Studies on the Respiratory Metabolism of Isolated Human Adipose Cells

By DAVID GALTON

*New England Medical Center Hospitals and the Department of Medicine,
Tufts University School of Medicine, Boston, Mass. 02111, U.S.A.*

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1. Some metabolic characteristics of fat cells isolated from 50 patients were examined. 2. The respiratory activity of human fat cells was of the same order of magnitude as cells obtained from the rat, whether the comparison was based on the molar triglyceride content of cells or the nitrogen content of tissue. 3. There was no difference in the respiratory metabolism of fat cells isolated from three separate age groups: 20–40, 40–60 and 60–80 years. 4. The respiratory activity of human adipose cells was partially inhibited by malonate (0.1 M), arsenite (1 mM) and iodoacetate (1 mM). 5. Oxygen consumption by human adipose cells could not be stimulated by adding glucose or glucose plus palmitic acid to the medium. However, stimulation of oxygen uptake with these substrates was observed after the tissue had been preincubated for 5 hr. in a bicarbonate buffer. 6. After the tissue had been preincubated for 5 hr., there was a linear relationship between the oxygen uptake by isolated cells and the amount of oleic acid added to the incubation medium. Stimulation of oxygen uptake by oleic acid (0.95 μ mole/ml.) was dependent on the presence of glucose (5–6 mM) in the incubation medium. 7. Of the total [14 C]palmitic acid metabolized by human adipose cells, over 99.8% of the radioactivity was recovered in the neutral-lipid fraction and less than 0.2% in carbon dioxide. Under conditions where oxygen uptake is stimulated by [14 C]palmitic acid, the radioactivity of the 1,2-diglyceride pool increased 20-fold.

Previous studies have indicated that the basal rate of lipogenesis in human adipose tissue is about 10- to 100-fold slower than in adipose tissue of a fed rat weighing 150–300 g. (Gellhorn & Marks, 1961; Hirsch & Goldrick, 1964). This, combined with the poor response of human adipose tissue to insulin *in vitro* (Hirsch & Goldrick, 1964) and lipolytic hormones such as adrenaline (Hamosh, Hamosh, Bar-Moar & Cohen, 1963), has led to the opinion that human adipose tissue may not play such an active role in energy metabolism as does rat adipose tissue.

The present experiments were designed to examine the metabolic activity of human adipose cells by manometry. It was found that the respiratory activity of cells isolated from human adipose tissue was of the same order of magnitude as rat adipose cells whether compared on the basis of molar triglyceride content of cells or on the basis of nitrogen content of tissue. The major oxygen-consuming reactions in adipose tissue are fatty acid synthesis, re-esterification of fatty acids to triglycerides and β -oxidation of fatty acids. Incubation conditions were selected to minimize the rate of fatty acid synthesis by use of a phosphate buffer

that decreases synthesis by about 50% (Zahnd, Dagenais & Thorn, 1960), and by adding to the medium free fatty acids that after acylation with CoA produce inhibition of fatty acid synthetase (Porter & Long, 1958). The oxygen consumption by human adipose cells was measured under these conditions with increasing concentrations of fatty acids in the medium. Since the paths of disposal of fatty acids that utilize oxygen are β -oxidation and esterification to triglycerides an estimate of the ratio of activity of these pathways was made by tracer studies; this then made it possible to relate the consumption of oxygen to the energy requirements for re-esterification of fatty acids, as has been suggested by Ball & Jungas (1961) for rat adipose tissue. Additional evidence is presented to support this proposal and a maximal estimate of this pathway obtained.

MATERIALS AND METHODS

Source of tissue. Female rats of the Charles River breed weighing 250–275 g. were fed *ad libitum* on a Purina diet till the time of death by decapitation. Parametrial adipose

tissue was removed and treated in the same manner as the human tissue.

Human subcutaneous adipose tissue was obtained during abdominal surgery from a total of 50 patients (24 females, 26 males) whose ages ranged from 15 to 81 years and body weights from 106 to 202 lb. Patients with diabetes and jaundice were excluded. Preoperative treatment involved starvation for 8 hr., premedication with Nembutal and general anaesthesia induced by Pentothal and maintained with either cyclopropane and oxygen or halothane and nitrous oxide with oxygen. The adipose tissue was removed most frequently at the time of the first incision though on some occasions the tissue was obtained just before wound closure.

