Oxygen consumption of human endometrium during the menstrual cycle measured in vitro using an oxygen electrode

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Summary. Oxygen consumption of human endometrial tissue was measured in vitro in air and high oxygen gas phases using an oxygen electrode. The oxygen consumption increased throughout the proliferative phase, peaking around ovulation and then decreasing during the secretory phase. This pattern persisted whether the oxygen consumption was expressed on a dry weight, DNA or protein basis. Storage of the tissue and mincing produced lower values of oxygen consumption.

Introduction

Previous studies of the in-vitro oxygen consumption of human endometrium in the various phases of the human menstrual cycle have given contradictory results. Raab (1929) reported cyclic changes but neither Adler (1930) nor Dreyfus (1940) could confirm his observations. Stuermer & Stein (1952) obtained a peak in consumption around the time of ovulation but the increase was not statistically significant. All these studies employed manometric techniques involving long incubation periods uncontrolled for the possible utilization of oxygen by bacteria. Okagaki & Richart (1970) avoided these problems by using an oxygen electrode that allowed rapid uptake measurements and added antibiotics to the incubation medium. While they obtained a small but statistically significant increase in oxygen uptake in the secretory phase, their studies utilized minced endometrial tissue, a procedure known to reduce tissue oxygen consumption significantly. Because of the unsatisfactory nature of all previous investigations the present study was designed to measure in vitro the oxygen consumption of intact strips of human endometrium by using an oxygen electrode and to explore the effects of tissue preparation, gas phase, and the tissue bases for calculating the oxygen uptake. The oxygen uptake results are necessary to provide a basis for comparison with observations, currently being made, on the effects of steroid contraception and intrauterine devices on endometrial oxygen consumption.

Materials and Methods

Strips of endometrium were obtained from women by curettage, by dissection of the uterus after hysterectomy, or by suction endometrial biopsy (Vabra technique). The only samples used were those obtained from women with a histologically normal endometrium and regular menstrual cycles but without an intrauterine device in situ and with no use of oral contraception or other hormone therapy within the previous 3 months.

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Immediately after removal, the endometrium was placed in ice-cold gassed (95% O₂, 5% CO₂ v/v) Krebs-bicarbonate-saline (KBS, Krebs & Henseleit, 1932) for storage and transport to the laboratory. A sample was taken for histological study for assessment of abnormality or disease and to date the endometrium by the criteria of Noyes, Hertig & Rock (1950). Seven groupings of the endometrium were used: early proliferative, Days 1–4; mid-proliferative, Days 5–8; late proliferative Days 9–12; ovulatory, Days 13–16; early secretory, Days 17–20; mid-secretory, Days 21–24; late secretory, Days 25–28. Occasionally, when there was difficulty in discriminating between two adjacent groups, the patient’s last recorded menstrual dates were used to clarify the dating. Despite this, there may still have been some overlap between adjacent groups, especially in the ovulatory group, because the indicators of ovulation cannot be observed histologically in the endometrium until 24–36 h after the event.

At the laboratory the tissue was washed free of blood in fresh, ice-cold, oxygenated KBS, blotted on damp filter paper to remove excess fluid, divided into 20–100 mg samples and weighed to the nearest 0.1 mg. One sample was placed in an oven at 140°C for 1 h (the temperature and time to produce a constant dry weight) and then reweighed to give the dry weight to wet weight ratio. Oxygen consumption was measured in another sample with an oxygen electrode (Yellow Springs Instruments, model 53 Biological Oxygen Monitor). The respiration chamber contained 3 ml KBS maintained by a water jacket at 37°C (Lauda thermostat) and was allowed to equilibrate for 5 min. The KBS was saturated with pre-warmed and pre-humidified 95% air/5% CO₂ (v/v) or 95% oxygen/5% CO₂ (v/v). The oxygen electrode was placed into the chamber forming a tight seal and a permanent record of its output was recorded on a pen recorder (Servoscribe). Because the electrode consumes a small amount of oxygen at constant rate, a control slope of oxygen consumption without the tissue present was measured. This was then subtracted from the slope recorded with the endometrial sample in the chamber (Price, 1980). The endometrial sample was incubated for 15 min. The initial slope was used for calculation. The oxygen consumption of the endometrium was expressed as μl oxygen/mg dry weight/h.

