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Oxygen supply, body size, and metabolic rate at the beginning of mammalian life

D. Singer [a,*], A. Ince [b], B. Hallmann [b]

[a] *Department of Pediatrics, University Clinics, Josef-Schneider-Str. 2, 97080 Würzburg, Germany*
[b] *Department of Pediatrics, University Clinics, Robert-Koch-Str. 40, 37075 Göttingen, Germany*

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Abstract

To test the relationship between hypoxia/ischemia tolerance and metabolic rate in neonatal tissues, isolated unperfused hearts of neonatal, juvenile, and adult mice were studied by microcalorimetry and microrespirometry. Additionally, microslices of mouse hearts were prepared and studied in a microcalorimeter under different oxygenation conditions. Neonatal hearts had a slower hypoxic/ischemic decline in heat output than adult organs, correlated with a higher uptake of physically dissolved oxygen from the incubation solution. In the slice experiments, the neonatal samples were found to exhibit a higher metabolic activity which enables them to maintain, at low pO_2, a similar metabolic rate as the adult tissue at high pO_2. This corresponds to the fetal adaptation to low intrauterine oxygen tensions and might be a common basis for the elevated neonatal hypoxia/ischemia tolerance as well as for the postnatal increase in metabolic rate up to the level to be expected from body size.

Keywords: Neonate; Body size; Metabolic rate; Oxygen supply; Hypoxia tolerance

1. Introduction

Following a general biological law, called Kleiber's rule, the specific metabolic rate of animals increases with decreasing body mass [1,2]. This has often been explained by the thermoregulatory needs of smaller mammals which, due to their higher surface-to-volume ratio, lose more heat than larger species and, thus, need a higher endogenous heat production to maintain their body temperature constant. However, it is now clear that the same rule holds also true for poikilothermic animals, such as fish or reptiles, which do not exhibit autonomic thermoregulation, and that, from an evolutionary point of view, the overall metabolic size relationship must have been a favorable precondition rather than a secondary consequence of homeothermy. Thus, recent papers favor the substrate transport through the "fractal" surface of tissues as a possible cause of metabolic size relationship [3–5] although it remains uncertain how this mathematical explanation could be applied to the whole body level.

Even though the exact mechanisms are not fully understood, the metabolic size relationship does exert important effects on many physiological processes. It has been supposed, for instance, that the total life span of mammals and birds is inversely proportional to their specific metabolic rate, with the total amount of energy spent during lifetime being more or less constant among species [6,7]. Although this hypothesis is far from being widely accepted, it has been shown by

* Corresponding author. Tel.: +49-931-201-27290; fax: +49-931-201-27288.
E-mail address: d.singer@mail.uni-wuerzburg.de (D. Singer).

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microcalorimetry that at least in ischemic tissues, the metabolic decline is the faster, the higher the specific basal metabolic rate of the species is [8,9]. Hence, the metabolic increase associated to low body weight seems to be a relatively poor precondition for surviving periods of food or oxygen deficiency.

In view of these effects, it is not surprising that some of the smallest mammals with their particularly high specific metabolic rates are able to temporarily deviate from the regular metabolic size relationship in response to seasonal scarcity of food or transitory lack of oxygen. This has been found in hibernators which, during the winter season, reach a uniform minimal specific metabolic rate that equals the specific basal metabolic rate of the very largest mammals (such as elephants or whales) and may thus reflect a common limit of metabolic reduction in mammals [10–12]. In parallel, it has long been known that the mammalian fetus "behaves like an organ of its mother" and exhibits a specific metabolic rate much below the one expected from its own body mass [13–16] (Fig. 1). Moreover, it has been shown that in mammalian neonates that are born in a very immature state, the maintenance of the "inappropriately" low metabolic rate even after birth goes along with a fully aerobic metabolism in spite of highly immature lungs and with a high growth rate in spite of limited food availability [16–18]. Hence, both in hibernation and in fetal/neonatal life, reduction of metabolic demands appears to be an important adaptive response to seasonal or developmental restrictions in energy supply [18,19].