Tissue preparations. After removal, the adipose tissue (1–5 g.) was washed and transferred to 3 ml. of albumin-bicarbonate buffer (see below) without chilling. When preincubation was performed the tissue was transferred to 3 ml. of bicarbonate buffer and incubated at 37° for 5 hr. under a gas phase of CO₂+O₂ (5:95). The bicarbonate buffer contained NaCl (127 mm), KCl (2.7 mm), CaCl₂ (1.4 mm), MgCl₂ (0.5 mm), NaHCO₃ (12 mm) and NaH₂PO₄ (4 mm). The albumin-bicarbonate buffer was prepared fresh daily and contained 4% (w/v) of fraction V bovine albumin (lot no. A21908; Armour Laboratories, Kankakee, Ill., U.S.A.). The pH of the buffer was adjusted to 7.7 with 3N-NaOH and then the buffer was gassed with CO₂+O₂ (5:95) until its pH had fallen to 7.4.

A mixture of isolated fat cells and cell clumps was prepared by a modification of the procedure of Rodbell (1964) in which the tissue (0.5–1 g.) was incubated for up to 1 hr. in albumin-bicarbonate buffer containing approx. 10 mg. of bacterial collagenase (Worthington Biochemical Corp., Freehold, N.J., U.S.A.). At the end of the incubation the digested tissue was washed twice in bicarbonate buffer and transferred to either Warburg flasks or 1 oz. polyethylene bottles (Packard Instrument Co., Downers Grove, Ill., U.S.A.).

Manometry. Incubations were carried out in conventional Warburg vessels that had been treated with silicone and of approx. 17 ml. total capacity. The main chamber of each flask contained 2 ml. of modified Krebs-Ringer phosphate buffer containing NaCl (150 mm), KCl (6.2 mm), CaCl₂ (0.8 mm), KH₂PO₄ (0.1 mm), MgSO₄ (0.1 mm), phosphate buffer (1.2 mm), glucose (5.6 mm) and fraction V bovine albumin (4%, w/v). This phosphate buffer contained approx. 0.2 μ mole of titratable fatty acids/ml., which were combined with the albumin; the pH was adjusted to 7.4 with 3N-NaOH. The centre well contained 0.2 ml. of 17% (w/v) KOH and a wick made from Whatman no. 1 filter paper. The final addition to the flask was 200–500 mg. of adipose cells. The flasks were then incubated at 37° in a water bath and shaken at 100 strokes/min. After a 15 min. period of equilibration readings were taken at hourly intervals for up to 4 hr.

Tracer studies. Other incubations were performed in 1 oz. polyethylene vials (Packard) containing 2 ml. of the same albumin-phosphate buffer. The appropriate additions were made and the vials incubated for up to 1 hr. in a Dubnoff shaker at 37° under an air phase.

Materials. [1-¹⁴C]Palmitic acid was obtained from the New England Nuclear Corp. (Boston, Mass., U.S.A.) and approx. 0.3 μ C was added per flask to make a final specific activity of 0.5 μ C/ μ mole. Unlabelled palmitic acid was

added as the sodium salt. It was stored at room temperature and before use was heated until it was optically clear and then added with a preheated pipette. Oleic acid (0.1 M) in the form of a complex with albumin in phosphate buffer was stored at room temperature. Appropriate dilutions were made before use.

Assay procedures. The production of ¹⁴CO₂ was determined by the method of Fain, Scow & Chernick (1963) and labelled lipids by the procedure of Rodbell (1964) after separating fatty acids from neutral lipids by a modification of the procedure of Borgström (1952) and Bally, Cahill, Leboeuf & Renold (1960). The main difference from previous methods was the use of hexane as the upper non-polar phase. Tracer studies with [1-¹⁴C]palmitic acid and unlabelled palmitic acid as carrier showed that over 99.5% of the palmitic acid was removed from the hexane phase. Radioactivity was measured in a Tri-Carb liquid-scintillation counter (Packard) with Bray's (1960) scintillation solution. Correction for quenching was made by the use of internal standards.

Free fatty acids were estimated by a modification of the procedure of Dole & Meinertz (1960). Triglycerides were measured by titration of the fatty acids produced by saponification with 2 ml. of ethanolic KOH (1 ml. of saturated KOH/20 ml. of 95% ethanol).

Nitrogen was assayed by the micro-Kjeldahl method.

