Glycerokinase activity in human brown adipose tissue

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Abstract Brown adipose tissue (BAT) is known to be responsible for heat production in newborn and adult hibernating mammals. In rats and mice, BAT has been demonstrated to possess a much higher glycerokinase activity than white adipose tissue (WAT). It has been speculated that this high activity may cause the futile cycle of triglyceride breakdown and resynthesis to be activated, thus contributing to heat production. However, at present very little information is available regarding the location, function, and quantitative importance of BAT in adult human subjects. Our objective in this study was to locate BAT in human subjects and to characterize it biochemically, especially with respect to the enzyme glycerokinase. We have looked for histologically identifiable BAT in 32 human subjects and found it in 12 subjects. Most of the BAT samples were obtained from perirenal adipose depots in children undergoing surgery. Some of the samples were almost totally comprised of BAT cells, whereas others were a mixture of BAT cells and WAT cells. The glycerokinase activity per gram of tissue was higher in BAT than in WAT in all the subjects where the above comparison was made. The activity per mg protein or per μg DNA was higher in most BAT samples. In one pure BAT specimen, the basal lipolytic rate and the lipoprotein lipase activity were measured and they were both higher in BAT than in the WAT obtained from the same patient. These results show that human brown adipose tissue possesses an enzymatic profile very similar to that of rodent brown adipose tissue.— **Chakrabarty, K., B. Chaudhuri, and H. Jeffay.** Glycerokinase activity in human brown adipose tissue. *J. Lipid Res.* 1983. **24:** 381–390.

Supplementary key words brown adipose tissue • white adipose tissue • human • glycerokinase • lipoprotein lipase • basal lipolytic rate • hibernoma

It has been known for at least two decades that brown adipose tissue (BAT) serves a thermogenic function. BAT, found abundantly at various sites (1) in newborn mammals including man, helps the neonates maintain body temperature without shivering. However, after birth, BAT gradually disappears from these sites or changes into tissue histologically indistinguishable from white adipose tissue (WAT). It was presumed that it also loses its thermogenic capacity in adult nonhibernating mammals. Recent evidence appears to indicate that man does have some residual BAT at certain sites (2–4) and that it may indeed have a physiological function of burning off excess food energy intake in the form of heat (5). Although a great number of experiments have been carried out in animal BAT, no significant information is available at present about the enzymological profile of human BAT or its thermogenic function.

Various laboratories including ours have reported significantly higher glycerokinase activity in rat BAT, compared to WAT (6–8). The presence of glycerokinase in BAT suggests the possibility of a futile cycle of triglyceride hydrolysis and reesterification of glycerol contributing to thermogenesis (9). In the present study, we have looked for BAT in a number of human subjects at various anatomical locations. In most of our adult subjects, we were unable to find an appreciable amount of BAT. However, in 12 samples, mostly from children, histologically identifiable BAT was found. In most of these specimens, BAT was demonstrated to have a higher capacity of converting glycerol to α-glycerophosphate relative to WAT. The significance of this observation is discussed.

METHODS AND MATERIALS

Source of adipose tissue

The clinical details about the human subjects who served as donors of the BAT specimens used in this study are listed in **Table 1.** The conditions of removal and tentative identification of BAT varied from specimen to specimen. One almost pure BAT sample was removed during the autopsy of a 6-month-old baby (subject #3). It was a small dark brown lobulated mass, distinct from the surrounding white adipose tissue in the axilla of the infant and could be easily identified as BAT. All other specimens were obtained during surgery. The brown fat tumor from a 60-year-old woman (subject #1) was also distinct, large, relatively nonvascular, creamy

Abbreviations: BAT, brown adipose tissue; WAT, white adipose tissue.

TABLE 1. Clinical data on subjects

Subject	Age	Sex	Medical History	Circumstances of Tissue Procurement	Description of Brown Adipose Tissue Obtained[a]
1	60 yr	F	Obese (183% IBW), diet-controlled diabetes, hypertension	Surgery, excisional biopsy of hibernoma from posterior axillary area; subcutaneous WAT from same area	95% Type I, 5% Type II
2	14 yr	M	Obese (134% IBW), systemic hypertension, pheochromocytoma	Surgery, adrenalectomy, removal of benign adrenal tumor; Med.: phenoxybenzamine; perirenal BAT, subcutaneous WAT	95% Type I, 5% Type II
3	6 mo	M	Acute bilateral bronchopneumonia; cause of death unknown	Autopsy; axillary BAT, WAT not obtained	100% Type I
4	7 mo	F	Multiple genetic abnormalities, tracheoesophageal fistulae, choanal atresia, seizure disorder	Surgery, Nissen fundoplication; Med.: Dilantin, phenobarbital; omental BAT, subcutaneous WAT	5% Type I, 90% Type II
5	2 yr	M	Cleft palate, cardiac problems	Surgery, colostomy closure; omental BAT, no WAT	20–30% Type I
6	8 days	F	Congenital ureteropelvic junction obstruction	Surgery, rt. pyloplasty; perirenal BAT, abdominal subcutaneous WAT	20–25% Type I
7	8 days	M	Hepatic hemangioma, anemia, heart murmur	Surgery, hepatic resection; perirenal BAT, abdominal subcutaneous WAT	20–25% Type I
8	17 yr	F	Healthy; death by gunshot wound	Surgery while on respirator, kidney donor; perirenal BAT, perirenal WAT	15% Type I, 5% Type II
9	5 yr	F	Sickle cell disease	Surgery, elective splenectomy; perirenal BAT, abdominal subcutaneous WAT	5–10% Type I
10	18 yr	M	Healthy, obese (135% IBW); death by gunshot wound	Surgery while on respirator, kidney donor; perirenal BAT, perirenal WAT	10% Type I, 5% Type II
11	14 yr	M	Obese (136% IBW); cause of death brain tumor	Surgery while on respirator, kidney donor; perirenal BAT, abdominal subcutaneous WAT	5% Type I, 5% Type II
12	5 yr	M	Prune-belly syndrome	Exploratory uretral surgery; perirenal BAT, abdominal subcutaneous WAT	100% Type II

