notes on methodology

New device for preparing thin slices of adipose tissue for metabolic studies in vitro

J.-D. Faulhaber, H.-U. Klor, and H. Ditschuneit

University of Ulm, Center of Medicine and Pediatrics, Division of Metabolism and Nutrition, 79 Ulm, Germany

Summary A new microtome is described which allows the rapid preparation of equal slices of well-defined thickness of fresh human tissue, especially adipose tissue. Presetting the microtome for a section thickness of 500 μm, we found a variation of about 5% with human adipose tissue. Slices of human adipose tissue sliced by the microtome showed a higher sensitivity to insulin and a better reproducibility of results than slices prepared freehand.

Supplementary key words fresh-tissue microtome · human adipose tissue · lipogenesis · lipolysis

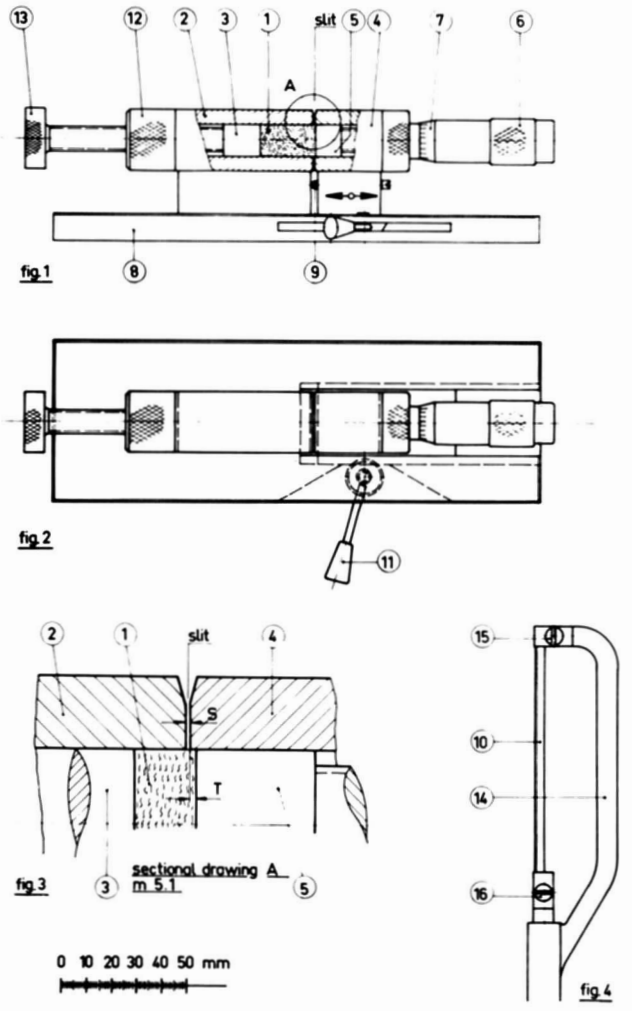
In vitro investigations of the metabolism of adipose tissue have been done mainly with the epididymal fat pad of the rat (1). This tissue is often used because it is easily accessible, and its shape, consisting of several tips of adipose tissue at the epididymis, enables the preparation of approximately identical pieces without further slicing. In some respects, results of in vitro investigations on human adipose tissue are hardly comparable to those obtained with rat adipose tissue (2–8). The difficulties in the experimental use of human adipose tissue are due to the considerable variations that are found even under the most exacting experimental conditions. A major factor leading to such variations is the difficulty of preparing slices of uniform thickness. Most investigators use scissors or a razor blade for freehand slicing of the subcutaneous or peritoneal fat obtained during a laparotomy (4, 8–12).

Using the procedure of freehand slicing, tissue contusion and laceration are nearly inevitable. From our own experience, the tissue cylinders obtained by fat biopsy (13) are altered in the same way. Even the preparation of very homogeneous fat cell suspensions isolated from human adipose tissue according to the method of Rodbell (14) does not lead to more consistent results as it does with rat adipose tissue. Gries and Steinke (8) were able to prove that insulin has a greater effect on human adipose tissue slices than on isolated fat cells. Moreover, human fat cells tend to rupture after isolation from the tissue, as seen by the appearance of confluent fat droplets at the surface of the incubation medium (8, 15).

The fresh-tissue microtome of Stadie and Riggs (16) is not commonly used for slicing adipose tissue. Adipose tissue, which consists of liquid lipids and tough connective tissue, requires a special cutting technique. We have therefore developed a fresh-tissue microtome which allows the rapid preparation of equal slices of well-defined thickness from adipose or other soft tissues.