Thin-layer chromatography. Isolated cells were incubated for $\frac{1}{2}$ hr. with [1-¹⁴C]palmitic acid under conditions where palmitic acid stimulated oxygen uptake. At the end of incubation lipids were extracted with 35 ml. of chloroform-methanol (2:1, v/v) by the procedure of Folch, Lees & Sloane-Stanley (1957). After phase separation the chloroform layer was evaporated under nitrogen to approx. 0.5 ml. and then made up to 1.5 ml. with benzene. Triglycerides were separated from mono- and di-glycerides on a silicic acid column (100–200 mesh; Fisher Scientific Co., Fair Lawn, N.J., U.S.A.) by the method of Scow, Stricker, Pick & Clary (1965). The chloroform eluent (containing the diglycerides) was fractionated by thin-layer chromatography on SilicAR gel-7G (Mallinckrodt Chemical Works, New York, N.Y., U.S.A.) with light petroleum (b.p. 30–60°)–diethyl ether–acetic acid (60:40:1, by vol.). The plates were sprayed with aq. 50% (v/v) H₂SO₄ to detect the components of the chromatogram; glycerides were identified by the use of markers (triolein, diolein, mono-olein and oleic acid; lot 26451; K & K Laboratories, New York, N.Y., U.S.A.). The *R_F* values of the separated classes were: monoglycerides, 0.09; diglycerides, 0.73; triglycerides, 0.96. The segment of silica gel corresponding to diglycerides was scraped from the plate directly into 10 ml. of Bray's (1960) solution and the radioactivity in this fraction was measured in a liquid-scintillation spectrometer (Packard). Quenching was again corrected by the use of internal standards.

Calculations. All statistical comparisons were based on means of paired experiments and tested for significance of the difference where necessary by a non-parametric sign test (Siegel, 1956). Values are based on the total fatty acid content of adipose cells added to each flask with the assumption that 1 g. of human adipose cells contains approx. 1 m-mole of triglyceride [D. Galton, unpublished work: 1 g. of human adipose tissue contains 700 $\pm$ 38 (5) μ moles of triglycerides]. Results are expressed as μ moles of substrate/100 μ moles of triglyceride of adipose cells/hr. or μ moles of substrate/100 mg. of adipose cells/hr.

RESULTS

When manometry was to be performed the collagenase digestion of the tissue was allowed to proceed until the tissue had broken into isolated cells and small cell clumps. This was done because isolated cells placed in Warburg flasks from the start of the experiment tended to break after 4 hr. incubation. However, when cell clumps were employed the connective-tissue stroma provided some protection for the fat cells and the clumps were small enough to allow adequate equilibration of oxygen between the tissue and gas phases. After about 2 hr. incubation all cell clumps had broken into isolated cells.

The respiratory activity of human adipose cells is of the same order of magnitude as that of rat cells whether compared on the basis of molar triglyceride content of cells or nitrogen content of tissue (Table 1). Fig. 1 shows the time-course of oxygen utilization by human fat cells. The decline after about 2 hr. was attributed to a decrease in number of respiring fat cells due to cell breakage. The oxygen consumption was a linear function of the amount of human cells added to each flask. There was no difference in the respiratory metabolism of isolated cells obtained from subjects of different ages. Oxygen uptakes (in $\mu\text{l./100 mg.}$ of adipose cells/hr.) given as means $\pm$ s.e.m. (no. of observations) for preparations from subjects aged 20–40, 40–60 and 60–80 years and incubated under the conditions described in Table 1 were 3.3 ± 0.8 (3), 3.6 ± 1.5 (14) and 3.5 ± 1.6 (12) respectively.

The effects of three metabolic inhibitors on the oxygen utilization by human adipose cells were examined. Arsenite (1 mM), iodoacetate (1 mM) and malonate (0.1 M) inhibited oxygen uptake by cells incubated under the conditions given in Table 1 by 39, 48 and 43% respectively (results based on means of four to six experiments with each inhibitor).

Table 2 demonstrates that the respiratory

metabolism of human cells is independent of an external source of substrate (glucose and glucose with palmitic acid) until after 5 hr. preincubation. On further incubation after this period, glucose with palmitic acid stimulated oxygen uptake to a greater extent than glucose alone.

