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# Exposure to ethanol induces oxidative damage in the pituitary gland

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**Abstract**

Chronic exposure of pubertal male rats to ethanol results in a decline in serum testosterone and decreased or inappropriately normal serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels suggesting a functional defect in the pituitary. The molecular mechanisms behind this disorder are undefined. A role for ethanol-induced oxidative damage in the pathophysiology is supported by studies in liver, muscle, and heart of experimental animals, but there is limited evidence in the pituitary. We examined markers of oxidative damage to lipids and proteins in pituitaries from rats consuming ethanol for 5, 10, 20, 30, and 60 days in addition to markers of damage to nucleic acids in pituitaries after 60 days of ethanol exposure. There were increases in 8-oxo-deoxyguanosine immunoreactivity, a marker of oxidative damage to nucleic acids, and an overall increase in malondialdehyde and 4-hydroxynonenal, markers of lipid peroxidation. Protein carbonylation and protein nitrotyrosination, markers of protein oxidation, were significantly increased after 30 days and 60 days of ethanol consumption, respectively. After 60 days of ethanol exposure, TUNEL assay revealed that cell death in the ethanol-treated pituitaries was not significantly different from that in the pair-fed controls at the time of examination. We also measured serum testosterone, FSH, and LH after ethanol consumption for 5, 10, 20, 30, and 60 days. Through 5 to 60 days of ethanol exposure, testosterone levels were consistently lower whereas LH and FSH were inappropriately unchanged, suggesting pituitary malfunction. These results provide evidence for ethanol-induced oxidative damage at the pituitary level, which may contribute to pituitary dysfunction. 

*Keywords:* Lipid peroxidation; Protein oxidation; Nucleic acids oxidation; Ethanol; Adolescent; Pituitary

## 1. Introduction

Chronic ethanol ingestion is marked by a number of biomedical and physiological changes. In the male reproduction system, it is well documented that chronic ethanol suppresses the hypothalamic–pituitary–gonadal axis. The initial reports of more than 25 years ago have been confirmed by more recent studies (Cicero, 1981, 1982; Dissen et al., 2004; Emanuele & Emanuele, 2001; Kim et al., 2003; Van Thiel et al., 1979). Both acute and chronic ethanol exert effects at the level of the hypothalamus, pituitary, and testes. The attenuation of serum testosterone after ethanol exposure has been shown in humans and animals (Van Thiel et al., 1979; Widenius et al., 1987). Although ethanol results in a fall in serum testosterone levels, the expected increase in luteinizing hormone (LH) and follicle stimulating hormone (FSH) is not seen, implying that ethanol has an effect on the hypothalamic–pituitary unit. Ethanol exposure to male rats has been reported to decrease pituitary LH content (Kim et al., 2003), and increased (Salonen et al., 1992) or unchanged (Halloran et al., 1993) mRNA levels have been described. Based on the experimental evidence, it appears that ethanol acts on the hypothalamus to depress the release of LH releasing

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hormone, resulting in subsequent decreases in the LH, FSH, and testosterone (Ching et al., 1988; Dees & Kozlowski, 1984). Inhibition of pituitary FSH and LH secretion by ethanol has been demonstrated as well (Emanuele et al., 1994; Pohl et al., 1987; Schade et al., 1983).

Several mechanisms may be involved in the ethanol inhibition of pituitary LH and FSH secretion. A recognized mechanism of ethanol action is its ability to enhance oxidative stress. Hydroxyethyl free radical formation in biological systems in the presence of ethanol has been detected by spin trapping techniques (Knecht et al., 1995; Reinke, 2002). Ethanol is metabolized to acetaldehyde through alcohol dehydrogenase in cytosol, through cytochrome P-450 II E1 (CYP2E1) in the microsomes, and through catalase in the peroxisomes (Lieber, 1997a; Lucas et al., 1990). Further oxidation of acetaldehyde to acetate is accompanied by the generation of free radical/reactive oxygen species (ROS) (Mira et al., 1995; Quintanilla & Tampier, 1992). Ethanol can enhance ROS formation through induction of CYP2E1 in the liver (Cederbaum, 1998) and in the brain (Montoliu et al., 1995). Ethanol can also induce lesions between complex I and complex III of the mitochondrial electron transport chain, and enhance superoxide anion production (Bailey et al., 1999; Cunningham & Bailey, 2001). Indirectly, chronic ethanol may augment oxidative stress by decreasing antioxidant defenses such as reducing glutathione (GSH) peroxidase and altering GSH homeostasis (Oh et al., 1998; Rouach et al., 1997). Supplementation of antioxidants has been shown to prevent ethanol-induced injury in liver, brain, heart, and skeletal muscle (Lieber, 1997b; Mansouri et al., 2001).

