Measurements of adipose tissue respiration in a closed chamber using an oxygen sensor: methodological considerations

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Abstract A new polarographic system for measurement of oxygen consumption in vitro has been constructed to minimize different types of errors. The system was built of polyamide-6, Monax glass, stainless steel, and rubber to diminish oxygen leakage and gas capacitance properties. With these materials the system was gas-tight. Nevertheless, the incubation chambers had oxygen capacitance properties that had to be corrected for mathematically. Thus, in order to obtain more exact absolute values of the respiratory rate, the apparent respiratory slopes were continuously corrected for ΔpO_2. Furthermore, the initial slope observed in the absence of biological material was also taken into account. Using these variables, the apparent slope (mm Hg/min) could be adjusted by subtracting the following correction factor: correction factor = $0.00306 \times \Delta pO_2 + 0.198 \times$ (initial slope). This correction gave a high degree of linearity and an overall error on the order of 10%. Without the described correction for the oxygen capacitance, the true respiratory rate may be underestimated by up to 50%. — **Hallgren, P., S. Korsback, and L. Sjöström.** Measurements of adipose tissue respiration in a closed chamber using an oxygen sensor: methodological considerations. *J. Lipid Res.* 1986. **27:** 996–1005.

Supplementary key words adipocytes • oxygen consumption

Oxygen sensors are used to follow rapid changes in metabolic rate. They have been used to measure oxygen consumption in many tissues and species (1–5). Studies have been performed on whole animals (6), tissue slices (1–3), free cells (5, 7–13) as well as subcellular elements (14). Electrode characteristics (15–17) and comparisons between polarographic and manometric techniques (18, 19) are well documented in the literature but astonishingly little interest has been devoted to errors and artifacts due to chamber material or tubings used for medium transport. Few reports have studied corrections for "initial slopes" without any biological material present in the incubation chamber (20) or the influence of variations in pressure and temperature (19). To our knowledge, no studies have documented linearity conditions with respect to the amount of tissue incubated.

In our hands, commercially available equipment gave rising pO_2 values when the chambers were filled with nitrogen-equilibrated media. This was true not only when the chambers were preequilibrated at pO_2 = 150 mm Hg but also at pO_2 = 0 mm Hg. We also observed a slow decline in pO_2 values in the absence of biological material when the chambers were filled with air-gassed media. If not taken into account, phenomena of this kind will probably bias the calculated rates of oxygen consumption. This study was undertaken in order to minimize artifacts and to calculate corrections for unavoidable systematical errors.

METHODS

An aluminium box (wall thickness 4 mm, dimensions: 30 × 37 × 22 cm) was constructed to fit inside a commercially available climate incubator (type 911134-T 288, ASSAB, Stockholm, Sweden). The flow of medium to and from three incubation chambers (Monax glass, vol 10 ml) within the box could be regulated from the front of the incubator. The thermostat regulation was improved and three extra fans were mounted within the incubator. Changes in temperature within the box were thus limited to ± 0.01°C. Bottles with incubation media and the incubation chambers with their electrodes (E9001, Radiometer A/S, Köbenhavn, Denmark) were all kept within the aluminium box, usually at a temperature of 37.00°C. The temperature inside the box and inside the incubation chambers was registered with an electronic thermometer (S 1223, Systemteknik, Stockholm, Sweden) to the nearest 0.001°C using Pt_{100} sensors. The electrodes were connected to PHM-73 oxygen analyzers (Radiometer).

The medium was gassed, either with gases of a known fixed composition or with gases obtained from a specially constructed gas mixing device. This equipment made it possible to mix air, CO_2, N_2, and/or O_2 in any combination. The system is based on thermal mass flow controllers.

One dual sensor for CO_2 (max. flow rate 10 ml/min) and air (max. 200 ml/min) and one dual sensor for O_2 (max. 200 ml/min) and N_2 (max. 200 ml/min) were used (type 5841, Brooks Instruments, Veenendal, the Netherlands). The sensors were used together with two modulator control units (type 4250, Brooks Instruments).

For correct gain adjustments at pO_2 levels diverging from the atmospheric partial pressure, an unchanged pO_2 is necessary during the medium transport from the medium bottle to the incubation chamber (see below). Preliminary experiments with commercially available polyethylene or Teflon tubing showed large changes in medium pO_2 even at moderate oxygen gradients. From the literature it was found that the relative gas diffusion coefficients for polyamide-6, Teflon, and polyethylene were 1, 5, and 200, respectively (21). Unfortunately, polyamide-6 tubing is not commercially available, but Rehau Plastics, Hamburg, West Germany kindly produced such tubing with an inner and outer diameter of 1 and 1.6 mm, respectively. With this tubing, pO_2 changed less than 3 mm Hg per m at an oxygen gradient of 150 mm Hg and a medium flow rate of 0.5 ml/min. At oxygen gradients usually used (15–20 mm Hg) the change in medium pO_2 during transportation was less than 0.5 mm Hg.

