The Incorporation of ^{14}C-Glycerol into Adipose Tissue Lipids of Weanling Rats with Hypothalamic Obesity (36711)

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Adipose tissue triglyceride stores are in dynamic equilibrium between esterification and hydrolysis to free fatty acids (FFA) and glycerol. For a long time it was assumed that only FFA could be utilized by the tissue whereas glycerol, due to lack of glycerokinase, was not utilized (1, 2). An exception to this observation was the finding of glycerokinase activity in adipose tissue of obese-hyperglycemic mice (3). The presence of this enzyme in the adipose tissue of *obob* mice was considered to be pathogenetically important in the development of metabolic obesity in contrast to regulatory obesity which could be accounted for entirely by hyperphagia (4). Recently, however, the incorporation of labelled glycerol into triglycerides was demonstrated in the adipose tissue of normal rats (5) and the use of a sensitive assay of glycerokinase activity (6) established its presence in adipose tissue of normal mice as well as its enhancement by insulin (7). This latter finding could explain the increased glycerokinase activity in intact adipose tissue (3) and isolated adipose cells (7) of *obob* mice which are grossly hyperinsulinemic (8, 9).

The presence of hyperinsulinemia in weanling rats with experimentally induced hypothalamic obesity prompted the present study in which the utilization of glycerol by adipose tissue from these animals was measured as a reflection of glycerokinase activity.

Materials and Methods. Handling of animals and operative procedure. Weanling male rats (19–21 days), weighing 50–60 g, were obtained from Sprague-Dawley Co., Madison, WI. After 3–4 days of acclimatization, the rats were anesthetized with Evipal (Winthrop Lab), body weight and length were measured, and stainless steel electrodes (0.25 mm in diameter and insulated except for 0.2 mm of the tip) were positioned in the ventromedial nuclei of the hypothalamus (10). In one group of rats (VMN), bilateral electrolytic lesions were produced by passage of a direct anodal current of 1.5 mA for 10 sec (15 mC). In a control group, the same operative procedure was followed but without current flow. Both groups of rats were fed laboratory chow (Teklad, Inc., Winfield, IA) and tap water *ad libitum* until sacrificed. Food intake was the same for each group of rats.

Fourteen to 28 days after hypothalamic operations, the rats were again weighed and measured[1] under light ether anesthesia. On the following day they were sacrificed by decapitation. The brain of each animal was examined and lesions verified as described previously (12). Rats which failed to show bilateral symmetrical lesions in the ventromedial hypothalamic nuclei were excluded from final statistical analysis, as were those in which the lesions extended beyond the VMN or into the median eminence.

Incubation procedures. Approximately 100 mg of epididymal fat was weighed, incubated for 1 hr in 1 ml of Krebs–Ringer bicarbonate buffer containing 50 mg (5%) bovine albumin (fraction V) and 0.25 μCi (5.55×10^5 dpm) of glycerol-U-^{14}C (Amersham, sp act, 9 mCi/mmole). In other experiments the time course of glycerol-U-^{14}C incorporation into adipose tissue lipids was studied by incubating 100 mg of adipose tissue in 10 ml

[1] In most cases the obesity syndrome was well developed and rats with an obesity index (11) greater than 312, as opposed to 305 or lower in control animals, were chosen for the studies.

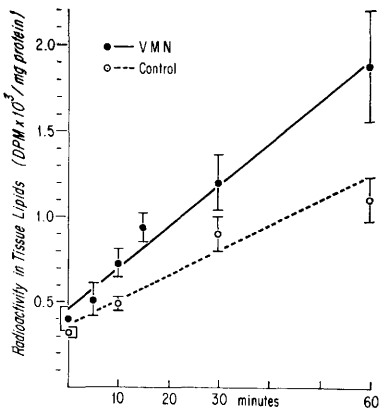

FIG. 1. Time course of glycerol-U-^{14}C incorporation into adipose tissue lipids. Tissues were incubated for different time periods in medium containing nonradioactive glycerol (10 μmoles/ml) and glucose (5 μmoles/ml). The data presented are the means ± SEM (6 VMN and 3 control animals). The nonparallelism in the slope between the two groups of animals was significant ($p < .05$).

of medium containing 5 μmole of glucose and 10 μmoles of glycerol/ml in addition to the above mentioned constituents; a 15 min preincubation allowed equilibration of glycerol between medium and tissue prior to the addition of glycerol-U-^{14}C (0.25 μCi/ml). Timing of the incubation began with the addition of tracer. Pieces of tissue from obese animals were incubated for 0, 5, 10, 15, 30 and 60 min while tissue from controls, due to the scarcity of adipose tissue, only for 0, 10, 30 and 60 min. Incubation was carried out at 37° in a Dubnoff shaking incubator, in an atmosphere of O_2:CO_2 (95:5).

