Energetics of Anaerobic Sodium Transport by the Fresh Water Turtle Bladder

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ABSTRACT  Certain of the metabolic events associated with anaerobic sodium transport by the isolated bladder of the fresh water turtle have been investigated. The data suggest that energy for this transport arises from glycolysis and that endogenous glycogen was the major and perhaps the sole source of substrate. The rate of anaerobic glycolysis, as determined by lactate formation, correlates well with the rate as determined by glycogen utilization. Using lactate formation as the index of anaerobic glycolysis, a linear relationship was observed between glycolysis and net anaerobic sodium transport. In the absence of sodium transport, glycolysis decreased by approximately 45 per cent. Tissue ATP concentrations were maintained at about the same level under anaerobic as under aerobic conditions. Finally if it is assumed that in the conversion of glycogen to lactate anaerobically, 3 moles of ATP are generated per mole of glucose residue, an average of over 15 equivalents of sodium were transported for every mole of ATP generated.

The urinary bladder of the fresh water turtle is among the specialized biologic membranes that actively transport sodium transcellularly in vitro. However, in contrast to similar structures such as the proximal renal tubule, the frog skin, and the toad bladder, the turtle bladder transports sodium (transepithelially) almost as well in the absence of oxygen as in its presence (1). Presumably, therefore, glycolysis plays a major role in the synthesis of free energy for anaerobic electrolyte transport.

In the present studies, an effort has been made to define the quantitative relationships between anaerobic glycolysis and anaerobic sodium transport in the isolated turtle bladder. Because glycolysis is a more primitive, and certainly less complex, pathway for energy production than mitochondrial-linked oxidative pathways, investigation of the turtle bladder could contribute importantly to the understanding of the coupling between metabolic energy and active transport.
METHODS

The urinary bladders of fresh water turtles (Pseudemys scripta elegans) were suspended as diaphragms between the two symmetrical halves of lucite chambers. Twenty-five ml of Ringer's solution were added to each hemichamber in the usual experiment. The composition of this solution is the same as that described previously (1) except that in the present experiments, neither glucose nor any other substrate was added to the media. After the bladders were mounted, they were maintained in a short-circuited state except for the brief intervals required to measure potential difference every 10 to 15 minutes.

Initially the Ringer's solutions were oxygenated, using either room air or 100 per cent oxygen as the gas phase. After steady state conditions were achieved (1), measurements were obtained over a 60 to 90 minute period under aerobic conditions in the majority of the experiments. Thereafter, the gas phase was changed to 100 per cent nitrogen, and unless specifically indicated in the text, the nitrogen was continued throughout the remainder of each experiment. The nitrogen was passed through an oxygen trap (2) before it entered the Ringer's solutions in order to remove trace quantities of oxygen. Fifteen to 20 minutes were allowed for deoxygenation of the Ringer's to occur. Then measurements were made over a 60 to 90 minute period under anaerobic conditions.

Net sodium transport was estimated as the Na\textsuperscript{22} influx (from mucosa to serosa) minus the Na\textsuperscript{24} efflux (from serosa to mucosa). The two flux rates were determined simultaneously as described previously (1). Lactate formation was measured in both serosal and mucosal bathing media during the aerobic control periods and again during the anaerobic periods. Penicillin and streptomycin were added to the bathing solutions in the initial experiments. However, in the absence of substrate in the bathing media, no differences could be detected in the rate of lactate formation with or without the antibiotics. The lactate was measured (3) by the conversion of DPN to DPNH in the presence of LDH in an alkaline medium. Pyruvate formed in the reaction was removed by adding hydrazine. The DPNH was measured using a Farrand fluorometer.

A separate series of experiments was performed for the measurement of ATP and glycogen concentrations. For the ATP measurements individual bladders were cut into 9 to 12 segments approximately 1 cm\textsuperscript{2} in area. These were divided into three groups, each of which contained 3 to 4 segments totalling about 100 mg wet weight. One group was processed immediately to obtain control values. The other two groups were transferred into Erlenmeyer flasks containing 10 ml of Ringer's solution. The flasks were incubated at room temperature (approximately 22°C) in a Dubnoff metabolic shaker. For the first hour 100 per cent O\textsubscript{2} was used as the gas phase in all flasks. Thereafter the gas phase was changed to 100 per cent nitrogen in half of the flasks while oxygen was continued in the other half. The second phase of these experiments was continued for 1 to 2 hours.

