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Resistance of the Male Gonad to a High Galactose Diet

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Summary

Rats were fed a 50% galactose diet during pregnancy and nursing, and the testes were later examined and hormone levels determined in male offspring. Exposure to galactose for various periods during pregnancy, throughout the entire gestation, or postnatally to nursing mother until pups were 5 wk of age produced no significant differences from control testicular weight, seminiferous tubular diameter, or microscopic appearance of the testis when the offspring became adult (66 or 127 d). Serum leuteinizing hormone, follicle stimulating hormone, and testosterone levels were determined 127 d postnatally and no significant differences from controls were observed in any of the treatment groups. Blood glactose-1-phosphate levels in animals receiving the 50% galactose diet were comparable to levels observed in human galactosemia. The resistance of the rat testis to a high galactose diet is consistent with the infrequency of testicular insufficiency in human galactosemia, and contrasts with the prenatal female gonadal sensitivity to galactose previously demonstrated in the rat and the high frequency of ovarian failure in human galactosemia.

Abbreviations

FSH, follicle stimulating hormone
gal-1-p, galactose-1-phosphate
LH, luteinizing hormone

With early diagnosis through newborn screening programs and institution of appropriate treatment, many patients with inherited metabolic disorders have survived with good long-term health and are now reaching childbearing age. Individuals with some inborn errors of metabolism have borne children (17), but their long-term fertility is largely unstudied.

Galactosemia, secondary to a deficiency in galactose-1-phosphate uridyl-transferase activity is inherited as an autosomal recessive trait and is characterized by mental retardation, cataracts, hepatosplenomegaly, and renal tubular dysfunction (19). The frequent association between this form of galactosemia and premature ovarian failure, even when a galactose-restricted diet is started early in infancy, is well documented (9, 11, 21). Endocrinologic studies in affected women are consistent with hypergonadotropic hypogonadism. In contrast, testicular dysfunction is uncommon in galactosemia. Kaufman et al. (11) studied eight male patients, and found no evidence of hypothalamic, pituitary or testicular dysfunction. Steinman et al. (21), however, reported elevated levels of basal and stimulated FSH in three of their older patients (age, 21–24-yr-old), suggesting an abnormality in seminiferous tubular function.

Administration to rats of a 30–50% galactose diet is known to produce cataracts, liver abnormalities, and aminoaciduria resembling the human galactosemia syndrome (18, 20). We have shown previously, using this animal model, that offspring of rats fed a high galactose diet during pregnancy have significantly reduced numbers of oocytes (1). The data suggests that prenatal toxicity from galactose and/or its metabolites may contribute to the premature ovarian failure characteristic of human galactosemia. In this paper, we report, using the same animal model and similar experimental design, the effect of galactose treatment on male reproductive function.

MATERIALS AND METHODS

Galactose, gal-1-p, galactose dehydrogenase, and alkaline phosphate were purchased from Sigma Chemical Co. (St. Louis, MO), and a 50% galactose diet and corresponding regular rodent diet without galactose (NIH-07) from Zeigler Bros., Inc. (Gardners, PA). NIH-07 regular rodent diet consits of 24.5% protein, 5.2% fat, 3.7% fiber, and approximately 50% carbohydrate. The

50% galactose diet is prepared by mixing an equal weight of galactose and NIH-07 diet. Sprague-Dawley rats were obtained from the Veterinary Resources Branch, NIH.

Treatment of rats. Rats were housed in an animal room with a 14 h:10 h day:night cycle and had free access to tap water. One group was exposed to galactose prenatally, that is pregnant rats received a 50% galactose diet *ad libitum* at various times during pregnancy: from d 1 postconception to gestational d 9, d 9 to 14, d 15 to 20, or during the entire gestational period. Another group was exposed to galactose only postnatally: the diet was given to lactating mothers at birth and continued until weaning at 27 d of age. After weaning, the pups were separated from the mothers and received the 50% galactose diet until 5 wk of age. In addition, a group of rats was treated with galactose both prenatally and postnatally, from d 1 postconception until 5 wk of age. This group is not discussed further because prenatal plus postnatal galactose treatment was very toxic and no male offspring survived for study.

Control animals received a normal diet instead of the high galactose diet. As an additional control, rats were pair fed by food weight to rats receiving the galactose diet for the entire period of gestation.

All offspring were weaned 27 d after birth. Male and female rats were grouped in separate cages. Body and testicular weights of the offspring were determined at the indicated times.

Assay of galactose and gal-1-p. Galactose and gal-1-p levels were determined by a fluorometric method (6) on whole blood or whole amniotic fluid (including amniotic and exfoliated fetal cells) samples from animals maintained on the galactose or control diet for at least 4 d. Fetal blood and amniotic fluids samples were assayed by pooling material from five individual fetuses.

Testicular histology. Testes were fixed in Bouin's medium for 24 h, placed in 70% ethanol, processed and embedded in paraffin, and sections stained with hematoxylin-eosin. Seminiferous tubular diameter in testes of adult rats was evaluated at 66 d and 127 d using a Zeiss microscope (final magnification, ×100) with the aid of an eyepiece micrometer. Thirty randomly selected circular cross sections per slide were counted. Slides were coded and during analysis the observer was not aware of specimen origin.

