Lipids in Newborn of Alloxan-diabetic Rats

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SUMMARY

Total fats, phospholipids, cholesterol, neutral fat and total water content have been determined in newborns of alloxan-diabetic rats and in control newborns. The newborns were divided into two groups: one with overweight newborns (> 5.30 gm.), the second with mainly average and abnormally small newborns (< 5.30 gm.). The overweight newborns of alloxan diabetic rats showed higher neutral fat content and lower cholesterol content than the controls. No significant differences in total fats, phospholipids and water content were demonstrated between the groups. The findings are discussed in the light of our present knowledge about hormonal disturbances in newborns of alloxan-diabetic rats, whose lipogenesis might be influenced thereby. Previously it has been shown that newborns of alloxan-diabetic rats are longer than control rats at equal body weight. An increase in the neutral fat content without increase of other lipids suggests increase of depot fat. From these findings it may be concluded that the overweight newborns of alloxan-diabetic rats are abnormally long as well as abnormally obese. DIABETES *14*:724-28, November 1965.

Diabetic rats can bear large as well as small offspring.[1,2,13,15,23] Discussing hyperglycemia and increased insulin production by the fetus as a possible explanation of the elevated birth weight, Hultquist[13] suggested that increased accumulation of fat in the fetuses of diabetics, analogous to "insulin fattening," might be involved. Angervall[1] studied the length and water content of newborns of alloxan-diabetic rats and found that they were longer but did not demonstrate any significant difference in water content by comparison with controls at equal body weights. As fat has much less water content than "lean body mass,"[4-6] these findings do not suggest changes in fat deposition in newborns of alloxan-diabetic rats but rather that these newborns can be heavier by virtue of enhanced growth.

From investigations in newborn infants of diabetic mothers, Osler[19] concluded that, at birth, these infants are obese and thus have relatively low total body water. Furthermore Osler pointed out that the increased glycogen stores in these infants must be regarded as closely related to the obesity.

In newborns of alloxan-diabetic rats the presence seems probable of hyperinsulinism as well as of other endocrine disturbances[1] capable of modifying lipogenesis and lipolysis.

Against this background, we deemed it interesting to determine the total content of various lipid fractions in newborns of alloxan-diabetic rats.

MATERIALS AND METHODS

Forty newborns from eighteen alloxan-diabetic rats (Ax series) and thirty-one newborns from nine control rats (C series) were used. Each series was divided into two subgroups, one with newborns weighing more than 5.30 gm. (Ax_1 and C_1) and the second with newborns weighing less than 5.30 gm. (Ax_2 and C_2). All rats ate freely of rat bread and drank tap water.

The pregnant rats were given alloxan on the tenth, eleventh or twelfth day of pregnancy in a single dose of 0.100 or 0.125 mg. per gm. body weight in the form of a subcutaneous injection in one of the thighs of freshly prepared 5 per cent alloxan monohydrate solution in physiological saline. All then had glycosuria varying between 2 and 5 gm. per twenty-four hours.

The newborn rats were weighed, immediately decapitated and placed in 250 ml. Erlenmeyer flasks. The carcasses were then homogenized in 5 ml. of water for five minutes with a Bühler homogenizer equipped with a four-bladed knife of 20 mm. diameter revolving at 40,000 rpm. The homogenization vessel was cooled by running tap water. The homogenate was poured back into the Erlenmeyer flask and the homogenization vessel carefully rinsed with 100 ml. of a mixture of equal volumes of chloroform and methanol which was then transferred to the Erlenmeyer flask and left for thirty minutes at room temperature before being further processed as described by Folch et al.[10]

Aliquots of the washed chloroform infranatant were taken for determination of weight, Lieberman-Burchard chromogenes[8] and lipid phosphorus.[24]. Lieberman-Burchard chromogenes were estimated as cholesterol, and lipid phosphorus was converted to phospholipids by multiplying by twenty-five. The residual weight after sub-

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traction of the weights of cholesterol and phospholipids was assumed to represent glycerides.

