11. Jackson, W. B., Am. Heart J., 1958, v56, 222.
12. Tobian, L., Janecek, J., Tomboulian, A., Ferreira, D., J. Clin. Invest., 1961, v40, 1922.

Received June 28, 1965. P.S.E.B.M., 1966, v121.

Lipid Metabolism of Two Highly Inbred Strains of Mice.* (30774)

RICHARD W. HANSON† AND PAUL F. FENTON
Department of Biology, Brown University, Providence, R. I.

Reports from this laboratory(1,2,3) have demonstrated differences in the metabolic patterns of 2 highly inbred strains of mice. One of the most marked differences noted was the ease with which the A/Fn strain mouse became obese in response to diets containing up to 50% fat. The I/Fn, when fed the same diets, remained lean(4). This difference was, to a substantial degree, due to the greater food intake of the A strain mouse and particularly to the breakdown in food intake regulation as diets of increasing caloric density were fed. However, pair-feeding of the 2 strains still permitted the A strain to deposit significantly more carcass fat(5).

These observations led us to investigate the metabolic capacities of intact animals and of adipose tissue and liver *in vitro*. The results suggest that the A strain mouse is able to channel more lipid into depots while the I strain is more dependent upon fat oxidation to meet its energy requirements.

Experimental. Male mice of the A/Fn and I/Fn strain, 4-8 months of age, maintained on Purina Laboratory Chow were used throughout this study.

Free fatty acid (FFA) release. Animals were killed by cervical dislocation, the epididymal fat pads removed, weighed and incubated in calcium-free Krebs-Ringer phosphate buffer, pH 7.4, containing 3% bovine serum albumin (Fraction V). The tissue was preincubated for 30 minutes then transferred to fresh buffer for a period of 3 hours at 38°C. Portions of the medium were taken for FFA determination by the method of Dole and Meinertz(6). FFA levels were determined on plasma collected from both strains.

Ketone body formation by liver slices. Liver slices were prepared from the left lateral lobe, weighed and placed in a Warburg flask containing Krebs-Ringer phosphate buffer, pH 7.4, with 3% bovine serum albumin and 93 μg of octanoic acid per 3.0 ml of buffer. This concentration of octanoic acid was shown experimentally to be optimal for ketone body formation by liver slices *in vitro*. The tissue was incubated at 38°C for 3 hours. Ketone bodies were determined by the method of Lyon and Bloom(7) on 1.0 ml portions of the incubation medium.

Glycogen determination. Tissue glycogen was isolated from the left lateral lobe of the liver by digestion in hot 30% KOH followed by precipitation with ethanol. The precipitated glycogen was centrifuged and then dissolved in 1 N H_2SO_4. Glycogen was determined colorimetrically by the method of Fales(8).

Fat oxidation in vivo. Animals of both strains were placed in a metabolism cage suitable for collecting $C^{14}O_2$. Respiratory $C^{14}O_2$ was drawn through the system by suction and trapped in two 25% KOH traps. The temperature in the chamber was maintained at 30°C. Animals were lightly anesthetized with ether, weighed and fed by stomach tube 0.25 ml glyceryl tri (palmitate-1-C^{14}) (New England Nuclear Corp.) dissolved in olive oil. In experiments measuring the effect of carbohydrate on tripalmitin oxidation, the same concentration of fatty acid was administered along with 0.25 ml of a 25% glucose-50% starch solution. The animals were then placed

* Supported by a grant from Nat. Cancer Inst. (C1995).

† This work is part of a thesis submitted to the Department of Biology, Brown University, in partial fulfillment of the degree of Doctor of Philosophy and was supported in part by a Predoctoral Fellowship of USPHS. Present address: Fels Institute, Temple Univ. Med. School, Phildelphia, Pa.

TABLE I. Plasma FFA and FFA Release *in vitro* by Epididymal Fat Pads from A and I Strain Mice.