The direct measurement of ROS generated from ethanol can be achieved by electron spin resonance spectroscopy (Knecht & Mason, 1993; Mason & Knecht, 1994). The drawback of this technique is its relatively low sensitivity because of the low steady state concentrations of ROS formed in the tissue. Another approach to quantify ROS is based on the indirect analysis of ROS damage products in tissues. Oxidative damage to DNA is typically quantified by measuring 8-OHdG in tissues (Kasai, 2002). The end products of the peroxidation of polyunsaturated fatty acids, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), are used as markers to assess ROS-induced lipid peroxidation (Esterbauer, 1991). The most extensively studied oxidative modification of proteins is the formation of carbonyl groups in amino acid residues, which can be identified through reaction with 2,4,-dinitrophenylhydrazine (DNP) (Amici et al., 1989; Berlett & Stadtman, 1997; Stadtman & Berlett, 1998). Other specific ROS modifications of proteins, such as the hydroxylation of aromatic and hydrophobic amino acids and the conversion of tyrosine residues to nitrotyrosine or dityrosine are markers of oxidative stress as well (Davies et al., 1999; Dean et al., 1997).

The purpose of this study was, by using the ROS damage markers, to examine the oxidative injury from chronic ethanol consumption on the pituitary and to correlate it with ethanol-induced secretion changes in FSH, LH, and testosterone in male animals as they progress through puberty. These measurements could provide the first evidence for oxidative damage in the pituitary caused by ethanol, a finding of potential functional significance.

## 2. Materials and methods

The project was approved by the Institution Animal Committee on Animal Research at Loyola University at Chicago Medical Center and Veterans Affairs Hospital. All procedures are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

### 2.1. Animals

Three groups of male Sprague–Dawley rats, 35 to 60 days old at the start of the experiment and purchased from Harlan Labs, Indianapolis, IN, were used for these studies. The animals were housed with 12-h light:12-h darkness regime at 22–24°C. The ethanol-fed animals were treated via a pair-feeding regime. The Lieber–DeCarli diet is a nutritionally complete liquid diet with 36% calories as either ethanol (fed to the ethanol group) or dextrimaltose (given to the pair-fed controls). The pair-fed control group was given the same amount of nonethanol containing (i.e., 36% dextrimaltose) diet as its ethanol imbibing mate, only 1 day later. The third group was given regular rat chow ad libitum. The feeding periods were 5, 10, 20, 30, and 60 days.

### 2.2. Blood ethanol levels

Blood ethanol levels were determined using a kit purchased from Sigma (St Louis, MO; 332C) following the protocol for serum samples.

### 2.3. Hormone determinations

The plasma levels of testosterone, FSH, and LH were measured. The testosterone radioimmunoassay (RIA) was done using commercially available kit (Diagnostic Systems; Plantation, FL) according to the manufacturer's instruction. The LH and FSH RIAs were done utilizing the materials supplied by the NIDDK and the National Hormone and Pituitary Program as previously described (Emanuele et al., 2002).

### 2.4. Determination of lipid peroxidation by slot blotting

The pituitaries were removed and homogenized in a buffer containing 10 mM Hepes, 137 mM NaCl, 4.6 mM KCl, 1.1 mM $KH_2PO_4$, 0.6 mM $MgSO_4$, 1.1 mM EDTA, and pH 7.4. This buffer was supplemented with a protease