Construction and mode of action. The construction and mode of action of the microtome can be seen in Figs. 1–5. The piece of tissue (*1*) is placed into the cylinder (*2*) and pushed forward by the piston (*3*) into the countercylinder (*4*). The optimal size of a piece of adipose tissue obtained by surgery is 10 × 10 × 30 mm. The thickness of the slice (*T*) is determined by the distance of the counterpiston (*5*) from the slit (*S*) between cylinders *2* and *4* (see detail *A*, Fig. 3). The micrometer screw (*6*) serves to adjust the counterpiston (*5*). This adjustment can be done with an accuracy of 10 μm. At each rotation of the micrometer screw the counterpiston moves 100 μm, read off on the scale (*7*). In the closed position (as opposed to the open position necessary to remove the finished tissue slice and shown in all figures), i.e., when cylinder *2* has been brought to countercylinder *4*, the slit serves to guide the razor band (*10*) during the cutting procedure. The width of the slit is regulated by screw *9*. Cylinder *2* is fixed but countercylinder *4* is movable on a sliding carriage that can be locked by an eccentric disc turned by lever *11*. By unscrewing the cap (*12*), piston *3* is removed from cylinder *2* in order to put the adipose tissue in place, after which the tissue is pushed forward with a forceps to the counterpiston *5*. Piston *3* is replaced, and by turning spindle *13* it presses the tissue into the countercylinder. The air trapped between the tissue and the counterpiston escapes via the slit. Since the adipose tissue is not compressible and is totally enclosed by the cylinders, the resistance while turning screw *13* for moving the tissue increases rapidly after air has been pressed out of the cylinders. This indicates the optimal pressure for slicing. Tissue damage has never been observed. The adipose tissue now fills the total volume of the two cylinders, and a tissue slice of well-defined thickness can be cut by a to-and-fro motion of the razor band in the slit. Since countercylinder *4* can be drawn back from cylinder *2* after unlocking with lever *11*, the tissue slice can be easily removed with a forceps or a wire loop. Now cylinder *4* is brought back close to cylinder *2* and is refixed; by turning the spindle (*13*), the piston (*3*) pushes more tissue forward into countercylinder *4*, thus preparing a section for the next slice.

At first, a razor blade was used for cutting the slices. Since the bores in the razor blade lacerate the tissue dur-



FIGS. 1–4. Fresh-tissue microtome and cutting instrument. Fig. 1, side view, partially sectional; Fig. 2, plan view; Fig. 3, enlargement of area *A* (5:1) from Fig 1. *1*, tissue; *2*, cylinder; *3*, piston; *4*, countercylinder; *5*, counterpiston; *6*, micrometer screw; *7*, scale; *8*, baseboard; *9*, screw for adjusting width of slit; *11*, lever for locking countercylinder in position; *12*, cylinder cap; *13*, spindle for moving piston *3*. Fig. 4, cutting instrument. *10*, razor band fixed by screws *15* and *16* into frame *14*; *17*, screw for adjusting tension of razor band.

ing the to-and-fro motion, we now use a razor band.[1] It is fixed in the frame (*14*) by screws *15* and *16*. The tension of the razor band is adjusted by screw *17* (Fig. 4). The cylinder and the countercylinder were made from Plexiglas; all other parts were made from stainless steel.

Results. Table 1 shows the weight, the surface area, and the thickness of 10 consecutive slices. The microtome was set for a thickness of 500 μm. Every slice was weighed immediately after cutting. In order to determine the surface area of the slice, the surface of counterpiston *5* was covered with graph paper (1 mm²/square). After slicing, the surface area of the tissue was calculated by subtracting the amount of uncovered squares from the total area, which was 123 mm². Assuming the specific weight of adipose tissue to be 1.0 g/cm³, we calculated the thickness of the slice according to the equation: thickness = volume (mm³)/surface area (mm²).