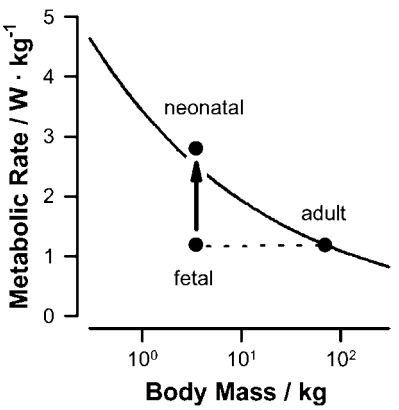

Fig. 1. Schematic diagram of metabolic size allometry and perinatal metabolic adaptation: According to Kleiber's rule, the specific metabolic rate of mammals increases with decreasing body mass. As an adaptation to the limited intrauterine oxygen supply, the fetus has a similar metabolic rate as an adult ("behaves like an organ of its mother"). Only after birth, the metabolic rate increases up to the level to be expected from body size.

The same mechanism might also play a role in the increased hypoxia tolerance of neonates, protecting them against the imminent risks of birth asphyxia [20–22]. In fact, it has repeatedly been found that the mammalian neonate, when exposed to hypoxia, is able to reduce its metabolic rate without apparent signs of oxygen debt [22–25]. This is reminiscent of the "hypoxic hypometabolism" or "oxyconformism" known from overwintering amphibia and intertidal invertebrates [26,27], yet contrasts to the usual invariability of metabolic demands in adult mammals.

Based on these considerations, it was the aim of this calorimetric investigation to study the interrelationship between hypoxia/ischemia tolerance and metabolic rate as well as between metabolic rate and oxygen supply in neonatal tissues.

2. Materials and methods

To record "dying curves" of unperfused (hypoxic/ischemic) organs, isolated hearts of neonatal (postnatal day 0, P 0), juvenile (postnatal day 6/7, P 6/7; postnatal day 13/14, P 13/14), and adult mice were incubated in air-equilibrated Ringer's solution at 25 °C and examined by either microcalorimetry (2277 Thermal Activity Monitor, ThermoMetric, Sweden) or microrespirometry (RE K1-1 N, Biolytik, Germany). The heart was chosen as the experimental organ since in earlier studies, it had proven to widely parallel the whole body metabolic level. An incubation temperature of 25 °C was selected to avoid the metabolic decline being too fast for proper observation. Further details of the microcalorimetric technique are described elsewhere [8,9,28]. The microrespirometric instrument was equipped with a polarographic oxygen sensor and provided voltage data that reflected oxygen tension values and had to be transformed, in a few mathematical steps, into oxygen consumption rates [29]. Since the microrespirometric measuring chamber differed from the microcalorimetric ampoules in both fluid volume and gas content, the respirometric data are to be considered as analogous,

but not as mathematically comparable (in terms of calorimetric/respirometric ratio) to the calorimetric results.

To study aerobic tissue metabolic rates, 300 μm samples from neonatal, juvenile, and adult mouse heart were prepared using a tissue slicer (752M Vibroslice, Campden Instr., UK) and measured by microcalorimetry in oxygen bubbled (pO_2 = 500 mmHg) Hanks' solution at 25 °C. By the incubation of very thin slices in a fully oxygenated nutritional solution at lowered temperature, aerobic conditions, according to Warburg's formula [30], were met. To study the effects of different oxygen tensions, analogous measurements on neonatal and adult samples were done in air (pO_2 = 150 mmHg) and argon ($pO_2 \approx$ 75 mmHg) bubbled solutions.