The medium to be used is slowly (15 ml/hr) perfused through the incubation chamber (vol, 10 ml) the night prior to an experiment. The principal flow scheme for one of the three incubation chambers is shown in **Fig. 1.** The rectangle represents the aluminium box. Incoming gas is humidified (at a) and then equilibrated with the medium (b). The gas leaves the system (at d) 20 cm below the water surface. A constant pressure of 20 cm of water thus drives medium through the stopcock (e), the incubation chamber (c), point g, and the three-way stopcock (h). A peristaltic pump (i) works as a braking flow regulator. At any given flow rate the system produces a constant pressure at the oxygen electrode which is essential for proper signal function. During this procedure stopcock f is closed. K represents the oxygen electrode.

Before the experiment the chamber is perfused at a rate of 0.5 ml/min for 2 hr. Meanwhile the base-line value of the PHM instrument (m) (type PHM73 Radiometer) is established by disconnecting the electrode. The chamber is then closed at e and h, and the gain of the PHM instrument is adjusted to the pO_2 of the medium. The medium pO_2 was calculated according to the formula:

$$pO_2 = (p_{atm} - p_{H_2O} + Pd) \times C \qquad Eq.\ 1)$$

where p_{atm} = atmospheric pressure during experiment, mm Hg; p_{H_2O} = water vapor pressure at 37°C (47 mm Hg); C = the fraction of oxygen in the gas; and Pd = driving pressure through system, 200 mm H_2O = 15 mm Hg.

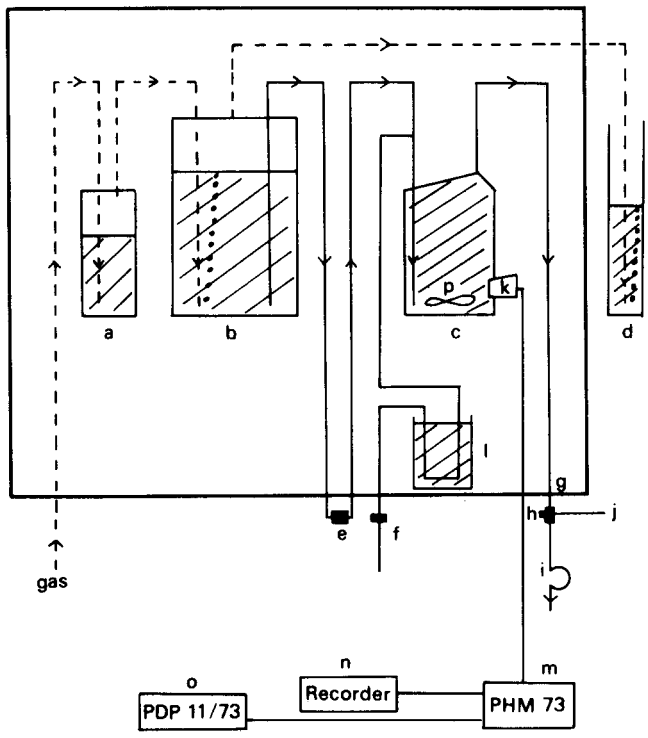

Fig. 1. Principal flow-scheme of the polarographic system for measurement of oxygen consumption in vitro. a, Gas humidifier; b, medium reservoir; c, incubation chamber; d, gas outlet 20 cm below water surface; e, f, h, stopcocks; point g; i, peristaltic pump; k, electrode; l. beaker with water for heat equilibration of media from outside; m, oxygen analyzer; n, recorder; o, computer; p, magnet. Broken lines, gas flow; unbroken lines, medium flow, both through polyamide-6 tubing.

When the medium was gassed with air-CO_2 mixtures:

$$C = (1 - a) \times 0.20946 \qquad Eq.\ 2)$$

where a = fraction of CO_2 in air; and 0.20946 = the fraction of oxygen in dry air.

Usually, Parker 199 medium with 5.5 mM glucose (SBL, Stockholm, Sweden) with or without 4% bovine albumin (fraction V, Sigma, St. Louis, MO) was used. Cephalexine (Kefiex, Lilly, Indianapolis, IN) and tobramycin (Nebcina, Lilly) was added in the concentrations 100 mg and 8 mg per liter, respectively. When indicated, Ringer's solution buffered with Tris (7.15 g of HEPES/l, Flow Laboratories, Ayrshire, U.K.) was used. The linearity of electrodes was tested by mixing known proportions of N_2 and air-gassed media (22).

With the system closed, a basal slope is followed for 30 min (cf. c in Fig. 2). Five ml of medium is then withdrawn at f by opening stopcocks f and h (the latter in the j-h-g direction). The medium is withdrawn at a constant rate of 1 ml/min using a reversible syringe pump. Free fat cell suspensions are introduced (1 ml/min) at f in a volume of

1-2 ml and medium is then reintroduced (1 ml/min) at f until the last air-bubble has left the top of the chamber. The reintroduced medium passes through 25 cm of tubing in a beaker containing 200 ml of water (1) equilibrated with the temperature of the box. Stopcocks f and h are then closed and respiration is registered during constant use of a magnetic stirrer (p). The same procedure is used for adipose tissue slices except that they were introduced by removing the stopper for a short moment.

The system is sterilized with 0.5% glutaric aldehyde. During this procedure the exact individual chamber volume (electrode mounted and magnetic stirrer present) is determined to the nearest 0.1 ml. All work with cells and medium is performed under aseptic conditions.

