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Impact and Reversibility of Chronic Ethanol Feeding on the Reproductive Axis in the Peripubertal Male Rat

Nicholas V. Emanuele,[1,3,4–6] Nancy LaPaglia,[1,5] Wayne Vogl,[7] Jennifer Steiner,[1,5] Lidia Kirsteins,[1,5] and Mary Ann Emanuele[1,2,4–6]

Departments of [1]Medicine and [2]Molecular and Cellular Biochemistry, the [3]Molecular Biology Program, and the [4]Division of Research on Drugs of Abuse, Loyola University Medical Center, Maywood, IL; [5]Research and [6]Medical Services, V.A. Hospital, Hines, IL; and [7]Department of Anatomy, Faculty of Medicine, University of British Columbia, Vancouver, Canada

Teenage drinking continues to be a major problem in the United States as well as abroad. A significant depression in serum testosterone in adolescents who consume EtOH has been well described. In the male rodent model, a similar fall in testosterone has been reported, and prevention with the opiate blocker naltrexone has been demonstrated. To explore further the impact of chronic EtOH exposure on the reproductive axis in peripubertal rats, we designed this study specifically to define whether or not there was recovery after abstinence by examining reproductive hormones and their genes during and after EtOH exposure. Peripubertal male rats 35 d old were fed an EtOH-containing diet or a calorically matched control diet for 60 d. A third group was fed the control liquid diet ab libitum. EtOH was then withdrawn and all animals were fed standard rat chow and water ad libitum for an additional 3 mo. The EtOH-imbibing animals were found consistently to weigh less than their pair-fed mates and liquid diet ad libitum animals. Serum testosterone levels and testicular weights were significantly decreased by EtOH whereas serum estradiol levels were higher, suggesting enhanced peripheral conversion by EtOH. Spermatogenesis, assessed by histological parameters, was unaltered by EtOH. Serum luteinizing hormone levels were not different among the groups. Hypothalamic luteinizing hormone–releasing hormone mRNA levels were unaffected by EtOH. During the 3-mo recovery period, all the changes reversed, with a significant increase noted in testosterone. All other parameters remained the same among the groups. Thus, although chronic EtOH exposure in the peripubertal age period results in significant reproductive alterations, there is complete recovery on withdrawal.

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Author to whom all correspondence and reprint requests should be addressed: Mary Ann Emanuele, MD, Loyola University Medical Center, Division of Endocrinology and Metabolism, 2160 South First Avenue, Building 117, Room 11, Maywood, IL 60153.

E-mail: Memanue@wpo.it.luc.edu.

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Introduction

The impact of EtOH on male rodent reproductive and growth hormones has been intensively studied in adults, and changes in all three levels of the hypothalamic-pituitary-gonadal axis have been described. A consistent fall in testosterone with luteinizing hormone (LH) levels that are inappropriately normal have been reported whereas luteinizing hormone–releasing hormone (LHRH) alterations have been variable *(1–6)*. However, these same parameters have not been as well described in the adolescent animal *(6,7)*. With the well-known rise in teenage drinking *(8–11)*, it is imperative that hormonal alterations, the possibility of their recovery with abstinence, and the clinical implications be assessed. Before preventive measures can be determined, an in-depth investigation of the alterations in the hormonal milieu of this vulnerable age group needs to be conducted. In the present study, peripubertal male rats were exposed to EtOH during their critical growth and reproductive development. The gonadal steroids testosterone and estradiol were measured. Testicular structure was examined. These parameters were correlated with serum LH and pituitary mRNA for LHβ. At the level of the hypothalamus, the mRNA for LHRH was evaluated. On reaching adulthood, EtOH was withdrawn, and recovery of these same parameters was assessed. The findings and implications of this study are highly relevant to the human adolescent who imbibes EtOH in his teens, and reverts to abstinence in early adulthood.

Results

Blood EtOH

At the conclusion of 2 mo of feeding, EtOH levels were 102 ± 16 mg/dL in the EtOH-fed animals at the time of sacrifice and undetectable in both pair-fed and ad libitum groups.

Animal Weights

All animals steadily gained weight throughout the study. However, the EtOH-fed group consistently weighed less than either the pair-fed or ad libitum groups. Beginning after the first week, the EtOH group weighed significantly ($p < 0.001$) less, and this continued throughout the feeding period and until the fourth week of the recovery period. The animals, however, appeared healthy, with no evidence of malnourishment or illness based on their behavior. The EtOH animals had comparable weights by wk 4 of the recovery period (Fig. 1). Despite the fact that the pair-fed animals received the same number of calories daily as the EtOH animals, their weights were indistinguishable from the ad libitum group.

This pattern has consistently been noted in all of our past chronic-feeding studies, and may be related to subtle malabsorption. The EtOH animals invariably had more stool pellets in their cages each day. In other studies using this same feeding paradigm, our laboratory has noted a statistically significant increase in total stool from the EtOH-fed rats compared with their pair-fed mates after 2 wk *(12)*.

