

Hepatocyte thermogenesis revisited

Dallas Clark¹, Menno Brinkman¹, John Phillips², Tony Grivell², and Michael Berry²

¹CSIRO, Division of Human Nutrition, Kintore Avenue, Adelaide, South Australia

²Department of Medical Biochemistry, Flinders University of South Australia, Bedford Park, South Australia

Abstract - Aerobic studies with isolated rat hepatocytes have shown that as the concentration of extracellular glucose was raised from 10 up to 80 mM rates of lactate formation and the ratios of heat produced per unit of oxygen consumed were also increased. These results suggest that there is a relationship between the formation of lactate and the heat/oxygen (kJ/mol) ratio.

INTRODUCTION

During the last fifteen years microcalorimetry has been employed to assess actual rates of heat production in cells and tissues which can have very high rates of oxygen consumption. The preparations used in these investigations include intact bundles of skeletal muscle¹, hepatocytes from euthyroid and hyperthyroid rats² and hormonally-stimulated isolated brown fat cells^{3,4}. Although the fully dissipative, aerobic catabolism of glucose by these cellular systems should produce 470 kJ of heat per mol of oxygen consumed⁵ it is frequently found that these and other mammalian cell preparations produce 500 to 800 kJ of heat per mol of oxygen⁵. Gnaiger & Kemp⁵ have argued that ratios higher than 470 kJ/mol result from the anaerobic formation of lactate (and/or other glycolytic end-products) during the aerobic catabolism of glucose.

We have recently demonstrated that the accumulation of lactate by isolated rat hepatocytes required the presence of supraphysiological concentrations of extracellular glucose⁶. As the concentration of added hexose was increased from 20 through 40 to 80 mM, rates of lactate formation were also increased: these rates were not linear and reached steady state levels of ~1.7, ~2.8 and ~3.5 mM, with 20, 40 and 80 mM glucose respectively, after 50-60 minutes of incubation⁶. The differences in rates of lactate formation, with the rat hepatocyte preparation, provide a model which can be used in attempts to determine whether high heat/oxygen ratios result from anaerobic processes.

MATERIALS AND METHODS

Collagenase, bovine serum albumin (fraction V) and enzymes for metabolite determination were from Boehringer Mannheim (F.R.G.). The albumin was defatted by the method of Chen⁷ before use. All other chemicals were of the highest quality available.

Hepatocyte preparation and incubation. Isolated liver parenchymal cells were prepared from male Hooded Wistar rats (250-280g body wt.) that had been deprived of food for 24 hours, by a modification⁸ of the method of Berry & Friend⁹. The hepatocytes (~30 mg dry wt.) were incubated in 4.0 ml of a balanced bicarbonate buffered medium^{10,11} containing albumin, 2.25% (w/v) under a gas phase of O₂:CO₂ (95:5) in a modified LKB model 10700 batch-type microcalorimeter^{4,12} which was operated at 37°C. Consumption of oxygen was

determined polarographically in a Yellow Springs Instrument model 53 O₂ electrode assembly at the same time as the measurements of heat production^{4,12}. At the completion of the incubation period (30 minutes) samples taken from the microcalorimeter were deproteinised with an equal volume of cold 1M-perchloric acid and were neutralised⁸. Metabolites were measured by standard enzymatic techniques¹³ using a Cobas FARA automated analyser (Roche Diagnostics, Basle).

The dry weight of each liver cell preparation was determined gravimetrically and hepatocyte numbers were counted in an improved Neubauer haemocytometer. Wet weights of the hepatocyte preparation were obtained by multiplying the dry weight by 3.77¹⁴. On average 1g wet wt. of liver cells contained ~1.20 x 10⁸ hepatocytes.

RESULTS

The endogenous rates of heat production and oxygen consumption and the ratio of heat produced per mol of oxygen consumed (Table 1) are close to the values expected for hepatocytes prepared from rats which have been deprived of food for 24 hours¹⁵.

TABLE 1. The effects of increasing glucose concentration on lactate formation, heat production, oxygen consumption and the ratio of heat production to oxygen consumption in isolated rat hepatocytes.