3. Results

The microcalorimetric "dying curves" of isolated organs clearly show that the hypoxic/ischemic decline

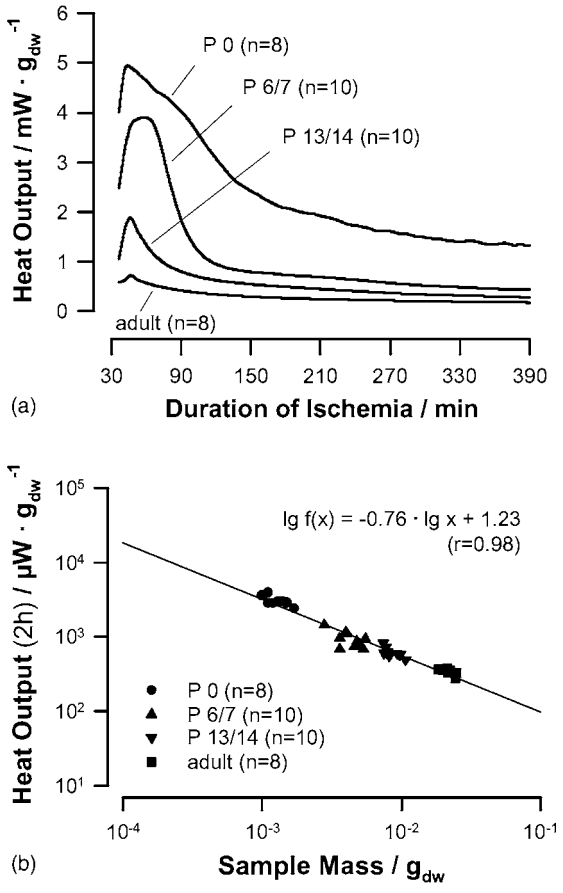

Fig. 2. (a) Microcalorimetric records of isolated, unperfused hearts from neonatal (P 0), juvenile (P 6/7; P 13/14), and adult mice. The ischemic decline in heat output is much slower in the neonatal than in the juvenile or adult organs (where the final baseline is already attained at the beginning of measurement). (b) When the results are plotted on log scales, a linear relationship between heat output and sample mass (dw: dry weight) is found, typical of the "crowding effect".

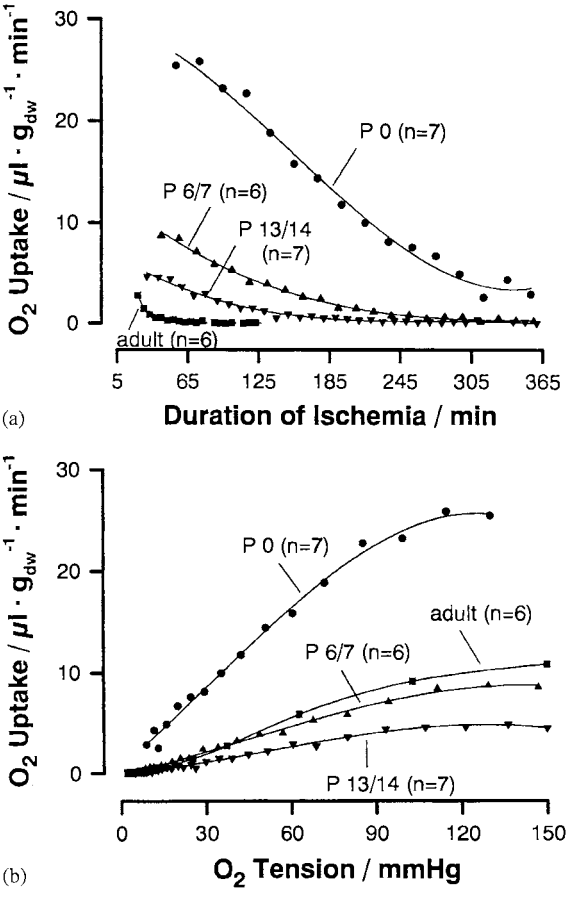

Fig. 3. (a) Microrespirometric records of isolated, unperfused hearts from neonatal, juvenile, and adult mice. The oxygen uptake is much higher in the neonatal than in the juvenile or adult organs (where the physically dissolved oxygen in the incubation medium is used up within a few minutes). (b) When the oxygen uptake rates are plotted against oxygen tensions, it becomes clear that the neonatal heart, at low pO_2, has a similar oxygen uptake as the adult heart at high pO_2.

in heat output is slower in neonatal than in juvenile or even in adult organs where the final baseline is already attained at the beginning of measurement (Fig. 2a). When the heat output rates after 2 h of hypoxia/ischemia are plotted against sample size on log scales, a linear relationship with a relatively steep negative slope of −0.76 is obtained (Fig. 2b), reflecting a strong effect of sample size on tissue heat output.

