Rates of Net Absorption of Fat when Fed Alone or with Essential Nutrients[1] (35940)

DAVID L. TROUT AND EMILY S. CONWAY

Human Nutrition Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705

Laboratory rats which have not been exposed to high-fat diets have usually been used to measure the rates at which test meals of fat (triglyceride) can be digested and absorbed (1–7). Published values for the rates of fat absorption obtained under these conditions (1–7) are only high enough to account for about half the energy required for rapid growth. However, it is known (8, 9) that diets providing far more than 50% of calories as fat can support rapid growth in the rat. Apparently, briefly starved rats, when given a 100% fat meal, do not absorb the fat as rapidly as do growing rats fed a nutritionally complete high-fat diet. The present study sought first to test out this inference. The study also indicated how promptly, after first receiving a high-fat diet, do rats become able to absorb fat at the rate required for rapid growth.

Used in a part of this study was the nonionic surface-active agent, WR-1339, formerly called Triton (an oxyethylated tertiary octylphenol polymethylene polymer). This drug inhibits the removal of triglyceride from the blood and thus induces hyperlipemia (10). Evidence is presented that the response of blood lipids to this drug may be used to estimate the rate of fat absorption under special conditions.

Methods. Net fat absorption was measured under the following three conditions: (i) in starved animals given a test meal of fat; (ii) in growing animals fed a high-fat diet *ad libitum*; (iii) in starved animals provided for 24 hr with the same high-fat diet. Olive oil, one of the dietary fats known to be most readily digested and absorbed by the rat (5, 6), was used for the 100% fat meal. The high-fat diet contained the following (g/100 g): partially hydrogenated vegetable oil (Crisco), 67; lactalbumin, 25; vitamin mix in glucose,[2] 2; and Jones–Foster (11) salt mix, 6. The Crisco contributed 85% of calories.

Male animals of a Wistar-derived stock were obtained commercially for these studies.[3] They were fed a moderately low-fat chow diet for at least 1 week.[4] Animals designated as "starved" were deprived of food for 22 hr. Either olive oil or high-fat diet was then made available at the end of a short metal tunnel from which food could not easily be scattered. Olive oil was offered in an amount of 7.5 g/kg of body weight. This is a larger amount than is usually tube-fed in absorption studies and more than most of our rats would consume in several hours. The high-fat diet was fed *ad libitum* for 24 hr. The "*ad libitum* fed" animals received the high-fat diet for an 11–17 day period.

Net fat absorption was determined by measuring how much fat was ingested and how much of the latter could be accounted for in feces and as extra total (mostly esterified) fatty acids in the digestive tract. In studies involving the refeeding of starved rats, the digestive tracts were dissected out intact, and the combined total fatty acids (TFA) of the tract and ingesta were measured. This measurement was made for con-

[1] Presented in part at the Annu. Meet. Fed. Amer. Soc. Exp. Biol. 55th, Chicago, IL, Apr., 1971.

[2] Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[3] A "specific pathogen-free" Wistar-derived stock was obtained from Manor Research, which has been reorganized as Purina Laboratory Animals.

[4] D and G Research Animal Laboratory Diet, Rat and Mouse baked biscuit, the Price Wilhoite Co., Frederick, Maryand.

trol animals, which had been starved for 22 hr, and for test animals which were killed after a specified period of refeeding. The extra TFA above control levels was converted to grams of unabsorbed fat on the basis that 1 g of olive oil or Crisco yielded, respectively, 3.45 or 3.40 mmoles of TFA. In the study involving *ad libitum* consumption of the high-fat diet, diet intake was measured and feces were collected during the final 96 hr of the 11–17 day feeding period. The digestive tracts were assumed to have contained essentially the same quantity of fat at the beginning and end of the test period. Fat intake was corrected for fecal lipids, and the difference was taken to represent net fat absorption.

The estimated fat absorption of each animal was adjusted to a common basis, *i.e.*, a body weight of 250 g. This correction, which was usually less than 4%, was carried out on the basis that fat absorption is directly related to body surface (1), as estimated by Lee's formula (12).

The above method of estimating fat absorption tended in two ways to yield low values. First, it failed to include the "endogenous" component of fat absorption, *e.g.*, of fatty acids brought into the digestive tract in bile. Secondly, fatty acids were not identified as absorbed until they had passed out of the intestinal wall into the blood.