Serum Testosterone and Testicular Weights

Serum testosterone levels were significantly decreased in the EtOH-imbibing animals compared with their pair-fed mates ($p < 0.001$), as noted in Fig. 2A. However, after 3 mo of recovery, testosterone levels were actually higher in those animals that had been EtOH exposed than in the pair-fed controls ($p < 0.05$, Fig. 2B). The pair-fed animals had testosterone levels not significantly different than the ad libitum animals during either the feeding or recovery periods. Testicular weights followed an identical pattern with a significant decrease after 2 mo of EtOH feeding ($p < 0.05$) and return to levels comparable to the pair-fed and ad libitum animals with 3 mo of abstinence. After the end of the 2-mo feeding period, the weight of the testes averaged 1.68 ± 0.03 g in the EtOH-fed animals, significantly less than either pair-fed (1.76 ± 0.02 g) or ad libitum rats (1.73 ± 0.06 g). At the end of the recovery period, the testes weight of the EtOH animals had increased to that of the others. Specifically, testes weights were 2.01 ± 0.03 g, 1.96 ± 0.02 g, and 1.97 ± 0.05 g in the EtOH, pair-fed, and ad libitum rats, respectively. The ratio of testes to body weight after the 2 mo of feeding was 5.1×10^{-3} for the EtOH group, 4.4×10^{-3} for the pair-fed group, and 4.2×10^{-3} for the ad libitum group. Thus, the testicular weight loss was roughly proportional to body weight loss in the EtOH-fed animals. After 3 mo of recovery, the ratio was identical for all three groups at 3.9×10^{-3}.

Serum Estradiol

Interestingly, serum estradiol levels in the EtOH-exposed animals were significantly elevated after 2 mo compared to both the pair-fed control and ad libitum ani-

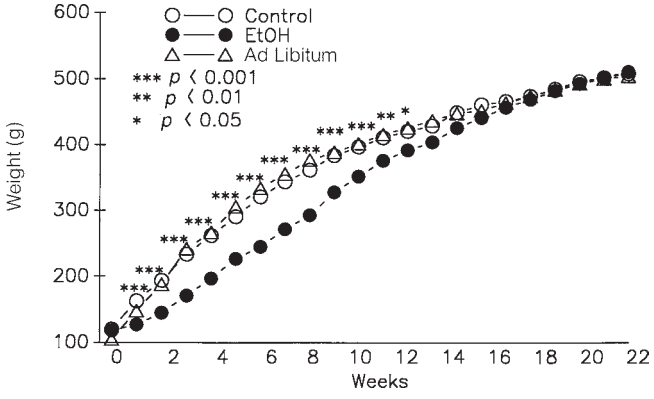

Fig. 1. Animals were weighed weekly throughout the feeding period and for 3 mo after changing to standard rat chow and water (recovery period). The EtOH-exposed animals weighed significantly less throughout most of the 2 mo of the study diet, but 4 wk after withdrawal had achieved weights similar to the other two groups. There was no difference between the pair-fed control and liquid diet ad libitum animals at any time during the study or in the recovery period.

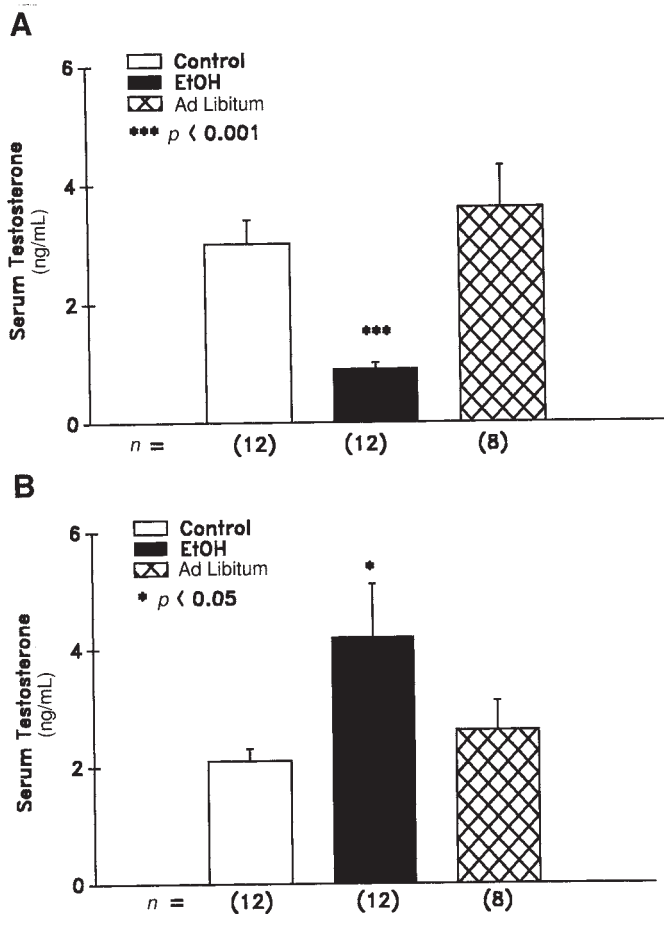

Fig. 2. Serum testosterone was significantly decreased ($p < 0.001$) in the EtOH-fed animals **(A)** with a significant ($p < 0.05$) rebound noted after withdrawal **(B)**. Testosterone levels were not different between the other two groups.

mals ($p < 0.01$) (Fig. 3A). Similar to testosterone, estradiol returned to control levels with 3 mo of abstinence (Fig. 3B).

Serum LH

Serum LH levels were not statistically different among the three groups at any time during feeding or recovery (Fig. 4).

Pituitary LHβ mRNA

After 2 mo of EtOH ingestion, the message for LHβ was significantly increased in the EtOH-exposed animals compared to the pair fed group ($p < 0.01$). There was no statistical difference between the EtOH and ad libitum or between the pair-fed and ad libitum groups (Fig. 5A). In the recovery study, there was a significant fall in pituitary β-mRNA levels in the EtOH and ad libitum groups compared to the pair-fed control group ($p < 0.01$, Fig. 5B).