At the end of incubation, the tissue was washed thoroughly, blotted and homogenized in 1 ml of Krebs–Ringer bicarbonate buffer. To determine the oxidation of glycerol by adipose tissue, a plastic well was suspended from the rubber stopper of the incubating flask into which 0.2 ml of Hyamine was injected at the end of 1 hr incubation whereas 0.5 ml of 3 *N* H_2SO_4 was injected into the medium. Incubation flasks were shaken for another hour, the rubber stopper was carefully removed, the outside of the well was wiped free of splashed medium, and the well was dislodged into a counting vial containing toluene phosphor (13).

Analytical procedures. An aliquot of the homogenate, representing 20 mg of tissue, was extracted for lipids (14). Radioactivity in half the volume of the lipid-containing organic phase was determined by liquid scintillation counting in toluene phosphor. In one experiment, another aliquot of the lipid extract (representing 10 mg of tissue) was evaporated under N_2, reconstituted in a small amount of hexane, and fractionated by thin-layer chromatography (hexane:diethyl ether:glacial acetic acid, 90:10:1), using adsorbosil-5 (Applied Science Lab) as adsorbent. Fractions corresponding to various lipid components were identified with iodine vapor, scraped from the plate and collected in counting vials where they were counted in a suspension of 4% thixotropic gel–toluene phosphor.

The rest of the homogenate was centrifuged at 20,000*g* for 15 min, the aqueous phase collected and its glycerol concentration, as well as that of the incubation medium, measured (2). To determine the radioactivity of this glycerol, an aliquot of the aqueous supernatant (representing 10 mg of tissue) as well as a 100 μl aliquot of the incubation medium were extracted with 5 ml of chloroform to remove lipids and placed in Bray's phosphor (13). All radioactivity was

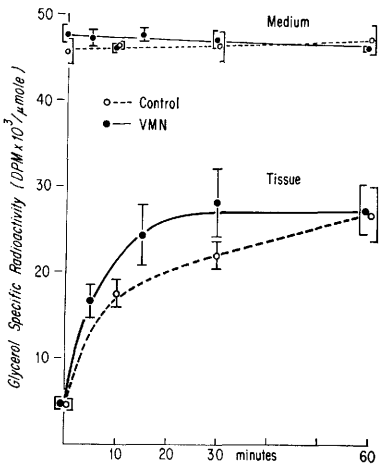

FIG. 2. Time course of specific radioactivity of ^{14}C-glycerol (dpm/μmole glycerol) in incubated tissue and incubation medium. The data presented (means ± SEM) are derived from the same experiment shown in Fig. 1.

TABLE I. Effect of VMN Lesions on the Incorporation of ^{14}C-Glycerol into Adipose Tissue Lipids During a 1 hr Incubation (Means ± SEM).

Animals	Radioactivity of tissue lipids (dpm × 10^3/mg protein)	Sp radioactivity of tissue glycerol (dpm × 10^3/μmole)
VMN (17)[a]	15.78 ± 1.92[b]	7.34 ± 0.76
Control (13)	8.01 ± 0.64	9.02 ± 0.88

[a] Number of animals used is given in parentheses.

[b] $p < .005$ (VMN vs control).

counted in a liquid scintillation counter and disintegrations per minute (dpm) were calculated by the channels ratio technique. Results were expressed per milligram of tissue protein which was determined on a 50 μl aliquot of tissue homogenate by a modification (12) of a Folin phenol method (15).

Results. ^{14}C-Glycerol was incorporated into adipose tissue lipids of both VMN and control animals and was linear with time. The regression lines calculated for both groups showed that the rate of incorporation was greater in the VMN than in control tissue (Fig. 1). In these experiments, 10 μmoles of nonradioactive glycerol and 5 μmoles of glucose were added to the incubation medium, and the specific radioactivity of medium glycerol remained constant through incubation for both VMN and control tissues. However, the specific radioactivity of tissue glycerol, while rising during the early part of incubation, plateaued by the end of one hour at a level similar for VMN and control rats (Fig. 2).

Similarly, when nonradioactive glycerol was omitted from the incubation medium, adipose tissue of VMN rats showed greater incorporation of ^{14}C-glycerol into lipids as compared with control in the presence of comparable specific radioactivity of tissue glycerol[2] (Table I). Thin-layer chromatography revealed that most of the radioactivity incorporated into tissue lipids of VMN and control rats was found in the triglyceride fraction. However, both the absolute and relative radioactivity of diglycerides was higher in adipose tissue of VMN as compared with control rats (Table II).