The microrespirometric records reveal that the oxygen uptake of the unperfused organs (by diffusion from the incubation medium) is higher in the neonatal than in juvenile or even in adult samples where the physically dissolved oxygen is used up within a few minutes (Fig. 3a). Obviously, the time course of oxygen consumption rate is influenced by the differing ratio of sample and chamber volumes. However, the higher "residual aerobiosis" of neonatal as compared to adult organs is still found when the oxygen uptake, in a volume-independent manner, is plotted against oxygen tension (Fig. 3b).

In contrast to the organ "dying curves", the heat output records of tissues slices are much more stable, reflecting the different incubation conditions (aerobiosis instead of ischemia). They indicate the specific heat output at the tissue level being higher in the neonatal than in juvenile or even in adult samples (Fig. 4a). When representative heat output values (read after 90 min of incubation) are plotted against body mass on log scales, a linear relationship is found with a relatively smooth negative slope of −0.33, similar to the −0.25 typical of the overall metabolic size relationship (Fig. 4b). When the same experiments are performed at lower oxygen tensions, a reduction of metabolic rate is to be observed in both adult and neonatal samples which might be partly due to methodological reasons (impairment of oxygen diffusion into the slices). However, there is no doubt that at any given pO_2, the metabolic rate is higher in the neonatal than in the adult tissue (Fig. 5).

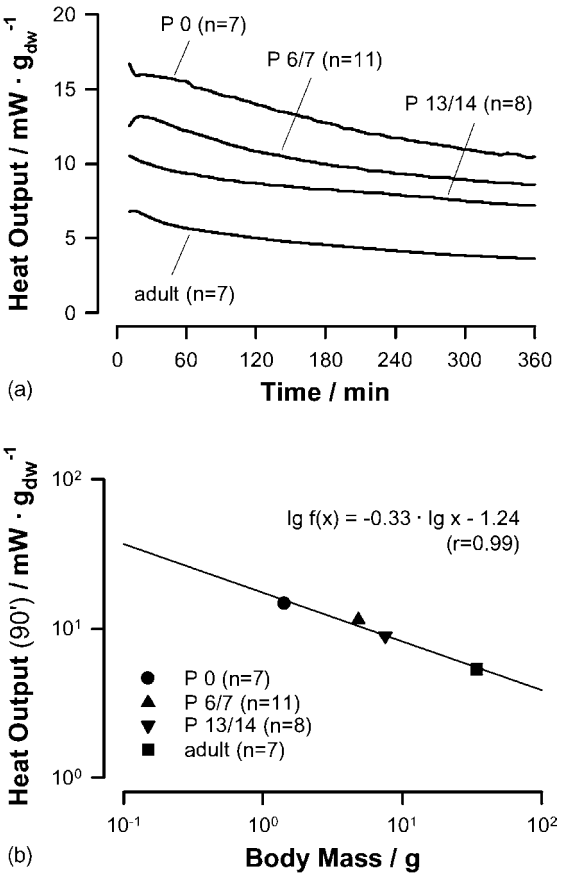

Fig. 4. (a) Microcalorimetric records of 300 μm myocardial slices from neonatal, juvenile, and adult mice. Note the slow decline in heat output, reflecting the mostly aerobic incubation conditions. The tissue heat output is higher in the neonatal than in the juvenile or adult samples. (b) When the results are plotted on log scales, a linear relationship between heat output and body mass is found, corresponding to the overall metabolic size allometry.