Additions to medium	Lactate formation (umol/min per g wet wt.)	Heat production (J/min per g wet wt.)	O ₂ consumption (umol/min per g wet wt.)	Ratio (kJ/mol)
None	0.12 ± 0.03	1.02 ± 0.03	2.14 ± 0.06	-477 ± 15
10mM Glucose	0.15 ± 0.06	1.16 ± 0.05	2.36 ± 0.37	-492 ± 35
20mM Glucose	0.91 ± 0.12	1.35 ± 0.05	2.62 ± 0.12	-515 ± 35
40mM Glucose	1.64 ± 0.23	1.45 ± 0.07	2.58 ± 0.13	-562 ± 52
80mM Glucose	1.68 ± 0.25	1.48 ± 0.08	2.45 ± 0.10	-604 ± 45

As the concentration of extracellular glucose was increased through 10, 20, 40 and 80 umol/ml both the rate of lactate formation and the rate of hepatocyte heat production continued to increase although both measurements had nearly plateaued at the highest glucose concentration (Table 1). This was not the case with the oxygen consumption data which increased following the addition of 10 and 20 mM glucose, plateaued with 40 mM glucose and then appeared to decrease in the presence of 80 mM glucose (Table 1). The differences between the rates of heat production and oxygen consumption are reflected in the heat/oxygen ratios which increased from -477 kJ/mol (the negative sign indicating energy loss from the system) in the absence of added substrate, through -515 kJ/mol with 20mM glucose up to -604 kJ/mol in the presence of 80mM glucose.

In these experiments there was a nearly linear relationship between the molar lactate/oxygen ratios⁵ and the heat/oxygen ratios during the endogenous incubations and the metabolism of 10, 20 and 40 mM glucose ($y=132.7x + 474.9$; $r^2=0.964$; Fig. 1).

DISCUSSION

The ratios of heat produced per unit of oxygen consumed for hepatocytes incubated in the absence of added substrates (Table 1; Ref 15) are very similar to the theoretical, thermochemically derived heat/oxygen ratios for the fully dissipative, aerobic catabolism of glucose (-469 kJ/mol), palmitate (-434 kJ/mol) and protein to urea (-438 kJ/mol)⁵. However, the addition of supraphysiological (40 and 80 mM) concentrations of glucose to these hepatocyte preparations resulted in marked increases in the heat/oxygen ratios (Table 1).

The question which needs to be addressed is why should an increase in extracellular glucose produce such a marked effect on the heat production/oxygen consumption ratio?

Gnaiger and Kemp⁵, using data from a number of different laboratories, have suggested that high negative heat/oxygen ratios can result from the production of lactate, or other glycolytic end-products, during anaerobic glycolysis under aerobic conditions. They have pointed out that the net production of lactate from glucose in a bicarbonate buffered system is accompanied by a dissipative, catabolic, enthalpy change of -63 kJ/mol of lactate⁵.

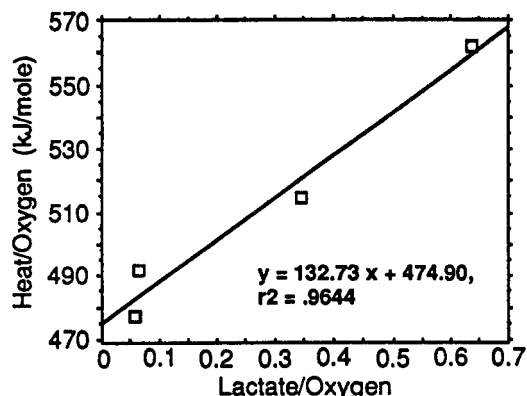


Fig. 1 Relationship between the heat to oxygen and lactate to oxygen ratios

The present investigation, like our previous study with supraphysiological concentrations of extracellular glucose⁶, has shown that there is a marked increase in the initial rate of lactate production and its accumulation as the concentration of extracellular glucose was raised. Furthermore the relationship between total lactate accumulation and the ratios of heat produced to oxygen consumed with 0, 10, 20 and 40 mol glucose was nearly linear ($r^2 = 0.96$; $P < 0.01$; Results not shown). These data indicate that heat/oxygen ratios more negative than -470 kJ/mol could result from lactic acid formation during the aerobic catabolism of glucose by isolated rat hepatocytes. However, an enthalpy change of -63 kJ per mol of lactate formed⁵ accounts for only ~40% of the observed increase in the heat production/oxygen consumption ratios, or ~50% when the accumulation of pyruvate is also considered.

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