Two portions of the study involved measurement of the response of blood lipids to WR-1339 at specified intervals after olive oil was fed. The drug was obtained from Ruger Chemical Co., Irvington-on-Hudson, New York, and was diluted with 0.16 *N* NaCl. It was injected into a lateral tail vein at a dose of 400 or 800 mg/kg and in a volume of 2 or 4 ml/kg. Bloods were obtained by decapitation and collected in heparinized beakers.

Fecal lipids were extracted after an acid hydrolysis of nonlipid material and determined gravimetrically (13). Plasma total fatty acids and those in the neutral lipid fraction were determined by standard methods of extraction, saponification, and titration (14, 15). The total fatty acids of the digestive tract were also determined by a similar procedure, after tracts were dispersed in methanol by use of a Virtis homogenizer and further extracted through the addition of chloroform.

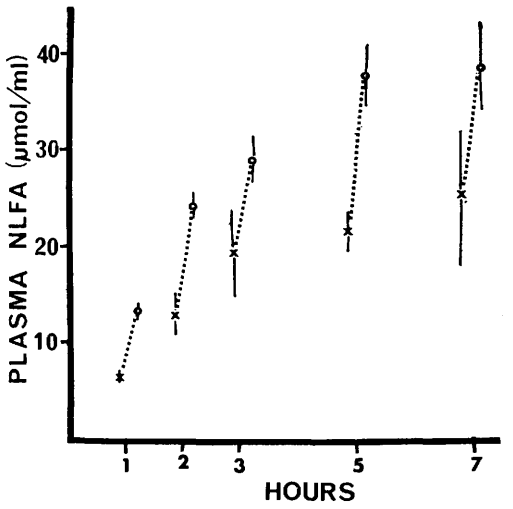

FIG. 1. Neutral lipid fatty acids of plasma (μmoles/ml) at specified intervals after the start of the fat meal, either 20 min after the iv injection of WR-1339 (○) or in untreated animals (×).

Results. Preliminary studies were carried out to determine a time after the offering of olive oil when fat absorption was relatively rapid and constant.

In a first experiment, rats were decapitated or injected iv with 400 mg/kg of WR-1339 at specified intervals after the olive oil was offered. The fatty acids of the neutral lipid fraction of blood plasma were then measured at 0 and 20 min after the injection of WR-1339. Results, as shown in Fig. 1, indicated that, even without receiving WR-1339, rats became severely lipemic. The rate of accumulation of plasma lipid after an injection of this drug appeared to increase for several hours after the start of the test meal. Results were interpreted as indicating a lag in fat absorption.

The rate of lipid accumulation after WR-1339 treatment was further investigated during the 4–8 hr interval after the start of the olive oil meal (Table I). In this study, animals were given a larger dose (800 mg/kg) of WR-1339 and were allowed a longer time in which to accumulate blood lipid (80 min) than in the previous work. Initially, we were unable to measure the response to WR-1339

TABLE I. Accumulation of Total Fatty Acids in Plasma of WR-1339-Treated Rats During 4–8 hr Interval After Start of a Fat Meal.[a]

Time Interval (hr) after start of fat meal	Plasma total fatty acids (μmoles/ml $\pm$ SE)[b]		
	Starting[c]	Final (80 min post-WR)	Accumulation
4[b]–5.33	28 $\pm$ 5.1	94 $\pm$ 6.3	66
5.33[b]–6.67	27 $\pm$ 3.5	100 $\pm$ 4.0	73
6.67[b]–8	20 $\pm$ 4.3	95 $\pm$ 4.2	75
Av	—	—	71

[a] 100% olive oil.

[b] Standard error.

[c] At start of each time interval, blood was taken from six rats; and WR-1339 was injected into 8 other rats which were then killed 80 min later.

under these conditions, because we could not obtain a representative sample of the extremely fatty blood plasma. The problem was clumping of the chylomicrons during centrifugation in the cold. This problem was solved, however, by collecting and centrifuging bloods at room temperature, and total fatty acids (TFA) were then measured. Data shown in Table I indicated that the rate of TFA accumulation after WR-1339 treatment was relatively constant during the 4–8 hr interval and averaged 71 μmoles/ml/80 min.

The net absorption of fatty acids from the digestive tract was observed during the first 4 and 8 hr after the start of the test meal of olive oil. Results are shown in Table II. Again, there appeared to be a delay in the rate of net fat absorption. During the second 4 hr period, absorption was calculated to have occurred at a rate of almost 4 g of olive oil/24 hr/250 g rat.

