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INFLUENCE OF MATERNAL DIETARY PROTEIN AND FAT LEVELS ON FETAL GROWTH IN MICE

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The influence of maternal dietary fat and protein levels during gestation on fetal growth was determined in LAF1/J mice. Dams were fed diets containing various levels of casein (ranging from 6 to 20%) and fat (either 5 or 15%). On the 18th day of gestation, all animals were sacrificed. Products of conception were examined, and maternal liver and fetal body compositional analysis was performed. It was found that low protein diets did not depress caloric intake, indicating that observed effects are due to differences in respective nutrient intakes rather than altered caloric intakes. Both the dietary protein and fat levels significantly influenced the fetal cellular growth (cell number rather than cell size) of LAF1/J mice. In addition, dietary fat influenced protein adequacy for satisfactory fetal cellular growth of these mice, probably by altering protein utilization. Optimal fetal cellular growth was obtained with the diet containing 20% casein and 15% fat as indicated by increased fetal protein, DNA, and RNA contents. Normal maternal liver lipid content in animals fed the 20% protein and 15% fat diet also indicates that this diet is optimal for gestational performance of LAF1/J mice.

INDEX WORDS: Maternal diet, fetal growth, protein, fat, pregnancy, mouse.

INTRODUCTION

During the past several years, many reports have appeared dealing with the effects of maternal nutritional restriction during the periods of gestation and lactation on growth and development of the young (Burke, *et al.*, 1943; Lechtig, *et al.*, 1975; Zeman, 1967; Zeman and Stranbrough, 1969). Particularly, the effects of protein deprivation during pregnancy on prenatal growth and development have been extensively studied. Rats have been used almost exclusively in animal investigations and a 12% protein diet with 5% fat is recommended for satisfactory reproduction of rats (National Academy of Sciences/ National Research Council, 1978). It has been presumed that nutrient requirements of mice are similar to those of rats (Franklin, *et al.*, 1932), and that mice can take the place of rats in nutritional studies (Bosshardt, *et al.*, 1946). Mice have seldom been used in prenatal growth studies because the nutritional requirements of mice during gestation are poorly defined. Even though 18% dietary protein was recently recommended for satisfactory reproductive performance of mice (National Academy of Sciences/National Research Council, 1978), there is debate concerning the dietary protein level which gives optimal reproductive performance, possibly due to differences in fat content of

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Table 1

Composition of Diets[1,2]

Diet	Fat (%)	5			15		
	Casein (%)	6	12	20	6	12	20
		% diet					
Casein		6.0	12.0	20.0	6.0	12.0	20.0
Methionine		.3	.3	.3	.3	.3	.3
Corn oil		5.0	5.0	5.0	15.0	15.0	15.0
Corn starch		51.3	51.3	51.3	51.3	51.3	51.3
Sucrose		31.4	25.4	17.4	21.4	15.4	7.4
Salt mixture[3]		5.0	5.0	5.0	5.0	5.0	5.0
Vitamin mixture[4]		1.0	1.0	1.0	1.0	1.0	1.0
Caloric density (kcal/g diet)		4.01	4.01	4.01	4.51	4.51	4.51

[1]All diets fed ad libitum.

[2]Prepared in a 2% agar gel (1:1 dry diet-water).

[3]Rogers-Harper mineral mixture, catalog #170760, Teklad test diet, Madison, Wisconsin.

[4]Vitamin fortification mixture, catalog #40060, Teklad test diet, Madison Wisconsin.

dislocation (The Universities Federation for Animal Welfare, 1976) on day 18 of gestation. At the time of dissection, maternal livers and all fetuses were excised, weighed, and immediately frozen at −25°C for subsequent compositional analysis. Uteri of dams were carefully examined for the presence of implantation sites to determine whether the sites were normal or in the process of resorption. Since no effect of litter size on the fetal weight was observed (correlation coefficient between litter size and fetal weight was .06) in this study, all pregnant animals were eligible for the study.