Both protein and DNA were estimated in endometrium taken from patients at various stages of the menstrual cycle. The samples (20–100 mg wet weight) were chopped into small pieces and then homogenized, with a teflon pestle and glass homogenizer (Adelphi Ltd) at 2500 r.p.m., in 1–2 ml ice-cold KBS for 10 1-min periods, allowing cooling intervals of 1 min between each homogenization. During the whole procedure the homogenate temperature was maintained below 4°C. The protein of the homogenate was measured by the method of Lowry, Rosebrough, Farr & Randall (1951). For the analysis of DNA, the endometrial sample (20–100 mg wet weight) was similarly homogenized but in 0.5 ml perchloric acid. The DNA content was estimated by the method of Burton (1956). The total protein and total DNA were expressed as a percentage of the tissue dry weight.

Statistical analysis

The results are expressed as the mean ± s.e.m. and the means were compared by Student’s paired or unpaired t tests as appropriate. Significance was assumed at P < 0.05. The endometrial oxygen consumption at the different phases of the menstrual cycle was compared by an analysis of variance and the differences between groups were located by the least significant range test (LSR) as detailed by Sokal & Rohlf (1969):

\[
\text{LSR} = Q \sqrt{\text{MS}_{\text{within}}} \left( \frac{\sqrt{n_1 + n_2}}{\sqrt{2n_1n_2}} \right)
\]

where Q is obtained from tables (Rohlf & Sokal, 1969), MS_{within} is taken from the analysis of variance tables in which n₁ and n₂ are the numbers of the observations in each of the two groups.
compared. The results were taken as significant when the computed difference between two means exceeded the actual difference of the two means. The test was applied at $P = 0.05$.

**Results**

**Validation of technique**

**Storage.** The effect of storage of the endometrium in oxygenated, ice-cold KBS after removal from the patient on the tissue uptake was investigated. Oxygen consumption was measured 30, 60, 120 and 240 min after removal using air or oxygen for the gas phase. The results are shown graphically in Text-fig. 1. The pattern was similar for both oxygen and air. Between 30 and 120 min there was a sharp fall in consumption to 50–60%, while between 120 and 240 min there was a slower decrease. Paired $t$ tests of the consumption at 30 and 60 min showed small but significant declines of 8.2% in air ($P < 0.05$) and 12.4% in oxygen ($P < 0.01$). Because the consumption of oxygen continually decreases after removal of the tissue from the patient it was measured in all subsequent experiments between 20 and 30 min after removal.

![Text-fig. 1](image)

**Text-fig. 1.** The effect of storage of the whole endometrial strip in ice-cold oxygenated KBS on oxygen consumption. The measurement was carried out in oxygen- (n = 5) and air- (n = 5) gassed incubation medium. Values given are mean ± s.e.m.

**Tissue integrity.** Samples of endometrium obtained at different stages of the menstrual cycle were subdivided and the oxygen consumption of similar weights of tissue was measured as a whole strip or as tissue minced into small fragments. The oxygen consumption of the minced tissue was reduced by 37.9% ($P < 0.01$) in the air phase and 32.8% ($P < 0.01$) in the oxygen phase.

**Addition of glucose to the incubation medium.** When glucose was added at a concentration (4.4 mmol/l) similar to that in blood and endometrial luminal fluid (Douglas & Garrow, 1970), there was no significant effect on the endometrial oxygen consumption within the period of the measurements (up to 15 min).

**Size of the endometrial strip.** To determine whether the size of the endometrial sample influences the oxygen consumption, regression lines and coefficients of correlation were calculated for tissues (20–100 mg in air- and oxygen-gassed incubation medium) at each of the phases of the normal menstrual cycle. At no phase of the menstrual cycle did the size of the endometrial sample show any statistical correlation with the measured oxygen consumption.

