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Prenatal Cocaine, Alcohol, and Undernutrition Differentially Alter Mineral and Protein Content in Fetal Rats

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CHURCH, M. W., K.-L. C. JEN, M. A. PELLIZZON AND P. A. HOLMES. *Prenatal cocaine, alcohol, and undernutrition differentially alter mineral and protein content in fetal rats.* PHARMACOL BIOCHEM BEHAV **59**(3) 577–584, 1998.—Alcohol exposure and undernutrition during pregnancy have been associated with altered fetal body composition. Recent observations suggest that cocaine exposure during pregnancy may impair delivery of nutrients to the fetus and could thereby alter body growth and composition. Such effects are important because they can adversely influence physical and neural development. Consequently, we investigated the dose-dependent effects of cocaine on fetal body composition in an animal (rat) model and compared such effects with those caused by prenatal alcohol exposure and undernutrition. Pregnant Sprague–Dawley rats received either 20, 30, 40, or 50 mg/kg cocaine HCl (SC) twice daily from gestation days 7 through 19. Pair-fed (undernutrition) and untreated control groups and a group receiving 3.0 g/kg alcohol (PO) twice daily served as comparison groups (n = 11 to 14/group). Females were sacrificed on gestation day 20. One male and one female fetus was removed from each dam. The fetuses were minced, dehydrated, defatted, and analyzed for content of protein and the minerals Zn, Ca, Fe, Mg, K, and Na. In terms of concentration per unit of fat-free dry solids, male fetuses in the cocaine groups showed significant decreases in protein compared to untreated controls (15 ± 3 to 20 ± 2 mg/g vs. 24 ± 4 mg/g, $p = 0.01$). There was a significant treatment effect for Ca ($p < 0.05$), reflecting a trend for decreased Ca concentrations in the fetuses of the cocaine and undernutrition groups. Male fetuses in the alcohol group had significantly elevated Mg levels compared to male fetuses in the other groups (3.0 ± 0.8 vs. 1.0 ± 0.2 to 2.3 ± 0.7 mg/g, $p < 0.05$). There were some sex differences, with female fetuses having significantly lower concentrations of Mg, Fe, K, and higher protein concentrations than male fetuses. Although the effects were few and modest, these results suggest that prenatal cocaine, alcohol, and undernutrition can differentially alter fetal body weight and composition and, therefore, adversely influence fetal development. © 1998 Elsevier Science Inc.

Body composition Fetal development Mineral content Prenatal alcohol Prenatal cocaine Prenatal undernutrition Protein content Teratology

SEVERAL recent observations suggest that prenatal cocaine exposure may impair the delivery of nutrients to the fetus. For example, cocaine exposure during pregnancy reduced placental sodium transport in mice (23), caused hyponatremia in human neonates (4,12), decreased uterine blood flow in rodents and sheep (13,32), reduced amino acid uptake in rat and human placentae (3,11,27), decreased the percentage of body fat content and the whole-body weights of rat fetuses and human infants (5,16), altered bone composition in human and rat offspring (29,30), and caused maternal diuresis, appetite loss, and undernutrition (9,10). Such conditions can cause various imbalances in fetal body composition, thereby compromising the neurological and physical development of the fetus and infant (19–21).

Consequently, we evaluated select aspects of fetal body composition in an animal model of prenatal cocaine exposure. Specifically, near-term rat fetuses that were prenatally exposed to cocaine were evaluated for sodium, calcium, iron, magnesium, potassium, zinc, and protein content. The effects of prenatal undernutrition and prenatal alcohol exposure were also evaluated for comparison purposes because both conditions can cause altered fetal body composition (18–21).

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In a previous study, we evaluated the current population of animals, but assessed different dependent variables (9). For example, the previous study reported decreased maternal food consumption and weight gain, decreased fetal weights, and increased fetal morbidities and mortalities in some of the treated groups described herein. The fetuses in the undernutrition, alcohol and high-dose cocaine groups also exhibited decreased percentages of total body fat content, but not percentages of body water, dry weights, or fat-free dry solids. Such alterations in fetal body fat content lead to the current follow-up study, which sought to investigate other possible alterations in fetal body composition.

METHOD

Subjects

Nulliparous female Sprague–Dawley rats (Harlan–Sprague–Dawley, Inc., Indianapolis, IN), aged 90–110 days at the time of mating, were housed in polycarbonate cages (45 × 23 × 20 cm) with wood chip bedding material. Rooms were temperature (22 ± 1°C) and humidity (40–50%) controlled with a timed light cycle of 14 h of light per day (0500 to 1900 h). Matching for weights, animals were assigned to an untreated control (UTC) group, one of four cocaine-treated groups, one of two pair-fed control groups, or an alcohol-treated (ALC) group (n = 11–14 dams per group).

