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Microcalorimetric determination of blood heat output in human neonates ☆

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Abstract

Based on earlier results on a body size relationship of blood heat output in different mammalian species, an intra-specific comparison of blood heat output in human neonates and adults was made. To avoid taking additional blood samples from neonates, a resuspension technique was developed, allowing the use of material left over from clinical routine sampling. In 13 adult volunteers, microcalorimetric heat output ($\bar{x} \pm$ SEM) was 48.1 ± 0.8 μW $(ml_{blood})^{-1}$ or 105.6 ± 2.2 μW $(ml_{cells})^{-1}$ in the whole blood and 47.3 ± 1.6 μW $(ml_{susp.})^{-1}$ or 104.0 ± 3.3 μW $(ml_{cells})^{-1}$ in the resuspended samples, respectively, proving that the resuspension procedure did not alter the thermal power values. Using this technique on blood samples from 38 neonates in their first week of life, a heat output of 77.2 ± 3.1 μW $(ml_{susp.})^{-1}$ or 151.8 ± 5.9 μW $(ml_{cells})^{-1}$ was found. This is significantly higher than in adults, and fits the body size relationship of blood heat output previously described for different mammalian species.

Keywords: Blood; Microcalorimetry; Neonate

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1. Introduction

As blood heat output arises from different cellular fractions [1], microcalorimetry has mostly been used to study the metabolism of *isolated* blood cells and their reactions to various states of illness [2–4]. However, in a recent microcalorimetric study on *whole* blood samples from different mammalian species, a decrease in heat output with increasing body size was found, corresponding to the size relationship of basal metabolic rate [5]. This intriguing result led to the question of whether a similar relationship could also be found within one and the same species. Therefore we decided to make an intra-specific comparison of blood heat output between human neonates and adults. To avoid additional blood sampling from neonates, a resuspension technique was developed, allowing the use of blood left over from clinical routine sampling.

2. Methods and materials

2.1. Microcalorimetry

Microcalormetric measurements were made on a four-channel ThermoMetric 2277 thermal activity monitor [6] in the static ampoule mode (5 ml stainless steel ampoules) at an incubation temperature of 37°C. Because of preparatory procedures and thermal equilibration, the time elapsing from blood sampling to onset of measurement was 30 min in the methodological and 60 min in the clinical investigation (see below). Heat output was monitored over 10 h and continuously recorded on a personal computer.

A typical microcalorimetric record of human blood heat output is shown in Fig. 1. The curve consists of a more or less steady "plateau" of heat output followed by a terminal decline in metabolic rate, the latter being associated with exhaustion of glucose reserves in the measuring ampoule. Mean plateau heat output was calculated from the total heat liberated during the plateau period (determined by integration of the thermal power curve) divided by the duration of the plateau [5]. The results are expressed in microwatts per ml of sample (blood or suspension) volume and transformed into microwatts per ml of cell volume to correct for biological or methodological differences in the hematocrit values.

2.2. Methodological investigation (resuspension technique)

To test the resuspension procedure, heparinized blood samples from 13 healthy adult volunteers were divided into two portions and simultaneously measured in the microcalorimeter with and without resuspension pretreatment. Resuspension consisted in centrifugation of the sample and replacement of the plasma by an equal amount of Hanks' balanced salt solution (NaCl 8000 mg l^{-1}, KCl 400 mg l^{-1}, $CaCl_2$ 140 mg l^{-1}, $MgSO_4 \cdot 7H_2O$ 200 mg l^{-1}, $NaHCO_3$ 350 mg l^{-1}, KH_2PO_4 60 mg l^{-1}, Na_2HPO_4 48 mg l^{-1}, glucose 1000 mg l^{-1}; supplied by Biochrom Seromed) [7].