To check the completeness of the extraction procedure, the precipitated tissue was collected from the filter and rehomogenized in extraction mixture in a Potter-Elvehjem apparatus. The additional lipid material weighed less than 2 per cent of that obtained initially. Hence the adopted method was considered adequate.

The water content of the newborns was estimated on the basis of dry weight by the following procedure. The newborns were homogenized as described above, frozen to —40° C., transferred to a freeze-drier for forty-eight hours and further desiccated under vacuum over phosphorus pentoxide to constant weight (which was reached after fourteen days).

Statistics. Newborns for lipid analysis were chosen so that the birth weights were practically identical in the corresponding groups (table 1). The regression of lipid weights upon births weight were computed for each of the four groups.

When no significant difference was demonstrated in mean lipid weights between the corresponding groups, covariance analysis was done according to the principles given by Hald.[12]

If the regressions are significant in the two groups and do not significantly differ, the difference in lipid weights ($\bar{y}_1 - \bar{y}_2$) is tested according to the formula:

$$t = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{s^2_{1,y.x}}{n_1-2} + \frac{s^2_{2,y.x}}{n_2-2}}}$$, where $s^2_{y.x}$ is the residual variance.

If none of the two regressions was significant, the difference in mean lipid weights is tested according to this formula:

$$t = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{s^2_{1,y}}{n_1-1} + \frac{s^2_{2,y}}{n_2-1}}}$$, where s^2_y is the variance of y.

If the regression is significant in only one of the two groups, say the second, the following formula is used:

$$t = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{s^2_{1,y.x}}{n_1-2} + \frac{s^2_{2,y}}{n_2-1}}}$$

It may be noted that the above formulas are derived from those given by Hald[12] and are valid for the special case in this investigation, namely that the mean birth weights ($\bar{x}_1$ and $\bar{x}_2$) do not differ significantly between the two groups.

RESULTS

Table 1 gives means and standard errors for birth weights and weights of total fats, phospholipids, cholesterol and neutral fat in groups 1 and 2 in the Ax and C series. Table 2 presents the equations for the regressive relationships between the various lipid fractions and birth weight. The figures for the weights of the various lipid fractions and the birth weights are plotted in figures 1, 2, 3 and 4.

Groups 1 (birth weights > 5.30 gm.). Student's *t* test revealed that, at a birth weight of 5.69 gm., the neutral fat content was significantly higher (about 34 per cent) in the Ax group than in the C group ($t = 3.01$; $P < 0.01$). The cholesterol content was significantly lower (9 per cent) in the Ax group than in the C group ($t = 2.25$; $P < 0.05$). The phospholipid content was also lower in the Ax group (about 9 per cent) but the

TABLE 1

Means and standard errors in the Ax and C groups for weights of total lipids, phospholipids, cholesterol, neutral fat and birth weights.

Groups*	Total lipids (mg.)	Phospholipids (mg.)	Cholesterol (mg.)	Neutral fat (mg.)	Mean birth weight (gm.)
Ax group 1	83.36±2.23 (n=21)	44.94±1.96 (n=21)	8.15±0.24 (n=21)	31.00±1.51 (n=21)	5.69±0.05 (n=21)
Ax group 2	68.62±1.83 (n=15)	39.03±1.58 (n=15)	7.45±0.43 (n=15)	22.16±1.60 (n=15)	4.99±0.06 (n=15)
C group 1	81.59±2.50 (n=13)†	49.20±2.43 (n=14)	8.88±0.22 (n=14)	23.51±1.98 (n=13)†	5.69±0.07 (n=14) 5.67±0.08 (n=13)
C group 2	68.58±1.44 (n=15)	39.74±1.32 (n=15)	7.97±0.22 (n=15)	21.23±1.48 (n=15)	4.97±0.08 (n=15)

*The groups 1 comprise newborns weighing more than 5.30 gm., the groups 2 newborns weighing less than 5.30 gm.
†One neutral fat sample lost.

