Turnover and Oxidation Rates of Blood Glucose and Heat Production in Sheep Exposed to Heat

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ABSTRACT

Five shorn sheep were used to study effects of heat exposure (30°C, relative humidity 70%) on metabolism of blood glucose and heat production. The isotope dilution method was applied for determination of metabolism of blood glucose in a thermoneutral environment (20°C, relative humidity 70%) and on the 4th and 10th days of heat exposure. Concentrations of free fatty acids and thyroxine of plasma decreased during heat exposure with no reduction in feed intake. At 20°C, glucose turnover rate, percentage of glucose oxidized, and contribution of exhaled carbon dioxide derived from glucose to the total carbon dioxide production were 6.2 mg/kg·h⁻¹ per min, 34%, and 11%. Glucose turnover rate decreased significantly during heat exposure, but the proportion of glucose oxidized was unchanged. Heat production decreased almost parallel to the glucose turnover rate during heat exposure, resulting in an unchanged contribution of carbon dioxide derived from glucose to the total production of carbon dioxide. Blood glucose metabolism and heat production of sheep decrease, but the contribution of blood glucose to heat production remains unchanged during exposure to 30°C.

INTRODUCTION

Homeotherms are able to increase heat loss and reduce heat production to maintain a constant body temperature at high ambient temperatures. Sheep have the great advantage of heat tolerance because the wool coat provides protection against the heat of solar radiation (20). Panting and evaporation from the respiratory tract is the most important mechanism for heat loss in the sheep.

In ruminants only small quantities of glucose are absorbed from the alimentary tract, and most of the energy is supplied as volatile fatty acids produced by fermentation of ingested dietary carbohydrates in the rumen (2). This accentuates the importance of gluconeogenesis in ruminant metabolism. Glucose turnover rates in sheep have been studied extensively (3, 4, 8, 10, 13, 16, 23). However, there are few published data on the kinetic pattern of glucose metabolism in sheep at high or low environmental temperatures (1, 17, 22). Effects of high ambient temperatures on glucose metabolism may be of special importance for productivity of ruminant livestock with their reliance upon gluconeogenesis. Our report (17) showed that both pool size and turnover rate of blood glucose tended to decrease on the 4th and 10th days of heat exposure (30°C) in sheep. However, differences were not clear cut because of the small experiment.

The present study was to investigate the contribution of blood glucose to heat production as well as to confirm the tendency for decreased turnover rate of blood glucose of sheep exposed to heat.

MATERIALS AND METHODS

Experimental Animals

Five Corridale ewes, weighing 32 to 44 kg, were shorn closely (under 1 cm) before the experiment. The animals were kept in metabolic cages placed in a controlled environment cham-
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ber with temperature 20 ± 1°C and relative humidity 70 ± 5% for more than a week. Subsequently the temperature in the chamber was elevated to 30 ± 2°C with a relative humidity of 70 ± 5%, and the animals were exposed to this condition for 10 days. Each ewe was given 400 g of the ration (alfalfa hay cube 50%, commercial concentrate 50%) twice a day at 0900 and 1800 h. The ration contained 2.75 Mcal digestible energy per kilogram, 14% crude protein, and 15% crude fiber. Water was given ad libitum. Two of five animals were used twice for the experiment with an interval between the two experiments of heat exposure of at least 6 mo. For more than a month before the experiment, ewes were trained to wear a face mask each day so that they would remain unstressed for collections of exhaled gas. Polyethylene catheters were inserted into both jugular veins at least a day before blood sampling commenced.

Experimental Procedure

Physiological responses were observed and blood samples (10 ml) were collected just before the morning feeding for 4 days in the thermoneutral period (20°C) and for 9 days of heat exposure, starting on the 2nd day. Heat production was measured by collecting exhaled gas through the face mask into a Douglas bag. Collections were daily throughout the experiment for 10 min between 6 and 7 h after morning feed.

Measurement of metabolism of blood glucose using the isotope dilution method was on the 4th day of the 20°C period and the 4th and 10th days of heat exposure. On the experimental day, 25 μCi of [U-14C]glucose was injected through one catheter into the jugular vein 3 h after morning feeding. Immediately after the priming injection, labeled glucose was infused constantly for 5 h at .25 μCi/min (.5 μCi/ml of saline). Venous blood (5 ml) and exhaled gas samples were taken at intervals of 30 min for the last 2 h of the infusion period. Blood samples were centrifuged at 0°C, and plasma was frozen at −20°C until analysis. An aliquot of the exhaled gas was collected into 200 ml of 1 N NaOH to determine the specific activity of CO2.