Strain	Plasma FFA (μmoles/ml) Fed	Plasma FFA (μmoles/ml) Fasted 24 hr	FFA release *in vitro* (μmoles released/ 100 mg tissue/hr) Fed	FFA release *in vitro* (μmoles released/ 100 mg tissue/hr) Fasted 24 hr
A	.882* ± .087 (4)	1.522 ± .107 (6)	.818 ± .032 (3)	1.030 ± .101 (6)
I	.610 ± .034 (4)	1.011 ± .068 (7)	.843 ± .076 (7)	1.033 ± .146 (8)
p	<.05	<.01	n.s.†	n.s.

* Mean value ± standard error; No. of animals shown in parentheses.
† Not significantly different.

in the metabolism chamber and at 2-hour intervals the KOH traps were changed and a portion plated as barium carbonate on filter paper discs 2.5 cm in diameter. The samples were counted in the proportional region with a windowless flow counter and corrected for self-absorption(9).

After the $C^{14}O_2$ collection period, the animals were killed by cervical dislocation and saponified in 200 ml of 15% KOH-50% ethanol under reflux. An aliquot was acidified and extracted 3 times with petroleum ether (B.P. 30-60°C). A portion of the ether extract was pipetted onto a lens paper disc cut to fit a 2-inch aluminum planchet. The planchet and contents were heated to dryness and counted at infinite thinness.

Results. A comparison of plasma FFA levels and rate of *in vitro* FFA mobilization by epididymal fat pads from fed and fasted A and I strain mice is shown in Table I. FFA release from isolated fat pads was the same for fed and fasted animals of both strains when the results were expressed on the basis of 100 mg of tissue. It must be remembered, however, that the I strain animal, although nearly equal in weight to the A, is characterized by smaller fat pads(5). Therefore, the intact A strain mouse by virtue of its larger fat stores may be capable of releasing more fatty acids into circulation. Indeed, the amount of plasma FFA was found to be greater in both fed and fasted A than in comparable I strain animals.

The oxidation of fatty acids to ketone bodies by liver slices and its relationship to the liver glycogen levels in both strains is shown in Fig. 1. The slope of the regression line for the I strain is −28.80 compared with −4.38 for the A. Decreases in the glycogen content of the I strain liver of 50% caused a comparable increase in ketone body production. This inverse relationship of liver glycogen and ketone body formation was evident, although not as marked, in the A strain.

The rate of tripalmitin-C^{14} oxidation to $C^{14}O_2$ *in vivo* by A and I strain mice and the effect of simultaneously feeding 25% glucose-50% starch was determined (Fig. 2A and 2B). The A strain mouse oxidized the administered fat at a fairly constant rate from the second to the tenth hour with 15% left unoxidized after 24 hours (Table II). Tripalmitin oxidation began more rapidly in the I strain mouse, reaching its maximum between 2 and 6 hours, while less than 2% of the label was detected upon saponification of the animal 24 hours after feeding. The simultaneous feeding of 25% glucose-50% starch and tripalmitin-C^{14} resulted in an alteration of the pattern of fat oxidation of both strains (Fig. 2B). Carbohydrate feeding stimulated fat oxidation over the first 4 hours in the A strain mouse. During the same period (0 to 4 hours) the oxidation of tripalmitin-C^{14} in the I strain was depressed by feeding carbohydrate, with the maximum rate of fat oxidation occurring between the sixth and twelfth

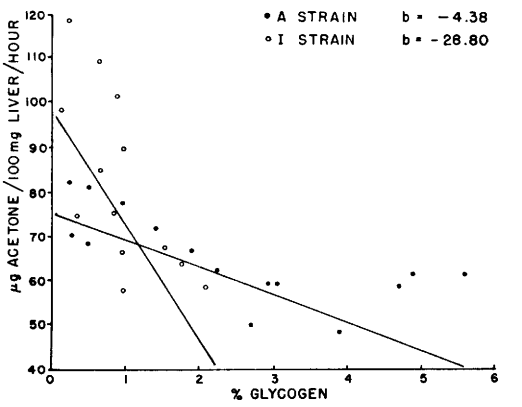

FIG. 1. Relationship between liver glycogen levels and ketone body production by liver slices from A and I strain mice. Each point represents one animal.