The morning on which a sperm plug was found was designated gestation day 0 (GD0). Starting on GD7 and continuing to GD19 (inclusive), the rats in the cocaine-treated groups were given twice daily SC injections of 20, 30, 40, or 50 mg/kg cocaine hydrochloride (HCl) dissolved in normal saline (2% solution). The first dose was administered between 0900 and 1000 h and the second dose between 1500 and 1600 h.

To obviate problems associated with skin ulcerations, cocaine solution volumes greater than 0.30 ml were injected into multiple sites. Although some skin ulcerations did occur in the higher dose groups, only a few required treatment with antibiotic ointment and healed rapidly. Gestation days 7–19 include the major periods for organogenesis and CNS development in the rat. Hereafter, the cocaine-treated groups will be referred to as C20, C30, C40, and C50.

Cocaine doses were selected on the basis of several considerations. First, there was a desire to use not just one or two doses, but a broad range of doses. This would permit evaluation of cocaine's dose-dependent effects. Second, we sought to use doses that would unequivocally span the range of toxicity. This meant using doses that would extend above and below the thresholds of the no observable effects levels (NOELs) for various maternal and fetal parameters. Third, our dose levels were similar to previous studies (6–10). For data on the blood serum cocaine levels of the animals used in the current study, the reader is referred to our previous publication (9).

The two pair-fed control groups (PF40 and PF50) were pair-fed to the C40 and C50 groups according to standard procedures (9,10). There was no need to pair-water animals in these groups because pair-fed animals drink as much or less water than their cocaine-treated cohorts (9,10). Pair-fed animals also received twice daily injections of isotonic saline solution (0.85% NaCl) that were isovolumetric to the cocaine solutions administered to the C40 and C50 animals (9,10). The PF40 and PF50 groups allowed us to assess the effects of undernutrition and handling stress that accompanies cocaine treatment (9,10).

The alcohol-treated (ALC) group received 3.0 g/kg alcohol (via oral gavage) twice daily from GD7 through GD19. This alcohol dose has a maternal and fetal toxicity level similar to 50 mg/kg cocaine HCl (twice daily) in the laboratory rat as assessed by maternal weight gain and food consumption and pup birth weights (9,10). The ALC group served as a comparison drug group. For data on the blood alcohol levels of the animals used in the current study, the reader is referred to our previous study (9).

The untreated control (UTC) group received no treatment and received no handling except for daily weighing. The UTC group was the normal control group to which all other groups were compared (9,10).

Animals in all groups were weighed daily at 0830 to 0900 h. Records were kept on changes in maternal body weight, food consumption, and water consumption throughout gestation. Food consisted of a special rodent chow (Teklad 10% pregnancy diet, Harlan–Sprague–Dawley, Inc.), which was started on GD0. On the morning of GD20, animals were sacrificed by carbon dioxide inhalation. The pups were removed from the uteri, gender was determined, and the pups weighed. For data on maternal weight, food, and water consumption, the reader is referred to our previous publication (9).

Fetal Body Composition Determination

Fetal body composition was determined chemically using the following procedure: immediately after removal, one male and one female fetus from each litter were selected at random and placed in separate preweighed beakers, minced with scissors, and weighed to the nearest 0.001 g. This measurement constituted the body weight or wet weight (WW). The minced tissues were desiccated to constant weight at 100°C in a convection oven. Dry weight (DW) was the weight after dessication (i.e., the weight of dry solids). Total body water (TBW) was calculated by subtracting dry weight from wet weight (TBW = WW − DW). After dessication, the dried solids were transferred to preweighed Whatman No. 1 filter paper and weighed to the nearest 0.0001 g. The filter paper with sample was loosely folded and inserted in a Tecator 15220034 thimble. Total body fat (TBF) was extracted at 105°C in a Soxhlet apparatus, using 45 ml of petroleum ether for each sample. Boiling and rinsing times were both 40 min. The coefficient of variation for this method of extraction was 3.4%. Fat-free dry solids (FFDS) weight or lean body bass was determined by subtracting the weight of the total body fat from the dry weight (FFDS = DW − TBF) (9). For data on fetal DW, TBW, TBF, and FFDS, the reader is referred to our previous publication (9).

Protein content was analyzed by the Lowry method (22) using 0.01 g of FFDS from each fetus. For mineral determinations, 0.03 g of FFDS sample from each fetus was wet washed with concentrated nitric acid, followed by 30% hydrogen peroxide. After complete dryness, 2 ml of 0.01% nitric acid was added to each sample. Calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), and zinc (Zn) analyses were performed using a Perkin–Elmer Model 5000 atomic absorption spectrophotometer (Perkin–Elmer, Norwalk, CT). The hollow cathode lamps of the respective elements were operated under standard conditions with wavelengths set at Ca = 422.7 nm, Mg = 285.2 nm, Na = 589.0 nm, K= 766.5 nm, Fe = 248.3 nm, and Zn = 213.9 nm. Standards were prepared for each element from certified standard solutions.