In studying the metabolic characteristics of human adipose tissue the best results were obtained with a slice thickness of at least 500 μm. As shown in Table 2, the rate of conversion of [U-^{14}C]glucose to lipids by slices of human adipose tissue increased with increasing thickness of the slice up to 500 μm. From 500 to 1000 μm, the rate of conversion remained constant. Apparently, the thinner the slice the larger the proportion of damaged tissue. The higher metabolic activity in thicker slices may therefore be explained by a decreasing number of damaged cells in the section. According to the theoretical considerations of Warburg (17), there is an upper limit of slice thickness. If this upper limit is exceeded, an equal supply of sub-

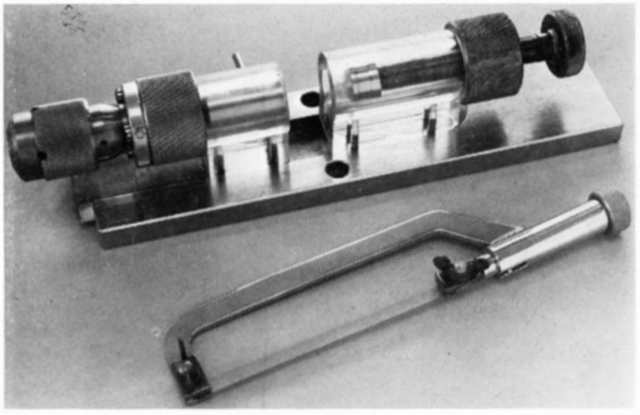


FIG. 5. Microtome and cutting instrument.

TABLE 1. Weight, surface area, and calculated thickness of 10 consecutive slices of subcutaneous human adipose tissue from the abdominal wall

Section No.	Weight	Surface Area	Calculated Thickness[a]
	mg	*mm²*	*μm*
1	63	116	545
2	60	119	505
3	56	113	495
4	56	115	487
5	58	117	495
6	59	119	495
7	61	121	505
8	60	114	526
9	60	117	512
10	57	111	513
Mean	59	116	508
Range	56–63	111–121	487–545

[a] The microtome was set for a thickness of 500 μm.

[1] The razor band is available in uncut lengths of 20 m from Gillette Roth Büchner GmbH., Berlin-Tempelhof, Germany, or as the Techmatic razor band to be found in every drugstore.

TABLE 2. Influence of slice thickness on conversion of [U-^{14}C]glucose to lipids by human adipose tissue

Slice Thickness (machine setting)	[U-^{14}C]Glucose into Lipids
μm	*nmoles/3 hr/g lipid*
100	172 ± 30
200	270 ± 58
300	346 ± 72
400	380 ± 68
500	396 ± 65
600	419 ± 78
700	396 ± 80
800	402 ± 71
900	410 ± 82
1000	422 ± 76

Human adipose tissue slices of increasing thickness (100 mg wet wt) were incubated in 1.0 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2.0% bovine albumin, 10 mM glucose, and tracer amounts of [U^{14}-C]glucose, at 37°C for 3 hr. Each value represents the mean of three individuals, determined in quadruplicate, ± SD.

TABLE 3. Lipolysis in human adipose tissue slices prepared freehand or by microtome

Norepinephrine	Glycerol Release	
	Microtome	Freehand
	nmoles glycerol/3 hr/100 mg lipid	
None	189.0 ± 19.7 (10.4%)	203.9 ± 46.1 (22.6%)
0.05 μg/ml	476.2 ± 52.9 (9.1%)	509.4 ± 128.6 (25.3%)

Human adipose tissue slices (100 mg wet wt, 500 μm thick) were incubated in 1.0 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2.0% bovine albumin, at 37°C for 3 hr. Each value represents the mean ± SD (n = 10); the coefficient of variation is in parentheses. Glycerol was determined by the method of Wieland (19).

strates and oxygen to all cells is not guaranteed. On the other hand, Fuhrman and Field (18) showed that for rat liver the lower limit of slice thickness is quite important. Below and above a range of 480–620 μm there was a sharp decrease in respiration. Our experiments on lipogenesis indicate that in human adipose tissue the lower limit of slice thickness is even more critical than the upper one, as there was no change in metabolic activity in slices that were between 500 and 1000 μm thick.

In order to demonstrate the advantages of the microtome in comparison with freehand slicing, we have studied lipolysis and lipogenesis of human adipose tissue. The lipolytic activity of human adipose tissue prepared with both methods is recorded in Table 3. With both methods the same mean values were obtained, but there was a much higher coefficient of variation with the freehand method.

