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Influence of dietary zinc deficiency during development on hepatic CYP2C11, CYP2C12, CYP3A2, CYP3A9, and CYP3A18 expression in postpubertal male rats☆

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Abstract

The present study investigated the effect of dietary zinc deficiency during the developmental period on hepatic cytochrome P450 (CYP) expression in postpubertal male rats. Twenty-one-day-old weanling male Wistar rats were randomly assigned to one of the following dietary groups: zinc-adequate (31 mg zinc/kg diet); marginal zinc-deficient (3 mg zinc/kg diet); severe zinc-deficient (1 mg zinc/kg diet); or pair-fed control for either the marginal or severe zinc-deficient group. All rats were killed at 63 days of age. Compared with the corresponding pair-fed controls, marginal zinc deficiency decreased CYP2C11-mediated testosterone 2α- and 16α-hydroxylase activities by 43 and 42%, respectively, whereas severe zinc deficiency reduced each of these activities by approximately 60%. The decrease in CYP2C11 activity was accompanied by a reduction in CYP2C11 protein and mRNA levels, as assessed by immunoblot and reverse transcription–polymerase chain reaction (RT–PCR) assays, respectively. Additional RT–PCR analysis indicated that severe zinc deficiency decreased CYP3A2 and CYP3A18 mRNA levels by 49 and 43%, respectively, whereas it increased CYP2C12 (253%) and CYP3A9 (238%) mRNA expression. Plasma testosterone concentration was decreased by 67% in the marginal zinc-deficient group when compared with the corresponding pair-fed control group. By comparison, it was below the limit of quantification (0.2 ng/mL) in the severe zinc-deficient rats. Overall, these results indicate that dietary zinc deficiency during the developmental period feminized the hepatic gene expression of the sexually dimorphic CYP2C11, CYP3A2, CYP3A18, CYP2C12, and CYP3A9 in postpubertal male rats.

Keywords: Cytochrome P450; CYP2C11; CYP2C12; CYP3A2; CYP3A9; CYP3A18; Growth hormone; Testosterone; Zinc

1. Introduction

Zinc is an essential trace mineral, and it is important in numerous biochemical and physiological functions. These include its catalytic and structural role in a large number of zinc metalloenzymes, its role in the function of numerous zinc-finger proteins involved in gene expression and regulation, its involvement in maintaining immunocompetence, and its function as an antioxidant [1–4]. Studies with rodents have shown that zinc confers protection against hepatotoxicity induced by various drugs and other xenobiotics, including carbon tetrachloride [5], bromobenzene [6], acetaminophen [7], and pyrrolizidine alkaloids [8]. In both humans and animals, dietary zinc deficiency results in growth retardation, hypogonadism, immune defects, alopecia, anorexia nervosa, dermal lesions, and abnormal fetal and postnatal development [1,3].

CYP is a superfamily of hemeproteins that catalyze the biotransformation of xenobiotics such as drugs and environmental pollutants and endogenous substances such as steroid hormones and fatty acids. Several hepatic CYP enzymes are expressed in a sex-dependent manner in rats. For example, CYP2C11 [9], CYP3A2 [10], and CYP3A18 [11, 12] are male-specific, whereas CYP2C12 [13,14] and CYP3A9 [15] are female-specific. The pattern of pituitary GH secretion has a major influence on hepatic expression of these sex-dependent CYP enzymes. In adult male rats, GH

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Abbreviations: CYP, cytochrome P450; GH, growth hormone; and RT–PCR, reverse transcription–polymerase chain reaction.

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is secreted by the pituitary gland in a pulsatile, intermittent manner characterized by high peaks of plasma GH followed by an interpulse period of very low or undetectable levels [16]. In female rats, GH is secreted in a continuous manner. The male pattern of GH secretion stimulates CYP2C11 expression [17], whereas the female pattern of GH secretion induces CYP2C12 [13] and CYP3A9 [12]. In addition, hepatic expression of CYP2C11 [18], CYP2C12 [13], CYP3A9 [11,19], and CYP3A18 [11,19] is regulated developmentally, and full expression of these enzymes is not reached until late in puberty.

Zinc is important in the production, storage, and secretion of a number of hormones, including GH. In a previous study, it was reported that plasma GH levels were reduced in pubertal and adult male rats fed a severe zinc-deficient diet (1 mg zinc/kg diet) when compared with both the *ad libitum*-fed and pair-fed control groups [20]. Given that GH plays a major regulatory role in the developmental expression of these CYP enzymes [21], we hypothesize that a deficiency in dietary zinc during the peripubertal period will interfere with the normal sex-differentiation of this group of enzymes. CYP enzymes are important catalysts of drug biotransformation. Therefore, potential consequences of alteration in hepatic CYP expression by dietary zinc deficiency may include reduced therapeutic efficacy or enhanced systemic toxicity of drugs that undergo CYP-catalyzed biotransformation.