Randomly chosen fetuses from each mother were assayed for deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein, fat, and ash contents. The nucleic acids were isolated by a modification of the method of Schmidt-Thanhouser (Fleck and Munro, 1962). DNA and RNA were measured by the modified-Ceriotti colorimetric method (Hubbard, *et al.*, 1970; Ceriotti, 1955) with calf thymus DNA and calf liver RNA, respectively, as standards (Sigma Chemical Co., St. Louis, Missouri). Protein was measured colorimetrically (Leshner and Litwin, 1972) using bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri) as the standard. The ratios of RNA to DNA and protein to DNA were calculated as means of assessing cellular growth of the fetus. Total lipids of maternal livers and fetuses were determined by the method of Folch, *et al.* (1975). The ash content of each fetus was measured gravimetrically after complete combustion in a muffler furnace at 550°C for 8 hours (Horwitz, 1975).

Table 3

Analysis of Variance for Maternal Characterics of Mice Fed Diets Containing Varying Levels of Protein and Fat[1,2]

Source	Mean Squares: Food Intake	Mean Squares: Caloric Intake	Mean Squares: Liver Lipids
Fat (F)	1.863** (1)	0.0001 (1)	734.72** (1)
Protein (P)	0.020 (2)	0.3495 (2)	94.57** (2)
FxP	0.145 (1)	2.6822 (1)	0.18 (2)
Error	0.101 (28)	1.8680 (28)	10.65 (30)

[1]Mean squares presented with level of significance.

[2]Degrees of freedoms are in parentheses.

**p<0.01

and litter size) were expressed in terms of percentage of implantation, however, percentage resorptions and percentage litters of implantation were markedly affected by fat content in diet. This is due to the fact that by presenting data in this way it is possible to compensate for variation in number of total implantations (Table 4), which tend to occur among animals independently of changes in dietary parameters. The effect of dietary fat on percentage resorptions and percentage litters of implantations varied with the level of dietary protein. When

Table 4

Gestational Performance of Mice Fed Diets Containing Varying Levels of Protein and Fat[1]

Diet	Fat (%)	5			15		
	Casein (%)	6	12	20	6	12	20
Number of dams		10	12	4	10	10	4
Number of implantations[2]		10.5±0.7	10.4±0.3	9.8±1.3	10.2±0.7	10.2±0.9	9.7±0.6
Resorptions of implantations							
Number of resorptions per dam		1.4±0.4	1.0±0.3	1.0±0.4	2.1±0.4	2.0±0.4	0.3±0.2
% resorptions of implantations per dam		12.1±3.5	9.7±3.4	9.1±3.7	20.5±3.3	19.2±3.9	3.9±2.9
Litters of implantations							
Litter size		9.1±0.3	9.4±0.4	8.8±1.0	8.1±0.6	8.2±0.8	9.3±0.8
% litters of implantations per dam		87.9±3.5	90.3±3.4	90.9±3.7	79.5±3.3	80.8±3.9	96.1±2.9
Fetal weight (g)		1.00±0.03	1.04±0.03	0.99±0.05	0.93±0.04	1.01±0.02	1.03±0.05

[1]Results are means ± S.E.

[2]Number of resorptions per dam plus litter size.

Table 7

Analysis of Variance for Major Constituents of Mice Fed Diets Containing Varying Levels of Protein and Fat[1,2]

Source	Mean Squares: Protein	Lipids	Ash
Fat (F)	783.4* (1)	0.33 (1)	5.20 (1)
Protein (P)	1534.9** (2)	0.95 (2)	1.07 (2)
FxP	702.8* (2)	4.68 (2)	0.48 (2)
Error	142.8 (44)	1.62 (44)	5.89 (42)

[1]Mean squares presented with level of significance.

[2]Degrees of freedoms are in parentheses.