For the determination of ATP, the tissue segments in each group were transferred into 10 ml of distilled water and placed in a boiling water bath for 10 minutes. The volume then was reconstituted to 10 ml and ATP concentrations were measured.
using the firefly technique (4). Values were expressed as micrograms per milligram of wet weight.

A similar system was used for glycogen determinations, except that larger segments of bladder (2 to 4 cm²) were used. After incubation, the tissues were homogenized and the glycogen was hydrolyzed to glucose in 2 N H₂SO₄ (5). Glucose then was determined by a glucose oxidase method. In a number of glycogen experiments, lactate was measured in the Ringer's solutions in order to compare glycogen utilization with lactate formation.

**RESULTS**

Fig. 1 depicts the results of 18 bidirectional sodium flux studies. The net flux (i.e. influx minus efflux) was only 16 per cent less under anaerobic than under aerobic conditions.

Lactate formation was measured in the serosal and mucosal bathing solutions in 25 experiments and the data are shown in Fig. 2. In 24 of the 25 studies, lactate formation increased after the gas phase was changed from 100 per cent O₂ to 100 per cent N₂. The mean value was $162 \times 10^{-3}$ moles (per 7 cm² of bladder per hour) in O₂ compared to $347 \times 10^{-3}$ moles in N₂. The increase was 114 per cent and is highly significant ($p < 0.01$).

In 8 experiments, oxygen was reintroduced after the anaerobic measurements were completed. In each experiment O₂ suppressed lactate formation. The results of these studies are summarized in Table I. In 2 experiments a second period of anaerobiosis was established and in both, lactate formation increased again.

1 The recovery of ATP, added to the tissue before boiling, averaged 104 per cent ($\pm 1.6 \text{ se}$).
2 Glucostat kits, Worthington Biochemical Company, Freehold, New Jersey.
3 Five of these 18 studies were included in the previous paper (1).
In order to investigate the relationship between sodium transport and glycolysis, measurements were made of the rate of lactate formation in an anaerobic system before and after removal of sodium ions from the bathing Ringer's solutions. Control measurements were made with conventional sodium Ringer's solution in both hemichambers. Thereafter the bladders were rinsed from 2 to 3 times with deoxygenated choline chloride Ringer's and fresh deoxygenated choline Ringer's was used as the bathing solution in contact with the two surfaces of the bladder. 100 per cent N\textsubscript{2} was maintained as the gas phase. The potential differences ($\text{PD}$) and the short-circuit current (S.C.C.) fell precipitously to zero after the choline chloride Ringer's was added. Values for lactate formation are shown in Fig. 3. In the presence of

![Figure 2](image)

**TABLE I**

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>100 per cent oxygen</th>
<th>100 per cent nitrogen</th>
<th>100 per cent oxygen</th>
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<tr>
<td>8</td>
<td>225</td>
<td>421</td>
<td>184</td>
</tr>
<tr>
<td>±56.2</td>
<td>±71.6</td>
<td>±21.9</td>
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</table>

Studies were initiated after the membranes had equilibrated in O\textsubscript{2} (1). Each of the gas phases was maintained for 60 minutes. Values represent the means and standard errors of means.
sodium transport the mean value was $380 \times 10^{-3}$ moles (per 7 cm$^2$ per hour) and in the absence of sodium transport the value fell to $208 \times 10^{-4}$ moles. The net decrease is 45.4 per cent. In 9 studies, the experimental sequence was reversed and measurements were made first with choline chloride Ringer's then with sodium Ringer's. Lactate formation increased in 8 of the 9 experiments by an average of 57 per cent. A decrease was noted in one experiment and for the group the increase averaged 36 per cent.