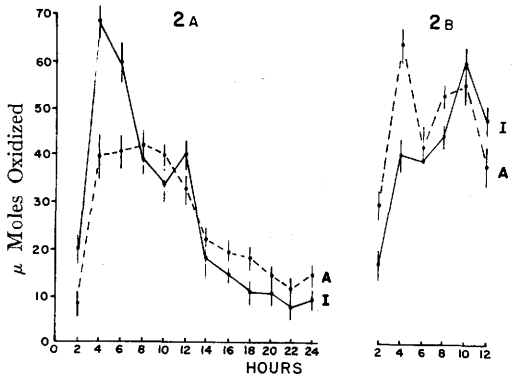

FIG. 2A. Tripalmitin-C^{14} oxidation to $C^{14}O_2$ *in vivo* by A and I strain mice. Each point is the mean of 3 animals. Vertical lines are the standard error of the mean. Animals used in this experiment were approximately the same age and closely matched in body weight. Tripalmitin-C^{14} (2 μc) was dissolved in olive oil and 0.25 ml administered (320 μ moles) orally.

FIG. 2B. Effects of carbohydrate on *in vivo* oxidation of tripalmitin-C^{14} to $C^{14}O_2$ by A and I strain mice. A 25% glucose-50% starch solution (0.25 ml) was administered orally after the tripalmitin-C^{14}. All other conditions the same as Fig. 2A.

hours. The amount of fat retained by both strains during this period did not differ significantly.

Discussion. Measurements of FFA mobilization *in vitro* indicate no strain differences in the rate of release if expressed on a unit weight basis. However, we have noted that in mice of the age used in this study (5-8 months of age) the A strain has a greater carcass fat content than the I. The greater plasma FFA concentration observed in the A strain animal may be due, in part, to the larger fat depots in this animal(5).

The concentration of FFA in the plasma is a balance between the rate of FFA release from the fat depots and the rate of removal by the other tissues. In the liver the metabolic pathway of fatty acids to CO_2 or ketone bodies is thought to be controlled by the intracellular levels of oxaloacetate and the activity of the enzyme citrate synthetase(10). In the present study we have demonstrated that ketone body production by liver slices is greater in the I than in the A strain mouse and is inversely related to the hepatic glycogen concentration. The mechanism of the glycogen effect on ketone body formation is not clear; however, it is tempting to speculate that glycogen contributes to the level of oxaloacetate in the liver cell.

The administration by stomach tube of tripalmitin-C^{14} to animals of both strains resulted in a greater rate of $C^{14}O_2$ output from the I strain mouse. The A strain animal oxidized less and retained more of the ingested lipid. Muscle of the I strain mouse has extremely high levels of glycogen that do not decrease significantly after a 24-hour fast(3). Lyon has made a detailed study of the muscle phosphorylase activity of the I strain mouse and has shown that phosphorylase *a* is not activated during fasting(11). The greatly diminished supply of carbohydrate available to the I strain muscle during fasting as well as the normally low levels of liver glycogen(3) may account for its greater dependence on fatty acid as a source of energy.

In the intact animal glucose spares the oxidation of fatty acids. Lewis *et al*(12) found that administered glucose was utilized preferentially by fasted rats and that fatty acid oxidation commenced after the exhaustion of available glucose. In the I strain mouse fatty acid oxidation is more rapid than in the A, and carbohydrate, fed with fat, has a more marked effect in depressing the conversion of tripalmitin-C^{14} to $C^{14}O_2$. This finding suggests a greater rate of fat oxidation by the I strain possibly due to the relative unavailability of muscle and liver carbohydrate in this animal. When carbohydrate and fatty

TABLE II. Effect of Carbohydrate on Retention of Tripalmitin-C^{14} by A and I Strain Mice.*

Strain	Tripalmitin-C^{14}	Tripalmitin-C^{14} 25% glucose, 50% starch
	(μmoles retained/24 hr)	
A	48.0† ± 6.2 (6)	63.0 ± 6.6 (3)
I	6.9 ± 5.1 (6)	54.6 ± 4.2 (3)
p	<.001	n.s.‡

* Animals received by stomach tube 320 μmoles of triglyceride containing 2 μc of tripalmitin-C^{14}.

