seems that the consequences of expressing UCP2 after reperfusion outweigh any potential protective effect of mitochondrial uncoupling. Additional studies are needed to clarify the detailed roles of UCP2 with respect to mechanism(s) of action, affected cell types, and consequences of regulation in the steatotic liver.

Acknowledgment—We thank Kathy Haines for the effort and support in keeping our laboratory running.

REFERENCES
1. National Center for Health Statistics Health (2006) Health, United States, 2006 with Chartbook on Trends in the Health of Americans, National Center for Health Statistics, Hyattsville, MD
2. Saadah, S. (2007) Nutr. Clin. Pract. 22, 1–10
3. Birnser, J. H., Wan, C., Cheng, G., Evans, Z. P., Polito, C. C., Fiorini, R. N., Gilbert, G., Haines, J. K., Schmidt, M. G., and Chavin, K. D. (2004) J. Surg. Res. 130, 97–101
4. D’Alessandro, A. M., Kalayoglu, M., Sollinger, H. W., Hoffmann, R. M., Reed, A., Knechtle, S. J., Pirsch, J. D., Hafez, G. R., Lorentzen, D., and Belzer, F. O. (1991) Transplantation 51, 157–163
5. Fernandez-Merino, F. J., Nuno-Garza, J., Lopez-Hervas, P., Lopez-Buenadicha, A., Moreno-Caparros, A., Quijano-Collazo, Y., Sanjuanbenito, A., and Vicente-Lopez, E. (2003) Transplant. Proc. 35, 1793–1794
6. Marsen, W. A., Wiesner, R. H., Rodriguez, L., Batts, K. P., Porayko, M. K., Hay, J. E., Gores, G. J., and Krom, A. R. (1996) Transplantation 62, 1246–1251
7. Chavin, K. D., Yang, S., Lin, H. Z., Chatham, J., Shacko, V. P., Hoek, J. B., Walajtys-Rode, E., Rashid, A., Cheng, C. H., Huang, C. C., Wu, T. C., Lane, M. D., and Diehl, A. M. (1999) J. Biol. Chem. 274, 5692–5700
8. Cortez-Pinto, H., Chatham, J., Shacko, V. P., Arnold, C., Rashid, A., and Diehl, A. M. (1999) J. Am. Med. Assoc. 282, 1659–1664
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9. Malhi, H., Gores, G. J., and Lemasters, J. J. (2006) Hepatology 43, Suppl. 1, S31–S44
10. Selzner, M., Rudiger, H. A., Sindram, D., Madden, J., and Clavien, P. A. (2000) Hepatology 32, 1280–1288
11. Pecqueur, C., Alves-Guerra, M. C., Gelly, C., Levy-Meyrueis, C., Couplan, E., Collins, S., Ricquier, D., Bouillaud, F., and Miroux, B. (2001) J. Biol. Chem. 276, 8705–8712
12. Rashid, A., Wu, T. C., Huang, C. C., Chen, C. H., Lin, H. Z., Yang, S. Q., Lee, F. Y. J., and Diehl, A. M. (1999) Hepatology 29, 1131–1138
13. Echtay, K. S., Roussel, D., St-Pierre, J., Jekabsons, M. B., Cadenas, S., Stuart, J. A., Harper, J. A., Roebuck, S. J., Morrison, A., Pickering, S., Clapham, J. C., and Brand, M. D. (2002) Nature 415, 96–99
14. Echtay, K. S., Winkler, E., Frischmuth, K., and Klingenberg, M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1416–1421
15. Echtay, K. S., Murphy, M. P., Smith, R. A., Talbot, D. A., and Brand, M. D. (2002) J. Biol. Chem. 277, 47129–47135
16. Krauss, S., Zhang, C. Y., and Lowell, B. B. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 118–122
17. Krauss, S., Zhang, C. Y., Scorrano, L., Dalgaard, L. T., St-Pierre, J., Grey, S. T., and Lowell, B. B. (2003) J. Clin. Investig. 112, 1831–1842
18. Chitturi, S., and Farrell, G. C. (2001) Semin. Liver Dis. 21, 27–41
19. Collins, P., Jones, C., Coudhury, S., Damelin, L., and Hodgson, H. (2005) Liver Int. 25, 880–887
20. Mattiasson, G., Shamloo, M., Gido, G., Mathi, K., Tomasevic, G., Yi, S., Warden, C. H., Castilho, R. F., Melcher, T., Gonzalez-Zulueta, M., Nikolich, K., and Wieloch, T. (2003) Nat. Med. 9, 1062–1068