Each oxygen electrode feeds an individual PHM instrument with information. Each instrument gives analogous signals to a recorder (Omin-Scribe, type B5217-2, Houston Instruments, Scandiametric AB, Solna, Sweden) and digital information to a monitor. At present, the system is computerized with a PDP 11/73 with an RSX-11M multiuser operative system.

Human or rat adipose tissue was used. In man, surgical biopsies from healthy subjects aged 42 ± 11 years and weighing 56 to 108 kg were studied. Epididymal pads of male Sprague-Dawley rats (200-350 g) (Anticimex, Lidingö, Stockholm) were utilized. Slices weighing 5-10 mg were either used directly for respiration experiments or incubated with collagenase (20 mg in 10 ml medium, type II, Sigma) for 45 min at 37°C in a metabolic shaker (180 rounds/min) to prepare free cells.

In cell separation experiments all vessels were siliconized. The collagenase-liberated fat cells were washed five times in fresh medium and floated. The infranatants were pooled and centrifuged at 5000 *g* for 10 min. Sedimented stromal-vascular cells were resuspended in 2 ml of medium. Adipocytes and stromal-vascular cells were related to the wet weight of tissue from which they originated. In control experiments the dry weight recovery of this procedure was higher than 90%.

In all other experiments the oxygen consumption was expressed in μl of O_2 per g of lipid $\times$ min^{-1} or per 10^6 fat cells $\times$ min^{-1}. After the respiration measurements, the lipid content of the incubation chamber was determined gravimetrically after heptane extraction according to the method of Dole and Meinertz (23). The number of incubated cells was calculated by dividing the lipid content by the mean fat cell weight (MFCW) (24).

Oxygen consumption was calculated according to the formula:

$$VO_2 = \frac{p_{atm} - pH_2O}{760} \times \alpha \times \frac{\Delta pO_2}{\Delta t} \times \frac{Vch - L \cdot 10^3/0.900}{L} \times \frac{273.15}{273.15 + 37} \qquad Eq.\ 3)$$

where VO_2 = oxygen uptake (μl O_2 $\times$ (g L)$^{-1}$ $\times$ min^{-1}), STPD; p_{atm} = atmospheric pressure during the experiment (mm Hg); p_{H_2O} = partial pressure of water vapor at 37°C (47 mm Hg); α = solubility coefficient of oxygen in the medium at 37°C = 3.0×10^{-5} μl $\times$ (μl medium)$^{-1}$ $\times$ (mm Hg)$^{-1}$; ΔpO_2 = change in partial pressure (mm Hg); Δt = change in time (min); Vch = chamber volume (μl); and L = lipid content of the chamber (g).

The first part of the formula corrects for the actual atmospheric pressure and the partial pressure of water vapor. The solubility coefficient, α, is equivalent to 0.02273 μl of O_2/μl of medium/760 mm Hg for a 155 mM NaCl solution at 37°C (25). The third part of the formula is the rate of oxygen disappearance in the medium. The fourth part of the formula takes into account the individual chamber volume (Vch) with its medium volume (Vch − L $\times$ 10^3/0.900, where 0.900 is the density of lipid) and lipid content (L). When L of the denominator is substituted with L/MFCW (where L and MFCW (mean fat cell weight) are expressed in μg) and the equation is multiplied by 10^6, the oxygen uptake is obtained in μl of O_2 $\times$ (10^6 cells)$^{-1}$ $\times$ min^{-1}. VO_2 is corrected to the volume at 0°C by multiplying by 273.15/(273.15 + 37). For conversions into μmol of O_2, results in μl of O_2 are divided by 22.4.

Ordinary linear and multivariate regression analyses were used. The error of the method was either calculated from double determinations according to Dahlberg (26) or as SD expressed in % of mean when determinations in triplicate were available.

RESULTS

A registration from a typical experiment is shown in **Fig. 2.** The system is perfused overnight (a) and then closed at b. After closure, the gain is set (b). The c part of the curve is the initial slope before the introduction of cells. Cells are introduced at d, and e is the reduction in pO_2 associated with respiration of the cells. The apparent rate of oxygen consumption (e) falls with time.

From Fig. 2 two important questions emerge. *1.* Should the initial slope (c) be subtracted from the biological slope (e)? *2.* Is the decreasing oxygen consumption with time real or artificial?

To answer these questions, the following experiments were performed. Oxygen consumption per min was linear with respect to the amount of incubated adipose tissue when calculated from uncorrected slopes (**Fig. 3a**). This was true at any incubation time and for the average slope over a complete experiment.

