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Influence of Dietary Fat, Fasting, and Acute Premature Weaning on *in Vivo* Rates of Fatty Acid Synthesis in Lactating Mice[1] (40338)

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Pregnancy and lactation necessitate alterations in carbohydrate and lipid metabolism to provide for fetal development and for milk production. The activities of lipogenic enzymes in rat liver and adipose tissue have been assayed to provide information on the rates of carbohydrate conversion to fatty acids in these organs during pregnancy and lactation. Reported changes in the activities of several lipogenic enzymes suggest that rates of fatty acid synthesis may be increased, decreased, or unchanged in liver (1–5) and adipose tissue (1, 5–7) of pregnant rats. Likewise, it is difficult from the reported data on lipogenic enzymes (1, 3–5, 8) to predict how lactation might alter *in vivo* rates of fatty acid synthesis in the liver. Activities of lipogenic enzymes, as measured *in vitro,* respond rather slowly to changes in flux of carbon to fatty acids; thus, it is possible that the activities of the enzymes measured did not reflect the dynamic metabolic changes which occur at the end of gestation and at the initiation of lactation.

The purpose of the present report was to evaluate the contribution of liver, adipose tissue, and mammary gland to *in vivo* fatty acid synthesis in pregnant and lactating mice; values for virgin mice were included for comparative purposes. The influence of dietary fat, fasting, and acute premature weaning on fatty acid synthesis in lactating mice was also investigated. Injection of tritiated water was utilized to obtain the *in vivo* estimates of rates of fatty acid synthesis independent of the source of the substrate (9).

Materials and methods. Female Swiss Webster[2] mice, 10 to 12 weeks of age, were housed in solid bottom cages with wood shavings for bedding. They were fed a stock diet[3] *ad libitum* unless indicated otherwise. Ambient temperature was 25 ± 2°. Male mice were placed with female mice for 48 hr; mice which became pregnant were used in subsequent experiments. Litter size was standardized to 10 pups within 24 hr postpartum.

In one experiment virgin and lactating mice were fed one of two semipurified diets for 5 days. The high-carbohydrate diet contained, in grams per 100 g: casein, 20.0; methionine, 0.3; mineral mix (10), 4.0; vitamin mix (11), 0.4; choline chloride, 0.2; cellulose, 5.0; corn oil, 5.0; and glucose, 65.1. The high-fat diet was formulated by replacing 43.1 g of glucose with tallow on an equal energy basis. The high-carbohydrate diet contained 21, 12, and 67% energy from protein, fat, and carbohydrate, respectively, whereas the high-fat diet contained 21, 51, and 28% energy from protein, fat, and carbohydrate, respectively.

In vivo rates of fatty acid synthesis were calculated by determining the rate of ^3H_2O incorporated into fatty acids. Each mouse was injected intraperitoneally with 0.2 ml of saline containing 1.5 mCi of ^3H_2O. Mice were killed at the times indicated under results. Plasma was collected and used to obtain an estimate of the body water specific activity. All removable adipose tissue was stripped from the mice. In virgin mice, the removable adipose tissue depots comprised 55% of total body fat. Adipose tissue, liver, and mammary gland (pregnant and lactating mice) were weighed and homogenized in an equal weight of water. Aliquots were saponified and fatty acids were extracted and counted as previously described (12). Results were calculated

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[2] Spartan Research Animals, Inc., Haslett, Michigan.

[3] Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois.

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as nanomoles of tritium incorporated into fatty acids per organ per time interval.

Results. The time sequence of ^3H_2O incorporation into fatty acids in liver, adipose tissue, and mammary gland of lactating mice was established (Fig. 1); rates of incorporation were approximately linear for the first 20 min in all three tissues. Rates of fatty acid synthesis in mammary gland were approximately double the rates observed in liver. Incorporation of ^3H_2O into fatty acids in adipose tissue occurred at a considerably slower rate than in mammary gland or liver. Ten minutes after injection of ^3H_2O, 30 ± 1, 1 ± 1, and 69 ± 1% of total fatty acid synthesis occurred in liver, adipose tissue, and mammary gland, respectively. Similar values were observed at 20 and 30 min. In subsequent experiments, mice were killed 15 min after injection of the tracer.