Hypothalamic LHRH mRNA

Neither EtOH exposure nor recovery had any impact on hypothalamic LHRH mRNA levels. There was no statistical significance among the groups immediately after dietary manipulation or after 3 mo of abstinence (data not shown).

Histology

No qualitative differences were observed among any of the groups in the material processed for standard histology. In all samples, the appearances of seminiferous tubules and interstitial tissues were similar and appeared normal (Fig. 6A,C,G,I,K). Stages VII to VIII, characterized by the occurrence of mature spermatids at the apex of the epithelium, were present in all samples (Fig. 6B,D,F,H,J,L).

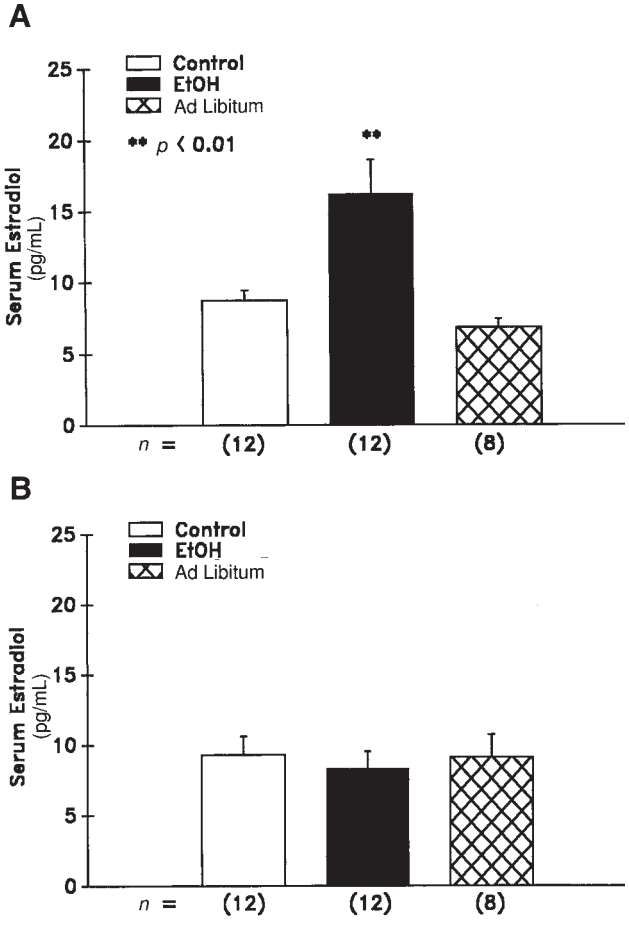

Fig. 3. Serum estradiol levels were significantly increased ($p < 0.01$) by EtOH **(A)** with return to control levels in the recovery period **(B)**. There was no difference between the pair-fed control and liquid ad libitum animals.

Discussion

The impact of acute EtOH exposure has been consistently demonstrated to result in testosterone suppression, with a significant decrease noted as long as 96 h after a single ip injection *(3)*. Although chronic EtOH has similarly been associated with testosterone inhibition in most, but not all studies, the exact locus of action has not been established *(1,2,4,12)*. Data exist demonstrating hypothalamic, pituitary, and gonadal effects, with more studies suggesting the testes as the prime target of alteration. Recently, reversal of this suppression was shown with the opioid blocker naltrexone *(13)* and nitric oxide synthase (NOS) inhibition in addition to other pharmacological intervention *(14–18)*. In the present study, we have further characterized this decrease in testosterone with chronic EtOH exposure in peripubertal male rats as they progress through puberty by assessing concomitant changes in estradiol as an index of testosterone conversion, in addition to changes in hypothalamic-pituitary reproductive hormone patterns.

The liquid diet utilized for these studies resulted in blood EtOH levels comparable to mild intoxication. Despite the pair-feeding procedure in which control animals were isocalorically matched to their EtOH-exposed partners, the EtOH rats did not gain weight at the rate that the pair-fed control or ad libitum group did. This EtOH-induced impairment of weight gain has been seen in other studies examining prepubertal and peripubertal rats *(12,19,20)*. In adult rats, decreased weight with EtOH compared to pair-fed rats has been seen in some *(21)*, but not all *(4,22,23)*, studies. The reason for impaired weight gain is not entirely clear. Serum levels of insulin-like growth factor (IGF-1), a potent growth factor, were not reduced in our previous data in male rats of this age *(1)*. The biological activity of IGF-1 can be modified, both enhanced and inhibited, by IGF binding proteins, which were not measured in our study. Interestingly, others have found that IGFBP-1, a protein that reduces IGF-1 biological activity, is increased by EtOH *(24)*. Furthermore, the ability of IGF-1 to activate its intracellular signal transduction pathways is inhibited by EtOH *(25,26)*.

The fall in serum testosterone confirms that of other chronic studies in prepubertal rats *(1,7,12)*. It is concordant with similar chronic-feeding studies in adult rats *(5,22,23)*.