**Site within the uterus.** For some patients a curettage was obtained from the anterior endometrium before hysterectomy and subsequently samples were dissected from four different sites of the posterior endometrium: (i) upper centre, (ii) lower centre, (iii) sides and (iv) just above the internal os. In the air phase there was no significant difference in the oxygen consumption.
consumption of endometrium taken from the upper or lower centre, or sides of the posterior aspect of the uterus or from curettage samples from the anterior aspect. The samples from just above the internal os had a significantly lower oxygen uptake (69.9 ± 9.2% of upper centre site value; n = 6, P < 0.05) than did samples from all the other sites. All the endometrial samples for the study of the oxygen consumption during the menstrual cycle were obtained by curettage or by dissection from sites high in the uterus.

**Oxygen consumption during the normal menstrual cycle**

Three methods for expression of the oxygen consumption were used in case the patterns detected were related to the strips of endometrium having a different number and size of cells per unit weight at the various stages of the menstrual cycle.

*Measured as per mg dry weight.* The endometrial oxygen consumption was higher in the 95% oxygen phase than in the air phase (P < 0.01), but the oxygen uptake pattern throughout the cycle was similar for both phases (Text-fig. 2). The oxygen consumption increased continuously through the proliferative phase, reaching a distinct peak around the time of ovulation. This was followed by a continuous decline through the secretory phase. The peak around the time of ovulation was significant for both the air and oxygen phases (Table 1) and, in

![Oxygen consumption graph](image)

**Text-fig. 2.** Human endometrial oxygen consumption (mean ± 1 s.e.m. for no. of observations indicated) during the normal menstrual cycle in air- and oxygen-gassed medium. EP, early proliferative; MP, mid-proliferative; LP, late proliferative; OV, ovulatory; ES, early secretory; MS, mid-secretory; LS, late secretory.

**Table 1.** Comparison by the least significant range test of the differences in oxygen consumption of endometrial tissues from patients at different stages of the menstrual cycle

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EP, early proliferative; MP, mid-proliferative; LP, late proliferative; OV, ovulatory; ES, early secretory; MS, mid-secretory; LS, late secretory.

* P < 0.05; NS, not significant.
general, adjacent groups were significantly different. The ratio of the mean consumption in the oxygen phase compared with that in the air phase was 3.57 for early proliferative-stage tissue; 1.66 for ovulatory-stage tissue; 1.81 for early secretory-stage tissue; 1.53 for mid-secretory and 1.64 for late secretory-stage tissue. Therefore apart from the early proliferative stage, the ratio was similar suggesting that the diffusion of oxygen across different thicknesses of endometrium at the various stages of the menstrual cycle was not a major cause of the differences.

Measured as per mg protein. When total protein was expressed as a percentage of the endometrial sample dry weight, there was a dramatic change during the normal menstrual cycle (Text-fig. 3a). Late secretory stage tissues could be separated into two distinct groups, with those from the last 2 days of the cycle being designated as very late secretory. The pattern obtained was qualitatively identical to that for the mg dry weight basis for both gas phases, although the rise during the proliferative phase was steeper and the fall in the secretory phase shallower, with a fall in the very late secretory phase (Text-fig. 4a).

![Graphs showing protein and DNA percentages](Text-fig. 3. Percentages of (a) protein and (b) DNA of the endometrial dry weight throughout the normal menstrual cycle (see Text-fig. 2 and text). Values are mean ± 1 s.e.m. for the no. of observations indicated.)
Text-fig. 4. Mean endometrial oxygen consumption throughout the normal menstrual cycle when based on (a) protein and (b) DNA content (see Text-fig. 3).

Measured as per mg DNA. The total DNA expressed as a percentage of the endometrial samples dry weight showed a pattern similar to that for the total protein (Text-fig. 3b) and the patterns for oxygen consumption on this basis were qualitatively identical to those obtained for the mg dry weight basis (Text-fig. 2) and mg protein basis (Text-fig. 4a) for both gas phases.