21. Teshima, Y., Akao, M., Jones, S. P., and Marban, E. (2003) Circ. Res. 93, 192–200
22. Li, L., Prabhakaran, K., Mills, E. M., Borowitz, J. L., and Isom, G. E. (2005) Toxicol. Sci. 86, 116–124
23. Li, L., Prabhakaran, K., Zhang, X., Borowitz, J. L., and Isom, G. E. (2006) Toxicol. Sci. 93, 136–140
24. Bodyak, N., Rigor, D. L., Chen, Y. S., Han, Y., Bisping, E., Pu, W. T., and Kang, P. M. (2007) Am. J. Physiol. 293, H129–H135
25. Uchino, S., Yamaguchi, Y., Furushashi, T., Wang, F. S., Zhang, J. L., Okabe, K., Khara, S., Yamada, S., Mori, K., and Ogawa, M. (2004) J. Surg. Res. 120, 73–82
26. Zhang, C. Y., Baffy, G., Perret, P., Krauss, S., Peroni, O., Grujic, D., Hagen, T., Vidal-Puig, A. I., Boss, O., Kim, Y. B., Zheng, X. X., Wheeler, M. B., Shulman, G. I., Chan, C. B., and Lowell, B. B. (2001) Cell 105, 745–755
27. Neil, D. A., and Hubscher, S. G. (2001) Transplantation 71, 1566–1572
28. Schnellmann, R. G. (1988) Am. J. Physiol. 255, C28–C33
29. Baffy, G., Zhang, C. Y., Glickman, J. N., and Lowell, B. B. (2002) Hepatology 35, 753–761
30. Chavin, K. D., Fiorini, R. N., Shafizadeh, S., Cheng, G., Wan, C., Evans, Z., Rodwell, D., Polito, C., Haines, J. K., Baillie, G. M., and Schmidt, M. G. (2004) Am. J. Transplant. 4, 1440–1447
31. Fiorini, R. N., Shafizadeh, S. F., Polito, C., Rodwell, D. W., Cheng, G., Evans, Z., Wan, C., Belden, S., Haines, J. K., Birnsrer, J., Lewin, D., Wadluk, K. R., Dunn, D. L., Schmidt, M. G., and Chavin, K. D. (2004) Am. J. Transplant. 4, 1567–1573
32. Ferguson, D. M., Gores, G. J., Ludwig, J., and Krom, R. A. (1991) Transplant. Proc. 23, 1552–1553
33. Gao, W., Connor, H. D., Lemasters, J. J., Mason, R. P., and Thurman, R. G. (1995) Transplantation 59, 674–679
34. Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B. S., Arsenijevic, D., Vallet, P., Hjelle, O. P., Ottersen, O. P., and Diem, K. R., Dunn, D. L., Schmidt, M. G., and Chavin, K. D. (2004) Am. J. Transplant. 4, 1567–1573
35. Bradbury, M. W., and Berk, P. D. (2004) Clin. Liver Dis. 8, 639–671
36. Mehta, K., Van Thiel, D. H., Shah, N., and Mobarak, S. (2002) Nutr. Rev. 60, 289–293
37. Stuart, J. A., Harper, J. A., Brand, M. K., Jekabsons, M. B., and Brand, M. D. (2001) J. Biol. Chem. 276, 18633–18639
38. Zhang, C. Y., Parton, L. E., Ye, C. P., Krauss, S., Shen, R., Lin, C. T., Porco, J. A., Jr., and Lowell, B. B. (2006) Cell Metab. 3, 417–427
39. Caldwell-Kenkel, J. C., Currin, R. T., Tanaka, Y., Thurman, R. G., and Lemasters J. J. (1991) Hepatology 13, 83–95
40. Ohno, A., Mochida, S., Arau, M., Hirata, K., and Fujiwara, K. (1994) Dig. Dis. Sci. 39, 861–865
41. Seifalian, A. M., Piasecki, C., Agarwal, A., and Davidson, B. R. (1999) Transplantation 68, 780–784
42. de Bilbao, F., Arsenijevic, D., Vallet, P., Hjelle, O. P., Ottersen, O. P., Bouras, C., Raffin, Y., Abou, K., Langhans, W., Collins, S., Plamondon, J., Alves-Guerra, M. C., Hauenerauer, A., Garcia, I., Richard, D., Ricquier, D., and Giannakopoulos, P. (2004) J. Neurochem. 89, 1283–1292
43. Mills, E. M., Xu, D., Fergusson, M. M., Combs, C. A., Xu, Y., and Finkel, T. (2002) J. Biol. Chem. 277, 27385–27392
44. Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B. S., Miroux, B., Couplan, E., Alves-Guerra, M. C., Goubert, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S., and Ricquier, D. (2000) Nat. Genet. 26, 435–439
45. Fulop, P., Derdak, Z., Sheets, A., Sabo, E., Berthiaume, E. P., Resnick, M. B., Wands, J. R., Paragh, G., and Baffy, G. (2006) Hepatology 44, 592–601