Data Analyses

The fetal body composition data were analyzed by two-way (group-by-sex) ANOVAs. When males and females from

the same litter are used, some investigators choose to treat sex as a repeated measure in statistical analyses. Because several of our dependent measures had results from only one gender of the male/female littermate pairs and because a between-subject analysis is more conservative than a repeated measures analysis, we treated sex as a between-subjects measure in all ANOVAs. When an ANOVA indicated a significant treatment effect, a post hoc test (Duncan's Multiple Range Test) was used to determine which treatment groups differed significantly from each other. Statistical significance was assumed for probability levels of 0.05 or less.

RESULTS

Total Amounts per Fetus

Table 1 shows the mineral and protein content data expressed in terms of the total amounts per fetus. This table also summarizes the statistical results and lists the number of male and female fetuses examined in each group.

Zinc. There was a significant group effect for zinc content. Post hoc analyses indicated that the ALC and PF50 male fetuses had significantly decreased zinc amounts. There was a significant sex effect, indicating that female fetuses had lower zinc amounts than their male cohorts. There was a significant group-by-sex interaction, reflecting the fact that the female fetuses (unlike the males) showed no significant group differences.

Calcium. There was a significant group effect for calcium content. Post hoc analyses indicated that the PF40 and PF50 male fetuses had significantly decreased calcium amounts. There were nearly significant trends for reduced calcium amounts in male and female fetuses as cocaine doses gradually increased. There were no significant effects for sex or the group-by-sex interaction.

Iron. There was a significant group effect for iron content. Whereas the post hoc test failed to identify any one group as having significantly altered iron amounts relative to the control groups at the criterion level of $p < 0.05$, female fetuses in the ALC, PF50, and C50 groups showed trends for decreased iron amounts. There was a significant sex effect, indicating that female fetuses had lower iron amounts than their male cohorts. There was no significant group-by-sex interaction.

Magnesium. There was no significant group effect for magnesium content. Female fetuses had significantly lower magnesium amounts than male fetuses. The group-by-sex interaction was significant, reflecting higher magnesium amounts in the ALC and PF40 male fetuses compared to their female counterparts.

Potassium. There was a significant group effect for potassium content, reflecting decreased amounts in the ALC, PF50, C30, C40, and C50 male fetuses. There was a significant sex effect, indicating that female fetuses had less potassium amounts than their male counterparts. There was no significant group-by-sex interaction.

Sodium. There was a significant group effect for sodium content. Post hoc analyses indicated decreased sodium amounts for male fetuses in the ALC, PF50, C40, and C50 groups and for the female fetuses in the ALC and PF50 groups and a nearly significant decrease for the C50 female fetuses. There were no significant effects for sex or the group-by-sex interaction.

Protein. There was a significant group effect for protein content. Post hoc analyses indicated lower protein amounts for the male fetuses in the ALC, PF40, C30, and C50 groups and for the female fetuses in the ALC, PF40, and PF50 groups and a trend for decreased protein amounts for the female fetuses in the C20, C40, and C50 groups. There was a significant sex effect, indicating higher protein amounts in the female fetuses. There was no significant group-by-sex interaction.

Weight. There was a significant group effect for fetal weight. Post hoc analyses indicated decreased weights for male and female fetuses in the ALC, PF50, and C50 groups. There were no significant effects for sex or the group-by-sex interaction.

Concentration Per Gram of FFDS

The mineral and protein amounts described above were influenced in part by fetal weights. That is, a growth retarded fetus is likely to have lesser amounts of body composition elements than the normal fetus merely because of its reduced body size. Consequently, mineral and protein contents were reanalyzed to control for this growth retardation factor. This was accomplished by determining mineral and protein content on the basis of concentration per gram of FFDS. The results of these analyses are presented in Table 2. When mineral and protein concentrations were analyzed thusly, many of the significant effects shown in Table 1 dissipated and some new effects emerged.

Zinc, iron, potassium, sodium. Briefly, there were no longer any significant group differences for zinc, iron, potassium, or sodium.