When using slopes from later parts of an experiment, the regression coefficient of the linearity equations decreased, reflecting the decreasing biological slopes with time (cf. Figs. 2, 3a). Without any corrections of the

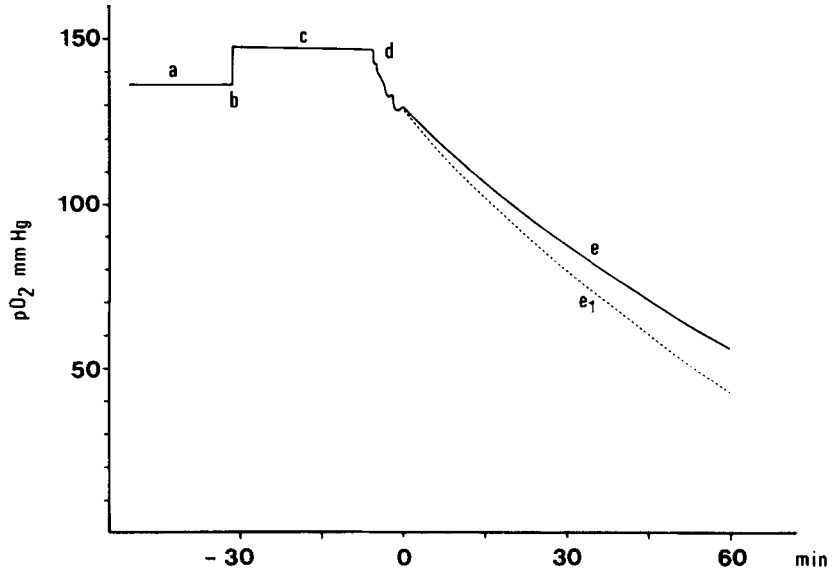

Fig. 2. Typical registration of pO_2 versus time. a, Perfusion 0.5 ml/min; b, gainsetting; c, initial slope; d, cell introduction; e, uncorrected biological slope; e_1, slope corrected according to equation 4, see text.

observed biological slope, the lines of equation gave intercepts close to origin (Fig. 3A). When the basal slope was subtracted in the calculations, negative intercepts were obtained (Fig. 3a).

The negative intercepts after correction for the basal slope indicated that the initial slope might be overestimated (Fig. 2, c part). Prolonged registrations of initial slopes did not reveal any decreasing tendency with time. The chamber is partially emptied (−5 ml) during the introduction of cells. When this emptying procedure was mimicked and the chamber was refilled without introducing cells, the following basal slope was reduced by 43.8 ± 6.9% (mean ± SD, n = 13). Biological slopes corrected for initial slopes × 0.562 also resulted in more negative intercepts when compared with uncorrected slopes, although the deviation from origin was less marked than in Fig. 3a.

In ten biological experiments there was a high correla-

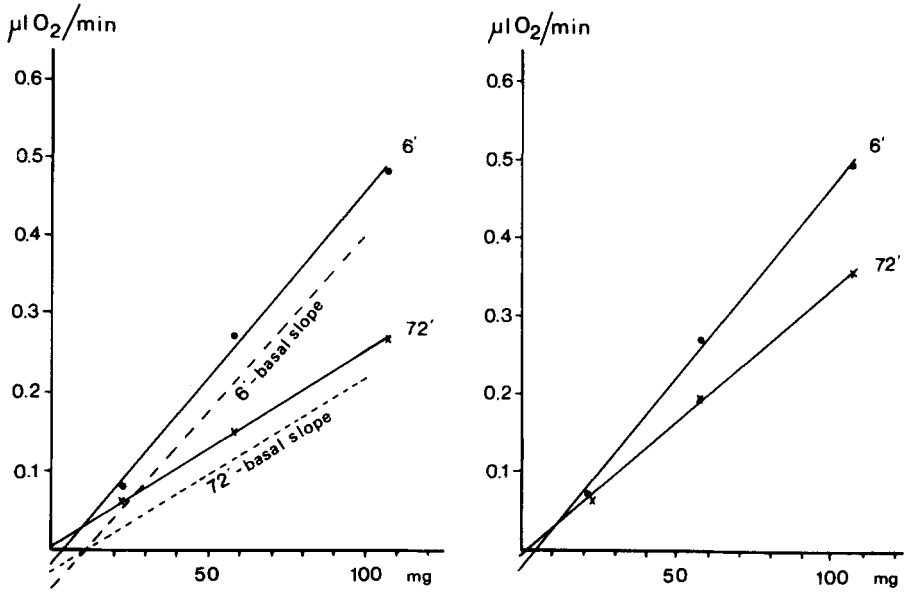

Fig. 3. The linearity of oxygen consumption with increasing amounts of free fat cell lipids (mg). Left panel, solid lines indicate uncorrected values at 6 and 72 min, respectively; dashed lines give the values after subtraction of the initial slope. Right panel, according to equation 4 (cf. e_1 in Fig. 2).

TABLE 1. Correlations between uncorrected biological slopes read after different periods of time from start in 10 consecutive experiments

	6'	12'	18'	24'	30'	36'	42'	48'	54'
12'	0.917								
18'	0.965	0.976							
24'	0.958	0.912	0.988						
30'	0.963	0.932	0.977	0.995					
36'	0.947	0.916	0.986	0.995	0.992				
42'	0.963	0.918	0.986	0.997	0.996	0.997			
48'	0.923	0.935	0.971	0.975	0.975	0.990	0.981		
54'	0.919	0.921	0.961	0.969	0.968	0.981	0.972	0.981	
$\bar{x}$	0.971	0.967	0.995	0.991	0.992	0.995	0.996	0.982	0.974

tion between uncorrected biological slopes obtained at different times after the introduction of cells into the incubation chamber (**Table 1**). When only relative values of oxygen consumptions are necessary, it should not matter whether an early, late, or a mean slope is chosen as the standard slope for all experiments. If, however, absolute values of the oxygen consumption are to be obtained, it is necessary to make clear whether the decreasing slope with time (cf. Fig. 2) is an artefact.