[a] The remainder of the fat cells in each tissue were of Type IIIb or Type IIIa (WAT cells).

beige in color, and had the texture and feel of liver rather than that of adipose tissue. In subject #2, a 14-year-old boy with an adrenal tumor, the adipose tissue surrounding the adrenal gland was dark brown and highly vascular, as evidenced by excessive bleeding during removal of the tumor during surgery. Whenever a small BAT specimen (10–100 mg) was removed directly during surgery, the characteristic dark coloration, vascularity, and lobulated appearance was used as a guide for selection of the tissue, and almost always, bleeding interfered with its detection. In individuals who had suffered brain-death (subjects #8 and #10), all the perirenal fat was removed along with the kidney during the surgery. As the kidney was being prepared for transplantation and all the fat surrounding the kidneys was being dissected out after its removal from the patient, it was noted that approximately 95% of the total perirenal fat was quite clearly white. Small dark areas that had more blood vessels and connective tissue than the surrounding WAT were selected for histological determination and simultaneous glycerokinase assay. It was relatively easier to identify BAT in these cases than to do so in situ.

Whenever possible, an adjacent WAT sample was removed and assayed for glycerokinase activity for comparative purposes. The tissue, after removal from axillary or perirenal areas, was transported to the laboratory on ice and the enzyme assay was immediately performed.

Adipose tissue from other areas such as around the esophagus, adjoining the mammary vessels, and around the ureter, removed during various types of surgery in adults as well as children, were also assayed. These areas were selected for investigation because of reported oc-

currence of BAT by other investigators. However, these specimens were indistinguishable from WAT visually, enzymologically, or histologically.

Histological appearance was the ultimate criterion used to designate a certain specimen as BAT. A small portion of each tissue assayed was fixed in 10% formalin in phosphate buffer, pH 7.4, processed, and embedded in paraffin sections. These were cut at 5–7 μm and stained with hematoxylin and eosin.

In some cases, the tissue was minced to 1 mm^3 and fixed in glutaraldehyde. This was post-fixed in osmium tetroxide, dehydrated, and embedded in Araldite. Thick 1-micron sections were examined to select the appropriate area and then thin sections were cut and examined under an H300 electron microscope.

The percentage of BAT cells in each specimen was assessed, in a blind fashion, where the histologist (B.C.) was unaware of the nature of the specimen. All the paraffin sections stained by hematoxylin and eosin were classified into three major types as follows.

Type I or pure BAT cells

These consist of cells with eosinophilic granular cytoplasm and oval nuclei. The cell borders are distinct. Electron micrographs of these cells exhibit lipid vacuoles, lysozomes, lipofuschin granules, pinocytic vesicles, and mitochondria. The characteristic association of these mitochondria to the lipid droplets is noted in Fig. 1B. The mitochondria are tubular in type. The lipofuschin granules have prominent granular and lipid components surrounded by a delineating membrane.

Type II or multilocular cells

These cells are intermediate in type between BAT and WAT. They have multiple tiny vacuoles interspersed in the eosinophilic cytoplasm giving a cobweb appearance. The nucleus is still oval to spindle and may be central or located at the periphery. The cell borders are distinct. The number of lipid vacuoles vary from few to numerous. Type II cells are encountered scattered between the eosinophilic granular Type I cells. They may form an island by themselves in a background of unilocular cells as in Fig. 3A and give a patchy appearance. These cells may also be very fine and scattered in white adipose tissue.

Type III or WAT cells

These are mature adipose cells. They consist of cells where the vacuoles coalesce to form a single vacuole. This single vacuole pushes the nucleus to one side giving the appearance of a signet ring. This type, in our observations, could be further classified into two types, Type IIIa and Type IIIb. In IIIa, the cells are smaller in size, although they have the same basic feature. We found this type of cell in perirenal depots in younger subjects and in axillary sites of some adults. In type IIIb, the cells are large and are typically found in most adult WAT depots.