Lactate accumulated on the opposite sides of the turtle bladders in an asymmetric fashion regardless of the experimental conditions. The values for serosal versus mucosal lactate accumulation are shown in Fig. 4. In sodium Ringer's solution, the serosal accumulation markedly exceeded the mucosal accumulation both in oxygen and in nitrogen. The same phenomenon was observed with choline chloride Ringer's solution.\(^4\)

The values for ATP concentrations of bladder segments incubated under aerobic and anaerobic conditions are shown in Table II. The mean value for the control tissues (i.e. those processed immediately after removal from the animals) was 0.25 µg per mg wet weight. The mean value for tissues from the same animals incubated in oxygenated Ringer's was 0.20 µg per mg wet weight, while the value for the tissues incubated under anaerobic conditions

\(^4\) A similar pattern has been reported by Leaf for the urinary bladder of the toad (6).
was 0.18. The difference between the aerobic and anaerobic values is not statistically significant ($p < 0.2$).

Fig. 5 depicts the glycogen concentrations of bladder segments. Aerobic incubation in substrate-free media was associated with a decrease in glycogen concentration (in comparison to control values). Furthermore glycogen utilization was greater in an anaerobic than in an aerobic environment; the difference between these two groups is statistically significant ($p < 0.01$).

The correlation between glycogen utilization and lactate formation in bladder segments is shown in Table III for 19 experiments. The glycogen values are expressed as micromoles of glucose residue and theoretically 1 mole

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**Figure 4.** The number of experiments in each group varied from 8 to 12. Measurements of lactate were made for 1 hour periods. Each value represents the mean ±1 standard error.

**Table II**

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Control</th>
<th>100 per cent $O_2$</th>
<th>100 per cent $N_2$</th>
</tr>
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<tbody>
<tr>
<td>8</td>
<td>0.25</td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.02</td>
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Control values for ATP concentrations were measured immediately after removal of the bladder from the animal. The other values represent ATP concentrations after 2 hours of incubation. (See text for details.) Values represent the means and standard errors of means.
of glucose should give rise to 2 moles of lactate if all of the lactate arises from glycogen and if none of it is further metabolized. The data show quite good agreement between the two expressions of anaerobic glycolysis. Thus the mean value for glycogen utilization was 10.4 μmoles per gm (1.88 μg/mg) while the mean value for lactate recovery was 21.9 μmoles/gm (1.97 μg/mg).

The relationship between net sodium transport and anaerobic glycolysis, as measured by lactate formation, is shown in Fig. 6. The two parameters were measured simultaneously under anaerobic conditions in 13 experiments. The rate of lactate formation is plotted on the ordinate against the rate of net sodium transport on the abscissa. Each value for sodium transport represents the mean of at least two 30 minute periods. The linear regression line is derived by the method of least squares and the correlation coefficient is 0.81. The function is statistically significant (p < 0.02).

DISCUSSION

The foregoing data indicate that glycolysis comprises the major, and perhaps the sole, source of energy for anaerobic sodium transport across the isolated turtle bladder. Furthermore in the absence of exogenous substrate, endogenous glycogen, which is located within the epithelial cells lining the bladder, serves as substrate for the anaerobic glycolysis.
That there is a direct coupling between anaerobic glycolysis and anaerobic transepithelial sodium transport is suggested by two observations. First, when sodium was removed from the bathing media (i.e., when the bladders were suspended in choline chloride Ringer's solutions), anaerobic glycolysis was diminished by approximately 45 per cent. Second, when the net rate of sodium transport was compared with lactate accumulation in the bathing media, a linear relationship was observed. Thus in group data a stoichio-

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Glycogen utilized (μmole/gm)</th>
<th>Lactate evolved (μmole/gm)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>9.5</td>
<td>22.6</td>
</tr>
<tr>
<td>2</td>
<td>11.7</td>
<td>22.7</td>
</tr>
<tr>
<td>3</td>
<td>15.8</td>
<td>41.0</td>
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<tr>
<td>4</td>
<td>13.4</td>
<td>29.4</td>
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<td>5</td>
<td>12.9</td>
<td>14.9</td>
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<td>6</td>
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<td>15</td>
<td>3.2</td>
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<td>16</td>
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<td>7.2</td>
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<td>18</td>
<td>14.6</td>
<td>28.8</td>
</tr>
<tr>
<td>19</td>
<td>1.4</td>
<td>2.6</td>
</tr>
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</table>

Mean 10.4 21.9
 (+1.2) (+3.1)

metric relationship exists between anaerobic glycolysis and net sodium transport despite the fact that the lactate values reflect total rates of glycolysis and not just that portion of anaerobic metabolism linked to sodium transport.