An increased leakage rate of oxygen into the system should be suspected when the oxygen gradient between the atmosphere and the chamber medium increases. Such a phenomenon should result in a graph such as that in Fig. 2 (e). In order to test this possibility, chambers were perfused with N_2-gassed media overnight. When the chambers were closed, no positive slope could be observed (**Fig. 4**). When medium was replaced by N_2 gas and the same medium was then reintroduced into the chamber, the slope was still approximately zero (Fig. 4). When, however, medium was replaced by air and then reintroduced into the chamber, the oxygen tension of the medium increased from 0 to 55 mm Hg corresponding to an uptake of about 16 μl of O_2. After this the pO_2 gradually decreased, indicating an oxygen uptake by the chamber walls. If a chamber, equilibrated at a low pO_2, is to take up O_2 from a medium with a higher oxygen tension, then oxygen might well be transported from the walls into the medium when the pO_2 of the medium decreases. Such a phenomenon may also explain the shape of the biological slope in Fig. 2.

In order to test this possibility, chambers were perfused overnight with media with a pO_2 = 150 mm Hg. The chambers were then closed and the initial slope was registered in the usual way. The medium of the chamber was then replaced with media having a pO_2 less than 150 mm Hg (**Fig. 5**). Under these artificial conditions, as pO_2 was decreased, the following slope became more positive (Fig. 5). The initial slope and the following artificial slope were also correlated (r = 0.41). Artificial positive slopes (y) were examined as the dependent variable using ΔpO_2 (positive value) and the initial (negative) slope as independent variables. The resulting equation was:

$$y = 0.00306 \times \Delta pO_2 + 0.198 \times \text{initial slope.} \quad \textit{Eq. 4)}$$

There was a good correlation between slopes calculated according to this formula and those artificial slopes actually observed (r = 0.93).

Uncorrected (negative) biological slopes corrected by subtracting artificial slopes with their signs calculated as above resulted in straightened biological slopes (Fig. 2, e_1) which, however, still had curvilinear shapes. Accordingly, regression coefficients of linearity experiments were less reduced when using late observations (cf. Fig. 3a and b).

In **Fig. 6** the results of four linearity experiments (cf. Fig. 3) are summarized. Expressed in % of the regression coefficient based on the 6-min values, the coefficient was

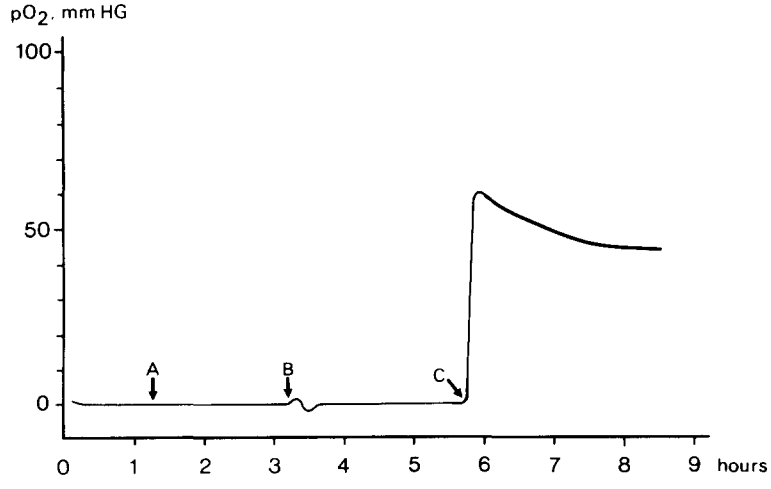

Fig. 4. Gas tightness and capacitance of the chamber. After perfusion overnight with a N_2-gassed medium, the chamber is closed at A. At B, 5 ml of medium is withdrawn and reintroduced. During withdrawal, the medium is continuously replaced with pure N_2 gas. At C, the same procedure as in B, but medium is replaced by air.

