Studies on the Composition of Adipose Tissue from the Genetically Obese Rats* (34049)

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(Introduced by E. B. Astwood)

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The genetically obese rat, called fatty, is a Mendelian recessive mutant in which obesity develops within the first few weeks of life (1). The affected siblings of each litter continue to accumulate fat throughout life. The distribution of this fat and other gross morphologic characteristics of these animals have been described by Zucker and Zucker (2), but there are no detailed studies on the composition of their adipose tissue. The present experiments have examined some aspects of their adipose tissue, looking specifically at the size of fat cells and the composition of the triglycerides in the subcutaneous fat. Lean littermates, or rats from the same strain made obese by the introduction of hypothalamic lesions served as controls for these studies.

Methods and Materials. Animals. The genetically obese rats used in these experiments were supplied through the kindness of Dr. Lois Zucker. Bilateral hypothalamic lesions were made in the hypothalamus of lean rats weighing 200–300 g as described previously (3). All rats were maintained in a temperature-controlled room and had free access to Purina Laboratory Chow and tap water.

Adipose tissue composition. Adipose tissue was removed under ether anesthesia from subcutaneous depots around the shoulder or hip and from the parametrial or epididymal fat pads. Weighed pieces of fat were placed in 15-ml centrifuge tubes with 3 ml of chloroform–methanol (2:1) and crushed. The extracts from the fat pieces were decanted and the tissue was washed two more times. The chloroform–methanol extracts from each tissue were pooled and washed with 3 ml of water. The chloroform layer was transferred to tared vials and dried at 60° to constant weight. DNA in the defatted residue was determined by the method of Ceriotti (4), as modified by Keck (5). A second piece of fat from each biopsy specimen was used to make fat cells by incubating it in 3 ml of Krebs-Ringer bicarbonate buffer containing approximately 10 mg of collagenase (Worthington). Aliquots of the fat cells were introduced into tared centrifuge tubes, weighed, and extracted with chloroform–methanol as described above. A second aliquot of the fat cells was placed in a drop of saline on a microscope slide and the size of 100–200 cells was measured with a calibrated eyepiece (American Optical) (6).

Gas chromatography. Pieces of subcutaneous fat from four fatties and four hypothalamic obese rats were placed in chloroform-methanol (2:1 v/v) and homogenized. The homogenate was washed three times with 0.2 *N* sulfuric acid and the remaining chloroform layer was dried under nitrogen. Each of the eight samples of triglyceride was incubated overnight with 5% sulfuric acid in methanol at 60° and extracted the next day with hexane. The samples were run on a Jarrell-Ash Gas chromatograph with a 4-ft stainless-steel column packed with 15% Hi-Temp DEGS on 80–100 mesh Gas Chrome P. The oven temperature was 190°, the hydrogen flame detector was 210°.

Calculations. Cell volume (cubic micra) $= \frac{\pi}{6}(\bar{x}^2 + 3\sigma^2)\,\bar{x}$ where $\bar{x}$ and σ are the mean and standard deviation for the diameter of the fat cells (7). Triglyceride (μg/cell)

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TABLE I. Dimensions and Triglyceride Content of Fat Cells from Obese and Lean Rats.

Animal	Body wt (g)	Source of fat: Subcutaneous Diameter (μ)	Subcutaneous Volume ($\bar{\mu}^3 \times 10^{-5}$)	Subcutaneous Triglyceride (μg/cell)	Parametrial Diameter (μ)	Parametrial Volume ($\bar{\mu}^3 \times 10^{-5}$)	Parametrial Triglyceride (μg/cell)	Epididymal Diameter (μ)	Epididymal Volume ($\bar{\mu}^3 \times 10^{-5}$)	Epididymal Triglyceride (μg/cell)
Genetic obese										
1	695	119.0 ± 2.9	9.6	0.72				124.0 ± 3.6	11.2	0.84
2	665	129.0 ± 2.9	12.0	0.90				145.6 ± 3.9	18.0	1.35
3	810	117.0 ± 2.5	8.9	0.67	130.8 ± 3.6	12.8	0.96			
4	595	117.0 ± 2.5	8.9	0.67	130.8 ± 3.6	12.8	0.96			
Hypothalamic obese										
1	492	107.0 ± 1.8	6.6	0.49	130.8 ± 2.2	12.6	0.95			
2	545	115.4 ± 3.4	8.6	0.64	139.2 ± 2.7	15.1	1.13			
3	535	129.0 ± 2.4	12.3	0.92	146.0 ± 2.7	17.0	1.27			
4	635	117.0 ± 2.2	8.8	0.66	139.2 ± 3.2	15.3	1.15			
Normal										
1	301				90.0 ± 1.7	4.0	0.30			
2	302				95.2 ± 1.9	4.8	0.36			
3	382							79.8 ± 1.7	2.8	0.21
4	407							79.8 ± 1.7	2.8	0.21

TABLE II. Triglyceride Content of Pieces of Fat and Aliquots of Fat Cells from Obese and Lean Rats.