In the present study, we examined the effect of dietary zinc deficiency during development on hepatic expression of GH-regulated CYP enzymes, such as the male-specific CYP2C11, CYP3A2, and CYP3A18 and the female-specific CYP2C12 and CYP3A9. The results obtained indicate that peripubertal dietary zinc deficiency feminized the hepatic expression of these sexually dimorphic enzymes in postpubertal male rats.

2. Materials and methods

2.1. Chemicals and reagents

The reference zinc solution for the atomic absorption spectrophotometry was obtained from Fisher Scientific. NADPH, RedTaq® DNA polymerase, 10 × PCR buffer (100 mM Tris–HCl, pH 8.3, and 500 mM KCl), and magnesium chloride were obtained from the Sigma Chemical Co. Trizol, dithiothreitol, dNTP mix, oligo(dT)$_{12–16}$ primers, deoxyribonuclease I, and SuperScript II reverse transcriptase were purchased from Canadian Life Technologies. Forward and reverse primers for CYP2C11, CYP2C12, CYP3A2, CYP3A9, CYP3A18, β-actin, and cyclophilin were custom-synthesized at the University of British Columbia Biotechnology Laboratory.

2.2. Animals

Twenty-one-day-old weanling male Wistar rats were purchased from the Animal Care Center at the University of British Columbia. The animals were housed individually in stainless steel cages, had free access to double-deionized water, and were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

2.3. Dietary treatment

Rats [21-day-old; body weight 35.8 ± 0.9 g, mean ± SEM for seven individual rats per group] were randomly assigned to one of the five dietary groups: (i) zinc-adequate; (ii) marginal zinc-deficient; (iii) pair-fed control for the marginal zinc-deficient group; (iv) severe zinc-deficient; and (v) pair-fed control for the severe zinc-deficient group. Rats in the zinc-adequate control, marginal zinc-deficient, and severe zinc-deficient groups were provided with the assigned diet *ad libitum.* Voluntary feed refusal is a characteristic sign of zinc deficiency [22], and feed restriction affects CYP expression [23]. Therefore, pair-fed rats also were included as secondary controls for the marginal and severe zinc-deficient groups. Each of the diets was a modified egg-white-based AIN-93G diet containing the same nutrients [24], but differed in the amount of zinc. The zinc contents in the zinc-adequate control, marginal zinc-deficient, and severe zinc-deficient diets were 31, 3, and 1 mg zinc/kg diet, respectively [24].

2.4. Isolation of liver microsomes and collection of blood samples

All rats were killed at 63 days of age. Livers were excised, washed in ice-cold 50 mM Tris/1.15% KCl (pH 7.5) buffer, and used immediately to prepare microsomes by differential ultracentrifugation [25]. The final microsomal pellet was suspended in 0.25 M sucrose, and aliquots of the suspension were stored at –75° until used. Blood was collected and plasma stored at –20° until used.

2.5. Hepatic microsomal protein and total CYP assays

Hepatic microsomal protein concentration was determined using the Bio-Rad Protein Assay Kit, with absorbance measured at 595 nm. Hepatic microsomal total CYP concentration was determined from the sodium dithionite-reduced carbon monoxide difference spectrum [26]. The extinction coefficient of 91 cm^{-1} mM^{-1} was used in calculating the concentration of total CYP.

2.6. Testosterone hydroxylase assay

The testosterone hydroxylase assay was performed as described previously [27]. Briefly, each 1-mL sample containing 50 mM potassium phosphate buffer (pH 7.4), 3 mM

Table 1
Nucleotide sequences of PCR primers

Gene		Primer sequence	PCR product (bp)	Reference
CYP2C11	Forward	5′-TGC-CCC-CTT-TTT-ACG-AGG-CT-3′	368	[32]
	Reverse	5′-GGA-ACA-GAT-GAC-TCT-GAA-TTC-T-3′		
CYP2C12	Forward	5′-TAT-AAA-CTC-AAT-ACG-TTC-TGA-G-3′	262	[32]
	Reverse	5′-TTT-TAC-ATT-AAC-TTC-AGA-AAC-TG-3′		
CYP3A2	Forward	5′-TTG-ATC-CGT-TGT-TCT-TGT-CA-3′	323	[33]
	Reverse	5′-GGC-CAG-GAA-ATA-CAA-GAC-AA-3′		
CYP3A9	Forward	5′-GGA-CGA-TTC-TTG-CTT-ACA-GG-3′	372	[11]
	Reverse	5′-ATG-CTG-GTG-GGC-TTG-CCT-TC-3′		
CYP3A18	Forward	5′-CAA-CTA-CGG-TGA-TGG-CAT-GT-3′	649	[11]
	Reverse	5′-CAC-TCG-GTT-CTT-CTG-GTT-TG-3′		
β-Actin	Forward	5′-TAT-GGA-GAA-GAT-TTG-GCA-CC-3′	786	[11]
	Reverse	5′-CCA-CCA-ATC-CAC-ACA-GAG-TA-3′		
Cyclophilin	Forward	5′-CTT-CGA-CAT-CAC-GGC-TGA-TGG-3′	265	[34]
	Reverse	5′-CAG-GAC-CTG-TAT-GCT-TCA-GG-3′		