Analytical Methods

Plasma glucose, free fatty acids (FFA), and thyroxine (T4) were determined by the same methods as in (17). Oxygen consumption and carbon dioxide production in exhaled gas were measured gas chromatographically (Yanaco, Model G 80). Heat production was calculated from oxygen consumption and carbon dioxide production as described by Young et al. (25). Therefore, estimates slightly overestimate true heat production. Isolation of glucose from plasma and measurement of radioactivity of glucose were as described in (13, 17). To measure radioactivity of exhaled CO2, a fraction (10 ml) of the alkali used as a CO2 trap was acidified with 6 N H2SO4 in an Erlenmeyer flask with a center well, and the liberated CO2 was trapped into 1 ml of Scintilamine-OH (Wako, Osaka, Japan) in the center well. After standing for more than 90 min, the Scintilamine-OH solution was transferred with 10 ml of scintillation solution (PPO: 2,5-diphenyloxazole, 5 g and POPPOP: 1,4-bis[2-(5-phenyloxazolyl)]benzene, 100 mg in 1 liter of toluene) into a counting vial, and the radioactivity of CO2 was measured by a liquid scintillation spectrometer (Packard, Model 3385). The amount of exhaled CO2 was determined by titrating a second fraction (10 ml) of the CO2-collecting 1 N NaOH with 1 N and .1 N HCl with phenolphthalein and methyl orange as indicators, respectively (1). Calculations of statistics of glucose metabolism were by the method of Bergman (3).

Statistical Analysis

Results are expressed as mean ± SD of five sheep. Significant differences in physiological responses and plasma constituents were tested by Student's paired t test comparing five means for 4 days at 20°C with those for each day of heat exposure. For plasma T4, however, measurements were only on 1 day at 20°C. Significant differences in glucose metabolism were tested by Student's paired t test comparing means at 20°C with those for the 4th and 10th day of heat exposure.

RESULTS

Physiological Responses and Blood Constituents

Each ewe consumed all the ration fed, and body weights changed little during the experi-
mental period. Respiration rate increased markedly \((P<.01)\) from 20 ± 4 resp./min at 20°C to 120 ± 59 resp./min on the 5th day of the heat exposure. Rectal temperature was elevated \((P<.01)\) from 38.7 ± .3°C at 20°C to 39.7 ± .7°C on the 4th day of the heat exposure. Heart rate did not change significantly.

At 20°C, heat production (Figure 1) changed little day to day, and the mean for 4 days was 4.1 ± .4 kcal/kg·75 per h. On the first day of heat exposure, heat production increased slightly, but through the later experimental period it was maintained at a relatively low level compared with that at 20°C.

Plasma FFA and \(T_4\) concentrations decreased significantly during heat exposure, but plasma glucose concentration remained unchanged (Figure 2).

**Blood Glucose Metabolism in Relation to Heat Production**

The concentration of plasma glucose was nearly constant, and specific activities of plasma glucose and exhaled CO₂ reached plateaus at 3 h after the beginning of the primed infusion of \([U-14C]glucose\) and thereafter in all experiments (Figure 3).

Characteristics of blood glucose metabolism are in Table 1. Turnover rate of blood glucose decreased significantly on both the 4th and 10th day of the heat exposure. However, the proportion of glucose that was oxidized was unchanged. Heat production decreased almost parallel to the blood glucose turnover rate, with a correlation coefficient of .64 \((P<.01)\). The contribution of exhaled CO₂ derived from glucose oxidation to total CO₂ production remained unchanged throughout the experimental period.

![Figure 1](image1.png)

*Figure 1. Changes in heat production following heat exposure in five sheep. Data are expressed as mean ± SD. *Significant at \(P<.05\) compared with thermoneutral mean.*

![Figure 2](image2.png)

*Figure 2. Changes in plasma glucose, free fatty acids (FFA), and thyroxine (\(T_4\)) concentrations following heat exposure in five sheep. Data are expressed as mean ± SD. **Significant at \(P<.01\) compared with thermoneutral mean. *Significant at \(P<.05\) compared with thermoneutral mean.*

![Figure 3](image3.png)

*Figure 3. Plasma glucose concentration and specific activities of plasma glucose and exhaled CO₂ during the infusion of \([U-14C]glucose\) (priming dose, 25 \(μCi\); infusion rate, .25 \(μCi/min\)). Data expressed as mean ± SD in five sheep exposed to 20°C (○) and 30°C with days 4 and 10 combined (●).*
TABLE 1. Plasma glucose concentration, glucose turnover, percentage of glucose oxidized, heat production, and percentage of exhaled CO₂ derived from blood glucose in five sheep in thermoneutral (20°C) and hot (30°C) environments.