Animals and site	Triglyceride (%)		
	Fat cells[a]	Pieces of fat[a]	p
Genetic obese			
Subcutaneous	62.6	84.7 ± 1.7	
Parametrial	65.5	87.3 ± 1.9	
Hypothalamic obese			<.05
Subcutaneous	65.9 ± 2.2	78.1 ± 2.2	
Parametrial	68.2 ± 1.6	87.9 ± 0.7	
Normal			
Parametrial Epididymal	64.6 ± 0.6	85.7 ± 0.6	

[a] Mean ± SEM for duplicate determinations on samples from each of four rats.

= cell volume × 0.82 × .915 (%T.G.× Density of tripalmitin).

Results. The diameter and thus the volume of parametrial and epididymal fat cells from the obese rats were significantly greater than the same parameter in fat cells from thin rats (Table I). Comparison of the fat cells from different depots in the same obese rat showed that the subcutaneous fat cells were smaller than the corresponding intra-abdominal fat cells.

The triglyceride content of pieces of fat from various sites did not differ except for the samples of subcutaneous fat from the animals with ventromedial lesions in which the percentage of triglyceride was significantly reduced (Table II). Weighed samples of fat cells contained approximately 65% triglyceride. Pieces of fat had more DNA than the corresponding fat cells whether expressed per gram of triglyceride or per fat cell (Table III). Moreover the DNA per fat cell was higher in fat cells from the obese animals than it was in fat cells from thin rats. The fatty acid composition of the subcutaneous biopsies from four genetically obese rats showed no significant differences in the frequency of any of the fatty acids (Table IV).

Discussion. An increase in the quantity of stored fat could occur by either one or a combination of two mechanisms; the number of adipocytes could increase; or the size of individual fat cells could enlarge. The studies of Hausberger(8), Salans, Knittle and

TABLE IV. Fatty Acid Composition of Subcutaneous Adipose Tissue from Obese Rats.

Fatty acid	Obese[a]	
	Genetic	Hypothalamic
14:0	1.3 ± 0.2	1.6 ± 0.3
16:0	25.5 ± 2.4	25.2 ± 1.4
16:1	11.0 ± 0.7	9.4 ± 0.9
18:0	1.7 ± 0.4	2.0 ± 0.3
18:1	43.6 ± 3.1	42.8 ± 1.1
18:2	16.6 ± 2.0	18.4 ± 0.7
18:3	0.8 ± 0.3	0.5 ± 0.9

[a] Mean ± SEM for four rats.

TABLE III. DNA Content of Fat Cells and Pieces of Fat from Obese and Lean Rats.

Animals and site	DNA content			
	Fat cells		Pieces of fat	
	(μg/g triglyc.)	(pg/cell)	(μg/g triglyc.)	(pg/cell)
Genetic obese				
Subcutaneous	134 ± 24	103	186 ± 29	142
Parametrial	124 ± 20	128	161 ± 38	163
Hypothalamic obese				
Subcutaneous	158 ± 20	121	225 ± 18	165
Parametrial	88 ± 30	100	143 ± 10	160
Normal				
Parametrial	61 ± 15	16.4	192 ± 26	53.5

Hirsch (9) and Bjurlf (10) have all suggested that the fat cells of obese subjects (man and animals) are larger in size and are increased in number. Our data on the genetically obese rat are in harmony with this conclusion. However, this enlargement is probably not the result of the genetic abnormality since a similar degree of hyperplasia and hypertrophy occurred in rats which became obese following the introduction of bilateral hypothalamic lesions. We would therefore conclude that in the fat rat, the increase in number and size of fat cells is secondary to a need to store more triglyceride.

Our results on fat cell size and composition compared favorably with that of other investigators (6, 7). Goldrick reports the diameter of epididymal cells in rats weighing 365–403 g to be between 82.9 and 83.4 μ (Table II, Ref. 7) which is in close agreement with our data (Table I). Similarly the content of triglyceride measured in pieces of fat from our rats is in keeping with his observations. The content of DNA, however, is higher and may reflect more complete recovery of DNA in our method.

The fatty acid composition of subcutaneous adipose tissue was similar in the two types of obese rats implying that the genetic defect causing obesity is not manifested in abnormal fatty acid content of subcutaneous adipose tissue. Comparison of our data on obese rats with data on epididymal adipose tissue from normal rats (11) shows a significantly higher content of linoleic acid (18:2) and a reduced quantity of oleic acid (18:1) in the subcutaneous adipose tissue of obese rats (Table IV). These differences might be due entirely to diet.

Summary. The size of fat cells and the composition of adipose tissue has been measured in normal and genetically obese (fatty) rats and in thin rats made fat with hypothalamic lesions. The fat cells of the obese rats were 50% larger in diameter than fat cells from normal rats. Subcutaneous fat cells in the obese rats were significantly smaller than the corresponding parametrial or epididymal fat cells. DNA content was higher in pieces of fat than in fat cells, and the content of DNA in cells from obese animals was higher than in the cells of normal animals. The fatty acid composition of the subcutaneous fat in the two types of obese rats was identical.

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