magnesium chloride, 0.25 mM testosterone, 0.3 nmol hepatic microsomes, and 1 mM NADPH was incubated at 37° for 5 min. The reaction was terminated by the addition of 6 mL dichloromethane. Subsequently, the internal standard (2.5 nmol 11β-hydroxytestosterone) was added. Testosterone metabolites were resolved by reverse-phase HPLC. The HPLC system, including the instrument, columns, solvent, and elution conditions, was the same as described previously [28].

2.7. SDS–PAGE and immunoblot assay

SDS–PAGE, electrophoretic transfer of proteins to nitrocellulose membranes, and densitometric quantification of immunoblots were performed as described previously [29]. CYP2C11 protein, which was purified from hepatic microsomes isolated from untreated, adult male Long-Evans rats [30], was used to construct calibration curves for the immunoblot assays. Rabbit anti-rat CYP2C11 monospecific polyclonal IgG was purified as described previously [30]. Immunodetection of hepatic microsomal CYP2C11 was conducted with anti-CYP2C11 monospecific IgG at a concentration of 15 μg/mL.

2.8. Isolation of liver RNA and RT–PCR

Total liver RNA was isolated using the Trizol reagent, according to the protocol of the manufacturer. The RNA pellet was suspended in 50 mL of sterile 10 mM Tris/1 mM EDTA buffer (pH 8) and stored at –70° until used. RT–PCR co-amplification of target and internal control cDNAs was performed as described previously [31]. Sequences for the forward and reverse primers [11,32–34] used in the present study are listed in Table 1.

2.9. Plasma zinc and testosterone assays

Plasma zinc concentration was determined by flame atomic absorption spectrophotometry as described previously [22]. In this assay, plasma samples obtained from zinc-adequate control, pair-fed control for the marginal zinc-deficient, and pair-fed control for the severe zinc-deficient rats were diluted 10-fold, whereas the samples obtained from marginal zinc-deficient and severe zinc-deficient rats were diluted 2.5-fold with 0.1 M nitric acid. Plasma testosterone concentration was measured by a solid-phase ^{125}I-radioimmunoassay (ImmuChem Direct Testosterone Kit, ICN Biomedicals, Inc.), according to the protocol of the manufacturer.

2.10. Statistics

The difference between the means of dietary treatment groups was analyzed by one-way ANOVA and, where applicable, was followed by the Newman-Keuls multiple range test (SigmaStat™ Statistical Software, Version 1, Jandel Scientific). Statistical significance was set *a priori* at $P < 0.05$.

3. Results

3.1. Assessment of zinc deficiency

The characteristic signs of zinc deficiency include dermal lesions around the mouth and the paws, alopecia, reduced feed intake, suppressed somatic growth, and decreased plasma zinc concentration [1]. In the present study, dermal lesions were observed in both marginal zinc- and severe zinc-deficient rats; the effects were more pronounced

Table 2
Effect of dietary zinc deficiency on feed intake, body weight gain, liver weight, plasma zinc concentration, and total CYP content

	Dietary zinc content (mg zinc/kg diet)				
	Zinc-adequate *ad libitum* control (31 mg/kg)	Marginal zinc deficiency		Severe zinc deficiency	
		Pair-fed control (31 mg/kg)	Zinc deficient (3 mg/kg)	Pair-fed control (31 mg/kg)	Zinc deficient (1 mg/kg)
Total feed intake (g)	Not determined	328 ± 37	358 ± 42	247 ± 5[a,b]	245 ± 8[a,c]
Body weight gain (g)	272 ± 8	129 ± 7[d]	80 ± 10[a,d]	91 ± 3[a,b,d]	29 ± 9[a,b,c,d]
Absolute liver weight (g)	13.6 ± 0.4	6.6 ± 0.4[d]	5.4 ± 0.4[d]	4.8 ± 0.4[a,d]	3.0 ± 0.3[a,b,c,d]
Relative liver weight (% body weight)	4.4 ± 0.1	4.0 ± 0.2	4.7 ± 0.3	3.8 ± 0.2	4.6 ± 0.3
Plasma zinc (μg/mL)	2.5 ± 0.1	2.5 ± 0.2	0.4 ± 0.1[a,c,d]	2.6 ± 0.3	0.5 ± 0.1[a,c,d]
Total CYP content (nmol/mg protein)	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.2 ± 0.2

Twenty-one-day-old male rats were provided with a zinc-adequate control diet, a marginal zinc-deficient diet, or a severe zinc-deficient diet as described in "Materials and methods." Rats in the pair-fed control for either marginal zinc-deficient or severe zinc-deficient groups were provided with the zinc-adequate diet at the same amount consumed by the zinc-deficient rats on the previous day. All rats were killed at day 63 of age. Results are means ± SEM for seven individual rats per treatment group.