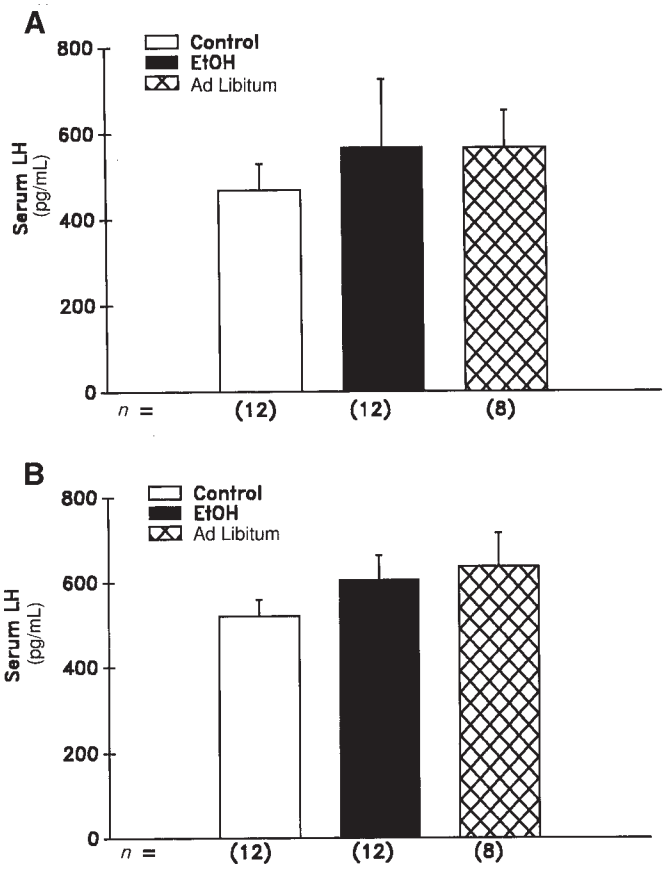

Fig. 4. Serum LH levels were not different among any of the groups either in the dietary feeding period **(A)** or the after change to standard rat chow **(B)**.

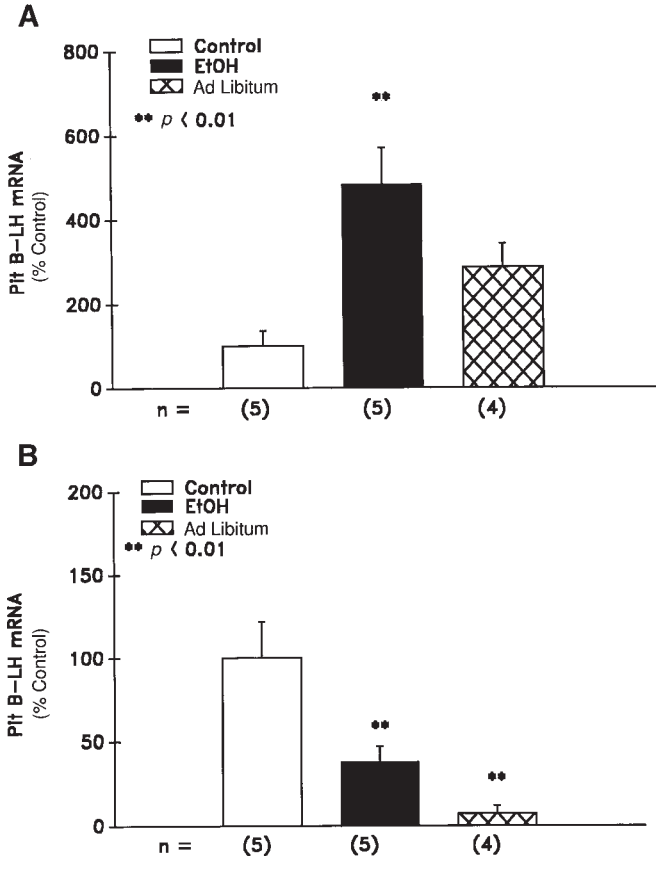

Fig. 5. Pituitary βLH mRNA levels were significantly increased ($p < 0.01$) with 2 mo of EtOH feeding **(A)** with a significant fall ($p < 0.01$) after EtOH was withdrawn **(B)**, perhaps reflecting testosterone fluctuations. Curiously, this message was also unexplainably decreased ($p < 0.01$) in the animals receiving liquid diet ad libitum in the recovery period **(B)**.

Numerous studies have shown similar marked testosterone suppression after acute EtOH treatment, but acute studies are not the focus of this article. While EtOH may have an effect on dampening the known pulsatility of reproductive hormones, which could only be determined by frequent sampling, great care was exercised to eliminate this possibility by sacrificing the animals in synchrony at 10:00 AM as described in Materials and Methods. The fall in testosterone is likely owing to decreased secretion as well as increased metabolism. A number of in vitro studies have established that EtOH and, perhaps more important, its metabolite acetaldehyde can decrease testosterone secretion *(27–31)*. The ability of EtOH to increase the clearance of testosterone was established in healthy humans without liver disease *(32)*, though when significant liver disease supervenes, testosterone clearance is actually decreased *(33)*. In rats, chronic EtOH feeding has been shown to accelerate the metabolism of testosterone by increasing the activity of important hepatic enzymes: microsomal 5α-reductase *(34)* and aromatase *(35,36)*. Aromatase converts testosterone to estradiol. Aromatase was likely increased by EtOH in our study since EtOH-treated animals had elevated estradiol levels. This EtOH-induced rise in circulating estrogens has been seen in other, though not all, human and animal studies *(22,32,35–38)*.

In our study, after the 3-mo period of abstinence from EtOH, not only were testosterone levels restored, they were actually higher in rats that had been fed EtOH compared with those that had not. In a study similar to ours, Cicero, et al. *(7)* also reported that testosterone levels that had been EtOH suppressed rose sharply after EtOH withdrawal. In addition, our study was the first to demonstrate that the EtOH-induced rise in estradiol is fully reversible with the cessation of EtOH.