This study was approved by the Human Subjects Review Committee of the University of Illinois Medical Center.

Assay of glycerokinase

BAT was homogenized in 5 vols (w/v) of 50 mM Tris–HCl buffer, pH 7.4, in a glass-glass homgenizer, and the homogenate was assayed according to a modification of the procedure by Newsholme, Robinson, and Taylor (10). We used a higher concentration of glycerol (167 mM) in our assay for optimal activity, compared to 0.3 mM used by the above authors. The homogenate (50 μl) was added to plastic microcentrifuge tubes (capacity, 0.4 ml) containing 25 μl of freshly prepared incubation media. The final composition of the reaction mixture, after the addition of the homogenate, was as follows: [1,3-^{14}C]glycerol (New England Nuclear, Boston, MA) 167 mM (0.6 μCi per assay, sp act, 0.05 mCi/mmol); Tris, pH 7.4, 67 mM; 2-mercaptoethanol, 17 mM; NaF, 17 mM; $MgCl_2$, 3.3 mM; EDTA, 0.67 mM, and ATP, 4 mM. The tubes were incubated for 60 min at 37°C and the reaction was stopped by adding 50 μl of ethanol. All assays were done in triplicate and the blank values were obtained by adding ethanol before adding the homogenate. After stopping the reaction, and centrifuging at 10,000 g for 10 min, an aliquot (10 μl) of the supernatant was spotted on the center of DEAE-cellulose ion-exchange paper (Whatman DE-81, diameter 2.3 cm). The papers were placed on a Pyrex Hydrosol microanalysis paper-holder (Millipore Corp, Bedford, MA) and washed with 500 ml of water to remove all the unbound glycerol. The dried papers containing the labeled product α-glycerophosphate, were counted in a liquid scintillation counter in 10 ml of scintillation fluid (3 g PPO, and 0.1 g POPOP/l toluene). The results were expressed as either μmol of glycerol phosphate formed per hr per g tissue, or per mg protein, or per μg DNA.

The DNA content of the adipose tissue homogenate was determined as previously described (11). Briefly, DNA was extracted with 5% trichloracetic acid from the acetone-ether powder of the adipose tissue homogenate and color was developed with 0.08% indole–4 N HCL 1:1 reagent at 100°C. The precipitate remaining after DNA-extraction was used to determine protein content according to Lowry et al. (12). In some cases, the specimen was too small to determine DNA or protein directly from the homogenate. In such cases, the protein residue, precipitated after addition of alcohol to stop the enzymatic reaction, was redissolved in alkali

and the protein content was determined as above. DNA content could not be determined in these cases.

Lipolysis

The endogenous lipolytic activity of one BAT specimen (Case #1, hibernoma) was measured using standard techniques. Approximately 100 mg of BAT tissue was preincubated for 30 min in 1 ml of Krebs Ringer bicarbonate buffer (pH 7.4) containing 40 mg of bovine serum albumin per ml at 37°C. After removing the medium, the tissue was reincubated with 1.0 ml of the above medium for 3 hr at 37°C in a Dubnoff Metabolic Shaker. The free fatty acids were analyzed (13) in the medium and in the chloroform–methanol 2:1 extract of the adipose tissue. Concentration of glycerol was measured in the medium using a radioenzymic assay of Newsholme and Taylor (14). The results were calculated as the free fatty acids and glycerol released in the medium per hr per g of tissue and as free fatty acids remaining in the tissue and compared with the same values for WAT.

Lipoprotein lipase

The lipoprotein lipase activity was measured from defatted acetone-ether powders of BAT (Case #1) and WAT using the method of Nilsson-Ehle, Tornqvist, and Belfrage (15). Human serum-activated [^{3}H]triolein (Amersham) was used as substrate and the radioactivity in the released tritiated oleic acid was measured.

RESULTS

Anatomic sites and histological description of BAT-enriched adipose tissue samples

We removed 32 adipose samples from various anatomic sites around esophagus, mammary glands, ureter, axillae, and kidneys. Of these, 24 samples, mainly from the perirenal and axillary sites, resembled BAT visually. However, only 12 of them were histologically identifiable as BAT cells. Table 1 lists clinical details of the twelve subjects and the anatomical location of the specimens. Most of the histologically confirmed BAT samples were from the perirenal fat of children undergoing surgery.

The other 12 specimens, which grossly resembled BAT, had no multilocular cells (Type I or Type II). They were obtained from perirenal (seven subjects; five adults and two children) and axillary adipose tissue (five subjects; all adults). Their brown color was apparently derived from an overabundance of blood vessels or the presence of scattered lymphocytes. Some specimens were totally comprised of smaller unilocular cells (Fig. 4A), which have been classified as brown fat cells by some authors (4, 16). Upon very close examination, we could not find any difference between these cells and WAT cells except for their small size. Therefore, we have classified them as WAT cells (Type IIIa in Methods) rather than BAT cells. However, we noted that their presence could be detected only at the sites where BAT usually occurs. All of the 12 specimens had glycerokinase activity (per mg protein or per μg DNA) similar to that in the adjacent WAT, thus confirming the fact that negligible BAT was present in these samples. A large percentage of the adult subjects (80%) and the two child subjects in which no BAT was found were obese. The typical mature WAT cells (classification Type IIIb) present at most adipose sites are seen in Fig. 4B.