Radioimmunoassay of LH/FSH and testosterone. LH and FSH were assayed by using the NIADDK double-antibody radioimmunoassay kits with NIADDK-rat-LH-RPI and NIADDK-rat-FSH-RPI as the standards. Testosterone was assayed by the technique of Dufau *et al.* (4).

Statistical analysis. Group comparisons were made with Student's *t* test for unpaired data.

RESULTS

Weight. When pregnant rats were treated with galactose for the entire period of gestation, there was a large (~30%) decrease in mean fetal body weight near term (Table 1). The group exposed to galactose only toward the end of pregnancy (d 15 postconception to d 20) also showed a significant but smaller decrease of fetal body weight. At birth the galactose diet was changed to a control diet and body weight again measured at weaning (27-d-old). The pups previously exposed to galactose showed catch-up growth and actually weighed more, on average, than the control or pair fed animals at weaning. The same trend persisted until adult life (66-d-old), and was significnt in the groups treated with galactose for first 10 d of gestation or for the entire period of gestation. By 127 d of age, the difference in body weights became smaller and statistically insignificant.

In the postnatal galactose treatment group, mean body weight was reduced to 45% of control at weaning. With cessation of galactose treatment at 35 d of age, mean body weight gradually increased and no significant differences in weight between control and treated groups were found at 66 or 127 d of age. These data

Table 1. *Body and testicular weight and seminiferous tubule diameter (mean ± SE)*

	Fetus* (19-d gestation) Body wt. (g)	Weanling† (27-d-old)		Adult† (66-d-old)			Adult† (127-d-old)		
		Body wt. (g)	Testis wt. (g)	Body wt. (g)	Testis wt. (g)	Seminiferous tubule diameter (μ)	Body wt. (g)	Testis wt. (g)	Seminiferous tubule diameter (μ)
Galactose									
D 1 postconception → d 9	...	...	...	328 ± 8.8‡	3.45 ± 0.13	290 ± 9.1	445 ± 29.5	3.60 ± 0.08	297 ± 14.5
D 9 postconception → d 14	...	79.2 ± 3.9‡	0.60 ± 0.03	288 ± 6.4	3.15 ± 0.11	280 ± 7.1	440 ± 9.1	3.53 ± 0.14	281 ± 8.2
D 15 postconception → d 20	2.71 ± 0.07‡	75.5 ± 1.9‡	0.55 ± 0.2	286 ± 15.1	3.28 ± 0.08	297 ± 12.3	465 ± 35.2	3.50 ± 0.14	291 ± 8.2
D 1 postconception → d 20	2.26 ± 0.03‡	80.8 ± 1.0‡	0.62 ± 0.03	323 ± 17.3‡	3.06 ± 0.22	297 ± 11.5	495 ± 21.2	3.6 ± 0.1	287 ± 9.0
Birth → 5 wk	...	27.6 ± 1.0‡	0.31 ± 0.05‡	271 ± 5.3	2.91 ± 0.37	287 ± 8.8	380 ± 30.6	3.2 ± 0.7	300 ± 7.4
Control	3.22 ± 0.09	50.7 ± 3.7	0.52 ± 0.4	278 ± 7.9	3.06 ± 0.12	294 ± 6.1	421 ± 26.2	3.46 ± 0.14	291 ± 10.3
Pair fed	...	56.3 ± 1.8	0.58 ± 0.1	269 ± 7.3	3.04 ± 0.07	290 ± 6.8	419 ± 20.0	3.3 ± 0.6	289 ± 10.6

* Two pregnant dams with 15 or more fetuses per group.
† Four or more animals per group.
‡ P < 0.05 Student's *t* test, unpaired data.

Table 2. *Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone concentrations (mean ± SE)*

	LH (ng/ml)	FSH (ng/ml)	Testosterone (ng/ml)
Galactose			
D 1 postconception → d 9	24.1 ± 8.8	395 ± 73.0	242 ± 12.3
D 9 postconception → d 14	28.3 ± 5.9	289 ± 32.9	237 ± 68.2
D 15 postconception → d 20	29.8 ± 3.8	295 ± 19.3	249 ± 48.6
D 1 postconception → d 20	34.4 ± 7.3	307 ± 26.6	632 ± 153*
Birth → 5 wk	32.0 ± 10.1	320 ± 40.1	290 ± 40.3
Control	38.3 ± 7.2	346 ± 8.3	314 ± 58.1
Pair fed	35.9 ± 13.6	344 ± 23.2	449 ± 54

* Includes one value of 937, $P < 0.05$ when compared with control group and $P > 0.1$ compared with pair fed group.

suggest that the reduction of body weight while animals receive galactose is temporary and reversible.