Despite the profound EtOH effect on Leydig cells (i.e., reduced testosterone), the seminiferous tubules and spermatogenesis were unaffected, as assessed by careful histological examination (Fig. 6). This was somewhat surprising since adult male alcoholic humans and adult male rats given EtOH develop seminiferous tubule damage *(19,22,37,39)*. Perhaps spermatogenic function of adults is more vulnerable to EtOH gonadal toxicity than our peripubertal males. To our knowledge, ours is the first study examining testicular histology in this age group.

In our study, in light of low serum testosterone, a high LH level would have been anticipated since testosterone exerts a negative feedback on LH secretion and gene expression.

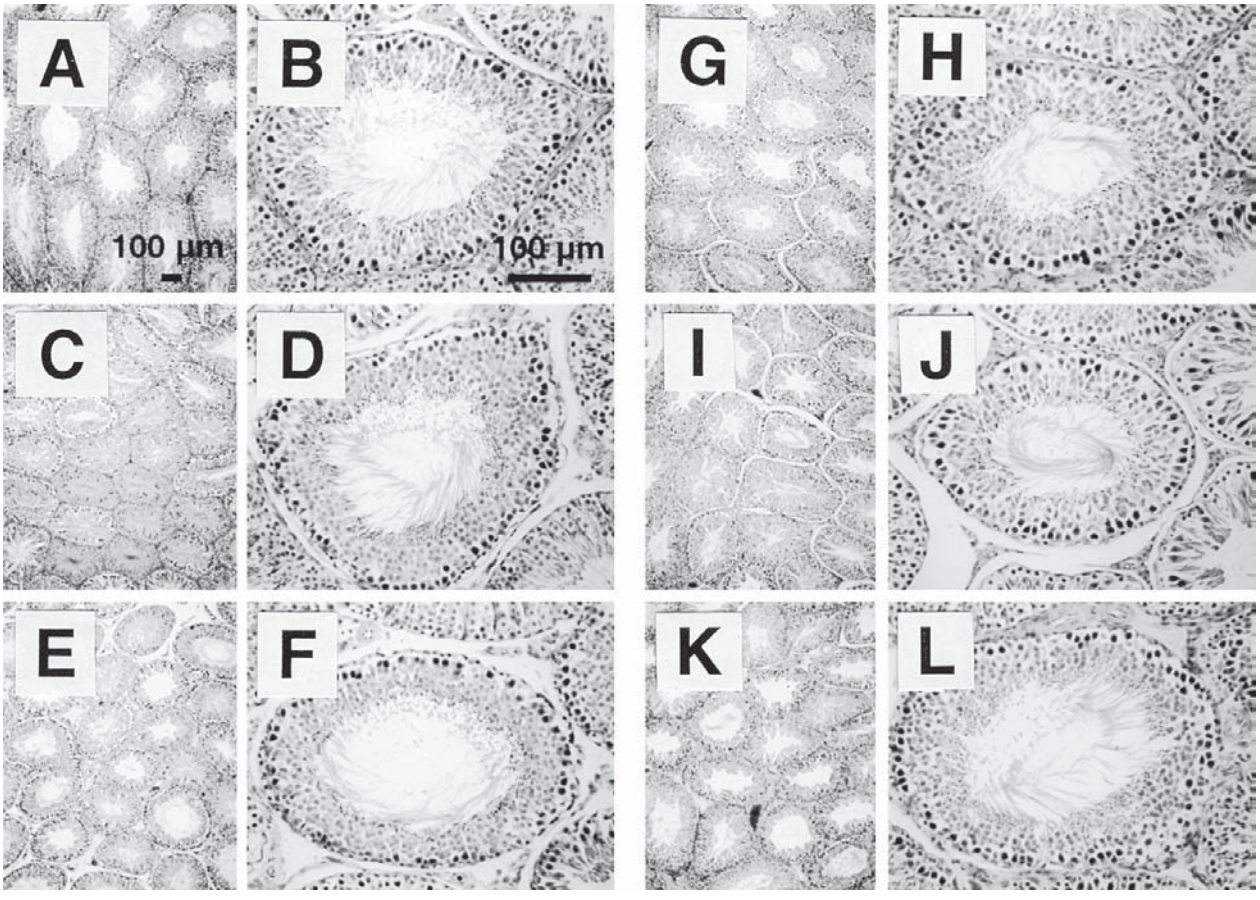

Fig. 6. Paired high and low magnification micrographs of testes from animals in two studies designed to investigate the effects of 3-mo recovery from 2 mo of EtOH feeding. **(A,B)** Pair-fed, **(C,D)** EtOH, and **(E,F)** ad libitum animals in the 3-mo recovery study; **(G–L)** animals sacrificed after 2 mo of EtOH feeding with no recovery. There were no apparent differences in the general histological appearance of the testes among the groups. All testes were fully spermatogenic, as indicated by the presence of late spermatids at the apex of the epithelium.

The fact that the LH was not elevated is compatible with the general notion that EtOH causes a suppression of hypothalamic-pituitary function *(5,7,20–23,32,35,37,38,40–43)*. We acknowledge that the secretion of LH is pulsatile and that our experimental design may have missed subtle changes in LH pulse amplitude and/or frequency.