In Table 1, all the cases with brown fat are classified according to the type and percentage of each type present. **Fig. 1A** shows a section of an axillary hibernoma (brown fat tumor) from a diabetic obese woman (Case #1). Its gross appearance was that of a well-encapsulated tumor and the cut surface was homogenous, lobulated, and light brown in color. Its histological appearance (17, 18) resembled pure BAT with lobules composed predominantly of large polygonal cells with eosinophilic granular cytoplasm (mainly Type I cells). Scattered in between these Type I cells, which had distinct cell borders and oval nuclei, were a few multiloculated or Type II cells. These Type II cells also had uniform oval nuclei, and a thin delicate membrane divided cells into multiple locules. The locules were represented by vacuoles in histological section because processing in alcohol and xylene dissolved the lipid.

Electron micrographs of the same hibernoma exhibited oval nuclei with smooth nuclear membranes. Nucleopores were unremarkable, but the cell membranes had pinocytic vesicles, which are not commonly seen in other types of cells. The cytoplasm was filled with numerous lipid droplets and mitochondria. The most important feature of BAT cells is, however, the close association of the mitochondria with the lipid vacuole, as seen in Fig. 1B. The mitochondria were tubular in shape and showed characteristic prominent cristae.

Fig. 2 shows a section from the periadrenal adipose tissue in a 14-year-old obese patient with pheochromocytoma. BAT in association with this tumor has been noted before (4). Grossly, this BAT appeared finely lobulated with color varying from grayish pink to dark brown and was richly supplied with blood vessels. They were almost all type I cells and essentially represented pure BAT, histologically resembling the cells in the hibernoma.

Fig. 3 shows a section of the BAT specimen from the perirenal region of a 17-year-old healthy girl, who had

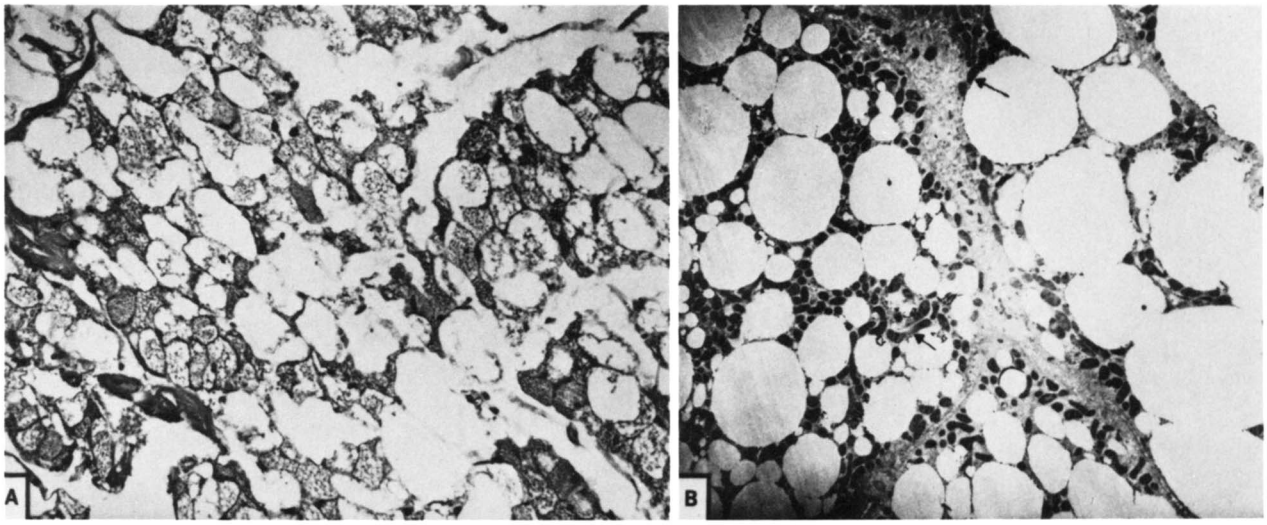

Fig. 1. Hibernoma of axilla. A. This photomicrograph shows portions of two adjacent lobules separated by a septum consisting of fibrous connective tissue. Each lobule is composed of several large eosinophilic cells with granular cytoplasm (H and E, ×110). B. Electron micrograph of the same tumor exhibits the peculiar close association of the lipid droplet with the tubular mitochondria (see arrows). This picture illustrates parts of cytoplasm of two adjacent cells with intervening cell membrane (×7100).