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>Concentration (mg/dl)</th>
<th>Turnover rate (mg/kg T S per min)</th>
<th>Oxidation rate (%)</th>
<th>Heat production (kcal/kg T S per h)</th>
<th>Percentage of exhaled CO₂ derived from blood glucose (%)</th>
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<td>Thermoneutral</td>
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<td>37.3</td>
<td>4.5</td>
<td>57.4</td>
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<td>On the 4th day of exposure to 30°C</td>
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<td>38.2</td>
<td>3.9</td>
<td>61.3</td>
<td>3.7</td>
<td>5.2</td>
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<td>On the 10th day of exposure to 30°C</td>
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<td>38.0</td>
<td>4.0</td>
<td>59.9</td>
<td>6.2</td>
<td>5.1</td>
<td><strong>34.7</strong></td>
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<td>3.5</td>
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</table>

*Significant at P<.05 compared with the thermoneutral mean.

**Significant at P<.01 compared with the thermoneutral mean.
DISCUSSION

Sheep exposed to hot environment (30°C, R.H. 70%) in a climatic chamber exhibited panting, hyperthermia, and lower heat production, although feed intake was not affected. These results mean that the environmental temperature in the experiment might be a little higher than the critical point of the thermo-neutral zone for shorn sheep. Johnson and Vanjonack (9) concluded in their excellent review that heat production of lactating cattle exposed to high environmental temperatures declined following an initial "overshoot". The slightly increased heat production on the first day of heat exposure in the present experiment was obtained between 3 and 4 h after initiation of heat exposure. The decrease in heat production during heat exposure is correlated closely with changes of blood concentrations of such calorigenic hormones as T₄, GH, catecholamines, and glucocorticoids (21). Therefore, it is likely that the decrease in heat production during heat exposure relates in part to the decrease of concentration of plasma T₄.

Concerning the response of the sympathetic nervous system in the heat exposed animal, Sasaki et al. (18) reported that the urinary excretion of catecholamines in hay fed sheep housed at 30°C for 1 wk did not exhibit a clear change, and hyperthermia also was not observed. Fiorica et al. (7) reported that the concentration of plasma catecholamines was not affected by acute heat exposure in the dog, a panting animal. Petrovic and Markovic-Giaja (15) reported that the increase in heat production after injection of noradrenaline was smaller for heat acclimated rats than for thermoneutral acclimated rats. Although we did not measure catecholamines, the concentration of FFA, which often are mobilized by catecholamines, was depressed. This indicates that sympathico-tonia did not develop from heat exposure.

The plasma concentration of glucose changed little both before and during the heat exposure period; however, turnover rate of blood glucose decreased significantly from 6.2 mg/kg⁻¹ per min at 20°C to 5.2 mg/kg⁻¹ per min on the 4th day (P<.01) and 5.1 mg/kg⁻¹ per min on the 10th day (P<.05) of exposure to 30°C. The turnover rate of blood glucose was smaller by approximately 15% where primed continuous infusion method of [U-¹⁴C] glucose was applied than when the single injection method was used (17) although nutritional and environmental conditions were the same in both experiments. The comparison of single injection and primed continuous infusion is discussed by several investigators. Scarey et al. (19) found in dog the former led to an apparent overestimate. However, Young (24) concluded in ruminants that the two methods of isotope administration gave the same result. Lindsay (12) reviewed glucose metabolism in ruminants and reported that glucose oxidation represents about 30 to 35% of turnover in various conditions, except in lactation (only 20%), and that glucose contributes 4 to 11% to respiratory CO₂. Means of the present experiment are within the limits of those.

The decreased turnover rate of glucose during heat exposure may result from decreased substrate supply, perhaps combined with a decreased hepatic capacity for gluconeogenesis. We succeeded in making the sheep consume all the ration given during the experimental period, important because glucose metabolism is influenced largely by energy intake (12). Positive correlation has been strong between production of propionate, a major precursor of blood glucose, in the rumen and entry rate of blood glucose (6). Kelley et al. (11) reported that volatile fatty acid production in the rumen of cattle was significantly less at 37.7°C than at 18.2°C, with a particularly marked decrease in propionate production. It is uncertain whether similar changes occurred in the present experiment in sheep exposed to 30°C, but the decrease in turnover rate of blood glucose might be related partly to decreased production of propionate in the rumen during heat exposure.

Gluconeogenesis is accelerated by cold exposure in the rat liver (14). However, information is scanty concerning the effect of heat stress on activities of enzymes of glucose metabolism. Chayoth and Cassuto (5) showed a marked reduction in activity of glucose-6-phosphatase [EC. 3.1.3.9] in the liver of heat acclimated hamsters.

The contribution of oxidized blood glucose to the total CO₂ production remained unchanged during heat exposure. The result has been the same in various conditions such as pregnancy (3), lactation (4), and cold exposure in sheep (22) in which the metabolic rates are accelerated markedly.

ACKNOWLEDGMENTS

The authors are most grateful to T. E. C. Weekes, The University of Newcastle, for his kind advice on the manuscript. The authors are grateful to M. Fujita and S. Takahashi for their analytical assistance and Y. Otomo for his skilled technical assistance.

REFERENCES