The results on βLH mRNA are of interest. During the period of EtOH exposure, at a time when testosterone was low (compared to pair-fed controls), the LH β-mRNA was actually increased, but it was not accompanied by elevated serum LH. After EtOH withdrawal, at the end of the recovery period, at a time when testosterone rebounded to higher levels than pair-fed rats, LH β-mRNA was significantly reduced, even though, again, this was not reflected in serum LH changes. The increased βLH mRNA during EtOH is likely owing to reduced negative feedback from reduced testosterone, and the decreased mRNA during EtOH recovery was probably owing to enhanced negative feedback from elevated testosterone. Salonen et al. *(4)* reported, in adult rats, that 5 wk of EtOH exposure resulted in increased βLH mRNA. Ours, however, was the first study to examine this in rats given EtOH during the pubertal period and the first to evaluate the issue after a 3-mo period of abstinence from EtOH. The lack of rise in serum LH despite high levels of LH β-mRNA during EtOH feeding may be owing to two factors. First, it is likely that EtOH reduced LH secretion. Second, it is possible that the LH β-mRNA was not directing efficient translation. In another study from our laboratory, Halloran et al. *(44)* showed that after acute administration, the subcellular distribution of LH message is drastically altered. EtOH reduced by about 50% the amount of β-LH mRNA associated with heavy polyribosomes, thus probably reducing translational rate. If chronic EtOH does the same thing, this may account for the paradox of high LH β-mRNA without similarly elevated serum levels. This point will require more study. In other articles from our group, acute EtOH caused a dramatic decrease in the steady-state levels of β-LH mRNA in castrated animals *(45)* and no change in gonadally intact male rats *(3)*. Thus, the effect of EtOH on LH β-gene expression is complex and highly dependent on the pattern of EtOH exposure as well as the animal's antecedent ste-

roid milieu. The reason for the very low levels of LH β-mRNA in the ab libitum fed animals at the end of recovery is totally unclear.

In the hypothalamus, mRNA levels for LHRH were unchanged in any of the three groups in either study. Overall, this lack of change in LHRH message supports our previous studies in which acute or chronic EtOH exposure was found to have no effect on this parameter. LHRH protein was not measured in the present study. This will have to be examined in future work to give a more complete picture of the effects of EtOH on this system.

This study demonstrates that the testosterone suppression seen after chronic EtOH ingestion is fully recoverable with abstinence in adolescent animals. Part of the lower testosterone levels may be attributable to enhanced conversion to estradiol by aromatase. The increased estradiol levels also returned to normal with cessation of EtOH. The hypothalamus remained unperturbed at least at the level of LHRH gene expression. Additional investigations will be necessary to explore further these findings.

Materials and Methods

Animals and Feeding

Sixty four 30 d-old male Sprague Dawley rats were purchased from Harlan Labs (Indianapolis, IN). In this colony, puberty begins as early as 35 d of age. Animals were allowed to become acclimated to their environment, which included a 12 h light/dark cycle for 5 d. The well-established Liber DeCarli liquid diet without EtOH was administered 4 d before beginning the study to allow the animals to become accustomed to the new diet . The EtOH group (n = 24) received 36% of their calories as EtOH, as previously reported *(12)*. The pair-fed group (n = 24) received an equal number of calories as their EtOH-fed mates, with dextramaltose substituted for EtOH. A third ad libitum group (n = 16) was given as much of the non-EtOH-containing liquid diet as they could consume, in order to observe whether there was any difference between the control pair-fed and ad libitum groups. Animals were weighed on a weekly basis and daily food consumption was measured. After 2 mo of feeding, half of the rats from each group were sacrificed by decapitation at 10:00 AM. The remaining rats were switched to a standard rat chow and water ad libitum for an additional 3 mo (recovery group). These animals were also decapitated at 10:00 AM at the conclusion of the 3 mo. Because of the known pulsatility of reproductive hormones, great care was taken to sacrifice the rats from each group in synchrony (i.e., one rat from the EtOH, one from the control, and one from the ad libitum group in successive order). Trunk blood was collected and serum separated and stored at –20°C for subsequent radioimmunoassay (RIA). Similarly, anterior pituitaries and hypothalami were quickly removed and stored at –70°C for further study *(1,12)*. All animal protocols were approved by the Joint Institutional Animal Care and Use Committee, Loyola University Stritch School of Medicine and Edward Hines Veterans Administration Hospital.

Radioimmunoassays

LH Radioimmunoassay

The LH RIA was conducted utilizing materials generously contributed by the National Institute of Diabetes, Digestive and Kidney Diseases, the National Hormone and Pituitary Program and University of Maryland School of Medicine, through Dr. A. F. Parlow, using methods previously described from our laboratory *(46)*. The National Institutes of Health standard used was RP3. The assay sensitivity was 156 pg/mL, and the inter- and intraassay coefficients of variation (CVs) were 5.2 and 4.0 %, respectively.

Testosterone RIA

The testosterone RIA was conducted utilizing a kit purchased from Pantex (Santa Monica, CA) following the suggested protocol. The assay sensitivity was 0.1 ng/mL and the inter- and intraassay CVs were 6.0 and 2.5%, respectively.

Estradiol (E_2) RIA

The estradiol RIA was conducted using a commercially available kit (Pantex). The assay sensitivity was 10 pg/mL, and the inter- and intraassay CVs were 5.1 and 4.2%, respectively.

Blood Ethanol Levels

Blood levels of EtOH were determined using a kit purchased from Sigma (St. Louis, MO; no. 332C) following the protocol for serum samples.