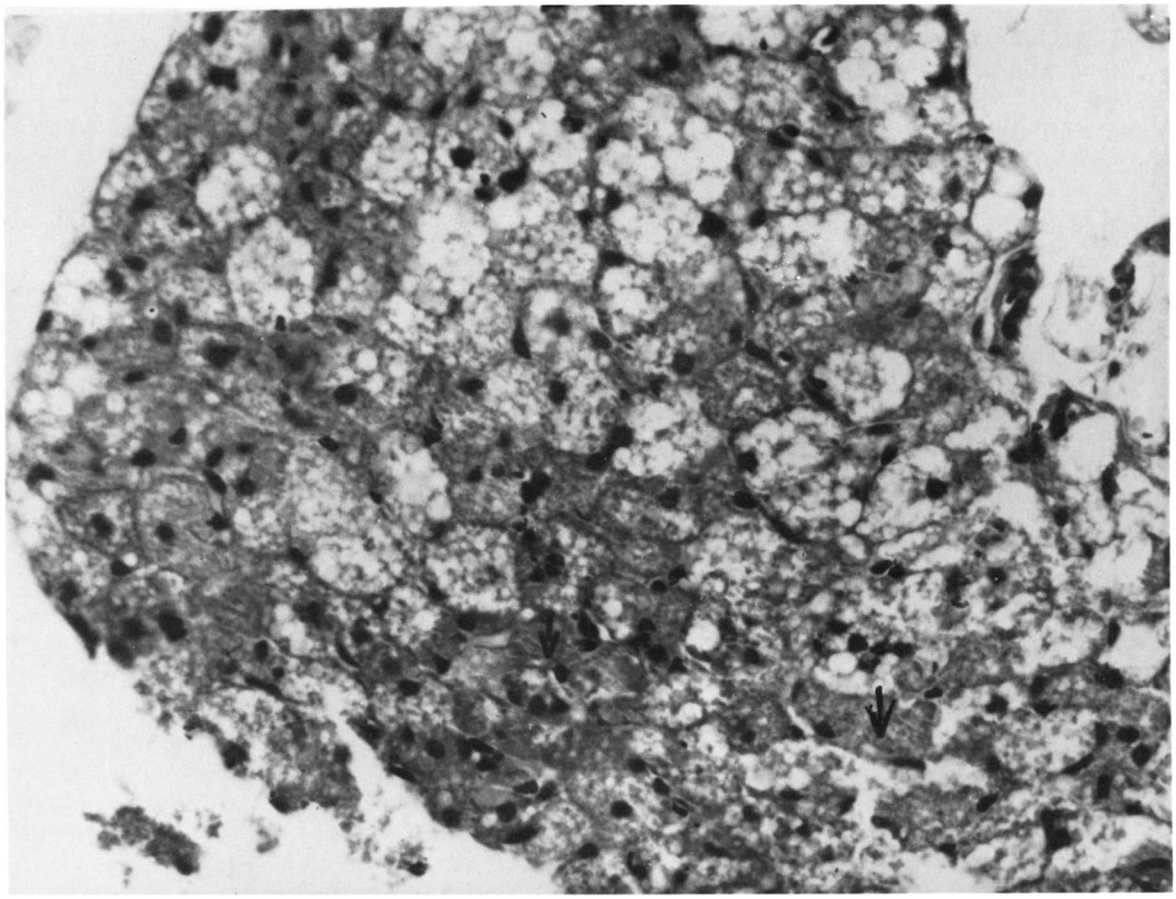

Fig. 2. Typical BAT (Type I) cells. This section from the adrenal region of a 14-year-old boy shows numerous large cells with eosinophilic granular cytoplasm. One such cell is indicated by an arrow. (H and E, ×275).

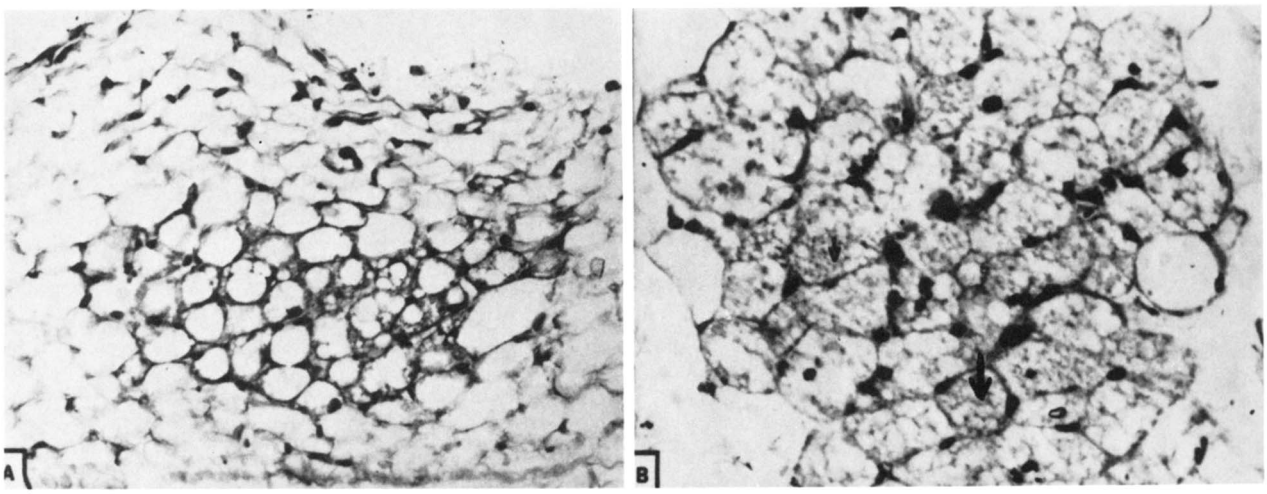

Fig. 3. Multilocular (Type II) cells. A. This photomicrograph from the perirenal region of a 17-year-old girl shows an island of multilocular cells in a sea of unilocular cells (H and E, ×110). B. Higher magnification of the Type II cells showing multiple lipid droplets within each cell. A typical Type II cell is indicated by arrow. (H and E, ×275).