Calcium. There was a significant group effect for calcium concentration. Post hoc analyses indicated that the PF40 and C40 male fetuses had decreased calcium concentrations. The data in Table 2 strongly suggested a general trend for reduced calcium concentrations in all pair-fed and cocaine-treated groups. Consequently, the calcium concentration data were reanalyzed by combining the male and female data within each treatment group and by collapsing both pair-fed groups into one pair-fed group and by collapsing all four cocaine-treated groups into one cocaine-treated group. This was done to increase statistical power. The subsequent ANOVA indicated a significant group effect: $F(3,179) = 2.35$, $p < 0.05$. Mean ($\pm$ SEM) values for the UTC, ALC, pair-fed, and cocaine groups were 9.5 (0.9), 9.3 (1.2), 7.5 (0.4), and 7.8 (0.3) mg/g FFDS, respectively. Post hoc analyses indicated that these pair-fed and cocaine groups had significantly reduced calcium concentrations compared to the UTC and ALC groups ($p < 0.05$, one-tailed tests).

Magnesium. There was a nearly significant group effect and a significant group-by-sex interaction for magnesium concentration. Because a previous study found elevated magnesium concentrations in alcohol exposed fetuses (18), we had a priori reasons to test this hypothesis. Post hoc analyses indicated significantly elevated magnesium concentrations in ALC male fetuses, but not in ALC female fetuses.

Protein. There was a significant group effect for protein concentration. Post hoc analyses indicated significantly reduced protein concentrations for the C30 male fetuses and the PF40 female fetuses. There was a nearly significant decrease for the PF40 male fetuses. A significant group-by-sex interaction reflected the fact that male fetuses in the C20, C30, C40, and C50 groups had significantly lower protein concentrations than their female cohorts (see Table 2).

Sex. There were significant sex effects for iron, magnesium, potassium, and protein concentrations, indicating lower concentrations of these three minerals and a higher concentration of protein in the female fetuses.

TABLE 1

TOTAL AMOUNTS PER FETUS (MEAN ± SEM)

Variable	Group								Probability Values		
	UTC	ALC	PF40	PF50	C20	C30	C40	C50	Group (G)	Sex (S)	G × S
Zinc (μg/fetus)											
Male	560 (44)†	341 (33)*	489 (32)	371 (38)*	440 (41)†	531 (29)	496 (42)	490 (36)	0.001	0.035	0.034
Female	438 (50)	318 (34)	456 (24)	358 (33)	580 (48)	414 (31)	417 (48)	400 (46)			
Calcium (mg/fetus)											
Male	42 (8)	28 (7)	25 (4)*	23 (3)*	30 (5)	29 (4)	26 (2)	27 (5)	0.015	NS	NS
Female	33 (3)	22 (3)	23 (2)	29 (2)	31 (4)	27 (2)	28 (3)	25 (4)			
Iron (mg/fetus)											
Male	1.7 (0.2)	1.3 (0.2)	2.2 (0.5)	1.8 (0.3)	2.2 (0.5)	1.9 (0.2)	1.4 (0.3)	1.6 (0.3)	0.037	0.008	NS
Female	1.9 (0.2)	1.1 (0.2)	1.7 (0.2)	1.0 (0.2)	1.5 (0.3)	1.6 (2)	1.4 (0.2)	1.1 (0.1)			
Magnesium (mg/fetus)											
Male	7.4 (1.5)	8.2 (2.2)†	8.9 (2.8)†	3.1 (0.4)	3.8 (0.5)	6.5 (1.8)	4.9 (1.0)	4.0 (1.1)	NS	0.001	0.05
Female	4.6 (0.6)	2.5 (0.2)	3.2 (0.2)	3.3 (0.3)	4.1 (0.4)	3.7 (0.5)	4.7 (0.4)	2.9 (0.3)			
Potassium (mg/fetus)											
Male	71 (4)†	40 (3)*	61 (4)	46 (6)*	62 (6)†	58 (5)*	59 (5)*	47 (3)*†	0.001	0.001	NS
Female	46 (4)	34 (2)	50 (3)	37 (2)	45 (3)	48 (2)	54 (3)	34 (3)			
Sodium (mg/fetus)											
Male	4.0 (0.3)	2.6 (0.1)*	3.5 (0.2)	2.7 (0.2)*	3.4 (0.1)	3.7 (0.2)	3.2 (0.3)*	2.7 (0.2)*	0.001	NS	NS
Female	3.5 (0.3)	2.5 (0.1)*	3.4 (0.2)	2.8 (0.1)*	3.5 (0.3)	3.2 (0.2)	3.5 (0.3)	2.9 (0.3)			
Protein (mg/fetus)											
Male	96 (16)†	59 (7)*	61 (8)*	87 (6)	76 (8)	55 (8)*†	70 (8)	58 (12)*†	0.001	0.001	NS
Female	129 (14)	64 (7)*	83 (3)*	92 (6)*	104 (10)	118 (10)	109 (5)	103 (13)			
Fetal weight (g/fetus)											
Male	4.0 (0.2)	2.8 (0.1)*	3.8 (0.1)	3.1 (0.1)*	3.8 (0.1)	3.7 (0.1)	3.8 (0.2)	3.1 (0.1)*	0.001	NS	NS
Female	3.9 (0.2)	2.6 (0.1)*	3.5 (0.1)	3.2 (0.1)*	3.7 (0.1)	3.3 (0.1)	3.8 (0.2)	3.0 (0.2)*			
Group size (*n*)											
Male	13	11	13	11	13	14	13	12			
Female	13	11	13	12	13	14	13	10			

* Different from untreated control (UTC), $p < 0.05$ (Duncan's Multiple Range Test).