*$p<0.05$, **$p<0.01$

Data concerning fetal cellular growth measurements are presented in Table 8, and the analysis of variance of these data is shown in Table 9. Fetal DNA and RNA contents were significantly affected by both fat and protein levels in diet. Further, a significant interaction of these two dietary factors, protein and fat levels, was noted on fetal DNA content. The influence of dietary fat on fetal DNA content was shown to vary with the level of dietary protein. When diets contained low protein (6% casein), fetal DNA content was decreased as the dietary fat level was increased from 5 to 15%. At the high levels of dietary protein (either 12 or 20%), however, fetal DNA content was increased as dietary fat level

Table 8

Parameters of Fetal Cellular Growth of Mice Fed Diets Containing Varying Levels of Protein and Fat[1,2,3]

Diet	Fat (%)	5			15		
	Casein (%)	6	12	20	6	12	20
Number of dams		10	4	4	4	4	4
DNA (mg/g fetus)		2.31±0.03	2.07±0.38	2.52±0.20	2.22±0.12	2.81±0.17	3.23±0.14
RNA (mg/g fetus)		6.85±0.14	7.80±0.42	9.73±0.10	7.80±0.40	9.17±0.25	9.90±0.50
Protein/DNA (mg/mg)		41.43±1.43	43.05±4.67	41.25±3.56	40.25±1.86	40.28±1.71	38.33±1.27
RNA/DNA (mg/mg)		2.96±0.06	3.23±0.28	3.56±0.15	3.00±0.16	3.04±0.25	3.08±0.18

[1]Results are means ± S.E.

[2]Wet weight basis.

[3]Two fetuses selected from each dam were analyzed for each assay, and each fetus was replicated two times.

et al., 1969) has been suggested as a possible cause for fatty liver in the case of protein deficiency. It has also been speculated that fatty changes in the liver on low protein diets might be due to a net increase in the amount of lipid per g of tissue caused by a reduction in liver cell size (Edozien and Switzer, 1978), rather than some changes in intracellular metabolism of the lipids. On the other hand, the observed reduction in the liver lipid accumulation caused by an additional fat in diet might be explained by a decrease in lipogenesis when animals are fed high dietary fat levels (Platka-Bird and Bennink, 1978).

The data on gestational performance of mice indicate a pronounced effect of an increase in dietary fat level on maintenance of pregnancy (percentages of resorptions and litters of implantations) and that the effect of dietary fat level depends on the level of dietary protein. When diets contained low levels of protein (either 6 or 12% casein), increasing the dietary fat content from 5 to 15% had an adverse effect on gestational performance (an increase in percentage of resorptions and a decrease in percentage litters of implantation). This is probably due to the fact that increasing the fat level lowered food intake, and consequently increasing fat content could exaggerate a deficient state of protein, as well as lead to a diminished intake of other nutrients such as vitamins and minerals. When diet contained sufficient amount of protein (20% casein), however, increasing the dietary fat content improved gestational performance, as indicated by a decrease in percentage resorptions and an increase in percentage litters of implantations. The improved outcome of gestation with increasing the dietary fat suggests that some fat, in addition to supplying the essential fatty acid, is needed for optimal gestational performance. Other investigators (Knapka, *et al.*, 1978) likewise noted that an increase in the fat content of the diet from 4 to 12% improved reproductive performance at the high level of dietary protein, as measured by increased litter sizes and weaning weights of pups in their study using other inbred strains of mice (BALB/cAnN, C3H/HeN, C57BL/6N, and DBA/2N.

As shown in Table 6, the protein content was low in fetuses from mice fed diets low in protein (6% casein) regardless of dietary fat level. However, the effect of increasing dietary protein level on fetal protein content was markedly affected by the levels of fats in diets. When diets contained low levels of fat (5%), an increase in fetal protein content was most evident with a large increase in dietary protein level (from 6% casein to 20% casein). At the high level of dietary fat (15%), however, the most marked increase in fetal protein content was noted with a small increase in dietary protein (from 6% casein to 12% casein). These results indicate that dietary protein utilization was affected by the dietary fat content as well as by the level of dietary protein.

A significant increase in fetal DNA content (Table 8), without a change in protein/DNA or RNA/DNA ratios, was found when the casein level was increased in either the low or high fat diets, indicating

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