Fig. 2 shows that, after preincubation of adipose tissue, oleic acid in the presence of glucose stimulated oxygen uptake by adipose cells and that the uptake of oxygen is a function of the oleic acid concentration in the medium up to about 1 mM. However, if glucose was omitted from the medium the oxygen uptake was 1.1 ± 0.3 (5) $\mu\text{l./100 } \mu\text{moles}$ of cell triglycerides/hr. in the presence of oleic acid (0.95 $\mu\text{mole/ml.}$), whereas in paired experiments with the same concentration of oleic acid the oxygen uptake was 3.2 ± 0.9 (5) $\mu\text{l.}$ when glucose (5.6 mM) was present. The disposal of fatty acids requiring oxygen might be to triglycerides via esterification or to carbon dioxide via β -oxidation. To assess the activity of each of these pathways, human adipose tissue was preincubated in an albumin—bicarbonate buffer for 5 hr. Isolated fat cells were then prepared and incubated for 1 hr. with glucose (5.6 mM), palmitic acid (1.2 $\mu\text{moles/ml.}$) and [^{14}C]palmitic acid (0.3 $\mu\text{C/ml.}$) under the conditions described in Table 2. Approx. 5% of the total fatty acids was metabolized by the cells, and 1.0 ± 0.7 (8) μmoles of palmitic acid/mole of cell triglycerides were converted into carbon dioxide and 600 ± 100 (8) $\mu\text{moles/mole}$ of triglyceride were converted into neutral lipids (mostly triglycerides).

To see whether the presence of fatty acids in the

Table 1. Comparison of the respiratory activity of rat and human adipose cells

Fat cells (450 μmoles of triglyceride/flask) were incubated for 2 hr. in Warburg flasks containing 2 ml. of albumin-phosphate buffer with glucose (5.6 mM). Results are given as means $\pm$ s.e.m. of nine experiments.

Species	O ₂ uptake	
	($\mu\text{l./100 } \mu\text{moles}$ of cell triglyceride/hr.)	($\mu\text{l./100 } \mu\text{g.}$ of tissue N/hr.)
Rat (250–275 g.)	4.7 ± 0.6	1.7 ± 0.2
Man	4.6 ± 0.5	2.3 ± 0.2

Fig. 1. Time-course of oxygen consumption by isolated human fat cells. Approx. 228 μmoles of triglyceride of human adipose cells were incubated in 2 ml. of an albumin-phosphate buffer with glucose (5.6 mM) and the oxygen uptake was measured over 4 hr. Points are means of 11 experiments with the s.e.m. represented between the bars.

Table 2. *Effects of glucose and palmitic acid on the respiratory activity of human fat cells before and after preincubation for 5 hr.*

Fat cells (465 μ moles of triglyceride/flask) were incubated in 2 ml. of albumin-phosphate buffer in the presence of glucose (5.6 mM) and palmitic acid (1.3 μ moles/ml.) where indicated. Oxygen uptake is the average rate over the final 2 hr. period of incubation. Results are expressed as means $\pm$ S.E.M. and the significance of the difference (*P*) was calculated by a non-parametric sign test (Siegel, 1956).

Time of incubation (hr.)	Substrate addition	No. of experiments	O ₂ uptake (μ l./100 μ moles of cell triglyceride/hr.)			<i>P</i>
			Control (without added substrate)	Substrate added	Stimulation (%)	
2	Glucose	5	4.7 $\pm$ 0.6	5.0 $\pm$ 1.0	+6	—
	Glucose + palmitic acid	4	3.7 $\pm$ 1.0	3.4 $\pm$ 0.8	−9	—
7	Glucose	7	2.5 $\pm$ 0.6	3.3 $\pm$ 0.5	+32	0.05
	Glucose + palmitic acid	7	2.6 $\pm$ 0.6	6.1 $\pm$ 0.8	+133	< 0.01

Fig. 2. Effect of oleic acid on oxygen uptake by isolated human fat cells after preincubation for 5 hr. Fat cells (480 μ moles of triglyceride/flask) were incubated in 2 ml. of albumin-phosphate buffer with glucose (5.6 mM). Different concentrations of oleic acid were added to the main chamber of the Warburg flask after preincubation for 5 hr. The numbers of experiments are indicated in parentheses.

incubation medium stimulated the esterification pathway, human adipose tissue was preincubated in an albumin-bicarbonate buffer for 5 hr. and then isolated cells were prepared from it. Approx. 1 m-mole of triglyceride of fat cells was then incubated with [1-¹⁴C]palmitic acid (1 μ mole/ml. and specific activity 0.3 μ C/ μ mole) with and without glucose (5.6 mM) for 30 min. The 1,2-diglyceride fraction was then separated by thin-layer chromatography and no difference was noted in the size of the diglyceride pool, by inspection, between the cells incubated with palmitic acid alone and cells incubated with glucose and palmitic acid. However, the radioactivity in diglycerides increased from 72 $\pm$ 34 (3) to 1504 $\pm$ 290 (3) counts/min. when glucose was present in the medium, and the incorporation of total radioactivity increased from 0.09 to 1.9%. This suggested that palmitic acid, in the presence of glucose, activates the esterification

pathway. It was also noted that no monoglycerides were detected in lipid extracts from human adipose cells.