† Different from female cohorts, $p < 0.05$ (Duncan's Multiple Range Test).

TABLE 2

CONCENTRATION PER GRAM OF FAT-FREE DRY SOLID (MEAN ± SEM)

	Group								Probability Value		
Variable	UTC	ALC	PF40	PF50	C20	C30	C40	C50	Group	Sex	G × S
Zinc (μg/g FFDS)											
Male	137 (7)	123 (9)	127 (8)	117 (11)	115 (11)	144 (7)	133 (10)	157 (14)	NS	NS	0.025
Female	113 (11)	119 (9)	129 (6)	113 (10)	157 (13)	122 (10)	109 (12)	131 (11)			
Calcium (mg/g FFDS)											
Male	10.6 (1.9)	10.4 (2.5)	6.4 (0.9)*	7.4 (0.7)	8.0 (1.2)	7.8 (1.0)	6.8 (0.6)*	8.6 (1.5)	0.035†	NS	NS
Female	8.4 (0.6)	8.3 (0.8)	6.4 (0.4)	9.8 (0.9)	8.3 (1.0)	8.0 (0.7)	7.3 (0.6)	8.2 (1.0)			
Iron (mg/g FFDS)											
Male	0.43 (0.06)	0.49 (0.07)	0.58 (0.11)	0.57 (0.09)	0.56 (0.13)	0.52 (0.04)	0.36 (0.06)	0.50 (0.07)	NS	0.03	NS
Female	0.50 (0.06)	0.43 (0.05)	0.50 (0.05)	0.34 (0.06)	0.41 (0.08)	0.47 (0.04)	0.38 (0.05)	0.36 (0.04)			
Magnesium (mg/g FFDS)											
Male	1.8 (0.4)	3.0 (0.8)*‡	2.3 (0.7)	1.0 (0.2)	1.0 (0.2)	1.7 (0.5)	1.4 (0.3)	1.3 (0.3)	0.065†	0.001	0.028
Female	1.1 (0.1)	1.0 (0.1)	0.9 (0.1)	1.1 (0.1)	1.1 (0.1)	1.1 (0.1)	1.2 (0.1)	1.0 (0.1)			
Potassium (mg/g FFDS)											
Male	17.7 (0.8)‡	14.8 (0.8)	15.7 (1.1)	14.7 (1.5)	16.0 (1.3)‡	15.6 (1.2)	16.0 (1.4)	15.3 (1.2)‡	NS	0.001	NS
Female	12.1 (0.6)	13.3 (0.8)	14.1 (0.8)	12.0 (0.7)	12.2 (0.7)	14.2 (0.5)	14.0 (0.7)	11.2 (0.6)			
Sodium (mg/g FFDS)											
Male	0.95 (0.05)	0.93 (0.04)	0.89 (0.04)	0.86 (0.03)	0.90 (0.03)	1.00 (0.04)	0.87 (0.04)	0.90 (0.05)	NS	NS	NS
Female	0.90 (0.03)	0.97 (0.03)	0.98 (0.03)	0.90 (0.04)	0.91 (0.07)	0.95 (0.04)	0.87 (0.03)	0.94 (0.05)			
Protein (mg/g FFDS)											
Male	24 (4)	21 (2)	16 (2)	28 (1)	20 (2)‡	15 (3)*‡	19 (2)‡	19 (4)‡	0.010†	0.001	0.001
Female	32 (3)	25 (3)	23 (1)*	29 (2)	28 (2)	35 (3)	28 (1)	33 (3)			

* Different from untreated control (UTC), $p < 0.05$ (Duncan's).

† Directional (one-tail) probability values.

‡ Different from female cohorts, $p < 0.05$ (Duncan's Multiple Range Test).

DISCUSSION

In utero growth retardation (IUGR) and altered body composition are associated with adversely affected physical and nervous system development and function (19,21,26). Prenatal cocaine, alcohol, and undernutrition can cause IUGR as well as physical and nervous system defects. Consequently, the present study investigated the differential effects of prenatal cocaine, alcohol, and undernutrition on the content of zinc, calcium, iron, magnesium, potassium, sodium, and protein in near-term fetal rats.