suffered a gunshot wound and was kept alive on a respirator until her kidneys were removed for a transplant operation. There were multiple small foci of BAT with a mixed population of Type I and Type II cells. Often only Type II or multilocular cells appeared as an island in a sea of unilocular cells (Fig. 3A). Although brownish or tan adipose tissue was selected from the entire perirenal fat for histological study, unilocular cells (Type III) by far predominated the BAT cells (Table I) even in this selected sample. Fig. 3B shows the island of multilocular cells in greater detail.

Fig. 4 shows histological sections of typical WAT cells. The gross appearance of WAT is quite different from that of BAT. It is usually pale yellow in color, feels resilient to touch, but melts into oily droplets when rubbed between fingers. Fig. 4A shows smaller unilocular cells from the perirenal region of a 17-year-old girl. They have been seen in posterior triangle of infants, and in older children with hypothermia (16). According to Mrosovsky and Rowlatt (16), unilocular cell structure is normal for BAT just before birth and transformation to multilocular structure may begin before the baby has started to respire or encountered extrauterine environment. However, histologically, the cells have a large single vacuole and the nucleus is pushed to one side. Except for their smaller size, they appeared similar to typical WAT cells found in adults (Fig. 4B). We did not note more distinct cell borders in type III A cells as an indication of increased quantity of cytoplasm and therefore did not classify them as BAT cells.

Glycerokinase activity in WAT and BAT

Table 2 lists the DNA content and the glycerokinase activity of BAT obtained from subjects listed in Table 1. Whenever WAT was available from the same patient, the above parameters were also measured in WAT. The DNA content of BAT was consistently higher than the DNA content of the corresponding WAT, thus confirming the often observed fact that BAT cells are smaller than WAT cells. Specimens 1 and 2, which were almost all BAT, had higher DNA content and higher glycerokinase activity compared to WAT than the other samples that were mixtures of BAT and WAT. The sample from subject #12 was composed of small, Type II cells and had the highest DNA content but a low glycerokinase activity. In all human BAT samples tested, the glycerokinase activity per g tissue was higher than the corresponding activity in adjacent WAT samples. In some WAT samples, we could not detect any activity. However, it should be pointed out that since the concentration of [1,3-^{14}C]glycerol was high and the specific activity was low, our assay system was not highly sensitive.

When the enzyme activity was expressed per mg protein, eight samples had higher activity in BAT-enriched samples, when compared to pure WAT samples. In two samples, the enzyme activity was almost identical in WAT and BAT. The activity when expressed per μg DNA was higher in BAT in five specimens and lower in two specimens.

When we compare these human data with those in the rat, certain similarities can be noted. When we measured glycerokinase activity in adipose tissue of a group of rats (average weight, 220 g), we found average values of 3.0 μmol of glycerol converted per hr per g of epididymal WAT in contrast to 49.0 μmol glycerol converted per hr per g of subscapular BAT, under the same assay conditions. Hence the activity was 16-fold higher