Measurements of ATP demonstrate the ability of the isolated bladder to maintain tissue ATP concentrations at the same level under anaerobic conditions as under aerobic conditions; although active sodium transport, a major

1 A close coupling between sodium transport and oxygen consumption has been established previously in several epithelial cell systems and a discussion of these data together with pertinent references is contained in a recent paper by Whittam (7).
source of energy utilization remains roughly the same in the anaerobic system. Hence, although measurements of ATP turnover are not available, this provides indirect evidence that ATP synthesis continued in the absence of molecular oxygen.

It is possible on the basis of the present data to examine the relationship between ATP and anaerobic sodium transport. In the 13 experiments in which bidirectional sodium flux rates and lactate accumulation rates were measured simultaneously (Fig. 6), the average value for net sodium transport was 3.16 μmoles/hr./7 cm². This corresponded to a mean value of 0.28 μmole of lactate for the same bladders during the same period of time. In the experiments using choline chloride Ringer’s it was found that slightly less than half of the total lactate formation was linked to sodium transport. Hence for purposes of the present calculations it will be assumed that out of the total of 0.28 μmole of lactate, 0.13 μmole was linked to sodium transport. Endogenous glycogen served as the source of the lactate; therefore, 3 moles of ATP

![Graph showing the relationship between sodium transport and lactate accumulation. The equation y = 29.8 + 80.1x with r = 0.81 (p < 0.02) is provided.](image)
were generated per mole of glucose residue metabolized, or 1.5 moles of ATP were formed for every mole of lactate generated. It would appear, therefore, that 3.16 moles of Na were transported for 0.195 mole of ATP. Hence over 15 equivalents of sodium would be actively transported per mole of ATP generated from anaerobic glycolysis. This must be considered as a rough approximation, since the utilization of preexisting cellular stores of ATP is not taken into account and, on the other hand, intracellular lactate was not measured. Nevertheless, it would appear that the cost of transporting sodium in this system may be extremely small; it also is evident that none of the existing models of active sodium transport will allow for this high a ratio between sodium ions and ATP (8).

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*This conclusion would obtain even if more than 50 per cent of the lactate produced were coupled to sodium transport (as is suggested by the intercept in Fig. 6).*
The Sodium Pump and Energy Regulation: Some New Aspects for Essential Hypertension, Diabetes II and Severe Overweight

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Summary. There is a growing evidence for that in modern societies the function of the cellular sodium-potassium pump (membrane-bound Na⁺/K⁺ ATPase) in several tissues in man cannot respond adequately to demands. This is not seen in any other free-living vertebrates on this earth. The clearly unphysiological very high intake of sodium-chloride (salt) and also alcohol is definitely playing an important role in the development of the common degenerating metabolic aberrations, e.g. essential hypertension, diabetes II and severe overweight, in man. The special and overall important role of the sodium-potassium pump for optimal cellular function and regeneration with special reference to the vascular tissues is presented and discussed.

The Cellular Balance of Electrolytes and Energy

With increasing age in man the body's stores of potassium, where 98% is situated intracellularly, are decreasing [1, 2]. In specific tissues — kidney and striated muscle — the fall is remarkably pronounced [3]. In parallel with the fall in intracellular potassium the concentration of phosphor is decreasing, whereas the concentration of calcium is increasing [3].