In an experiment designed to obtain an estimate of the overall metabolism of glycerol by adipose tissue of VMN rats, approximately one-half (48%) of the labeled glycerol was found in the tissue lipids, one-sixth (17%) in the supernatant, and one-third (32%) was oxidized to CO_2.

Discussion. The present data demonstrate that labeled glycerol is incorporated into adipose tissue lipids of weanling rats and are thus consistent with findings obtained by others from isolated fat cells (5) and homogenates of rat adipose tissue (16). The radioactivity recovered in adipose tissue lipids appears to represent true biological incorporation and not contamination since incorporation was negligible without incubation, increased linearly with time, and appeared in CO_2.

The data on glycerol incorporation reported in this paper show that less radioactivity was incorporated into adipose tissue lipids when both glucose and nonradioactive glycerol were added to the incubation medium despite the fact that the specific radioactivity of tissue glycerol was higher. This may be due to the presence of glucose in view of its suppressive effect on glycerol incorporation into adipose cell lipids of adult rats (5). When nonradioactive glycerol was added to the incubation medium, the specific radioactivity of tissue glycerol reached a plateau after 1 hr of incubation at which time the calculated rate of glycerol incorporation into lipids was 0.046 μmole/mg protein. It is not possible from these data to relate this rate to that of glycerol production during the process of lipolysis. However, earlier data obtained from similar experiments in this laboratory showed a rate of glycerol production of 0.27 μmole/mg protein/hr (12). Combining these two sets of data, we estimated that approximately 17% of the total glycerol produced was reutilized in the synthesis of glycerides. This is clearly an overestimate, since the value for total glycerol production does not account for the portion reesterified.

Adipose tissue of weanling VMN rats in-

[2] In the absence of incubation, negligible radioactivity was recovered in tissue lipids.

TABLE II. Incorporation of ^{14}C-Glycerol into Various Lipid Fractions During a 1 hr Incubation (Means ± SEM).

Animals	Monoglycerides and phospholipids	Diglycerides	Triglycerides	Free fatty acids
		Dpm × 10^3/mg protein		
VMN (11)[a]	0.29 ± 0.04	1.03 ± 0.13[b]	10.78 ± 1.04	0.12 ± 0.01
Control (8)	0.17 ± 0.02	0.21 ± 0.03	8.35 ± 0.81	0.08 ± 0.01
		Percentage of total radioactivity		
VMN (11)	2.23 ± 0.29	7.83 ± 0.48[b]	83.70 ± 1.07[c]	0.96 ± 0.07
Control (8)	1.80 ± 0.10	2.34 ± 0.46	88.42 ± 1.50	0.93 ± 0.19

[a] Number of animals used is given in parentheses.
[b] VMN vs control, $p < .001$; [c] $p < .025$.

corporated ^{14}C-glycerol into lipids at a faster rate than control. Assuming a similar steady state for tissue specific radioactivity, the amount of glycerol incorporated into lipids by the VMN adipose tissue was 0.07 μmole/mg protein/hr. Based on a glycerol production of 0.36 μmole/mg protein/hr by adipose tissue of VMN rats (12), approximately 20% of the total glycerol produced was reutilized for glyceride synthesis by adipose tissue of VMN rats. Thus, these figures indicate no greater rate of glycerol utilization relative to its production in adipose tissue of VMN rats, though the higher absolute rate of glycerol utilization in the presence of equal or higher production (12) suggests a faster turnover rate of glycerol in the VMN animals.

It is unlikely that differences in food consumption between VMN and control rats could account for the greater incorporation of ^{14}C-glycerol into tissue lipids of VMN rats since food intake has been shown to be similar in these two groups (17). It has been reported that glycerokinase activity of isolated fat cells from obese-hyperglycemic mice is much higher than that of their lean littermates, and correlates significantly with serum insulin levels (7). Moreover, the glycerokinase activity is decreased during fasting and in experimental diabetes. In the latter case, activity is restored by insulin treatment, the effect of which is blocked by actinomycin D. It thus appears that insulin plays a significant role in the induction of glycerokinase which, in turn, promotes the use of glycerol for glyceride synthesis. Since hyperinsulinemia is a feature of hypothalamic obesity in weanling rats (17) as it is in *obob* mice (8, 9), it is possible that the increased rate of glycerol utilization for glyceride synthesis reflects an induction of glycerokinase in adipose tissue of these animals.

Summary. Adipose tissue of weanling rats with hypothalamic obesity (VMN) incorporated labeled glycerol into lipids, mainly triglycerides, at a faster rate than control. It is postulated that hyperinsulinemia, an important feature of this obesity syndrome, contributes to the increased utilization of glycerol for lipid synthesis.

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