There were no significant differences in mean testicular weight between the control and galactose-treated groups except for those pups fed a 50% galactose diet postnatally. In this group, a 47% reduction of testicular weight was observed at weaning and body weight showed a 55% reduction. When the galactose diet was discontinued, both mean body and testicular weights returned to normal.

Testicular histology and seminiferous tubular diameter. Testicular histology and seminiferous tubular diameter were evaluated in testes of rats at 66 and 127 d of age. We extended our observations to 127-d-old rats because of the report (21) suggesting possible late testicular insufficiency in human galactosemia. The mean seminiferous tubular diameter in the galactose-treated groups did not differ significantly from the control or pair fed groups. Testicular histology, assessed by independent evaluation by two of the authors (Y.T.C. and D.R.M.), also did not differ between treatment and control groups.

Serum gonadotropin and testosterone concentrations. Serum LH, FSH, and testosterone levels were measured in the 127-d-old animals (Table 2). There were no significant differences between control and treated groups in LH or FSH concentrations. Including one value of 937 (Table 2), the mean serum testosterone level in the offspring of rats treated with galactose during the entire period of gestation was significantly elevated when compared with the control; however, this difference was not significant when comparison was made to the pair fed group.

Galactose and gal-1-P levels. Table 3 shows the representative galactose and gal-1-P levels in whole blood and whole amniotic fluid (which includes amniotic and exfoliated fetal cells) samples from animals maintained on high galactose or control diets for at least 4 d. In general, fetal blood levels of galactose and gal-1-P were slightly higher during galactose treatment than the cor-

Table 3. *Representative galactose and galactose-1-phosphate (gal-1-p) level, (mean ± SE)**

	Galactose (mg/dl)	Gal-1-P (mg/dl)
Prenatal		
Mothers (4)†	357 ± 56.4	14 ± 5.0
Amniotic fluid‡ (20)	416 ± 37.2	18 ± 7.1
Fetuses (20)	385 ± 79.7	15 ± 7.1
Postnatal		
Before weaning		
Mother (3)	44 ± 13.5	2.0 ± 0.7
Pups (8)	35 ± 11.1	1.8 ± 0.4
After weaning, pups (6)	420 ± 107.8	...
Control (10)	<1.5	<1.0

* Part of the data in this table was previously published (1).

† ( ), number animals; each point is the average of duplicate determinations; for determination of amniotic fluid and fetuses, five samples were pooled and four separate pools were analyzed.

‡ Includes amniotic and fetal cells.

responding maternal concentrations, and amniotic fluid levels tended to be even higher than the corresponding fetal and maternal concentrations. The high concentration of gal-1-P in amniotic fluids may have resulted from the presence of amniotic and fetal cells in our samples. Lactating mothers and their pups had lower levels of blood galactose during galactose exposure than did pregnant dams, their fetuses, and those pups maintained on galactose beyond weaning. Control animals had blood or amniotic fluid galactose and gal-1-P concentrations below the level of detection in this assay. The blood gal-1-P levels in rats treated with the high galactose diet were comparable to levels observed in human galactosemia.

DISCUSSION

In this study, exposure of rats to galactose for various periods during gestation, or postnatally to nursing mothers until pups were 5 wk of age, produced no significant alterations in testicular weight, seminiferous tubular diameter, microscopic appearance of the testis, or serum LH, FSH, and testosterone levels compared with pair fed animals when the offspring were adult (66 and/or 127 d). Whether or not the temporary decrease in testicular weight noted at the time of weaning in the group exposed to galactose postnatally through their nursing mothers is due to a selective galactose effect on the testis remains to be determined. Atrophy of seminiferous tubules and varying degrees of arrest of sperm maturation have been previously reported in rats receiving of a 70% galactose diet (3).

We have shown previously, using the same animal model and similar experimental design, that when pregnant rats were fed a 50% galactose diet there was a striking reduction in oocyte number in prenatally exposed offspring (1). The most prominent effects on oocyte number were noted after galactose exposure during the pre-meiotic stage of oogenesis. These results contrast sharply with the apparent lack of a corresponding effect of galactose or its metabolites on the testes. The comparative resistance of the prenatal or postnatal male gonad to a high galactose diet is consonant with the apparent infrequency of testicular insufficiency in human galactosemia.

Both this study and the previous study on ovarian toxicity (1) have shown a similar large decrease (~30%) in mean fetal body weight at term when pregnant rats were fed a 50% galactose diet. Human galactosemia, in contrast, showed a smaller (8.6–12%) but significant reduction of mean birth weight in galactosemic children compared with their unaffected siblings (8). The difference in the degree of intrauterine growth retardation is probably not due to protein-calorie malnutrition created by the 50% galactose diet. The galactose diet employed in our studies contains 12.25% protein, which meets the basic protein requirement (12%) for pregnant rats (15). Rats fed the galactose diet actually eat more than control rats. It should be noted, however, that the 50% galactose diet was prepared by mixing equal weights of galactose and control diet. Therefore the control and experimental diet in our study do not have the same nutrient composition. A study by Haworth *et al.* (7), using isocaloric diets, has demonstrated that rats fed a 40% galactose diet were not malnour-