DISCUSSION

The observation of similar values for the respiratory activity of human and rat adipose cells (Table 1) does not support previous reports that the rate of metabolism of human subcutaneous adipose tissue is less than that of the rat (body wt. 200–300 g.). The earlier studies assessed metabolic rates by measuring glucose and acetate incorporation into glycerides (Hirsch & Goldrick, 1964) or by release of free fatty acids stimulated with adrenaline (Hamosh *et al.* 1963). In human adipose cells oxygen uptake can be inhibited by about 50% with sodium malonate (0.1 M), which blocks the tricarboxylic acid cycle by inhibiting succinate dehydrogenase. This indicates that in human adipose tissue a major part of the oxygen uptake is used for the oxidation of the tricarboxylic acid-cycle intermediates. This is supported by the inhibition produced by sodium arsenite (1 mM), which, in this concentration, primarily blocks dithiol enzymes such as α -oxoglutarate dehydrogenase (Dawson, Elliott, Elliott & Jones, 1959) and therefore blocks the tricarboxylic acid cycle.

As others have found for rat adipose tissue (Jungas & Ball, 1960), substrates such as glucose did not stimulate oxygen uptake in isolated human cells (glutamate, pyruvate and fumarate were also tested, with negative results). However, when the tissue was preincubated for 5 hr. in a medium free from substrates (Table 2) it was found that the respiratory activity in control flasks fell from 4.7 to 2.5 μ l. of oxygen/100 μ moles of triglyceride of adipose cells/hr., though these values are not strictly comparable since they are not based on

paired experiments. After preincubation glucose stimulated oxygen uptake by about 30%, which suggests that endogenous substrates, probably glycogen, had been depleted so that the respiratory metabolism of fat cells became dependent on an exogenous source of substrate. In addition, palmitic acid in the presence of glucose greatly stimulated oxygen uptake in human fat cells (Table 2). The major paths of disposal of fatty acids that require oxygen are β -oxidation and esterification to triglycerides. When an estimate was made of the simultaneous activity of each of these pathways it was found that β -oxidation accounted for only about 0.16% of the total amount of palmitic acid utilized and 99.8% of the label was recovered in the neutral-lipid fraction. Therefore stimulation of oxygen uptake by fatty acids is possibly related to an increased rate of esterification. A relationship, which is not strictly linear, exists between oleic acid concentration in the medium and oxygen consumption by human adipose cells (Fig. 2). This suggests that the fatty acid concentration at the site of re-esterification in the cell may be proportional to the fatty acid concentration in the medium and that the esterification pathway may be maximally stimulated when the fatty acid concentration in the medium reaches about $1\text{ }\mu\text{mole/ml}$.

Further evidence that the stimulation of oxygen consumption by fatty acids is related to their rate of esterification is provided by the following observations. First, the stimulation of oxygen uptake was dependent on the presence of glucose; previous studies have shown that glucose metabolism is essential for re-esterification of fatty acids since it supplies the L- α -glycerophosphate for acylation by fatty acids (Bally *et al.* 1960; Raben & Hollenberg, 1960). Secondly, under conditions where oxygen uptake was stimulated by fatty acids the radioactivity of the 1,2-diglyceride pool increased 20-fold and this substance is a direct intermediate in the esterification pathway (Weiss, Kennedy & Kiyasu, 1960).

If this conclusion is accepted it is possible to make an estimate of the maximal rate of re-esterification. From Fig. 2 the re-esterification pathway is maximally stimulated when $0.5\text{ }\mu\text{g. atom}$ of

oxygen/100 μmoles of triglyceride of adipose cells/hr. is being utilized. Assuming a P/O ratio 3, and assuming that 7 'high-energy' phosphate bonds are required to esterify 1 mol. of glycerol with 3 mol. of fatty acids (the pyrophosphate produced from ATP probably cannot be re-utilized), then it follows that $0.5\text{ }\mu\text{g. atom}$ of oxygen will esterify $0.65\text{ }\mu\text{mole}$ of fatty acid and the maximal rate of re-esterification would be $0.65\text{ }\mu\text{mole}/100\text{ }\mu\text{moles}$ of triglyceride of adipose cells/hr.

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