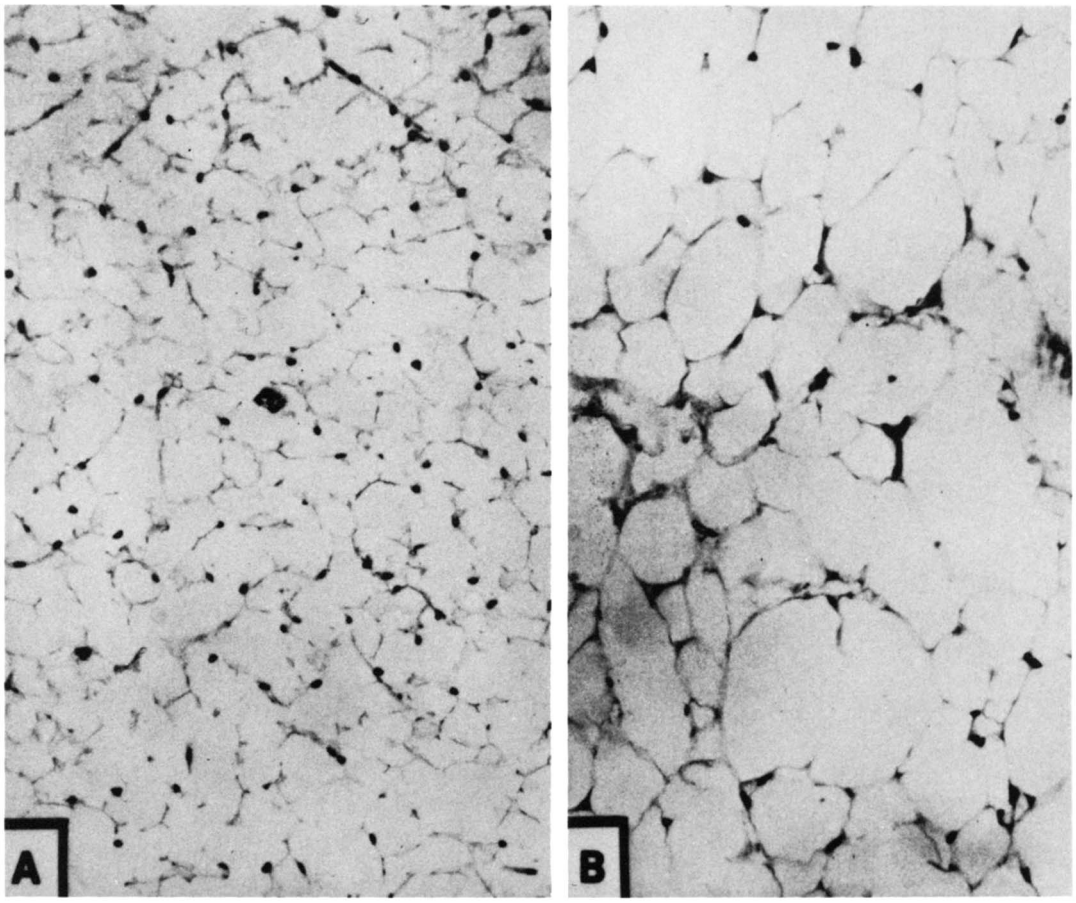

Fig. 4. WAT (Type III) cells. A. Photomicrograph of Type III A cells in the perirenal region of a 17-year-old girl. These are smaller unilocular cells with large single vacuole and nuclei pushed to one side. (H and E, ×110). B. Photomicrograph of Type III B cells in the axillary adipose tissue of a 57-year-old obese woman. Each cell is large, filled with fat, and has the typical signet-ring appearance of a mature adipocyte (H and E, ×110).

in BAT. Where the activity was expressed per mg protein, the corresponding values were 0.51 μmol per hr in WAT and 0.83 μmoles per hr in BAT. When μg of DNA was used as baseline, the values were 0.038 μmol per hr in WAT and 0.088 μmol per hr in BAT. The BAT/WAT activity ratio is highest when expressed per

TABLE 2. Activity of glycerokinase in brown and white adipose tissue (BAT and WAT) in humans

	Tissue Composition μg DNA/g Tissue		Glycerokinase Activity μmol Glycerol Converted/hr					
			per g Tissue		per mg Protein		per μg DNA	
Subject	WAT	BAT	WAT	BAT	WAT	BAT	WAT	BAT
1	84	266	0.182	4.00	0.034	0.250	0.002	0.015
2	58	388	0.015	4.39	0.038	0.360	0.003	0.011
3		62		10.25		1.55		0.170
4	13.6		4.59	25.09	0.685	0.720		
5				16.00		1.05		
6			4.30	9.40	0.203	0.388		
7			0.000	3.057	0.000	0.078	0.000	
8	10.8	34	0.540	2.960	0.135	0.740	0.050	0.088
9	7.2	69	0.000	0.154	0.000	0.159	0.000	0.012
10	8.6	38	4.200	9.160	1.400	1.720	0.488	0.242
11	16	45	1.291	5.880	0.688	0.954	0.082	0.129
12	110	485	0.789	1.815	0.150	0.140	0.007	0.004

TABLE 3. Lipolytic activity and lipoprotein lipase activity in human white and brown adipose tissue

	Lipolysis under Basal Conditions			Lipoprotein Lipase
	FFA in Medium	FFA in Tissue	Glycerol in Medium	FFA
White adipose tissue (subcutaneous tissue)	0.700[a]	0.316	<0.02	0.198
Brown adipose tissue (hibernoma)	2.170	6.870	0.670	0.700

[a] All values are expressed as μmol liberated per g adipose tissue per hr. The subject (#1) is described in Table 1.

g of tissue. This profile approximately fits human BAT specimens. There was great variation in enzyme activity in the human BAT specimens. Some of the most active human BAT specimens (cases #4 and #5) were almost as active as the rat subscapular BAT.

The DNA content of rat subscapular BAT (571 μg DNA/g tissue) was also 6-times higher than that of epididymal WAT (87 μg DNA/g tissue). In our human subjects, this ratio varied from 3- to 9-fold. Of course, some of the specimens were mixtures of WAT and BAT cells, leading to this variation. Hence it appears that at least in some human subjects, brown adipose tissue is similar to rat brown adipose tissue, with respect to its glycerokinase activity and DNA-content. Although our assay conditions use a high concentration of glycerol, we have tested the enzyme activity at various concentrations of glycerol and the activity in BAT (from hibernoma) was always several-fold higher than in adjacent WAT under substrate concentrations, ranging from 0.3 mM to 167 mM.

