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White adipocytes in primary culture. Long-term microcalorimetric and biochemical investigations.

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Abstract

A novel method was developed to facilitate long-term microcalorimetric and biochemical studies in white fat cells. Isolated rat adipocytes were primary cultured in a 3D-matrix of agar gel. Thermogenesis was measured continuously over 72h by using an LKB BioActivityMonitor. Glucose and lactate concentrations were determined in the medium of parallel cultures every 24h.

Glucose consumption and lactate production rates were constant over 72h whether employing aerobic (0.50 and 0.44 pmol/cell•h, respectively) or partial anaerobic culture conditions (0.41 and 0.57 pmol/cell•h, respectively). Aerobic (94 pW/cell) and partial anaerobic thermogenesis (39 pW/cell) were maintained during 72h. Addition of isoproterenol (10^{-5}M) increased aerobic heat production by 48%.

1. INTRODUCTION

In every living system energy is taken up and metabolized or stored. Via the thermogenic process heat is produced by cellular metabolism and subsequently dissipated into the environment. A diminished capacity of thermogenesis in relation to the actual energy intake leads ultimately to an increase of adipose tissue which is the major energy reservoir of the body [1]. Undoubtedly many investigators have been challenged to deal with cellular heat exchange. However, the present knowledge about energy expenditure on cellular level is chiefly based on animal experiments and mainly restricted to yield estimation of substrate exchange as measured in blood or occasionally in tissue samples. Direct microcalorimetry may represent a novel approach to characterize energy balance by direct determination of heat output of isolated cells allowing sensitive and continuous assessment of cellular heat production.

Until now microcalorimetric studies of isolated white adipocytes have only been employed in cell suspensions [2-6]. These cells, however, exhibit a short lifetime in suspension which invalidates long-term or repeated measurements [7]. According to a recent report the viability of white adipocytes can be maintained in gel cultures up to six weeks [8]. Thus, we employed these suggestions for long-term (72h) microcalorimetric investigations in rat adipocytes. In this report continuous measurement of heat production was combined with simultaneous assessment of glucose consumption and lactate and pyruvate production in fat cell cultures.

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2. METHODS

2.1. Materials

Epididymal and retroperitoneal adipose tissue was obtained from male Sprague-Dawley rats. Medium 199 was purchased from Gibco Ltd. and supplemented with HEPES (300 mg/l) and 10% fetal calf serum (Boehringer Mannheim). Collagenase type I was from Worthington Biochemical.

2.2. Primary culture

White fat cells were isolated according to a modification of the method of RODBELL [9;10]: Tissue samples were minced with scissors and incubated for 60 min at 37°C in culture medium containing 2 mg/ml collagenase. Isolated fat cells were filtered through a nylon mesh and subsequently washed three times with culture medium. Cell number was determined microscopically with a hemocytometer.

Gel cultures were prepared in glass ampoules. 150 µl of packed adipocytes were embedded in a 3D-matrix of agar gel which was prepared as described by FRESHNEY [11]. Then the ampoules were filled up to 1.5 ml ("aerobic culture conditions") or 2.25 ml ("partial anaerobic culture conditions") with agar-free medium. The gas phase was 1.5 or 0.75 ml air with 5% CO_2. Aliquots of isolated adipocytes were maintained in suspension cultures for comparison. These cultures were prepared by filling ampoules with 1.5 ml of cell suspension.

2.3. Microcalorimetry

Thermogenesis in fat cell cultures was measured for 72h with a thermopile heat conduction calorimeter (LKB BioActivityMonitor 2277) fitted with four ampoule measuring cylinders (LKB 2277-202) as described by WADSÖ and co-workers [12;13]. Under the experimental conditions used in the present work initial instability of the system hampers reliable assessment of heat production during the first 20 hours. Therefore we measured thermogenesis between 20 and 72h. The precision of the measurements was 9.0 % (C.V.).

The effect of isoproterenol on fat cell heat production was assessed in the following manner: Basal thermogenesis was monitored in 4 identical cultures simultaneously for 24h. Then the ampoules were taken out of the calorimeter. 50 µl of hormone solution were injected with sterile syringe and needle through the caps of two ampoules. 50 µl of medium without additives were injected into the remainig two ampoules. Then all ampoules were inserted into the calorimeter. After 60 minutes the measuring system was equilibrated and hormone induced heat production could be measured.