RNA Isolation and Reverse Transcriptase/Polymerase Chain Reaction Analysis: Hypothalamic LHRH mRNA Determinations

Total RNA was extracted from individual rat hypothalami according to an established procedure *(47)*. Four micrograms of RNA was reverse transcribed according to the following procedure. Thirteen microliters of RNA and diethylpyrocarbonate (DEPC)-treated H_2O and 1 mL of 0.5 mg/mL of oligo dT were incubated for 10 min at 70°C and then chilled on ice for 5 min. To each tube 2 mL of 10X polymerase chain reaction (PCR) buffer, 2 mL of 0.1 dithiothretol, 1 mL of 10 m*M* dNTP, and 1 mL of Superscript reverse transcriptase (RT) enzyme were added. Two additional samples were reverse transcribed and used as negative controls: one tube had no RNA (denoted H_2O blank) and the other contained RNA but RT was not added (denoted RNA blank). All tubes were then incubated at room temperature for 10 min, then at 42°C for 50 min, and finally at 95°C for 5 min. Five microliters of the cDNA was then used in the subsequent PCR reaction. Oligo primers were designed to span from exon 1 to 3 of the LHRH gene, which gives a 375-bp product. The sequences of the two oligo primers were as follows:

1. 5'CACTATGGTCA CCAGCGGGG
2. 3'AGAGCTCCTCGCAGATCCCTAAGA

Histone oligo, giving a product of 213 bp, was used to control for amplification and loading. The oligo primer sequences were as follows:

1. H3.3-1 CCACTGAACTTCTGATTCGC
2. H3.3-2 GCGTGCTAGCTGGATGTCTT

The PCR reaction tube containing 10 μL of 10X PCR buffer, 82 μL of DEPC H^2O, 5 μL of sample, 1 μL of *Taq* DNA polymerase, 10 μCi/μL of α^{32}P dCTP, and 1 μL of 10 pmol of each oligo was amplified for 1 cycle at 95°C for 3 min and 20 s, 60°C for 1 min and 30 s, and 72°C for 3 min and 20 s, followed by 23 cycles at 95°C for 50 s, 60°C for 1 min and 30 s, 72°C for 2 min and 20 s, and concluded with 72°C for 10 min. The products were electrophoresed on a 1.5% agarose gel, transferred on Nytran, and exposed to audioradiographic film. All lanes were scanned on a densitometer, and the relative amounts were compared.

Northern Blot Hybridization Analysis: Determination of Pituitary LHβ mRNA

Total RNA was extracted from pituitaries and the levels of LH mRNA were determined using Northern blot hybridization analysis. Ten micrograms of RNA were loaded per lane and run on a 1.5% agarose-formaldehyde gel and transferred onto Nytran membrane using a Stratagene ultraviolet oven. Hybridization was accomplished under stringent conditions in 50% formamide in a Robbins hybridization oven at 42ºC, and filters were washed in a 0.2X standard saline sodium citrate and 0.5% sodium dodecyl sulfate at 65°C. The filters were then exposed to X-ray film, and the autoradiographs were analyzed using a scanning densitometer. Histone 3.3 was used as a control for amplification and loading.

Testicular Histology

Testes were excised from animals and immersion fixed for 4 wk in standard buffered histological formalin. An approx 3-mm-thick layer was removed from the middle of each testis, perpendicular to the long axis of the organ, and the layer was then processed further for embedding in paraffin. Sections of 8 μm were cut from each paraffin block and then stained with hematoxylin and eosin.

Sections were evaluated qualitatively for the presence of any obvious morphological abnormalities in interstitial tissues and in seminiferous tubules. The presence or absence of spermatogenic stages VII–VIII were specifically determined in all samples.

Statistical Analysis

Statistical analysis was by two-way analysis of variance and individual group comparisons by student's unpaired *t*-test with $p < 0.05$ considered significant.