[a]Significantly different from the pair-fed control group for marginal zinc deficiency ($P < 0.05$).
[b]Significantly different from the marginal zinc-deficient group ($P < 0.05$).
[c]Significantly different from the pair-fed control group for severe zinc deficiency ($P < 0.05$).
[d]Significantly different from the zinc-adequate control group ($P < 0.05$).

in rats with severe zinc deficiency. In severe zinc-deficient rats, feed intake and body weight gain were reduced to 68 and 36%, respectively, of the levels in marginal zinc-deficient rats (Table 2). In addition, plasma zinc concentrations in the two zinc-deficient groups were decreased by > 80% when compared with the corresponding pair-fed control group. Consistent with previous findings [22,35], dietary zinc deficiency decreased absolute liver weight, but not relative liver weight (Table 2). Collectively, these data indicated that consumption of the zinc-deficient diets produced the expected somatic and pathophysiological effects.

3.2. Hepatic microsomal total CYP content and CYP2C11 enzyme activities

As shown in Table 2, hepatic microsomal total CYP content was not significantly different between the various treatment groups. Hepatic microsomal testosterone 2α- and 16α-hydroxylase activities are selective for CYP2C11 [21]. Therefore, these activities were employed as enzyme-selective catalytic markers. As shown in Fig. 1, feed restriction resulted in a reduction in CYP2C11-mediated enzyme activities. When compared with the *ad libitum*-fed control group, both testosterone 2α- and 16α-hydroxylase activities were decreased by 22% in the pair-fed control group for marginal zinc deficiency. By comparison, both of these enzyme activities were reduced by approximately 38% in the pair-fed control for severe zinc deficiency when compared with the *ad libitum*-fed control group. Dietary zinc deficiency resulted in a further decrease in CYP2C11-mediated enzyme activities. When compared with the corresponding pair-fed control groups, marginal and severe zinc deficiency decreased testosterone 2α-hydroxylase activity by 43 and 60%, respectively (Fig. 1A). Similar effects also were obtained for testosterone 16α-hydroxylase activity (Fig. 1B).

3.3. Immunoblot analysis of hepatic CYP2C11 protein content

To determine if the suppression of microsomal testosterone 2α- and 16α-hydroxylase activities by feed restriction and dietary zinc deficiency was accompanied by an alteration in hepatic CYP2C11 protein content, immunoblot assays were performed with rabbit anti-rat CYP2C11 monospecific antibody (Fig. 2A). Densitometric analysis of the immunoblots showed that CYP2C11 levels were decreased by 24 and 19% in the pair-fed control groups for marginal and severe zinc deficiency, respectively, when compared with the *ad libitum*-fed control rats (Fig. 2B). Furthermore, the marginal zinc- and severe zinc-deficient diets decreased CYP2C11 protein levels by 29 and 87%, respectively, when compared with the corresponding pair-fed control rats.

3.4. RT–PCR analysis of CYP mRNA expression

To determine if the decrease in CYP2C11 expression by severe dietary zinc deficiency occurred at a pretranslational level, RT–PCR analysis was performed (Fig. 3). CYP2C11 mRNA expression, expressed as a ratio of the optical density of the CYP2C11 band to that of the cyclophilin band (internal control), was decreased by 66% in the severe zinc-deficient group when compared with the corresponding pair-fed control group (Fig. 4). The mRNA levels of several other sex-dependent CYPs were also determined. Severe zinc deficiency decreased CYP3A2 (49%) and CYP3A18 (43%) mRNA levels, whereas it increased CYP2C12 and CYP3A9 mRNA expression by 253 and 238%, respectively.