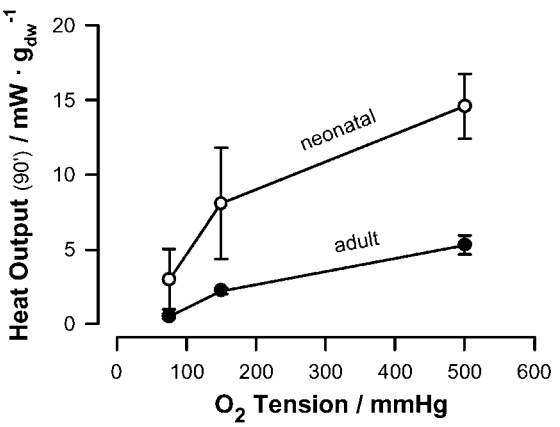

Fig. 5. Heat output rates of neonatal and adult mouse heart slices at differing oxygen tensions. At any given oxygen tension, the heat output rate is higher in the neonatal than in the adult tissue, so that the neonatal tissue, at low pO_2, has a similar metabolic rate as the adult tissue at high pO_2.

4. Discussion

The slow decline in the ischemic heat output of neonatal organs (Fig. 2a) reflects their elevated hypoxia/ischemia tolerance and contrasts to the aforementioned finding that the tissue survival time was shorter in small than in large mammals [8,9]. However, it is in accordance to the empirical fact that, in calorimetry, the specific (mass-related) heat output rates are usually higher in small than in large samples, apparently due to the better diffusion conditions and the proportionately higher degree of aerobiosis [9,31]. This is further supported by the strong linear relationship between sample size and specific metabolic rate (Fig. 2b), typical of the so-called "crowding effect". Obviously, this would mean that the apparent hypoxia tolerance of neonatal tissues might at least partly be due to a methodological artifact!

The respirometric records (Fig. 3a) seem to support these considerations, as they show the uptake of physically dissolved oxygen being higher in neonatal than in adult organs. When the oxygen uptake rates are plotted against oxygen tensions (Fig. 3b), it becomes evident that *the neonatal hearts, at low* pO_2*, maintain similar metabolic rates as the adult hearts at high* pO_2. Even if this should be primarily due to the "crowding effect" (whose oxygen diffusion theory would incidentally be confirmed by these data), this would not exclude that a similar geometrical effect could also play a role in vivo. Moreover, since the "crowding effect" refers to samples of differing size, yet of same origin (with the same inherent metabolic activity), this could indirectly confirm the maintenance of an "inappropriately" low (adult-like) metabolic rate in neonatal samples as the basis of their elevated hypoxia tolerance.

As a surprising contradiction to this assumption, the slice experiments revealed that, at least under fully aerobic conditions, the neonatal myocardium exhibits a significantly higher specific metabolic rate than the adult tissue (Fig. 4a). Moreover, when plotted on log scales, a linear relationship between heat output and body mass was found (Fig. 4b), corresponding to the overall metabolic size relationship. In other words, the neonatal tissue does not "behave like an organ of the mother", but already exhibits the metabolic rate to be expected from the neonate's own body mass—similar to what has been described by Krebs in tissue samples of different species [32]. Obviously, the higher metabolic rate would counteract the benefits of small organ size and, thus, indicates that the increased hypoxia tolerance of neonatal organs cannot or at least not only be explained by the "crowding effect". In fact, when the heat output rates of myocardial slices are measured under different oxygen tensions, it can be seen that *the neonatal samples, at low* pO_2*, maintain similar turnover rates as the adult samples at high* pO_2 (Fig. 5). This is in accordance to the respirometric organ experiments and suggests a metabolic rather than a purely geometrical adaptation.