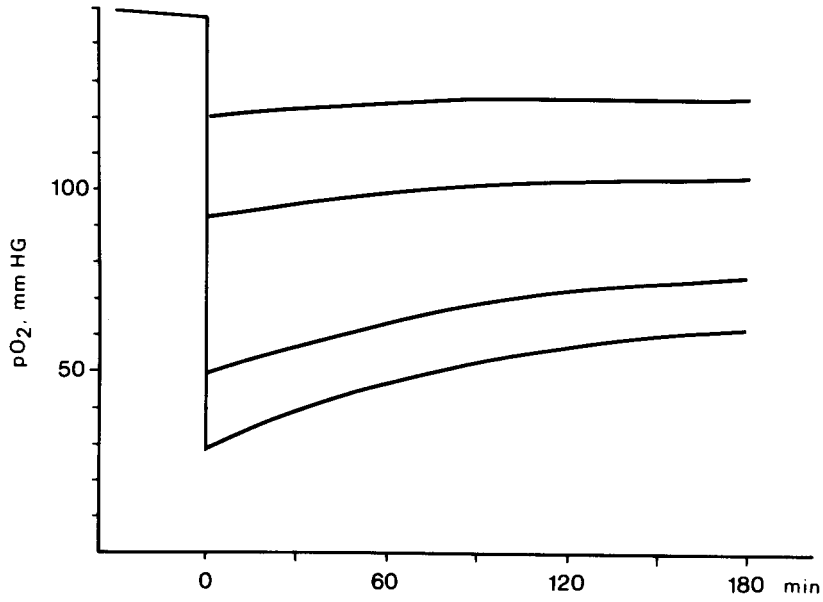

Fig. 5. Artificial slopes after lowering of medium pO_2 to different levels. The chamber is initially equilibrated at pO_2 150 mm Hg overnight.

reduced by 48% when the calculations were based on late uncorrected biological slopes (e curve in Fig. 2). When the biological slopes were corrected by subtracting calculated artificial slopes according to equation 4, the corresponding reduction of the regression coefficient was only 22% (Fig. 6). The new correction technique did not change the mean y-intercept significantly from the value obtained without corrections. The change in intercept was less pronounced than when just the initial slope was subtracted (Fig. 6). The error of the method was 7–11% both when the oxygen consumption per gram was calculated from uncorrected "biological slopes" and when calculated from "biological slopes" corrected for "artificial slopes." On the other hand, "biological slopes" corrected for "initial slopes" resulted in oxygen consumptions having a mean error of 24% in these four experiments (Fig. 6).

Incubation of adipose tissue slices impaired the linearity to some extent as compared to studies with free fat cells (**Fig. 7**). This was particularly the case for human adipose tissue. Fig. 7 also illustrates the difference in oxygen consumption between rat and human adipose tissue.

Adipose tissue boiled for 15 min had no respiratory activity (two experiments).

The linearity of the electrode, tested according to LeFevre (22) was excellent. Correlations between absolute and read values varied between $r = 0.993$ and 0.999 (tested every 10th mm Hg of pO_2 from 0 to 150 mm Hg).

The respiratory contribution of fat cells and stromal vascular cells (SVC) of adipose tissue was examined in cell separation experiments. Seventy five percent of the activity in adipose tissue slices was recovered in fat cells plus stromal vascular cells (**Fig. 8**). Free fat cells showed a respiration that was about five times higher than that of SVC expressed per unit of wet weight from which the cells originated. Fat cells and stromal vascular cells incubated in the presence of collagenase during the respiratory measurements did not show a reduced oxygen consumption as compared to cells examined in the absence of collagenase.

The error of the method was evaluated from all available double determinations (**Table 2**). As in Fig. 6, the error was larger when the results were calculated on "biological slopes" corrected for "initial slopes" and smaller when based on uncorrected slopes or slopes corrected for "artificial slopes." Using the latter correction procedure, the overall error was about 9% for measurements on free fat cells and 10 and 16% for measurements on rat and human adipose tissue slices, respectively. There was no correlation between the percentage of artifactual errors and the absolute oxygen consumption.

In rat adipose tissue the respiratory activity was in the order of 2.5 μl of $O_2 \times (\text{g lipid})^{-1} \times \text{min}^{-1}$ or 0.30 μl of $O_2 \times (10^6 \text{ cells})^{-1} \times \text{min}^{-1}$ while the corresponding figures for human adipose tissue were 0.40 and 0.16, respectively (Table 2).

DISCUSSION

Although the characteristics of oxygen sensors have been well documented (15, 16, 27) little attention has been paid to the properties of the incubation chambers them-