There were significant group differences for zinc, calcium, iron, potassium, sodium, and protein content when considered in terms of total amounts per fetus. Many of these effects were influenced in part by growth retardation in the treated animals, however. To control for this influence, fetal mineral and protein content were reanalyzed in terms of concentrations per gram of FFDS. When analyzed thusly, many of the initial group differences dissipated and new ones emerged.

One such effect concerned calcium concentrations. Prenatal cocaine exposure resulted in decreased calcium concentrations in FFDS. Although only the C40 males were significantly different by post hoc comparisons, there was a significant main effect for treatment groups that reflected a fairly consistent trend for low Ca levels in the other cocaine groups. When the data were reanalyzed after collapsing across gender and cocaine dose level, there was a significant Ca decrease for the cocaine condition. This result is consistent with observations of decreased calcium concentrations and altered bone composition in rats (29) and humans (28) prenatally exposed to cocaine. Decreased calcium concentrations were also observed in some prenatally undernourished fetuses, suggesting that the effect in the cocaine exposed fetuses may have been influenced by undernutrition.

Prenatal cocaine exposure was also associated with a significant reduction in protein concentration in the FFDS of male fetuses. This result is consistent with reports that cocaine impairs placental transfer of amino acids to the fetus (3,11,27). We are uncertain why this effect was only observed in the male rat fetuses. There is some literature indicating that male offspring are more adversely affected by pre- and/or postnatal undernutrition than their female cohorts (2,15,21, 24). This gender difference in sensitivity to undernutrition may be governed by hormonal differences.

Our data showed some indication that protein concentrations could be reduced by prenatal undernutrition as well. It is well known that deficient maternal nutrition can result in decreased protein levels in the fetuses (26,31). Prenatal cocaine exposure and undernutrition had no significant effects on zinc, iron, magnesium, potassium, or sodium concentrations in FFDS samples.

In a previous study, we observed decreased percent body fat content in the PF50, ALC, C40, and C50 rat fetuses (9). In terms of mechanisms, the reduction in fetal protein and body fat content in cocaine-exposed offspring suggests decreased protein and fat synthesis secondary to decreased placental transport of fatty acids and amino acids. This could have resulted from reduced supplies of these nutrients in the maternal blood secondary to decreased maternal food consumption, increased maternal/fetal energy demands, and the uterine and placental vasoconstrictions that accompany cocaine intoxication. Decreased transport of amino acids, for example, has been observed in both animal (27) and human placentae (3,11) following cocaine exposure. Likewise, the reduction in fetal calcium levels in such fetuses may have been due to decreased placental transport secondary to reduced maternal food consumption, uterine vasoconstriction, or even the chelation of Ca ions by cocaine (25). Reduced blood flow is one of the main mechanisms of IUGR (19,26,31).

It is also conceivable that the reduced protein, fat, and calcium levels may be secondary to fetal immaturity. That is, fetal weight, percent body fat, protein, and calcium concentrations increase with gestational age at the expense of body water (19). Thus, the relatively decreased levels of these former substances in the cocaine and pair-fed fetuses resemble those of chemically and physically immature offspring.

Proteins and fatty acids are important sources of calories and structural components of organs, the nervous system, and hormones. Calcium is needed for bone growth and the excitability of nerves and muscles. Deficiencies in these nutrients can, therefore, result in IUGR, decreased internal organ sizes, decreased cell numbers, decreased skeletal growth, limb reduction, decreased intelligence, immune deficiencies, and increased pre- and postnatal mortality (17,19–21,26,31).

One concern is the general lack of dose-dependent changes across the four doses of cocaine. The effects of prenatal cocaine are not always linear. For example, the animal literature suggests that a threshold dose is needed to cause significantly decreased fetal weight and body fat (9) and certain neurobehavioral effects (10), but that higher doses probably do not cause progressively greater effects on these parameters. This may also be the case with fetal protein, fat, and Ca concentrations.

Another concern is whether the drug treatments produced growth retardation and abnormal body composition that was similar across all pups in a litter or confined to a few pups that would have been born physically and behaviorally abnormal and perhaps nonviable. In past studies, we have noted fetuses and viable offspring with abnormalities such as anophthalmia, hearing loss, cephalic hemorrhages, dentofacial malformations, and/or limb malformations (6–10). Such abnormalities frequently occurred in just one or two littermates, while the remaining littermates were ostensibly normal. It seems reasonable, therefore, to assume that abnormalities in body composition may have been expressed strongly in only a few pups while other littermates were less noticeably affected.

Prenatal alcohol exposure was associated with significantly elevated magnesium concentrations in the FFDS samples of male fetuses. For unknown reasons, this effect was not echoed in their female counterparts. A previous study observed significantly elevated magnesium levels in prenatal alcohol-exposed rat fetuses. This previous study did not evaluate sex-dependent differences, however, (18). Prenatal alcohol exposure did not have significant effects on zinc, calcium, iron, potassium, sodium, or protein concentrations in the FFDS samples. Previous research on the effects of prenatal alcohol exposure on these body composition variables has been inconclusive (1).