Other enzymes in hibernoma

Specimen #1, the BAT hibernoma, was investigated more thoroughly than the other specimens, because of its larger size. The lipolytic activity and the lipoprotein lipase activity of BAT from the hibernoma specimen are depicted in **Table 3.** In both cases the BAT was more active than the WAT. In spite of the high glycerokinase activity, a considerable quantity of glycerol was released in the medium by BAT, indicating that all the glycerol released was not recycled. On the other hand, in WAT, no detectable glycerol was found in the medium. Either the lipolytic activity of this WAT specimen was very low or the low glycerokinase activity of the WAT, though minimal, was enough to convert most of the released glycerol into α-glycerophosphate.

DISCUSSION

There appears to be a definite link between the glycerokinase activity of BAT and its function, since the enzyme activity rises whenever BAT hypertrophies, e.g., during cold adaptation (19) or hyperinsulinemia (8, 20, 21). Our objective in undertaking this project was to locate human BAT and to investigate its glycerokinase potential. To our knowledge, significant enzymatic data regarding human BAT are not available at present.

The results show that in most cases studied, human BAT did have a higher glycerokinase activity than adjacent WAT. There was a great deal of variation from one subject to another, which is to be expected since the subjects had widely variant genetic and medical histories. Some of the children had multiple congenital abnormalities. Also, the "purity" of the BAT samples was difficult to define, since different types of cells were present. The glycerokinase activity expressed per μg of DNA appeared to be most meaningful because this value for WAT showed least variation in an individual when WAT was obtained from different sites, or within a population of different individuals. We were unable to measure cell numbers because of the extremely small quantities of tissue available. In two cases, we did observe a lower BAT glycerokinase activity per μg of DNA than the adjacent WAT. We are unable to offer any explanation for these exceptions. One of these patients was obese (case #10). The other (case #12) had very low activity and his BAT was histologically quite different from the other specimens. It had no Type I cells. There were also some scattered lymphocytes in this BAT specimen contributing to its high DNA content and the consequent low enzyme activity per DNA. It should be noted that some other obese patients (cases #1 and #2) had normal BAT (mainly Type I cells), with high glycerokinase activity.

Some of the most active human BAT specimens were as active as rat BAT specimens. Granted that values in some humans were often low, the range of enzyme activities are comparable in humans and rodents. The ratios of the enzyme activity and DNA content in BAT with respect to WAT were also comparable. Hence the capacity of human adipose tissue to convert glycerol to

α-glycerophosphate may not be as negligible as it was previously supposed.

One of our BAT specimens had a high lipoprotein lipase activity compared to WAT. These observations confirm similar data obtained in rat BAT (22). Hemon, Ricquier, and Mory (23) have recently proposed that the simultaneous presence of high lipoprotein lipase activity and high glycerokinase activity of rat BAT in a thermogenetically active state such as cold exposure or suckling, may mean a preferential utilization of plasma triglycerides and the reutilization of glycerol liberated by lipoprotein lipase. The basal lipolytic rate of the human BAT hibernoma specimen was also found to be high (Table 3). Rat BAT cells also have a high lipolytic rate, indicating a high hormone-sensitive lipase activity (19). Since the glycerol release by BAT is high because of its high lipoprotein lipase and high hormone-sensitive lipase activity, there may be a good reason for the high glycerokinase activity of BAT. The simultaneous presence of all these enzymes would contribute to a high rate of lipid turnover and subsequent production of heat.

We looked for BAT in both chilren and adults. Heaton (2) had reported the presence of BAT in human adults at a variety of sites. She used autopsy material and was able to thoroughly explore a variety of fat pads. We found it rather difficult to locate BAT during surgery because of the presence of bleeding and limited possibility of exploring an area. Most of the sites in adults where other investigators (2–4) have reported the presence of BAT appeared to have at least 90% WAT. The tissue specimens that appeared brown often did not have histologically distinguishable BAT cells. They did have a large number of blood vessels and scattered lymphocytes in some cases. Rothwell and Stock (5) have suggested that obese subjects may be deficient in BAT and/or its activity. It is difficult to distinguish BAT in obese patients owing to the large number of WAT cells diluting the BAT cells. Hence, it is conceivable that the predominance of obesity in the limited number of adult subjects we studied may have been responsible for our inability to find BAT in our subjects. We should point out that we did not preferentially choose obese patients for our study. Coincidentally, most of the patients undergoing radical mastectomy (for axillary tissue) or nephrectomy (for perirenal tissue) whose adipose tissue was available to us happened to be obese. Since other authors have found BAT in adult subjects (2–4) we are still continuing our search for BAT in adults. This preliminary study indicates that the total amount of BAT present in an adult human is rather small, and it is difficult to conceive that a deficiency in BAT plays a very major role in the development of obesity in adults.

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