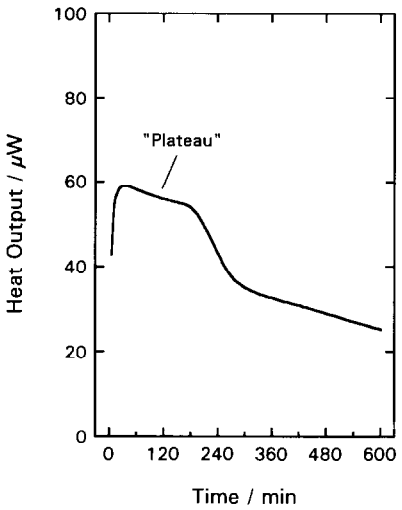


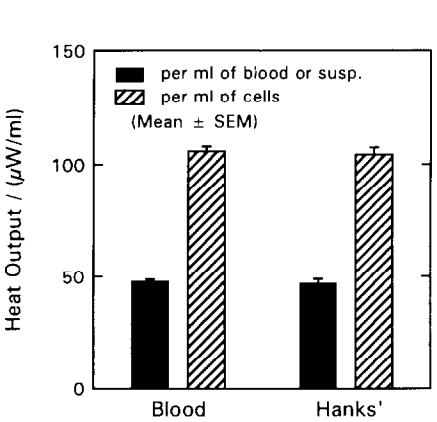
Fig. 1. Typical microcalorimetric record of adult human blood heat output (static ampoule conditions, sample volume 1 ml, hematocrit 45%).

To assure direct comparability between the whole blood and the resuspended samples, the identity of packed cell volume was controlled by microhematocrit determination (Hettich HAEMATOKRIT centrifuge). Moreover, several aliquots of each preparation were stored at 37°C for the measurement of glucose content (Beckman Glucose Analyzer 2) and pH value (Radiometer PHM 93 reference pH meter and GK2421C combined pH electrode) at regular intervals.

2.3. Clinical investigation (neonatal blood heat output)

Using this technique, routine blood samples from 38 neonates in their first week of life (mean age 3.9 ± 0.3 days, mean body weight 3361 ± 116 g) were studied by microcalorimetry. All were patients in the neonatal care unit of the Department of Pediatrics at the Göttingen University Clinics for various common neonatological problems, none of which affected blood heat output to a significant extent (unpublished data). In addition, to exclude unwarranted generalization of a phenomenon restricted to the first few days of life, samples from six infants aged 3–6 months (mean age 107 ± 13 days, mean body weight 6613 ± 321 g) were also studied. As in the methodological investigation, hematocrit (as well as initial glucose content) was determined in the resuspended samples to assure comparability with the whole blood conditions and to allow calculation of cellular heat output.

All results are given as mean values $\pm$ standard errors of the mean. Student's t-test for independent samples was used to assess statistical significance.



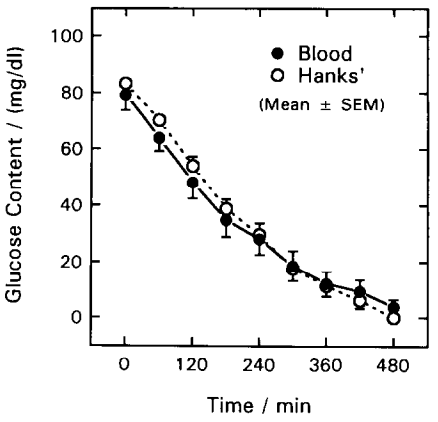


Fig. 2. Comparison between native and resuspended samples (adult human blood, $n = 13$): heat output rates, absolute (solid bars) and hematocrit-corrected (hatched bars).

Fig. 3. Comparison between native and resuspended samples (adult human blood, $n = 13$): time course of glucose content.

3. Results and discussion

3.1. Methodological investigation (resuspension technique)

Microcalorimetric measurements on adult whole body samples led to mean heat output values of 48.1 ± 0.8 μW $(ml_{blood})^{-1}$ or 105.6 ± 2.2 μW $(ml_{cells})^{-1}$, somewhat lower than previous data reported by Bandmann et al. [1] and by ourselves [5]. In the resuspended samples, which were successfully adjusted to the same packed cell volume as for the native samples (blood hematocrit $45.8 \pm 1.3\%$, resuspension hematocrit $45.5 \pm 1.1\%$), a heat output of 47.3 ± 1.6 μW $(ml_{susp.})^{-1}$ or 104.0 ± 3.3 μW $(ml_{cells})^{-1}$ was found. Thus, as illustrated in Fig. 2, neither the absolute heat output per ml of sample volume (solid bars) nor the corrected heat output per ml of cell volume (hatched bars) exhibited any significant difference between the two preparations.