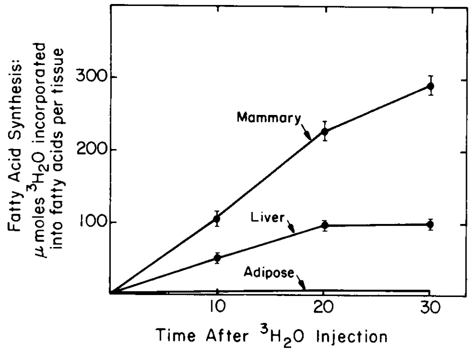

FIG. 1. *In vivo* rates of fatty acid synthesis in liver, adipose tissue, and mammary gland of lactating (3–6 days) mice. Mice weighed 45 ± 1 g; liver, adipose, and mammary gland weights averaged 3.0 ± 0.1, 0.6 ± 0.1, and 3.2 ± 0.1 g, respectively. Each mouse was injected intraperitoneally with 1.5 mCi of ^3H_2O and killed at the times indicated. Each point represents the mean ± SEM for 10 mice. Values for adipose tissue were very low; consequently, individual points were deleted.

Body weights of 18- to 19-day pregnant mice were heavier than body weights of lactating mice; virgin mice weighed less than either pregnant or lactating mice (Table I). A marked decrease in gastrointestinal tract fill contributed to the large loss of body weight in the fasted, lactating mice. Food intake was 50% higher in pregnant mice and 147% higher in lactating mice than in virgin mice (Table I).

Liver weight increased approximately 50% in the pregnant and lactating mice and fasting for 12 hr decreased liver weight (Table I). The *in vivo* rate of fatty acid synthesis in liver, expressed per gram, was 62% lower in pregnant mice than in virgin mice; but the rates per total liver were not significantly lower

TABLE I. *In vivo* RATES OF FATTY ACID SYNTHESIS IN LIVER, ADIPOSE TISSUE, AND MAMMARY GLAND OF VIRGIN, PREGNANT, AND LACTATING MICE.[a]

	Virgin		Pregnant	Lactating		
Parameter	Fed	Fasted	Fed	Fed	Fasted	Pups Removed
Body weight, g[b]	33 ± 1^{f}	33 ± 1^{f}	58 ± 2^{g}	45 ± 2^{h}	45 ± 1^{h}	44 ± 1^{h}
Changes in body weight, g[c]	$+0.4 \pm 0.3^{f}$	-3.2 ± 0.3^{g}	$+1.8 \pm 0.4^{h}$	-0.9 ± 0.7^{f}	-9.6 ± 0.7^{i}	$+0.6 \pm 0.9^{f,h}$
Food intake, g[c]	5.5 ± 0.4^{f}	2.0 ± 0.1^{g}	8.3 ± 0.3^{h}	13.6 ± 0.5^{i}	6.1 ± 0.9^{f}	12.4 ± 0.7^{i}
Liver						
Weight, g	1.9 ± 0.1^{f}	1.5 ± 0.1^{g}	2.9 ± 0.1^{h}	3.0 ± 0.1^{h}	2.4 ± 0.1^{i}	3.0 ± 0.1^{h}
FAS, nm/min[d]	1105 ± 256^{f}	221 ± 12^{g}	$644 \pm 97^{f,i}$	2359 ± 522^{h}	469 ± 60^{i}	1964 ± 304^{h}
Adipose						
Weight, g	2.3 ± 0.2^{f}	$2.1 \pm 0.3^{f,g}$	1.6 ± 0.2^{g}	0.7 ± 0.1^{h}	0.5 ± 0.1^{h}	$1.8 \pm 1.0^{f,g,h}$
FAS, nm/min[d]	741 ± 194^{f}	$247 \pm 41^{g,i}$	$115 \pm 37^{h,i}$	121 ± 26^{h}	57 ± 21^{h}	307 ± 66^{i}
Mammary gland						
Weight, g	—	—	2.0 ± 0.2^{f}	3.1 ± 0.2^{g}	2.0 ± 0.1^{f}	4.7 ± 0.2^{h}
FAS, nm/min[d]	—	—	227 ± 56^{f}	7059 ± 748^{g}	392 ± 218^{f}	2099 ± 426^{h}
Total FAS, nm/min[e]	2584 ± 744^{f}	468 ± 42^{g}	987 ± 169^{h}	9540 ± 1176^{i}	$919 \pm 264^{g,h}$	4370 ± 707^{f}
Percentage of total						
Liver	$60 \pm 4^{f,g}$	$50 \pm 4^{f,h}$	66 ± 3^{g}	24 ± 3^{h}	$62 \pm 5^{f,g}$	48 ± 4^{h}
Adipose	40 ± 4^{f}	50 ± 4^{f}	11 ± 2^{g}	1 ± 0.3^{h}	8 ± 3^{g}	7 ± 1^{g}
Mammary gland	—	—	23 ± 2^{f}	75 ± 3^{g}	$30 \pm 6^{f,h}$	45 ± 5^{h}

[a] Mean ± SEM for 10 mice. All mice were 10 to 12 weeks old. Mice were killed on the 18th or 19th day of pregnancy and on the 5th day of lactation. Food was removed 12 hr before the fasted mice were killed and pups were removed 12 hr before one group of lactating mice was killed. Means with the same superscript letter (*f* through *i*) are not significantly different ($P < 0.05$).