TABLE 2

The equations of regression between the various lipid fractions and birth weight

Series	Lipid fraction		Equation of regression	S_b	t_b	P for b
Ax	Total lipids	Group 1	$\bar{y}_x = 19.63x - 28.34$	6.49	3.02	<0.01
		Group 2	$\bar{y}_x = 14.60x - 4.26$	5.07	2.88	<0.02
	Phospholipids	Group 1	$\bar{y}_x = 13.96x - 34.45$	6.39	2.18	<0.05
		Group 2	$\bar{y}_x = 0.23x + 37.88$	6.28	0.04	>0.2
	Cholesterol	Group 1	$\bar{y}_x = 1.55x - 0.66$	0.80	1.93	<0.1
		Group 2	$\bar{y}_x = 1.04x + 2.29$	0.88	1.18	>0.2
	Neutral fat	Group 1	$\bar{y}_x = 0.64x + 34.65$	6.23	0.10	>0.2
		Group 2	$\bar{y}_x = 13.35x - 44.48$	4.50	2.97	<0.02
C	Total lipids	Group 1	$\bar{y}_x = 18.06x - 20.73$	6.27	2.88	<0.02
		Group 2	$\bar{y}_x = 13.19x + 3.03$	2.41	5.48	<0.001
	Phospholipids	Group 1	$\bar{y}_x = 8.40x + 1.42$	7.64	1.10	>0.2
		Group 2	$\bar{y}_x = 4.56x + 17.05$	3.56	1.28	>0.2
	Cholesterol	Group 1	$\bar{y}_x = 0.34 + 10.83$	1.46	0.24	>0.2
		Group 2	$\bar{y}_x = 2.18x - 2.55$	0.34	6.41	<0.001
	Neutral fat	Group 1	$\bar{y}_x = 9.28x - 29.06$	5.84	1.59	<0.2
		Group 2	$\bar{y}_x = 5.55x - 6.37$	3.95	1.41	<0.2

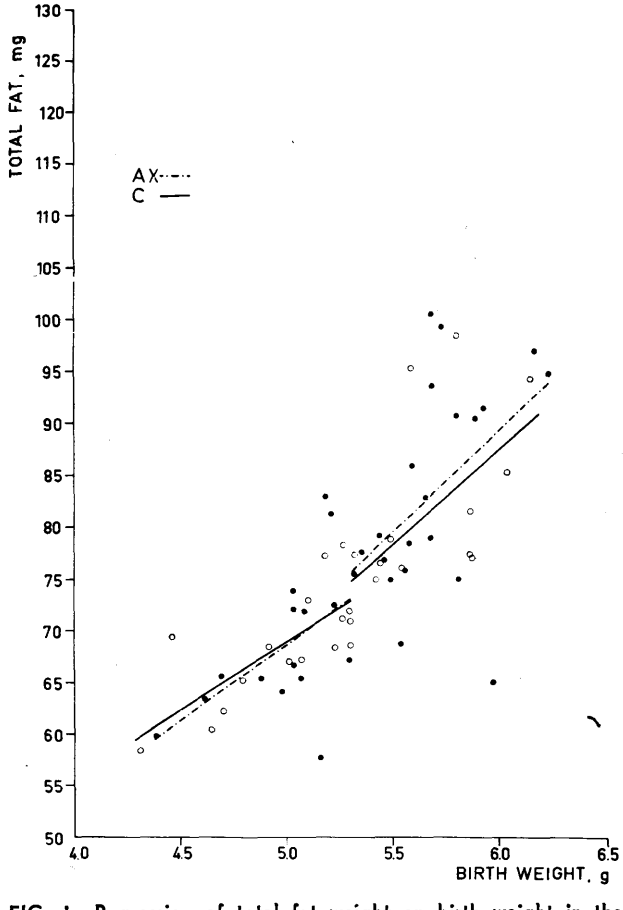

FIG. 1. Regression of total fat weight on birth weight in the Ax and C groups.