The favorable effect of the uniform microtome slices of human adipose tissue was even more evident in experiments on lipogenesis (Table 4). Besides the improvement

TABLE 4. Conversion of [U-^{14}C]acetate to lipids by human adipose tissue slices prepared freehand or by microtome

Insulin	Microtome	Freehand
ng/ml	*nmoles/3 hr/g lipid*	
0	71.4 ± 14.2 (19.9%)	57.7 ± 17.3 (29.9%)
2	201.3 ± 28.5 (14.2%)	100.6 ± 24.7 (24.5%)
4	251.4 ± 49.8 (19.8%)	139.7 ± 60.3 (43.2%)
8	257.9 ± 48.8 (19.0%)	169.3 ± 105.2 (62.2%)

Human adipose tissue slices (100 mg wet wt, 500 μm thick) were incubated in 1.0 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2.0% bovine albumin, glucose (10 mM), and sodium acetate (1 mM) with tracer amounts of sodium [U-^{14}C]acetate, at 37°C for 3 hr. Each value represents the mean ± SD (n = 6); the coefficient of variation is in parentheses.

in the coefficient of variation, one can observe a higher sensitivity of the tissue to insulin.

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REFERENCES

1. Renold, A. E., and G. F. Cahill, Jr., editors. 1965. Handbook of Physiology. Section 5: Adipose Tissue. American Physiological Society, Washington, D.C.
2. Burns, T. W., and P. Langley. 1968. Observations on lipolysis with isolated adipose tissue cells. *J. Lab. Clin. Med.* **72:** 813–823.
3. Burns, T. W., and P. Langley. 1970. Lipolysis by human adipose tissue: the role of cyclic 3′,5′-adenosine monophosphate and adrenergic receptor sites. *J. Lab. Clin. Med.* **75:** 983–997.
4. Owen, J. A., R. W. Lindsay, J. H. Saskin, and G. Hollifield. 1967. Response of human adipose tissue to endogenous serum insulin-like activity in vitro. *Metab. Clin. Exp.* **16:** 47–56.
5. Shrago, E., J. A. Glennon, and E. S. Gordon. 1967. Studies of enzyme concentration and adaptation in human liver and adipose tissue. *J. Clin. Endocrinol.* **27:** 679–685.
6. Shrago, E., T. Spennetta, and E. Gordon. 1969. Fatty acid synthesis in human adipose tissue. *J. Biol. Chem.* **244:** 2761–2766.
7. Brech, W. F., and E. S. Gordon. 1967. Die physiologische Rolle des Fettgewebes. *Klin. Wochenschr.* **45:** 905–917.
8. Gries, F. A., and J. Steinke. 1967. Comparative effects of insulin on adipose tissue segments and isolated fat cells of rat and man. *J. Clin. Invest.* **46:** 1413–1421.
9. Novák, M., V. Melichar, P. Hahn, and O. Koldovský. 1965. Release of free fatty acids from adipose tissue obtained from newborn infants. *J. Lipid Res.* **6:** 91–95.
10. Björntorp, P. 1964. The fatty acid release and lipolysis of human subcutaneous adipose tissue in vitro. *Metab. Clin. Exp.* **13:** 1318–1326.
11. Rath, R., and P. Petrasek. 1967. Release of nonesterified fatty acids from adipose tissue in relation to obesity. *J. Lab. Clin. Med.* **70:** 458–462.
12. Östman, J. 1965. A procedure for in vitro studies on fatty acid metabolism by human subcutaneous adipose tissue. *Acta Med. Scand.* **177:** 183–197.

13. Hirsch, J., and R. B. Goldrick. 1964. Serial studies on the metabolism of human adipose tissue. I. Lipogenesis and free fatty acid uptake and release in small aspirated samples of subcutaneous fat. *J. Clin. Invest.* **43:** 1776–1792.
14. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239:** 375–380.
15. Englhardt, A., F. A. Gries, H. Preiss, and K. Jahnke. 1969. Vergleichende Untersuchungen über Protein- und Lipidgehalt und die Aktivitäten von Enzymen der Glykolyse und des Pentosephosphat-shunts im Fettgewebe und isolierten Fettzellen Stoffwechselgesunder. *Horm. Metab. Res.* **1:** 228–234.
16. Stadie, W. C., and B. C. Riggs. 1944. Microtome for the preparation of tissue slices for metabolic studies of surviving tissues. *J. Biol. Chem.* **154:** 687–690.
17. Warburg, O. 1923. Versuche an überlebendem Carcinomgewebe. *Biochem. Z.* **142:** 317–333.
18. Fuhrman, F. A., and J. Field. 1945. Factors determining the metabolic rate of excised liver tissue. *Arch. Biochem.* **6:** 337–349.
19. Wieland, O. 1957. Eine enzymatische Methode zur Bestimmung von Glyzerin. *Biochem. Z.* **329:** 313–329.