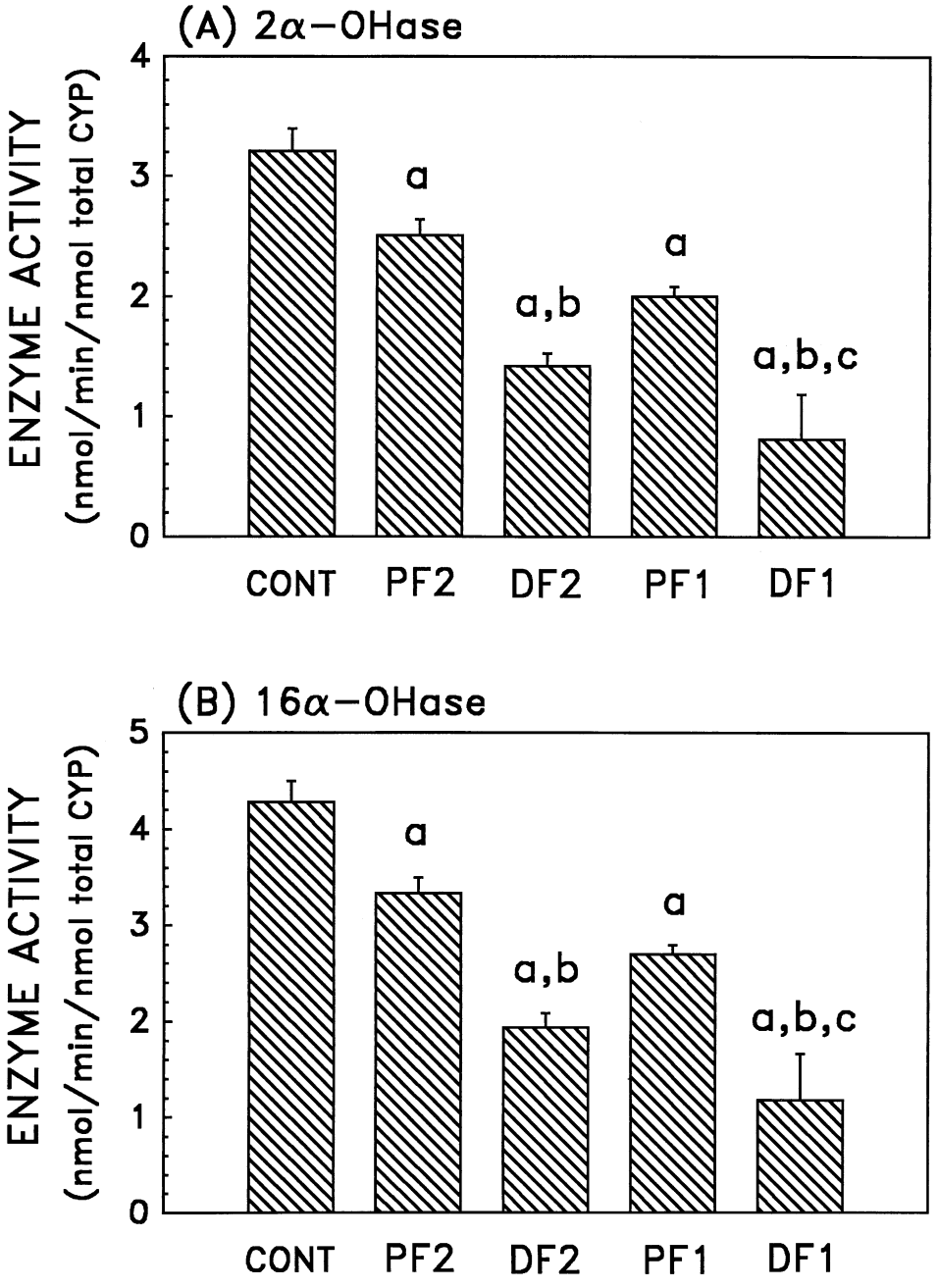

Fig. 1. Effect of dietary zinc deficiency on hepatic microsomal CYP2C11-mediated testosterone 2α- and 16α-hydroxylase activities. From days 21 to 63 of age, male rats were fed a diet containing various levels of zinc, as described in Table 2. Hepatic microsomes were prepared, and a testosterone hydroxylase assay was performed by an HPLC method [28]. Results are expressed as means ± SEM for seven individual rats per treatment group [except for the severe zinc-deficient (DF1) group, where there were samples from six rats]. Key: (a) significantly different from the zinc-adequate control (CONT) group ($P < 0.05$); (b) significantly different from the corresponding pair-fed (PF1 or PF2) control group ($P < 0.05$); and (c) significantly different from the marginal zinc-deficient (DF2) group ($P < 0.05$).

3.5. Plasma testosterone levels

Androgens are required for hepatic expression of sex-dependent CYP enzymes such as CYP2C11 [21], CYP3A2 [36], and CYP3A18 [12] in postpubertal male rats. Therefore, in the present study, we measured serum testosterone levels in rats fed the various zinc-containing diets. When compared to the *ad libitum*-fed control rats, plasma testosterone concentration was not reduced in the pair-fed control for marginal zinc deficiency, whereas it was reduced by 56% in the pair-fed control for severe zinc deficiency (Fig. 5). Marginal zinc deficiency decreased plasma testosterone levels by 67%, when compared with its pair-fed control group. By comparison, severe zinc deficiency resulted in plasma testosterone levels that were below the limit of quantification (0.2 ng/mL).