Effect of the age of the patients on endometrial oxygen consumption

Regression lines and coefficients of correlation were calculated for oxygen consumption, in air- and oxygen-gassed medium, of tissues at the various stages of the normal menstrual cycle from patients aged 18–50 years. There was no statistical correlation with the measured oxygen consumption at any phase of the menstrual cycle.

Discussion

The results show for the first time that there is a consistent pattern for endometrial oxygen consumption during the menstrual cycle with a distinct peak around the time of ovulation, regardless of whether an air or oxygen gas phase is used and the basis for calculation. As the pattern of oxygen consumption per mg dry weight was unaltered when expressed per mg protein or per mg DNA, the changes observed during the menstrual cycle are probably due to functional changes of the endometrial cells.

The increased endometrial oxygen consumption during the proliferative phase of the menstrual cycle occurs at a time of increasing oestrogen levels. Around the time of ovulation (when oxygen consumption peaks), 'giant' mitochondria with well-developed cristae are found (Wynn, 1977). If the endometrial oxygen consumption were sensitive only to the oestrogen level, a second peak would be expected around Day 16–18, coinciding with the secretion of oestrogen by the corpus luteum but we did not detect such a peak (Text-fig. 2). The decline of the oxygen uptake during the secretory phase may be due to the falling oestrogen concentrations, to an inhibitory effect of progesterone or to both.
The consumption of oxygen was significantly greater at each phase of the cycle when oxygen rather than air was used to gas the incubation medium, showing that the availability of oxygen was important in governing the oxygen usage by the endometrium. It is possible that this may be a factor in the lack of change in the low oxygen tension (about 15 mmHg) of the luminal bulk fluid of the human uterus during the menstrual cycle (Yedwab, Paz, Hommonnai, David & Kraicer, 1976). Alternatively, the increase in the in-vitro endometrial oxygen consumption at each phase of the normal menstrual cycle with the high oxygen incubation medium may result from the diffusion of oxygen through the endometrial strip being rate limiting. With the high oxygen tension, the diffusion may be sufficient to sustain the oxygen consumption whereas the lower oxygen tension may not allow adequate oxygen diffusion. However, if oxygen diffusion were rate limiting it would be expected that the thicker the endometrial strip, the greater the differential between the oxygen uptake in the high and low oxygen tension. The endometrial strip increased in thickness from the late proliferative to mid-secretory phase of the normal cycle but the calculated ratio of the oxygen uptake (high oxygen/low oxygen) appears to remain relatively constant throughout this period and is only higher in the early proliferative phase when the strip is thinner. This suggests that rate-limiting oxygen diffusion throughout the endometrial strip is not the overriding cause of the increased oxygen uptake in the high oxygen tension.

The findings from the present study indicate a lower oxygen consumption in endometrium taken from just above the internal os. This may explain some of the contradictions from previous studies in that the site of the endometrial sampling must be standardized. This lowered oxygen consumption may have biological relevance in that implantation usually occurs high in the uterus and rarely in the thinner endometrium near the cervix. This could be due to the endometrium in this area having an inadequate metabolic activity to allow implantation. Tsibris, et al. (1978) have reported that the unoccupied cytosolic hormone receptor levels in the human uterus are higher at the fundus than around the cervix at all times of the menstrual cycle and this is again consistent with functional differences within the uterus. It is not possible to compare our present finding of lack of change with age directly with that of Hackl (1973) who showed a decrease of oxygen consumption with increasing age in the mid- and late secretory phases of the cycle because he used a manometric method with a long incubation period.

Finally, our experiments have shown that intact tissue respires approximately 60% better than endometrium that has been minced or chopped. In the only other study that used an oxygen electrode (Okagaki & Richart, 1970) the endometrium was minced and measurements were made in an air-gassed medium 30–120 min after removal from the patients. The maximum values obtained (2 μl oxygen consumed/mg dry weight) were extremely low and we assume that these were due to the damaging effect of the mincing.

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References


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