[b] Body weight 24 hr prior to the time the mice were killed.

[c] During the last 24 hr of the experiment.

[d] Mice were injected intraperitoneally with 0.2 ml of saline containing 1.5 mCi of ^3H_2O 15 min prior to killing. Values represent nanomoles of ^3H_2O incorporated into fatty acids per minute per organ.

[e] Sum of values obtained in liver, adipose, and mammary gland.

because pregnant mice had enlarged livers. Rates of fatty acid synthesis in livers of lactating mice were double the rates observed in virgin mice. Fasting for 12 hr, as expected, decreased tritium incorporation into hepatic fatty acids in both virgin and lactating mice. Removal of pups from the lactating mice for 12 hr did not alter rates of fatty acid synthesis in the liver.

Weight of removable adipose tissue was lower in pregnant and lactating mice than in virgin mice (Table I). Removal of pups for 12 hr resulted in a twofold increase in adipose tissue weight in lactating mice, but the increase was not significant. Rates of fatty acid synthesis were highest in adipose tissue of fed virgin mice; pregnancy, lactation, and fasting decreased tritium incorporation in fatty acids. Removal of pups for 12 hr doubled the rate of fatty acid synthesis in adipose tissue of lactating mice.

Rates of fatty acid synthesis in mammary gland were quantitated in pregnant (18–19 days) and in lactating (5 days) mice (Table I). Only minimal quantities of tritium were incorporated into mammary fatty acids in the pregnant mice, whereas the rate of fatty acid synthesis in mammary glands of the lactating mice was rapid.

The quantity of fatty acids synthesized in the three organs examined was summed (Table I). The fed, lactating mice synthesized fatty acids at a rate nearly four times faster than observed in virgin mice and at a rate 10 times faster than observed in pregnant mice. Removal of pups for 12 hr reduced the total quantity of fatty acids synthesized by approximately 50% in lactating mice. Fasting the virgin and lactating mice markedly reduced the total quantity of fatty acids synthesized.

Approximately 50 to 60% of the fatty acid synthesis occurred in the liver of virgin mice, whereas in lactating mice only 24% of the total fatty acid synthesis occurred in the liver (Table I). The mammary gland accounted for 75% of total fatty acid synthesis in lactating mice. Adipose tissue was relatively unimportant as a site for fatty acid synthesis in pregnant and lactating mice.

The influence of a high-fat diet on fatty acid synthesis in lactating mice is presented in Table II. Values for virgin mice were included for comparison. Body weight, food intake, and liver weights were elevated in the lactating mice but adipose tissue weight was reduced relative to values observed in the virgin mice.

In agreement with the previous experiment, rates of fatty acid synthesis were elevated in the liver and depressed in the adipose tissue of the lactating mice relative to values obtained in virgin mice (Table II). High rates

TABLE II. EFFECT OF DIET ON *in vivo* RATES OF FATTY ACID SYNTHESIS IN LIVER, ADIPOSE TISSUE, AND MAMMARY GLAND OF VIRGIN AND LACTATING MICE.[a]

	Virgin		Lactating	
Parameter	Diet 1	Diet 2	Diet 1	Diet 2
Final body weight, g	35 ± 1^{c}	36 ± 1^{c}	43 ± 1^{d}	43 ± 1^{d}
Food intake, kcal/day	24 ± 1^{c}	26 ± 1^{c}	50 ± 3^{d}	56 ± 3^{d}
Liver				
Weight, g	2.2 ± 0.1^{c}	2.1 ± 0.1^{c}	2.9 ± 0.1^{d}	2.9 ± 0.1^{d}
FAS, nm/min[b]	2524 ± 364^{c}	605 ± 96^{d}	3622 ± 314^{e}	1017 ± 133^{f}
Adipose				
Weight, g	2.4 ± 0.2^{c}	3.2 ± 0.4^{c}	0.7 ± 0.1^{d}	0.6 ± 0.1^{d}
FAS, nm/min[b]	1204 ± 184^{c}	751 ± 107^{d}	323 ± 106^{e}	97 ± 28^{f}
Mammary gland				
Weight, g	—	—	3.9 ± 0.3^{c}	3.9 ± 0.2^{c}
FAS, nm/min[b]	—	—	10,200 ± 1413^{c}	6235 ± 1237^{d}
Total FAS, nm/min[b]	3728 ± 1086^{c}	1355 ± 328^{d}	14,145 ± 1748^{e}	7349 ± 1324^{f}
Percentage of total				
Liver	65 ± 5^{c}	45 ± 6^{d}	27 ± 3^{e}	16 ± 2^{f}
Adipose	35 ± 5^{c}	55 ± 6^{d}	2 ± 1^{e}	1 ± 1^{e}
Mammary gland	—	—	71 ± 3^{c}	83 ± 2^{d}