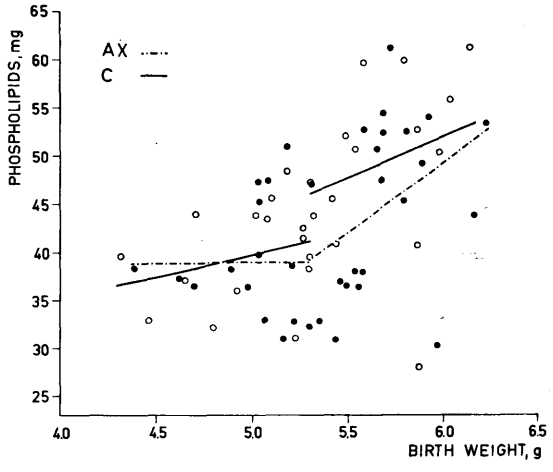

FIG. 2. Regression of the weight of phospholipids on birth weight in the Ax and C groups.

means were not significantly different ($t = 1.37$; $P < 0.2$), nor were the levels of the regression lines ($t = 1.51$; $P < 0.2$). No difference was demonstrated in total fats either between the mean weights in the Ax and C group or in the levels of the lines ($t = 0.719$; $P < 0.5$).

Groups 2 (birth weights < 5.30 gm.). No difference in means or in the levels of regression lines were demonstrated for the various lipid fractions. There existed a tendency to lower cholesterol content in the Ax group than in the C group also for the lighter newborns but the difference (in levels of the lines) was not signi-

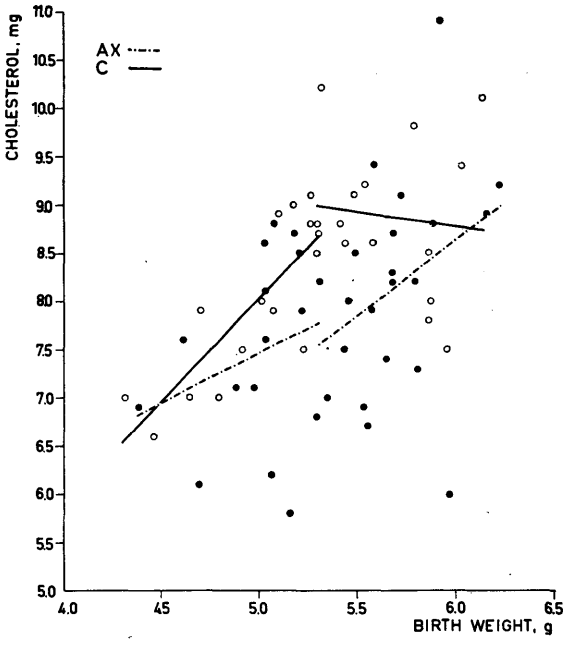

FIG. 3. Regression of cholesterol weight on birth weight in the Ax and C groups.

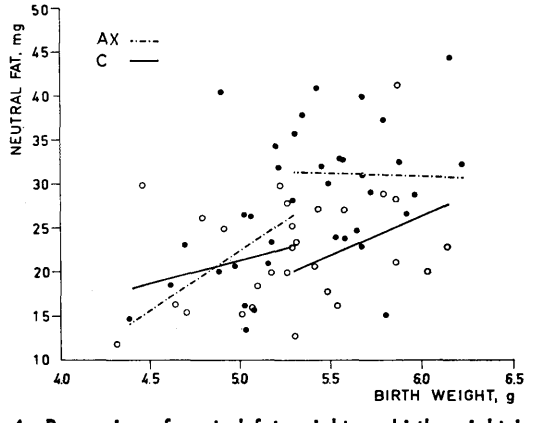

FIG. 4. Regression of neutral fat weight on birth weight in the Ax and C groups.

ficant ($t = 1.79$; $P < 0.1$).

The water content for twelve newborns in the C group (mean birth weight 5.65 ± 0.05 gm.) was 85.24 per cent ± 0.17 and for thirteen newborns in the Ax group (mean birth weight 5.68 ± 0.06 gm.) 85.24 per cent ± 0.24. Thus no difference in water content could be demonstrated between the groups at equal body weights.