To understand this adaptation, it is important to be aware of the fact that in the feto-placental circulation, the mean pO_2 is as low as 30 mmHg, corresponding to a stay in 6000–8000 m of altitude [21]. This is much below the 100 mmHg which, following the onset of lung breathing, are reached after birth. From this point of view, the "inappropriately" low metabolic rate of the fetus mentioned at the beginning should not only be regarded in terms of *reduced* demand. To the contrary, it is equally remarkable that the fetus is able to maintain *the same* specific metabolic rate as the mother *in spite of* a much lower oxygen tension. This requires a metabolic compensation which enables *the fetal tissue to maintain, at low* pO_2*, a similar metabolic rate as the adult tissue at high* pO_2. This, however, is exactly what has been found in the above experiments which, therefore, seem to reflect a fundamental metabolic adaptation in fetal/neonatal organs.

Starting from here, it may be speculated that the same metabolic compensation that results in the maintenance of a given metabolic rate at low pO_2, leads to an increase in metabolic rate with increasing pO_2 (Fig. 6). Thus, the onset of metabolic size relationship after birth would be the other side of the metabolic adaptation to low oxygen tensions during fetal life. In view of the fact that being born, from a respiratory point of view, is like being shuttled from the top of the Mount Everest to the sea level within a few hours, it might even be that the postnatal increase in oxygen consumption rate is not only a welcome result of improved supply, but also a mandatory defense strategy against oxidative stress [27,33,34]. Given this assumption, the metabolic rate

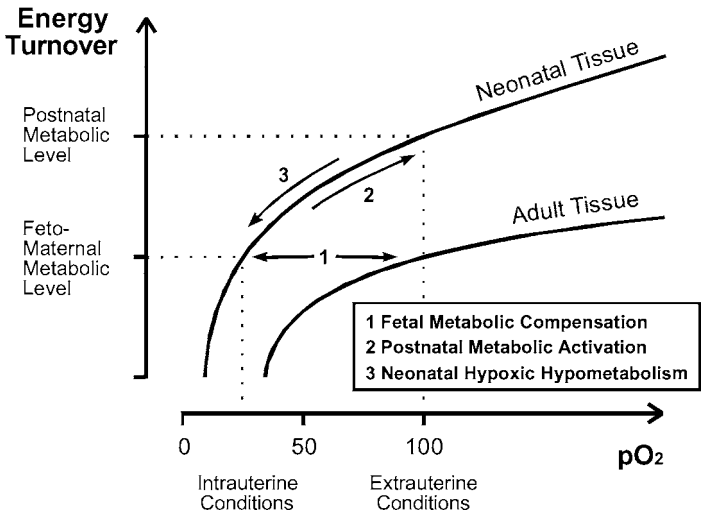

Fig. 6. Unifying hypothesis of perinatal metabolic adaptation, based on the findings in this study. The same metabolic adaptation that enables the fetus to maintain, at low pO_2, a similar metabolic rate as the adult at high pO_2 (arrow 1) might lead to the metabolic increase with increasing oxygen supply at birth (arrow 2) as well as to the "hypoxic hypometabolism" repeatedly found in neonates (arrow 3).

would have to maintain a constant tissue pO_2 in spite of increasing oxygen supply and, therefore, would necessarily become the higher, the smaller the species and the better the resulting diffusion conditions are [35].

On the other hand, there might be, in the case of neonatal hypoxia, a way back to the intrauterine conditions which allows the tissues to reduce their metabolic demands without suffering from an oxygen debt (Fig. 6). Hence, the increased hypoxia/ischemia tolerance of neonatal as compared to adult tissues would not result from an "inappropriately" low, but from an unusually flexible metabolic rate, according to what has been described as "hypoxic hypometabolism" [22,24,25]. This flexibility would reflect the dramatic changes in oxygen supply occurring at birth and would be lost as soon as the neonatal metabolism has been reorganized on a higher level, "appropriate" for body size.

In summary, although the physiological and biochemical mechanisms governing perinatal metabolic adaptation remain to be elucidated, the results presented here seem to explain two apparently unrelated phenomena on a common basis, namely, the elevated hypoxia/ischemia tolerance of neonates and the onset of metabolic size relationship at birth.

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