[a] Mean ± SEM for ten 10- to 12-week-old mice fed the respective diets for 5 days. Mice had been lactating for 5 days also. Means with the same superscript letter (*c* through *f*) are not significantly different ($P < 0.05$). Diet 1 was a high-carbohydrate diet and Diet 2 was a high-fat diet.

[b] See Table I.

of fatty acid synthesis were observed in mammary gland of the lactating mice. Consumption of the high-fat diet reduced tritium incorporation into fatty acids in liver and adipose tissue of both virgin and lactating mice. Similarly, rates of fatty acid synthesis were reduced by approximately 40% in the mammary gland of lactating mice consuming the high-fat diet.

Discussion. The rate of fatty acid synthesis from a combination of substrates can be estimated from the incorporation of labeled hydrogen from water (9). This is a particularly useful technique to compare *in vivo* rates of fatty acid synthesis in several organs as contrasted to the use of a carbon tracer since the source of carbon for fatty acid synthesis in each organ may vary (13, 14). Both liver and adipose tissue were important sites for fatty acid synthesis in the virgin mice. These results are in agreement with another report (15) and contrast with the chicken (16) where *de novo* fatty acid synthesis occurs almost exclusively in the liver and with the pig (17) where adipose tissue is the major organ for fatty acid synthesis.

In vivo rates of fatty acid synthesis were quantitated in late gestation. Even though food intake increased 50%, carbon flux to fatty acids was reduced by more than half in the pregnant mice. The energy demand for fetal development in late gestation would be expected to divert glucose from maternal utilization, thereby contributing to the lowered rate of fatty acid synthesis in the pregnant mice. Measurements of lipogenic enzyme activities in late gestation have not generally suggested such a marked reduction in fatty acid synthesis in pregnant animals. In fact, hepatic lipogenic enzyme activities in pregnant rats have often been reported to either not change (3, 4) or to increase (3, 5). These results illustrate that activities of lipogenic enzymes, as measured *in vitro* under optimal conditions, do not always reflect *in vivo* rates of fatty acid synthesis.

Mammary gland of the lactating mice exhibited an intense rate of fatty acid synthesis. Rates of fatty acid synthesis were also elevated in livers of lactating mice, but the quantity of fatty acids synthesized in adipose tissue was markedly depressed. These organ-specific responses during lactation allow the lactating mouse to direct dietary energy toward milk fat synthesis.

Smith *et al.* (8) have suggested that lipogenesis in the mammary gland does not respond to alterations in dietary fat, but others have obtained reduced rates of fatty acid synthesis in mammary gland preparation from rats fed high-fat diets (18) or fasted (19). Clearly, a short-term fast or consumption of a high-fat diet depressed *in vivo* rates of fatty acid synthesis in mammary gland of lactating mice. Abrupt weaning also depressed fatty acid synthesis in the mammary gland. Similarly, unilateral ligation of the teats reduced lipogenic enzyme activities in the ligated gland but not in the contralateral suckled gland of rats, suggesting that engorgement of the gland with milk has a direct effect on the lipogenic process (20).

Summary. In vivo rates of fatty acid synthesis in liver, removable adipose tissue, and mammary gland were obtained in mice. Liver contributed 60 to 65%, and adipose tissue 35 to 40% of the fatty acids synthesized in virgin mice fed a high-carbohydrate diet. Mice in the 18th and 19th day of gestation synthesized less than half the quantity of fatty acids synthesized in virgin mice, even though the pregnant mice consumed more food than the virgin mice. Rates of fatty acid synthesis were elevated more than threefold in lactating mice and 71 to 83% of the fatty acid synthesis occurred in the mammary gland of the lactating mice. Fasting for 12 hr or consumption of a high-fat diet for 5 days depressed rates of fatty acid synthesis in all three tissues examined. Removal of the pups for 12 hr decreased the rate of fatty acid synthesis in mammary gland and increased the rate in adipose tissue of lactating mice.

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