There were several significant sex-dependent differences. Compared to their male counterparts, female fetuses had significantly lower iron, magnesium, and potassium, but higher protein concentrations in the FFDS samples. These differences were quite consistent, occurring in all or almost all of the eight treatment groups. We are unaware of any previous studies that have investigated rat fetuses for such sex-dependent differences. Such differences may have been the result of sex-dependent differences in hormonal environments.

Due to economic constraints, the present study was able to assess only a few minerals. Because our results suggest that prenatal cocaine, alcohol, and undernutrition can differentially alter various aspects of fetal body composition, it would be worthwhile for future studies to investigate the influence of

such treatments on other minerals (e.g., iodine, chloride, manganese) and vitamins. Various vitamin deficiencies are known to occur in adults who abuse cocaine (30) and alcohol (14). Similar vitamin deficiencies may also occur in fetuses chronically exposed to such drugs.

Combined, the results of the previous and current studies indicate that the effects of cocaine, alcohol, and undernutrition on the developing fetus were similar in some respects, yet different in other respects. The cocaine and pair-fed treatment conditions were similar in that they caused decreased fetal weights, body fat, calcium, and protein concentrations. The alcohol treatment condition was different in its effects on calcium, magnesium, and protein content. Knowledge about the common and differential effects of each maternal condition on fetal body composition is important because such information can guide pre- and postnatal nutritional intervention. Such intervention would involve supplementation that provides the deprived nutrients (21). Because our results suggest that prenatal cocaine exposure is associated with decreased birth weight, body fat, protein, and calcium content, pre- and postnatal supplementation should provide at least extra fats, proteins, and calcium.

The results of the present study were admittedly modest and few. This is the first study to examine the issue of protein and mineral content in cocaine-exposed fetuses. Thus, these results should be regarded as preliminary and suggestive, not definitive. Yet the basic findings of the current study and our previous study that prenatal cocaine can reduce fetal weight, percent body fat, protein, and calcium concentrations are consistent with the malnutrition literature that suggests such effects reflect decreased nutrient supplies to the fetal and subsequent chemical and physical immaturity (19,21,31).