Parallel with the identical heat output rates, the glucose consumption, starting from similar values in resuspended (82.4 ± 1.9 mg dl^{-1}) as in whole blood samples (78.7 ± 4.9 mg dl^{-1}), was nearly identical in both cases (Fig. 3). Whereas no influence of the glucose concentration on the metabolic rate of blood samples (measured as heat output or glucose consumption) could be observed, there was, both in the whole blood and in the resuspended samples, an increase of plateau duration with increasing initial glucose content. However, although the average initial glucose content did not differ between the two preparations (apart from being more uniform in the resuspended samples), there was a somewhat longer plateau duration in the Hanks' (6.8 ± 0.2 h) than in the whole blood samples (4.5 ± 0.2 h) (Fig. 4). This might be correlated with a different course of mean pH values in the blood (from 7.49 at 60 to 7.35 at 480 min) and in the Hanks' samples (from 7.44 at 60 to 7.23 at

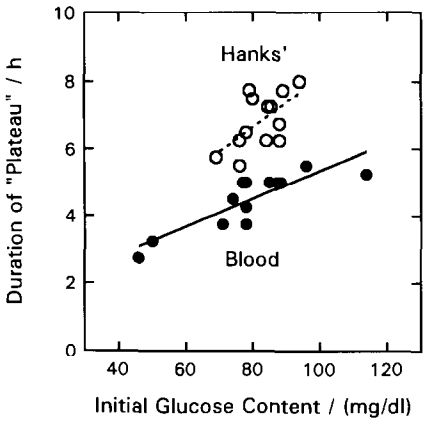


Fig. 4. Comparison between native and resuspended samples (adult human blood): relationship between initial glucose content and duration of "plateau", including the time delay from blood sampling to onset of measurement (30 min).

480 min), although the more rapid acidification of the Hanks' specimens would have been expected to suppress at least the red cell metabolism [8]. Different plateau duration in spite of equal glucose content and uniform heat production is perhaps a consequence of other factors (such as leucocyte stimulation) involved in whole blood calorimetry.

3.2. Clinical investigation (neonatal blood heat output)

The results of the clinical investigation are summarized in Fig. 5. In neonates, a heat output of 77.2 ± 3.1 μW $(ml_{susp.})^{-1}$ was found, which is significantly higher than in adults. This was due partly to the higher physiological packed cell volume (hematocrit) of neonates [9,10], amounting to $51.4 \pm 1.4\%$ in the Hanks' samples (as compared with $52.9 \pm 2.0\%$ in the whole load), but mainly reflected a higher cellular metabolic rate of 151.8 ± 5.9 μW $(ml_{cells})^{-1}$.

In the infant group, the heat output was 47.2 ± 2.8 μW $(ml_{susp.})^{-1}$ and thus seemed to have fallen to the adult level. However, this resulted from the physiological decrease of the hematocrit value during the first three months of life (to $28.0 \pm 1.0\%$ in the whole blood or $28.3 \pm 0.8\%$ in the Hanks' samples) [9,10], whereas the corrected heat output of 166.8 ± 9.8 μW $(ml_{cells})^{-1}$ was as high as or even higher than in neonates. The slight increase in cellular heat production could be due to an increased red cell regeneration rate and a younger cell population, as is known from patients with anemia [11,12].

The plateau duration was 4.9 ± 0.2 h in the newborn and 6.4 ± 0.6 h in the infant group (Fig. 6), and thus confirmed the previously described reciprocity of heat output and plateau duration in mammalian blood microcalorimetry [5]. This corresponds to the uniform initial energy (glucose) content in the resuspended as well as in the whole blood samples of any age group.