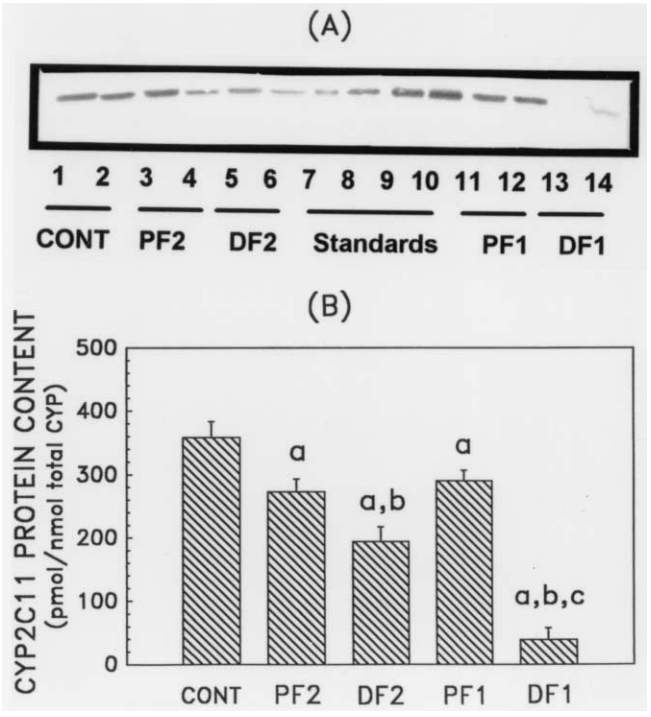

Fig. 2. Effect of dietary zinc deficiency on hepatic microsomal CYP2C11 protein content. From days 21 to 63 of age, male rats were fed a diet containing various levels of zinc, as described in Table 2. Hepatic microsomes (4 pmol total CYP/lane) and purified CYP2C11 standards were subjected to SDS–PAGE. Proteins were transferred electrophoretically onto nitrocellulose membrane. Shown in panel A is a representative immunoblot probed with rabbit anti-rat CYP2C11 monospecific IgG (15 μg/mL) followed by alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (1:3000 dilution). Immunoreactive CYP2C11 protein was detected as described in "Materials and methods." Lanes 1 and 2: zinc-adequate *ad libitum* control (CONT); lanes 3 and 4: pair-fed control (PF2) for the marginal zinc-deficient rats; lanes 5 and 6: marginal zinc-deficient group (DF2); lanes 7–10: purified CYP2C11 standards at 0.25, 0.5, 0.75, and 1 pmol/lane, respectively; lanes 11 and 12: pair-fed control (PF1) for the severe zinc-deficient rats; and lanes 13 and 14: severe zinc-deficient group (DF1). Shown in panel B are CYP2C11 protein content data expressed as means ± SEM for seven individual rats per treatment group (except for the DF1 group, where there were samples from five rats). Key: (a) significantly different from the zinc-adequate control (CONT) group ($P < 0.05$); (b) significantly different from the corresponding pair-fed (PF1 or PF2) control group ($P < 0.05$); and (c) significantly different from the marginal zinc-deficient (DF2) group ($P < 0.05$).