2.4. Biochemical analyses

In order to assist interpretation of the microcalorimetric measurements concentrations of glucose, lactate and pyruvate in the medium of parallel cultures were determined after 0, 24, 48, and 72h by known enzymatic methods [14-16]. Cell viability was determined by fluorescence microscopy after staining with acridine orange (0.1 % w/v) [17].

3. RESULTS

3.1. Cell viability

Viability of adipocytes in gel culture was >90% throughout the whole period of 72h as indicated by acridine orange staining. In suspension cultures however, adipocyte viability declined rapidly during the experiment. After 72h cell necrosis was widely apparent as jugded by microscopic examination.

3.2. Glycolysis

The rates of glucose consumption and lactate production of adipocytes in gel culture were constant during 72h as indicated by the linear time-course of glucose and lactate concentrations in the medium (Fig. 1). Under partial anaerobic culture conditions glucose consumption was 18% lower and lactate production was 30% higher than that under aerobic conditions. The ratio of glucose uptake to lactate production was 1 : 1.4 under partial anaerobic and 1 : 0.9 under aerobic conditions. Pyruvate production was negligible irrespective of culture conditions (Fig. 2).

3.3. Basal thermogenesis

The average unstimulated heat production of adipocytes in gel culture under partial anaerobic experimental conditions (n=9) was 58% lower than that under aerobic conditions (n=4). The initial measurements exhibited cellular heat production of 39 ± 9 pW under partial anaerobic and 94 ± 7 pW under aerobic conditions (mean ± SE; $p<0.005$). Throughout the remaining 50h heat production declined by 21% under partial anaerobic and 20% under aerobic conditions. The course of thermogenesis is illustrated in Fig. 3. In suspension cultures thermogenesis declined approximately exponential by 75% within 50 hours (data not shown).

3.4. Hormone induced thermogenesis

Heat production of adipocytes in gel culture under aerobic culture conditions was increased by 48 ± 8 % with 10^{-5}M isoproterenol (mean ± SE; n=10; $p<0.001$). Lower concentrations of isoproterenol (10^{-6}M; n=4) had no significant effect on thermogenis.

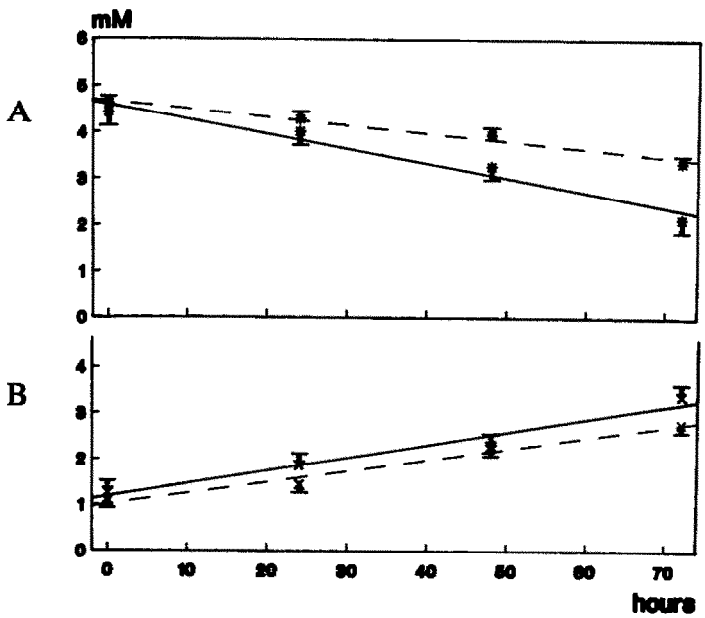

Figure 1. Time-course of glucose (A) and lactate (B) concentrations in the medium of fat cell gel cultures under aerobic (-----) and partial anaerobic (——) conditions (mean ± SD)