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REFERENCES

1. Abel, E. L.: Fetal alcohol syndrome and fetal alcohol effects. New York: Plenum Press; 1984.
2. Barnes, R. H.; Cunnold, S. K.; Zimmermann, R. R.; Simmons, H.; MacLeod, R. B.; Krook, L.: Influence of nutritional deprivation in early life on learning behavior of rats as measured by performance in a water maze. J. Nutr. 89:339–410; 1996.
3. Barnwell, S. L.; Sastry, B. V. R.: Depression of amino acid uptake in human placental villus by cocaine, morphine and nicotine. Trophoblast Res. 1:101–120, 1983.
4. Carlan, S. J.; Stromquist, C.; Angel, J.; Harris, M.; O'Brien, W. F.: Cocaine and indomethacin: Fetal anuria, neonatal edema, and gastrointestinal bleeding. Obstet. Gynecol. 78:501–503; 1991.
5. Chandra, R. K.: Nutrition and immunity: lessons from the past and new insights into the future. Am. J. Clin. Nutr. 53:1087–1101; 1991.
6. Church, M. W.; Dintcheff, B. A.; Gessner, P. K.: Dose-dependent consequences of cocaine on pregnancy outcome in the Long–Evans rat. Neurotoxicol. Teratol. 10:51–58; 1988.
7. Church, M. W.; Overbeck, G. W.; Andrzejczak, A. L.: Prenatal cocaine exposure in the Long–Evans rat: I. Dose-dependent effects on gestation, mortality and postnatal maturation. Neurotoxicol. Teratol. 12:327–334; 1990.
8. Church, M. W.; Overbeck, G. W.: Sensorineural hearing loss as evidenced by the auditory brainstem response (ABR) following prenatal cocaine exposure in Long–Evans rat. Teratology 43:561–570; 1991.
9. Church, M. W.; Morbach, C. A.; Subramanian, M. G.: Comparative effects of prenatal cocaine, alcohol and undernutrition on maternal/fetal toxicity and body composition in the Sprague–Dawley rat with observations on strain-dependent differences. Neurotoxicol. Teratol. 17:559–567; 1995.
10. Church, M. W.; Tilak, J. P.: Differential effects of prenatal cocaine and retinoic acid on activity level throughout day and night. Pharmacol. Biochem. Behav. 55:595–605; 1996.
11. Dicke, J. M.; Verges, D. K.; Polakoski, K. L.: Cocaine inhibits alamine uptake by human placental microvillous membrane vesicles. Am. J. Obstet. Gynecol. 169:512–521; 1993.
12. Dollberg, S.; Armon, Y.; Gur, I.; Litt, R.; Gale, R.: Hyponatremia in a neonate of a cocaine abusing mother. J. Toxicol. Clin. Toxicol. 27:287–292; 1989.
13. Fantel, A. G.; MacPhail, B. J.: The teratogenicity of cocaine. Teratology 26:17–19; 1982.
14. Fisher, S. E.; Karl, P. I.: Ethanol-nutrition interaction and the fetus. In: Abel, E. L., ed. Fetal alcohol syndrome: From mechanism to prevention, Chapt. 13. Boca Raton, FL: CRC Press, 1996:269–284.
15. Fleischer, S.F.; Turkewitz, G.: Effect of neonatal stunting on development of rats: Large litter rearing. Dev. Psychobiol. 12:137–149; 1979.
16. Frank, D. A.; Bauchner, H.; Parker, S.; Huber, A. M.; Kyei-Aboagye, K.; Cabral, H.; Zuckerman, B.: Neonatal body proportionality and body composition after in utero exposure to cocaine and marijuana. J. Pediatr. 117:622–626; 1990.
17. Gottesfeld, Z.: Fetal alcohol exposure and functional implications of the neuroimmune–endocrine networks. In: Abel, E. L., ed. Fetal alcohol syndrome: From mechanism to prevention, Chapt 6. Boca Raton, FL: CRC Press; 1996:113–144.
18. Greizerstein, H. B.; Abel, E. L.: Acute effects of ethanol on fetal body composition and electrolyte content in the rat. Bull. Psychonom. Sci. 14:355–356; 1976.
19. Hohenauer, L.: Oh, W.: Body composition in experimental intrauterine growth retardation in the rat. J. Nutr. 99:23–26; 1969.
20. Hurley, L. S.: Nutritional deficiencies and excesses. In: Wilson, J. G.; Fraser, F. G., eds. Handbook of teratology, vol. 1, Chapt. 7. New York: Plenum Press; 1979:261–308.
21. Lechtig, A.; Klein, R. E.: Pre-natal nutrition and birth weight: Is there a causal association? In: Dobbing, J., ed. Maternal nutrition in pregnancy: Eating for two? New York: Academic Press; 1981: 131–135.
22. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
23. Mahalik, M. P.; Gautieri, R. F.; Mann, D. E., Jr.: Mechanisms of cocaine-induced teratogenesis. Res. Comm. Subst. Abuse 5:279–302; 1984.
24. McConnell, P.; Uylings, H. B. M.; Swanson, H. H.; Verwer, R. W. H.: Sex differences in effects of environmental stimulation on brain weight of previously undernourished rats. Behav Brain Res 3:411–415; 1981.
25. Misra, A. L.; Mulé, S. J.: Calcium-binding property of cocaine and some of its active metabolites: Formation of molecular complexes. Res. Commun. Chem. Pathol. Pharmacol. 11:663–666; 1975.
26. Moghissi, K. S.: Relationship of maternal amino acids and proteins to infant growth and mental development. In: Moghissi, K. S.; Evans, T. N., eds. Nutritional impacts on women. New York: Harper & Row, Publ.; 1977:86–106.
27. Novak, D. A.; Beveridge, M. J.; Salhab, A. S.; Tebbett, I. R.; Shiverick, K. T.: Effect of chronic cocaine administration on amino acid uptake in rat placental membrane vesicles. Life Sci. 56:1779–1787; 1995.
28. Rico, H.; Costales, C.; Cabranes, J. A.; Escudero, M.: Lower serum osteocalcin levels in pregnant drug users and their newborns at the time of delivery. Ostet. Gynecol. 75:998–1000; 1990.

29. Seifert, M. F.; Church, M. W.: Long-term effects of prenatal cocaine exposure on bone in rats. Life Sci. 49:569–574; 1991.
30. Verebey, K.; Gold, M. S.: Psychopharmacology of cocaine: Behavior, neurophysiology, neurochemistry and proposed treatment. In: Morgan, D. W., ed. Psychopharmacology: Impact on clinical psychiatry. St. Louis, MO: Ishiyaku EuroAmerica, Inc.; 1985:219–241.
31. Widdowson, E. M.: The demands of the fetal and maternal tissues for nutrients, and the bearing of these on the needs of the mother to "eat for two." In: Dobbing, J., ed. Maternal nutrition in pregnancy: Eating for two? New York: Academic Press; 1981:1–16.
32. Woods, J. R.; Plessinger, M. A.; Clark, K. E.: Effects of cocaine on uterine blood flow and fetal oxygenation. JAMA 257:957–961; 1987.