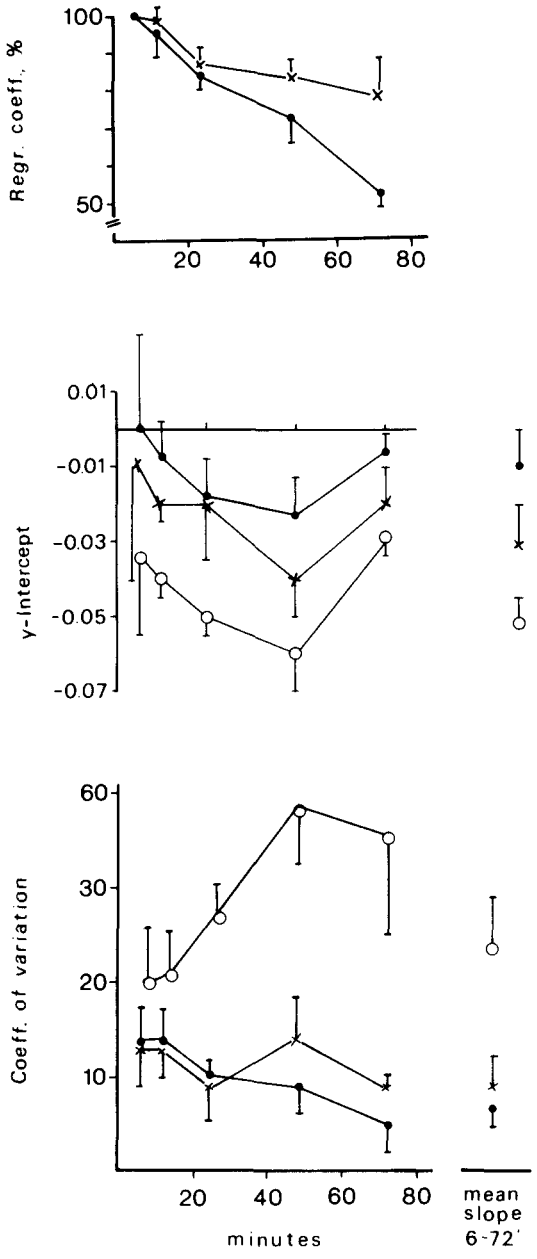

Fig. 6. Summary of four linearity experiments with free fat cells; for details, see legend to Fig. 3. The regression coefficients for ml of O_2 per min versus mg of lipid at different times are expressed in relation to the coefficient at 6 min (100%); mean ± SEM. (●), Results based on uncorrected biological slope (cf. e in Fig. 2); (X), correction according to equation 4; (○), results based on uncorrected slope minus initial slope.

selves. Most incubation chambers used are made of lucite or of glass with lucite lockers. The diffusion constant of lucite is some 30 times higher than that of polyamide-6 (28). Even when the lucite walls or stoppers are thick enough to prevent significant leakage of gas, the lucite material still has a capacitance function. As soon as the pO_2-equilibration between medium and lucite is disturbed, oxygen can be expected to diffuse along the new gradient. Our experiments have shown that similar shifts between medium and walls also occur—although less pronounced—when the chamber walls are made of Monax glass or quartz and stainless steel. The oxygen capacitance of the chamber was not increased by using rubber instead of stainless steel. This might indicate that the capacitance is mainly a surface phenomenon.

The slightest amount of visible air bubbles within our chambers reduced the apparent respiration rate by 10–30%. Some commercial chambers have an open communication between the chamber and the atmosphere via a narrow channel used for air-emptying purposes. Such a construction may further increase the oxygen capacitance.

In order to obtain correct settings of the gain, it is important to use tubing with minimal influence on pO_2 during transportation of medium to the incubation chamber. Since polyethene, or even Teflon, tubing introduces large errors, we use polyamide-6 tubing.

Various techniques for calibration of oxygen sensors have been described (22, 29, 30). During routine conditions, the pO_2 instrument was zeroed electrically by disconnecting the electrode. When testing the linearity of the electrodes, we used the method described by LeFevre (22). The linearity of the electrodes was excellent provided that oxygen diffusion was proportional to the change in medium pO_2.

Most studies have registered oxygen consumption for short periods of time, usually less than 10 min. Registrations as short as milliseconds have been used in some measurements (14). Also, large amounts of tissue in relation to the chamber volume have been used (2–4) resulting in steep slopes. Short registration periods and steep respiration slopes may explain why the capacitance influences have been overlooked. Although we have no data on the physical mechanisms of the capacitance phenomena, we present correction procedures that reduce its influence. These correction procedures reduce the influence of oxygen capacitance on the calculated rates of oxygen consumption. Finally, the suggested correction procedure improves the y-intercept in linearity experiments. It is probably necessary to adjust the coefficients of the correction equation presented here for each system used. Such adjustments are easily calculated after a number of "artificial slope" experiments (cf. Fig. 5). Without such corrections for oxygen capacitance, respiration may well be underestimated by 50%, especially at the end of an experiment.

In mammals, arterial and venous oxygen tensions are on the order of 150 and 40 mm Hg (31), whereas the extracellular tissue fluid has an oxygen tension of ca. 30 mm Hg (3, 32, 33). Warburg (34) demonstrated a relationship between oxygen tension and tissue oxygen uptake. He introduced the concept "critical oxygen tension"

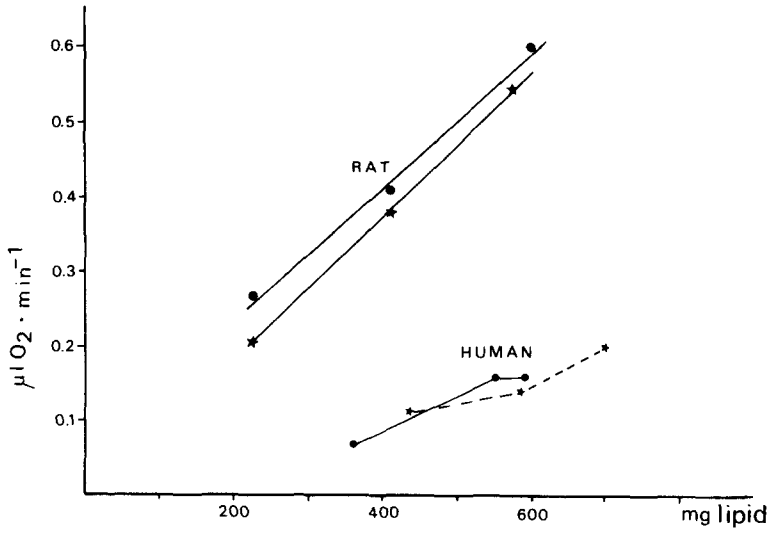

Fig. 7. Linearity experiments with rat and human adipose tissue slices. Epididymal fat from 200-g Sprague-Dawley rats and subcutaneous fat from the abdominal region of two 40- to 45-year-old women weighing 70 and 84 kg, respectively.