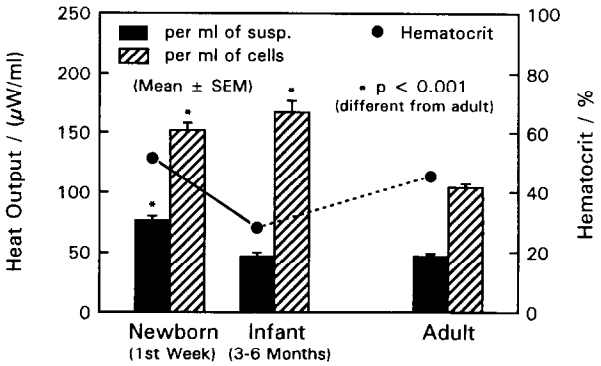


Fig. 5. Evolution of blood heat output rates (as related to the hematocrit values) in human neonates ($n = 38$), infants ($n = 6$), and adults ($n = 13$) (resuspended samples).

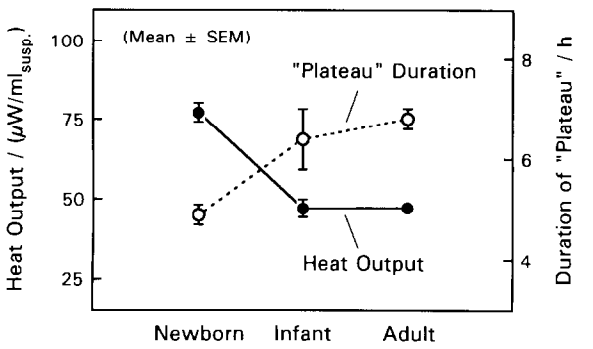


Fig. 6. Reciprocity of heat output and "plateau" duration in human blood microcalorimetry (resuspended samples). As in Fig. 4, the "plateau" duration includes the time interval between the sampling procedure and the start of microcalorimetric measurement, which in the clinical investigation amounted to 60 min.

Remarkably, when compared with previous results, the thermal power values found in human neonatal samples fit the interspecific body size relationship of blood heat output in different mammalian species (Fig. 7). In other words, the blood heat output of humans is higher in neonates than in adults, as expected from their lower body mass, and corresponds to the heat output found in small mammals of comparable size.

4. Conclusions

In view of the inter- and intraspecific body size relationship, it can be stated that blood heat output, although arising from different cellular fractions, seems to reflect the basal metabolic rate of the organism, as is known from tissue samples of large and small animals [13–15].

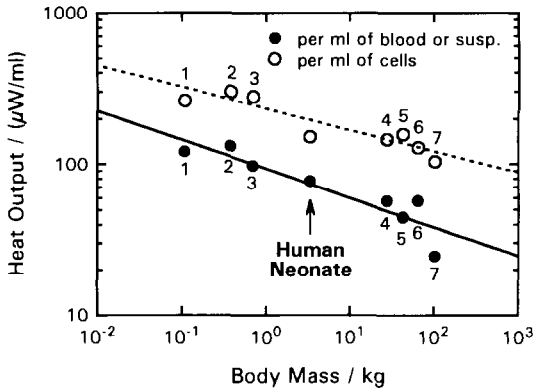


Fig. 7. Human neonatal blood heat output as compared with the mammalian interspecific size relationship. Numbers refer to the species investigated in an earlier study [5]: 1, hamster ($n = 8$); 2, rat ($n = 5$); 3, hedgehog ($n = 9$); 4, dog ($n = 5$); 5, swine ($n = 7$); 6, human ($n = 10$); 7, sheep ($n = 7$).

As blood heat output is known to increase with increasing erythropoiesis [11,12], it may be speculated that a high basal metabolic rate of the whole body goes along with a high red cell regeneration rate which, probably because of a larger proportion of younger cells, leads to a correspondingly higher blood heat output. Hence, intraspecific variations in blood heat output may not depend simply on body size, but also on maturation and growth.

Further research is needed to study the influence of methodological factors (such as sedimentation rate) on blood heat output determination in different species [16] and to identify the cellular components mainly responsible for size relationship. Moreover, it will be of interest to follow blood heat output throughout childhood and to test whether it really shows the same relationship to body size as is known for basal metabolic rate [17]. If this should be the case, blood microcalorimetry (provided that hematological disorders are excluded) could be used to detect pathological deviations of metabolic rate, similar to findings in adults with thyroid disease [18–20], and thus may attract diagnostic interest in clinical pediatrics.

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