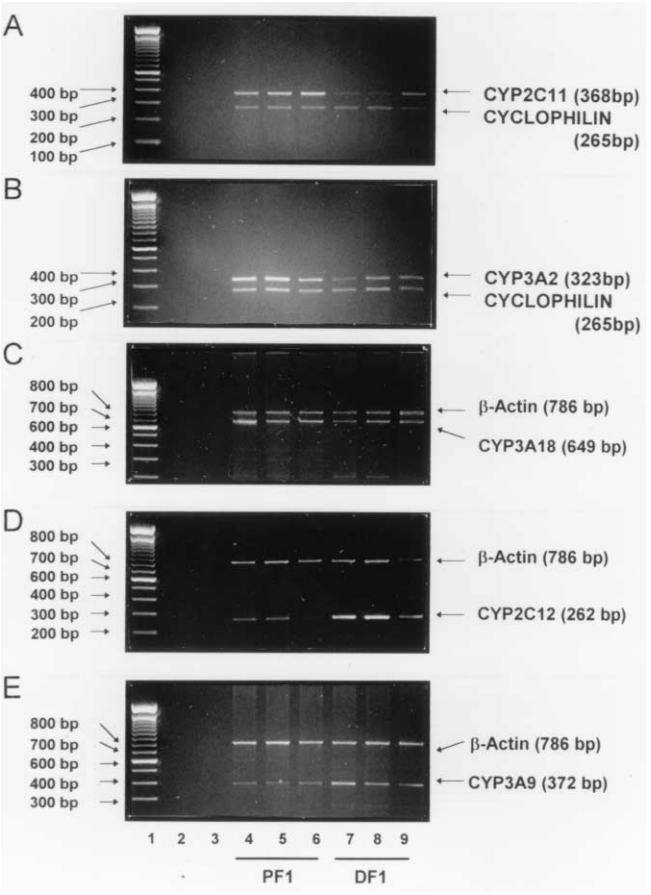

Fig. 3. RT–PCR analysis of hepatic CYP2C11, CYP3A2, CYP3A18, CYP2C12, and CYP3A9 mRNA levels in rats fed a zinc-deficient diet. From days 21 to 63 of age, male rats were fed a diet containing 3 mg zinc/kg of feed, as described in Table 2. Total liver RNA was isolated and reverse transcribed as described in "Materials and methods." CYP2C11 (panel A), CYP3A2 (panel B), CYP3A18 (panel C), CYP2C12 (panel D), and CYP3A9 (panel E) cDNA were co-amplified with β-actin or cyclophilin cDNA (internal control). PCR products were subjected to agarose (1.7%) gel electrophoresis. Shown are ethidium bromide-stained agarose gels. Lane 1: DNA ladder; lane 2: negative control (no primers); lane 3: negative control (no cDNA); lanes 4–6: pair-fed control (PF1) for the severe zinc-deficient group; and lanes 7–9: severe zinc-deficient group (DF1).

4. Discussion

Previous studies have shown a decrease in CYP-mediated aminopyrine *N*-demethylase activity in hepatic microsomes from male rats fed a zinc-deficient diet [22,37,38]. The present study provides the first demonstration that consumption of a zinc-deficient diet during the developmental period resulted in feminization of the mRNA expression of sexually dimorphic hepatic CYPs. Specifically, it suppressed the male-specific CYP2C11, CYP3A2, and CYP3A18 and increased the female-specific CYP2C12 and CYP3A9 in postpubertal male rats.

The previously reported effect of dietary zinc deficiency on hepatic microsomal aminopyrine *N*-demethylase activity [22,37,38] may be explained by our novel finding that CYP2C11-specific testosterone 2α- and 16α-hydroxylase activities were reduced in hepatic microsomes from male rats fed a zinc-deficient diet. The reason is that immunoinhibition experiments have indicated that CYP2C11 contributes substantially to hepatic microsomal aminopyrine *N*-demethylase activity in uninduced male rats [39]. To explain the decrease in this enzyme activity by dietary zinc deficiency, a posttranslational mechanism has been proposed. Based on the findings that dietary zinc deficiency increases hydrogen peroxide production in rat liver [38] and enhances the susceptibility of hepatic microsomal membranes to lipid peroxidation [40], it has been postulated that dietary zinc deficiency causes structural damage and impairment of CYP catalytic function by the increased gener-

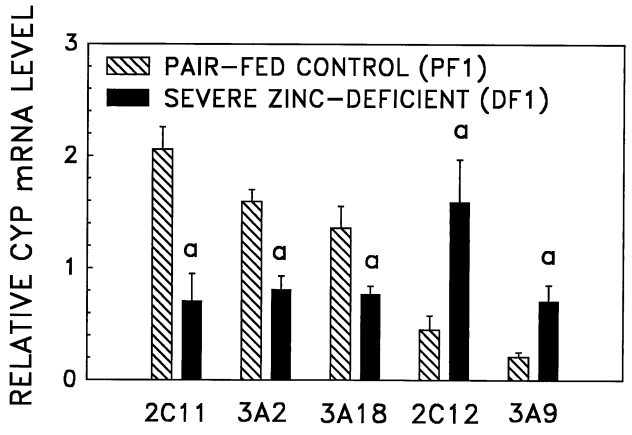

Fig. 4. Hepatic CYP2C11, CYP3A2, CYP3A18, CYP2C12, and CYP3A9 mRNA levels in male rats fed a severe zinc-deficient diet. Photographs of ethidium bromide-stained agarose gels (cf. Fig. 3) were subjected to densitometric analysis. Relative CYP mRNA expression was expressed as a ratio of the optical density of the CYP band to that of the internal control band. Shown are relative CYP mRNA levels (means ± SEM) for five individual rats per treatment group. Key: (a) significantly different from the pair-fed control group ($P < 0.05$).

ation of free radicals [22]. However, other mechanisms also may account for the decrease in CYP catalytic activity by dietary zinc deficiency. As shown in the present study, the mRNA expression of hepatic CYP2C11, CYP2C12, CYP3A2, CYP3A9, and CYP3A18 was altered in rats fed a zinc-deficient diet, leading to our conclusion that a pretranslational mechanism(s) is involved in the modulation of these CYP enzymes by dietary zinc deficiency. The precise molecular basis for this alteration in CYP expression is not known, but transcript initiation is a major control point in the regulation of CYP2C11 and CYP2C12 [21]. Recent studies have identified the GH-pulse activating signal transducer and activator of transcription 5b (STAT5b) [41] and GH-regulated nuclear factor (GHNF) [42] as transcriptional factors involved in the regulation of sexual dimorphic hepatic CYP gene expression. Whether zinc plays a role in these and other signal transduction pathways important in CYP expression is currently unknown.