In a further experiment, net fat absorption was estimated during the final 96 hr of a 11–17 day period of *ad libitum* consumption of the high-fat (67% Crisco) diet. These animals showed as rapid growth as did two other groups which received, respectively the previously mentioned chow diet and a modification of the high-fat diet (in which the lactalbumin was increased from 25 to 32.2% at the expense of Crisco). Table II shows that nonfasting animals fed the 67% Crisco diet absorbed fat at a rate of 5.8 g/24 hr/250 g rat—nearly 50% faster than was observed during the 4 to 8 hr interval after the test meal of olive oil.

The net absoption of fat from the intestinal tract was also measured in 22 hr fasted animals which were provided the same 67% Crisco diet for a 24 hr period. These animals consumed the diet at a higher rate than did the steadily growing group, and they showed a still higher net rate of fat absorption—7.4 g/24 hr/250 g animal.

Discussion. Present data provide a basis for comparing, under the same conditions, the net rate of fat absorption from the intestinal tract and the rate of fat accumulation in WR-1339-containing blood plasma. During the 4–8 hr interval after the start of the olive oil meal, plasma total fatty acids rose after WR-1339 injection many times as rapidly as have been reported for fasted animals (16, 17). The observed accumulation of total fatty acids (71 μmoles/80 min/ml plasma) was assumed to apply to the 250 g rat having a 10 ml plasma volume. If one makes a simplifying assumption, namely that the accumulating lipid was derived entirely from the digestive tract and was completely trapped in the plasma by WR-1339, the plasma data indicates an absorption of olive oil at a rate of 3.7 g/24 hr/250 g rat. This calculated rate is not different from the directly observed rate of net absorption (3.9 g/24 hr/250 g rat).

The above finding implies that, under at least some conditions, the plasma response to WR-1339 provides a useful indirect measure of fat absorption. However, caution is needed in using WR-1339 for this purpose. The problem derives from the mode of action of

TABLE II. Estimated Rates of Net Absorption of Dietary Fat Under Varied Conditions.

Expt. no.	Pretreatment	Body wt (g)		Period of measurement	Source of dietary fat	Fat consumed (g)	Fat recovered[a] in digestive tract and feces		Net absorption	
		Initial	Final				Gross	Net	Obs (g/rat)	Calc (g/24 hr/ 250 g rat)[b]
1	Starved (22 hr)	236	—	4	Olive oil	1.64	1.45	1.12	0.52	3.24 ± 0.30
		238	—	8	Olive oil	1.78	0.95	0.62	1.14	3.59 ± 0.18
		—	—	2nd 4 hr period	Olive oil	—	—	—	0.62	3.94 ± 0.34
2	67% Fat diet for 7–13 days	244	262	96	67% Fat diet	25.9	—[c]	2.5[c]	23.4	5.83 ± 0.25
3	Starved (22 hr)	238	254	24	67% Fat diet	11.80	4.80	4.48	7.32	7.38 ± 0.33

[a] "Fat recovered" is expressed as grams of olive oil or Crisco which would contain the observed quantity of total fatty acids. "Net" implies amount in excess of that found in the digestive tracts of control animals after being without food for 22 hr.

[b] Estimates of fat absorption of individual rats were adjusted to a 250 g of body weight on the basis that rate was assumed to be proportional to body surface. The variance about the mean of these values and about the mean value for 22 hr fasted controls was combined in calculating standard error.

[c] In Expt. 2, fecal lipids were measured; the digestive tracts and their contents were assumed not to have accumulated a significant quantity of fat during the 96 hr test period.

WR-1339.

WR-1339 is believed to absorb onto serum lipoproteins or chylomicrons, rendering them unsuitable substrates for the hydrolysis of triglyceride by tissue lipoprotein lipase (10). This reaction with chylomicrons appears to take at least 30 min to reach completion *in vivo* and even then does not entirely prevent the disappearance of chylomicron triglyceride from the blood (18). Apparently, WR-1339 is relatively ineffective in entrapping chylomicron triglyceride under conditions in which this lipid molecule has a short biological half-life.

In the present study, animals fed olive oil became severely lipemic. Apparently, mechanisms for extracting triglycerides of chylomicrons and lipoproteins were overloaded. Animals were also found (data not shown) to have high plasma free fatty acids, a condition known to inhibit synthesis of tissue lipoprotein lipase (19). Apparently, for these reasons, WR-1339 was moderately effective in entrapping chylomicron triglyceride in the blood plasma. However, there are reports that a small fat meal or the substitution of some dietary fat for carbohydrate in a test meal may fail to speed the accumulation of plasma lipid after WR-1339 treatment (16, 17).