† Mean value ± standard error; No. of animals shown in parentheses.

‡ Not significantly different.

acid are administered simultaneously to the I strain mouse, fatty acid oxidation is depressed. It is interesting to note that in the A strain mouse carbohydrate stimulates the oxidation of tripalmitin, a result difficult to interpret in light of the reports of a diminished fat oxidation when fat and carbohydrate are fed simultaneously(12,13).

This study points to two important differences in the lipid metabolism of the A and I strain animals. The A strain mouse is able to channel more ingested fat into depots (Table II) and upon fasting may liberate larger quantities of FFA into the plasma. The larger size of the fat stores in the A strain(4) may partly account for both the higher concentration of plasma FFA and, in turn, the greater hepatic lipid content of the fasted animal(5). Carbohydrate is probably not available in sufficient quantities to meet the requirements of the tissues of the fasting I strain mouse, forcing a greater reliance on fatty acid oxidation in this strain.

Summary. Differences in the metabolism of 2 highly inbred strains of mice were studied. There was a higher level of FFA in the plasma of the A/Fn strain mouse as compared to the I/Fn, although measurements of FFA release *in vitro* revealed no differences in the rate of mobilization per unit weight of adipose tissue. Ketone body production by liver slices was greater in the I, and in both strains was inversely related to the hepatic glycogen content. Tripalmitin-C^{14} oxidation to $C^{14}O_2$ *in vivo* was more marked in the I than in the A, while fat retention was greater in the A. The simultaneous feeding of a 25% glucose-50% starch solution markedly depressed CO_2 output from tripalmitin in the I strain and caused an enhanced output in the A.

1. Fenton, P. F., Dowling, M. T., Mershon, J. S., J. Nat. Cancer Inst., 1954, v15, 429.
2. Marsh, J. M., Fenton, P. F., Endocrinology, 1959, v65, 916.
3. Lyon, J. B., Jr., Fenton, P. F., Am. J. Physiol., 1956, v187, 415.
4. Fenton, P. F., Am. J. Clin. Nutr., 1960, v8, 748.
5. Fenton, P. F., Dowling, M. T., J. Nutr., 1953, v49, 319.
6. Dole, V. P., Meinertz, H., J. Biol. Chem., 1960, v235, 2595.
7. Lyon, J. B., Jr., Bloom, W. L., Can. J. Biochem. Physiol., 1958, v36, 1047.
8. Fales, F. W., J. Biol. Chem., 1951, v193, 113.
9. Gora, E. K., Hickey, F. C., Anal. Chem., 1954, v26, 1158.
10. Wieland, O., Weiss, L., Eger-Neufeldt, I., Adv. Enzyme Reg., 1965, v2, 85.
11. Lyon, J. B., Jr., Porter, J., Biochim. Biophys. Acta, 1962, v58, 248.
12. Lewis, K. F., Allen, A., Weinhouse, S., Arch. Biochem. Biophys., 1959, v85, 499.
13. Lassow, W. J., Chaikoff, I. L., ibid., 1955, v23, 23.

Received July 1, 1965. P.S.E.B.M., 1966, v121.

Urinary Excretion of Ingested α-Aminoisobutyric Acid-N^{15} in Dogs Treated with Growth Hormone or Corticotropin.* (30775)

H. C. CHOITZ AND O. H. GAEBLER
Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Mich.

In previous experiments on dogs(1), we found that excretion of N^{15} ingested as glycine, alanine, or ammonia was reduced by growth hormone (STH) and increased by corticotropin (ACTH). That STH increases incorporation of nitrogen from all of the above sources into tissue proteins was demonstrated in experiments on rats(2-4), but the possibility that this hormone also alters the rate of N^{15} excretion by influencing transport of utilizable substances was not excluded. Moreover, it would be difficult to prove directly that increased output of N^{15} in experiments with ACTH was due entirely to accentuated protein breakdown or gluconeogenesis. We have, therefore, studied effects of both

* This work was supported in part by a grant from Nat Inst. Health (AM-01362 END).