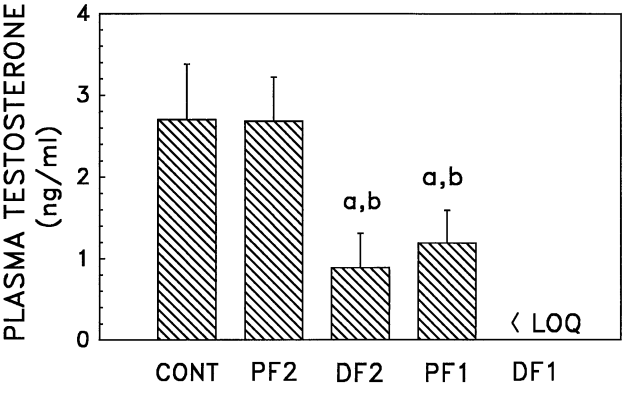

Fig. 5. Effect of dietary zinc deficiency on plasma testosterone concentration. From days 21 to 63 of age, male rats were fed a diet containing various levels of zinc, as described in Table 2. Plasma testosterone concentration was measured by solid-phase ^{125}I-radioimmunoassay as indicated in "Materials and methods." Results are expressed as means ± SEM for seven individual rats per treatment group. Key: (a) significantly different from the zinc-adequate control (CONT) group ($P < 0.05$); and (b) significantly different from the pair-fed control for the marginal zinc-deficient rats (PF2) ($P < 0.05$). Plasma testosterone concentration in the severe zinc-deficient (DF1) group was below the limit of quantification (LOQ), which was 0.2 ng/mL.

Androgen plays an essential role in regulating the expression of male-specific hepatic CYP enzymes. Castration and testosterone replacement studies have shown that this androgen is required for postpubertal expression of CYP2C11 [14,17,30], CYP3A2 [36], and CYP3A18 [12] in male rats. Therefore, the observed reduction in plasma testosterone concentration in rats fed a zinc-deficient diet could explain, at least in part, the concomitant decrease in CYP2C11, CYP3A2, and CYP3A18 expression. However, this change in androgen levels cannot account for the up-regulation of CYP2C12 and CYP3A9 expression by dietary zinc deficiency in male rats. According to previous studies with castrated prepubertal and postpubertal male rats, the absence of this hormone does not increase the expression of CYP2C12 [14] or CYP3A9 [12]. Therefore, an additional hormonal mechanism(s) must be involved in the overall modulation of the sexually dimorphic hepatic CYP enzyme expression by dietary zinc deficiency.

The pattern of pituitary GH secretion influences hepatic levels of the sexually dimorphic CYP enzymes. Hepatic CYP2C11 [17], CYP3A2 [43], and CYP3A18 [12] levels are abolished in male rats administered GH by continuous infusion as a means to mimic the female pattern of endogenous GH secretion. As little as 3% of the normal concentration of GH found in female rats is sufficient to decrease CYP2C11 and CYP3A2 expression [44]. By comparison, continuous GH infusion induces CYP2C12 [13] and CYP3A9 [12] in male rats. Based on these findings, alteration in the pattern of pituitary GH secretion could be a potential neuroendocrine mechanism for the suppression of CYP2C11, CYP3A2, and CYP3A18 and the concomitant increase in CYP2C12 and CYP3A9 levels observed in the present study. It is possible that in male rats fed a zinc-deficient diet during the developmental period, the deficiency of this essential trace mineral interferes with the establishment of the male-specific pattern of pulsatile, intermittent GH secretion that normally occurs during puberty [16]. As a result, the plasma GH profile remains continuous and is recognized as feminine by the hepatocytes. However, a detailed examination of the GH secretory dynamics in rats fed a zinc-deficient diet will be required to test this possibility.

In summary, dietary zinc deficiency during the developmental period resulted in a pretranslational suppression of the male-specific hepatic CYP2C11, CYP3A2, and CYP3A18 expression in postpubertal male rats. These changes were accompanied by a concomitant induction of CYP2C12 and CYP3A9, which are normally expressed at

significant levels only in postpubertal female rats. The mechanism responsible for these effects is not known, but based upon the overall pattern of modulation of CYP expression, it may involve perturbation of the hypothalamic-pituitary-gonadal axis, thereby affecting the sex-specific pattern of pituitary GH secretion and androgen production.

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