The present study confirmed the tentative inference from previous studies that, when starved briefly and fed fat alone, previously chow-fed rats do not absorb dietary triglyceride as rapidly as do growing animals receiving a high-fat diet. It was further shown that the relatively slow absorption of a fat meal is not due to the previously chow-fed animal being unable to absorb fat rapidly (Expt. 3). Nonlipid components of the high-fat diet appear to have contributed to the relatively rapid absorption of its fat content. The protein and perhaps also other ingredients brought about an increased fat consumption, an effect known to enhance fat absorption (1, 2), and may have facilitated micelle formation in the small intestine. The massive intake of the high-fat diet by starved animals appears to have been responsible for the particularly high rate of fat absorption observed in these animals.

The rate of absorption of a 100% fat meal observed in this study was larger than some investigators have found (3, 4, 6, 7). This may reflect special conditions of this study. Olive oil was the fat provided. Animals were not traumatized by prolonged fasting or by being force-fed. The early period of relatively slow fat absorption was excluded from the interval over which the average rate was measured. However, some investigators have reported rates of fat absorption similar to our reported value of 3.9 g/24 hr/250 g rat (1, 5). It may well prove possible to obtain still higher rates if methods of inducing an increased fat consumption and, particularly, of speeding the movement of fat into the duodenum are worked out.

Data from the present study indicate that, when a briefly starved animal is fed fat, the rate of fat absorption increases for several hours. The main finding is that, when the fat is fed as the main component of a nutritionally complete diet and is consumed in large amounts, animals reach within the first day a rate of fat absorption exceeding that required for rapid growth during *ad libitum* consumption of a high-fat diet.

Summary. The net rate of fat absorption, following a test meal of olive oil, was found to be 3.9 g/24 hr/250 g rat. When WR-1339 (800 mg/kg) was injected iv into rats similarly fed olive oil, fatty acids were found to accumulate in the blood plasma at a similar rate. These rates, however, were 30% lower than that observed during prolonged *ad libitum* consumption of a high-fat diet and were almost 50% lower than that found among fasted rats when fed the same high-fat diet.

1. Deuel, H. J., Hallman, L., and Leonard, A., J. Nutr. **20**, 215 (1940).

2. Bennet, S., and Simmonds, W. J., Quart. J. Exp. Physiol. **47**, 32 (1962).

3. Aberdeen, V., Shepherd, P. A., and Simmonds, W. J., Quart. J. Exp. Physiol. **45**, 265 (1960).

4. Shepherd, P., and Simmonds, W. J., Aust. J. Exp. Biol. Med. Sci. **37**, 1(1959).

5. Steenbock, H., Irwin, M. H., and Weber, J., J. Nutr. **12**, 103 (1936).

6. Thomasson, H. J., J. Nutr. **59**, 343 (1956).

7. Crocket, M. E., and Deuel, H. J., J. Nutr. **33**, 187 (1947).

8. Deuel, H. J., Meserve, E. R., Straub, E.,

Hendrick, C., and Scheer, B. T., J. Nutr. **33**, 569 (1947).

9. Konijn, A. M., Muogbo, D. N. C., and Guggenheim, K., Isr. J. Med. Sci. **6**, 498 (1970).

10. Schotz, M. C., Scanu, A., and Page, I. H., Amer. J. Physiol. **188**, 399 (1957).

11. Jones, J. H., and Foster, C., J. Nutr. **24**, 245 (1942).

12. Lee, M. O., Amer. J. Physiol. **89**, 24 (1929).

13. Ass. Offic. Agr. Chem. "Official Methods of Analysis," 9th ed., Sect 13.119, p. 176. Ass. Office Agr. Chem., Washington, DC (1960).

14. Trout, D. L., Aerosp. Med. **35**, 357 (1964).

15. Albrink, M. J., J. Lipid Res. **1**, 53 (1959).

16. Moore, W. T., Tidwell, H. C., and McPherson, J. C., Amer. J. Clin. Nutr. **16**, 135 (1965).

17. Otway, S., and Robinson, D. S., J. Physiol. (London) **190**, 321 (1967).

18. Otway, S., and Robinson, D. S., J. Physiol. (London) **190**, 309 (1967).

19. Nikkilä, E. A., and Pykälistö., *in* "Drugs Affecting Lipid Metaoblism" (W. L. Holmes, L. A. Carlson, and R. Paoletti, eds.), p. 239. Plenum, New York (1969).

Received May 4, 1971. P.S.E.B.M., 1971, Vol. 138.