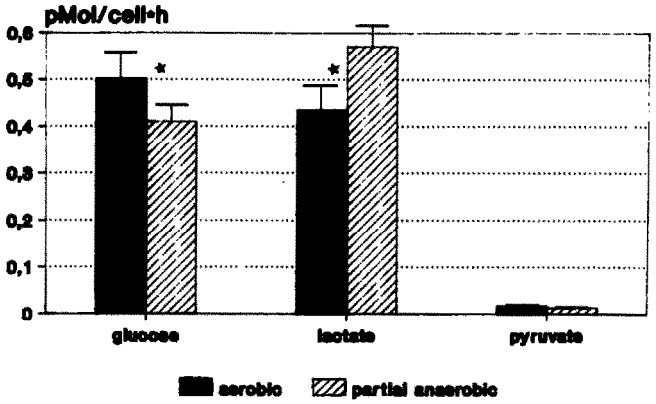

Figure 2. Glucose consumption and lactate and pyruvate production of fat cells in gel culture under aerobic and partial anaerobic culture conditions (mean ± SD; * $p < 0.05$)

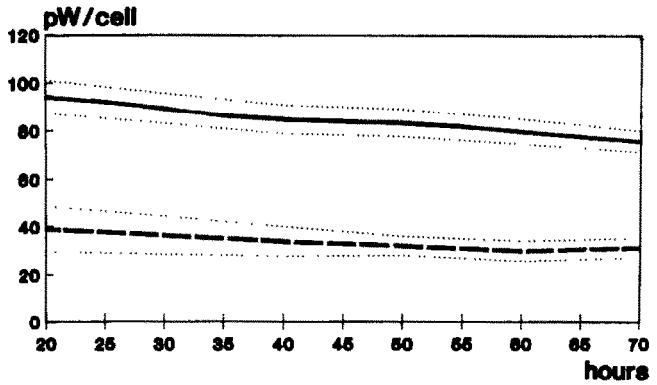

Figure 3. Cellular heat production of fat cells in gel culture under aerobic (——) and partial anaerobic (- - -) culture conditions (mean ± SE; $p < 0.001$)

4. DISCUSSION

The original method for culturing white fat cells in collagen gel [8] could not be entirely adopted in the present study. Collagen solution gels very slowly thus suspended cells gather at the surface without being fixed within the gel. Therefore as a minor modification, aliquots of packed adipocytes were enclosed in agar which gels much faster than collagen gel.

Under aerobic culture conditions rat adipocytes cultured according to this technique showed a somewhat higher glucose consumption and lactate production (Fig. 2) than in other studies performed in suspensions (0.3-0.4 and 0.2-0.3

pMol/cell•h, respectively). However, the percentage of glucose metabolized to lactate under aerobic conditions (44%) was the same as reported by other investigators (40-50%) [18-20].

Basal thermogenesis of gel-cultured adipocytes as measured in the present study is in line with previously reported short-term assessment of heat production in suspension cultures from rats (40 pW/cell) [2] and humans (49 pW/cell) [4;5]. Long-term microcalorimetry in fat cell suspensions however, revealed a sharp decline of heat production after 6-10h of culture. This was presumably due to the declining number of living cells rather than to a diminished cellular heat production. Occurrence of cell necrosis in suspension cultures as observed in the present study was in agreement to the theory of large cell necrosis [7].

An interesting observation in the present investigation was the lower heat production of adipocytes in gel culture during partial anaerobiosis as compared with aerobic conditions. This phenomenon can be explained by the decreased glucose consumption presumably associated with diminished glucose oxidation since lactate production is elevated. The apparent decrease in glucose oxidation is of the same relative magnitude as the decrease in thermogenesis since glucose degradation to lactate yields considerably smaller change in free energy than that to CO_2.

The addition of the ß-agonist isoproterenol to fat cells in gel culture resulted in an immediate increase of thermogenesis which turned back to the initial rate of thermogenesis after several hours. This effect is probably due to ß-adrenergic stimulation of triglyceride - fatty acid cycling as earlyer postulated [21;22].

The great strength of long-term microcalorimetry as described in the present study is the capability to refer heat production to prolonged biochemical, endocrinological and immunological processes wich may indeed control and regulate thermogenesis in the cell. An appropriate assessment of cellular energy expenditure requires metabolic steady state. Instability of the system is always accompanied with variations emerging from measurement at metabolic disequilibrium invalidating the assessment of thermogenesis. Actually in the present study the relative rate of heat production, glucose consumption and lactate production were virtually maintained over 72h indicating metabolic steady state and, thus, adequate measurements of thermogenesis. Indeed, epididymal and retroperitoneal rat adipocytes as used in the present work are certainly not representative for human fat cells with regard to their metabolic behaviour. Yet the results of this methodological study may assist in future evaluation of thermogenesis in human adipocytes in vitro.

Acknowledgements

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