below which tissue respiration falls (34). Caldwell and Wittenberg (35) found that the oxygen utilization of several tissues decreases progressively below an oxygen pressure of 100 mm Hg. The curvilinear shape of corrected biological slopes in our investigation are in line with the observations of Caldwell and Wittenberg. We observed a severely reduced oxygen consumption below an oxygen tension of 5 mm Hg.

During cell separation experiments, the dry weight recovery was greater than 90%, while the recovery of respiratory capacity was on the order of 75%. The reduced respiratory capacity did not seem to be related to the collagenase treatment. Per unit wet weight, the collagenase-liberated fat cells had an oxygen consumption that was about five times higher than the stromal vascular cells. We found no similar separation experiments in the literature for comparison.

In Sprague-Dawley rat adipose tissue, the respiratory activity was in the range of 1 to 4 μl of O_2 × (g lipid)$^{-1}$ × min^{-1} or 0.2–0.4 μl of O_2 × (10^6 cells)$^{-1}$ × min^{-1}. In

TABLE 2. Oxygen consumption and overall error of methods calculated from double determinations of rat and human adipose tissue according to three different procedures[a]

		Oxygen Consumption							
		Per g of Lipid						Per Cell	
		No Correction		Correction Basal Slope		Corrections: Basal Slope + pO_2[b]		Corrections: Basal Slope + pO_2[b]	
Tissue	Number of Double Determinations	μl of O_2 per g lipid · min	% Error	μl of O_2 per g lipid · min	% Error	μl of O_2 per g lipid · min	% Error	μl of O_2 per (10^6 cell · min)	Cell Size (μg)
Collagenase-liberated rat adipocytes	4	2.8 ± 0.9	7	2.4 ± 0.9	24	3.0 ± 1	9	0.30 ± 0.3	0.10 ± 0.08
Rat adipose tissue slices	7	1.8 ± 0.8	10	1.6 ± 0.8	15	2.1 ± 1	10	0.29 ± 0.09	0.14 ± 0.03 (n = 5)
Collagenase-liberated human adipocytes	2	0.29 ± 0.03	10	0.26 ± 0.02	8	0.29 ± 0.01	7	0.10 ± 0.01	0.37 ± 0.04
Human adipose tissue slices	8	0.44 ± 0.13	14	0.39 ± 0.14	20	0.47 ± 0.13	16	0.22 ± 0.07	0.48 ± 0.18

[a]This table does not reflect differences in oxygen consumption between collagenase-liberated cells and slices since double determinations from very different types of donors are used for the calculation of errors.
[b]Corrections according to equation 4.

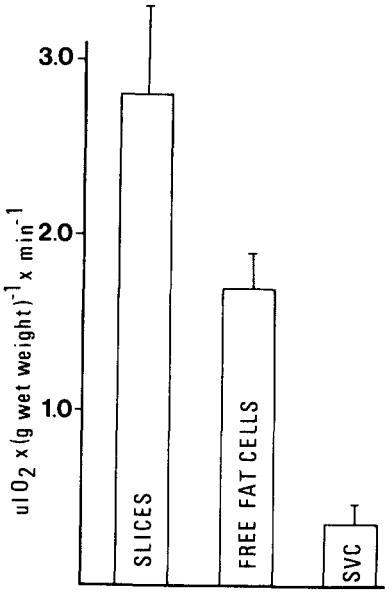

Fig. 8. Comparison of respiration in rat adipose tissue slices, free fat cells, and stromal vascular cells. Collagenase-liberated cells were separated as described in Methods. Oxygen consumption is expressed per g wet weight of original tissue. Dry weight recovery was 87–102%; mean ± SEM of five experiments using epididymal pads of 150-g Sprague-Dawley rats.

human adipose tissue, the corresponding values were 0.30–0.60 μl of $O_2 \times (g \text{ lipid})^{-1} \times min^{-1}$ or 0.15–0.30 μl of $O_2 \times (10^6 \text{ cells})^{-1} \times min^{-1}$. We have previously obtained corresponding values in rat adipose tissue using a microspectrophotometric technique measuring the oxygen consumption of single cells (11). Microcalorimetric measurements in human fat cells have shown a heat production of about 50 pW/cell (13). Recalculated to oxygen consumption (20 kJ/l O_2, 1 watt = 1 J/sec) this heat production corresponds to 0.15 μl of $O_2 \times (10^6 \text{ cells})^{-1} \times min^{-1}$. Thus three methods based on completely different physical principals give values for energy expenditure within the same range. There is a positive correlation between cell weight and oxygen consumption (11) and the human fat cells of the present investigation were much larger than the rat cells. After correction for cell weight, the metabolic activity per cell is 5–10 times higher in rat as compared to human adipocytes.

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