DISCUSSION

From previous investigations it is thus known that diabetic rats can bear large as well as small offspring (see above). Conceivable factors in fetuses of alloxan-diabetic rats that may inhibit or enhance fetal growth have previously been discussed.[1] It is pertinent that insulin—in fetuses of diabetic rats probably occurring in increased amounts—enhances adipose tissue lipogenesis and contributes to protein anabolism (and thus may promote fetal body growth) while cortisone increases lipolysis (and contributes to protein catabolism).[3,17,22,25,26] Pregnant alloxan-diabetic rats show adrenal hyperplasia and thymus involution and the fetuses adrenal hypoplasia, apparently induced by transplacental passage of large amounts of maternal corticosteroids.[1] This postulate is further supported by the study of Devecerski and Frawley[9] indicating that alloxan diabetes in nonpregnant rats produces not only enlargement of the adrenal cortex, but a quantitative increase in steroid production. Interestingly, the administration of large doses of ACTH or cortisone results in abnormally small newborns.[18] Hence there are reasons to divide the newborns in this investigation into groups with large and small ones.

The present investigation has shown that, at equal birth weights, the total neutral fat content is significantly higher and the total content of cholesterol significantly lower in the heavier newborns of alloxan-diabetic rats than in control newborns of the same weight. Compared with controls no significant difference was demonstrated for total fats and phospholipids in these heavier newborns, nor for any lipid fractions in the groups of smaller newborns.

The findings might be related to the endocrine disturbances existing in newborns of alloxan-diabetic rats and should be considered in the light of the fact that depot fat contains almost only neutral fat as far as lipids are concerned:[7] a higher neutral fat content in the heavier newborns of the alloxan-diabetic rats could thus imply increased depot fats, probably at least partly owing to enhanced insulin-induced lipogenesis. The observation of a higher neutral fat content in these newborns is in accordance with the conclusion of Osler[19] that diabetic women bear obese children. Notably, no difference was demonstrated in neutral fat or other lipid fractions between the smaller newborns of alloxan diabetic rats and the control newborns. These observations might bear out the opinion that the former newborns are under the influence of abnormally high amounts of corticosteroids that increase lipolysis and inhibit fetal body growth.

A higher fat content in newborns of alloxan diabetic rats would presumably mean a lower water content in these newborns. However, in the present investigation, no difference (in comparably small samples) was dem-

onstrated in water content between the overweight newborns of alloxan-diabetic rats and control newborns. It is conceivable that a relatively high content of water-binding intracellular glycogen contributes to normalization of the total water content of obese newborns of alloxan diabetic rats (cf. Osler[19]).

The significantly lower cholesterol content and the tendency to lower phospholipid content in the heavier newborns of the alloxan-diabetic rats are not fully understood. Goldwater and Stetten[11] have shown that the transplacental transfer of cholesterol as well as of fatty acids from the pregnant rat to the fetus is small. These authors administered labeled cholesterol and fatty acids to rats between the eighteenth and twentieth day of pregnancy and estimated that no more than 10 per cent of fetal cholesterol and 1.5 per cent of fetal fatty acids were derived as such from the maternal circulation. Korent and Shafrir[16] found an equally low transplacental transfer of labeled fatty acids in rats. These authors considered that placental lipolysis may give an additional tributary of FFA to the fetal circulation. Popják and Beeckman,[21] in similar studies in the rabbit, confirmed the rapid synthesis of lipids by the fetus but concluded that there was no perceptible transfer of cholesterol from the mother. The conclusions were similar for phospholipids. With P-32 as a label for inorganic phosphate and phospholipids, it was evident that the fetal phospholipids were synthesized primarily by the fetus.[20,21] Accordingly, the changes in the total content of lipid fractions in newborns of alloxan-diabetic rats are, probably at least partly owing to disturbances in the fetal lipid metabolism, possibly also owing to altered placental lipolysis.

The observation of higher neutral fat content in newborns of the alloxan-diabetic rats together with the observations that these newborns have similar water content as control rats but greater length at equal body weights,[1] may lead to the conclusion that the overweight newborns of alloxan diabetic rats are abnormally long as well as abnormally obese.

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