Acknowledgments

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References

1. Steiner, J., Halloran, M., LaPaglia, N., Emanuele, N. V., and Emanuele, M. A. (1997). *J. Endocrinol.* **154,** 363–370.
2. Dees, W. L., McArthur, N. H., and Harns, P. G. (1984). *Exp. Brain Res.* **54,** 197–202.
3. Steiner, J., Halloran, M., Jabamoni, K., Emanuele, N. V., and Emanuele, M. A. (1996). *Alcohol. Clin. Exp. Res.* **20,** 1368–1374.
4. Salonen, I., Pakarinen, P., and Huhtaniemi, I. (1992). *J. Pharm. Exp. Ther.* **260,** 463–476.
5. Adams, M. and Cicero, T. J. (1991). *Alcohol. Clin. Exp. Res.* **15,** 685–692.
6. Little P. J., Adams, M. L., and Cicero, T. J. (1992). *J. Pharm. Exp. Ther.* **263,** 1056–1061.
7. Cicero, T. J., Adams, M. L., O'Connor, L., Nock, B., Meyer, E. R., and Wozniak, D. (1990). *J. Pharm. Exp. Ther.* **255,** 707–715.
8. National Institute on Alcohol Abuse and Alcoholism (1990). DHHS Publication No. (ADM) 90–1656, Washington: Government Printing Office.
9. Meilman, P. W., Stone, J. E., Gaylor, M. S., and Turco, J. H. (1990). *J. Studies Alcohol* **51,** 389–395.
10. Bailey, S. L. and Valey, R. J. (1993). *J. Studies Alcohol* **54,** 555–565.
11. Milgram, G. G. (1993). *Studies Alcohol Suppl.* **11,** 53–61.
12. Emanuele, N. V., LaPaglia, N., Steiner, J., Kirsteins, L., and Emanuele, M. A. (1999). *Alcohol. Clin. Exp. Res.* **23,** 60–66.
13. Emanuele, M. A., LaPaglia, N., Steiner, J., Jabamoni, K., Hansen, M., Kirsteins, L., and Emanuele, N. V. (1998). *Alcohol. Clin. Exp. Res.* **22,** 1199–1204.
14. Adams, M. L., Forman, J. B., Kalicki, J. M., Meyer, E. R., Sewing, B., and Cicero, T. J. (1993). *Alcohol. Clin. Exp. Res.* **17,** 660–663.
15. Adams, M. L., Meyer, E. R., Sewing, B. N., and Cicero, T. J. (1994). *Alcohol. Clin. Exp. Res.* **18,** 959–965.
16. Lancaster, F. E. (1992). *Alcohol. Clin. Exp. Res.* **16,** 539–541.
17. Shi, Q., Emanuele, N. V., and Emanuele, M. A. (1998). *Alcohol. Clin. Exp. Res.* **22,** 1763–1770.
18. Shi, Q., Hales, D. B., Emanuele, N. V., and Emanuele, M. A. (1998). *Alcohol. Clin. Exp. Res.* **22,** 1754–1762.
19. Van Thiel, D. H., Gavaler, J. S., Lester, R., and Goodman, M. D. (1975). *Gastroenterology* **69,** 326–332.
20. Symonds, A. M. and Marks, V. (1975). *Biochem. Pharm.* **24,** 955–958.
21. Cicero, T. J. and Badger, T. M. (1977). *J. Pharm. Exp. Ther.* **201,** 427–433.
22. Van Thiel, D. H., Gavaler, J. S., and Cobb, C. F. (1979). *Endocrinology* **105,** 888–895.
23. Salonen, I. and Huhtaniemi, I. T. (1990). *Biol. Reprod.* **42,** 55–62.
24. Lang, C. H., Fan, J., Lipton, B. P., Potter, B. J., and McDonough, K. H. (1998). *Alcohol. Clin. Exp. Res.* **22**, 823–829.
25. Resnicoff, M., Cui, S., Coppola, D., Hoek, J. B., and Rubin, R. (1996). *Alcohol. Clin. Exp. Res.* **20,** 961–966.
26. Cui, S., Tewari, M., Schneider, T., and Rubin, R. (1997). *Alcohol. Clin. Exp. Res.* **21,** 1121–1127.
27. Orpana, A. K., Harkonen, M., and Eriksson, C. J. P. (1990). *Alcohol, Alcoholism* **25,** 499–507.
28. Badr, F. M., Bartke, A., Dalteric, S., and Bulger, W. (1977). *Steroids* **30,** 647–655.
29. Cobb, C. F., Ennis, M. F., Van Thiel, D. H., Gavaler, J. S., and Lester, R. (1978). *Gastroenterology* **75,** 958–965.
30. Ellingboe, J. and Varanelli, C. C. (1979). *Res. Commun. Chem. Pathol. Pharm.* **24,** 87–102.
31. Cicero, T. J., Bell, R. D., Meyer, E. R., and Badger, T. M. (1980). *J. Pharm. Exp. Ther.* **213,** 228–233.
32. Gordon, G. G., Altman, K., Southren, A. L., Rubin, E., and Lieber, C. S. (1976). *N. Engl. J. Med.* **295,** 793–797.
33. Southren, A. L., Gordon, G. G., Olivo, J., Rafii, F., and Rosenthal, W. S. (1973). *Metabolism* **22,** 695–702.

34. Rubin, E., Lieber, C. S., Altman, K., Gordon, G. G., and Southren, A. L. (1976). *Science* **19,** 563– 564.
35. Gordon, G. G., Southren, A. L., and Lieber, C. S. (1978). *Alcohol. Clin. Exp. Res.* **2,** 259–263.
36. Gordon, G. G., Southren, A. L., Vittek, J., and Lieber, C. S. (1979). *Metabolism* **28,** 20–25.
37. Van Thiel, D. H., Lester, R., and Sherins, R. J. (1974). *Gastroenterology* **67,** 1188–1199.
38. Van Thiel, D. H., Lester, R., and Vaitukaitis, J. (1978). *J. Clin. Endocrinol. Metab.* **47,** 499–507.
39. Loyd, C. W. and Williams, R. H. (1948). *Am. J. Med.* **4**, 315–330.
40. Emanuele, M. A., Halloran, M. M., Uddin, S., Tentler, J. J., Emanuele, N. V., Lawrence, A. M., and Kelley, M. R. (1993). In: *Alcohol and the endocrine system* (Zahkari, S. ed.) NIH Publication #23: Bethesda, MD.
41. Van Thiel, D. H. and Lester, R. (1978). *Alcohol. Clin. Exp. Res.* **2,** 265–270.
42. Mendelson, J. H., Mello, N. K., and Ellingboe, J. (1977). *J. Pharm. Exp. Ther.* **202,** 676–682.
43. Mendelson, J. H., Ellingboe, J., Mello, N. K., and Kuehnle, J. (1978). *Alcohol. Clin. Exp. Res.* **2,** 255–258.
44. Halloren, M. M., Emanuele, M. A., Emanuele, N. V., Tentler, J. J., and Kelley, M. R. (1995). *Endocrine* **3,** 469–473.
45. Emanuele, M. A., Tentler, J. J., Emanuele, N. V., Kelley, M. R. (1991). *Alcohol* **8,** 345–348.
46. Azad, N., Duffner, L., Paloyan, E. B., Reda, D., Kirsteins, L., Emanuele, N. V., and Lawrence, A. M. (1990). *Endocrinology* **127,** 1928–1933.
47. Kelley, M. R., Jurgens, J. K., Tentler, J. J., Emanuele, N. V., Blutt, S. E., and Emanuele, M. A. (1993). *Alcohol* **10,** 185–189.