Although there is a limited number of reports on other vertebrates' life span, the tissue content of potassium (Kₑ) and phosphate seem not to decrease with age in sheep, pigs and oxen [4, 5]. Among all vertebrates, adult man has the lowest blood concentration of potassium (mmol/l serum — 4.0 against 4.5–6.0 in animals), magnesium (0.9 against 1.2–3.0) and phosphate (1.0 against 2.0–2.5 [5]). The sodium-potassium pump first described by Schou [6] is the most important regulator of intracellular potassium and sodium — and hence probably calcium [7, 8]. The age-correlated decrease in Kₑ may therefore be a sign of a curbed Na⁺/K⁺ pump either directly or indirectly.

The marked and unphysiological high Na⁺/K⁺ ratio in our food today (2.0–7.0) is without doubt one of the most important factors, as convincingly demonstrated by Luft et al. [9]. However, other factors such as alcohol [10, 11] and the probably relatively low intake of magnesium [12] in modern food seem also to be of importance. In animals (vertebrates) the food has a low Na⁺/K⁺ ratio, usually around 0.01 to 0.10, and they do not develop arteriosclerotic diseases — not even the immobile and fat dairy cow! Already in 1959 Loepfer et al. [13] showed that aorta from oxen contained 12–1,600 mg K⁺/kg wet tissue, whereas the fat-free part of aorta from deceased humans contained much less with a falling scale for the degree of atheromatous lesions (0 atheroma 400 mg/kg; (+) 250 mg/kg and (++) 140 mg/kg).

In 1964 Whittam et al. [14] showed with brain cells and erythrocytes that ouabain specifically inhibited the intracellular production of ATP and also the oxygen consumption and this has been verified by later authors [15, 16]. We have repeated this with rabbit smooth muscle cells in culture [17] and found the same for ATP and lactate, but also that other energy-demanding processes like (³H)DNA production (proliferation) and protein synthesis (collagen) were inhibited and partly reversed by an increase of the potassium concentration in the medium from 4.0 to 6.0 mmol/l (Fig. 1). It has also been shown lately that in nucleated cells there is a direct and strong correlation between the intracellular concentration of potassium and the production of DNA [18] (Fig. 1). It is more and more evident that many intracellular enzymatic processes rely strongly on the intracellular concentration of K⁺ as well as for free Ca²⁺ [7, 18, 19].

Alcohol given chronically to man and apes (around 20% of the daily calory intake) does also depress ATP production in muscle or liver cells.
Essential Hypertension

There is increasing evidence that patients with essential hypertension have a more pronounced negative potassium balance than "normal" man [2, 21, 22] (Fig. 2). The finding that plasma-potassium is negatively correlated to BP \( (r = -0.55, P < 0.01) \) even in a small group of younger hypertensives [23] as well as in larger epidemiological studies in Japan [24] points to an inhibited Na\(^+\)/K\(^+\) pump as one of the most likely explanations for the negative correlation to BP. As a cellular explanation for essential hypertension, Tobian et al. [25], D'Amico [26] and Losse et al. [27] were the first to propose a disturbed intracellular balance of sodium and later reports have supported these findings [28–30] – however, not uniformly [31]. As a genetic marker, increased intracellular sodium in erythrocytes [27, 32], lymphocytes [33] and the whole body [29] has been increasingly supported. Several aberrations in membrane-bound cation transport systems have been proposed for essential hypertension [34, 35] but have not been confirmed, probably because of a too large in-patient variation. Usually, known membrane-acting external factors like the sodium-potassium intake, plasma potassium-magnesium, alcohol intake and other medical treatment have not been reported in most of the later studies.

We have found red cell sodium to be a very constant parameter [32] in both controls and in the offspring of essential hypertensive patients. In both populations potassium and sodium are significantly and negatively correlated and points to an abnormally low total function of the membrane-bound Na\(^+\) K\(^+\) ATPase system in offspring. In recent studies using the \(^{3}H\)-ouabain binding method described by Schmittinger [36] we have found [37], that both in offspring of hypertensives and in controls, the number of \(^{3}H\)-ouabain binding sites is highly negatively correlated to intracellular sodium \( (r = -0.6-0.7, P < 0.001) \) and positively correlated to intracellular potassium \( (r = 0.5-0.6, P < 0.01) \). The \(^{3}H\)-ouabain site number is significantly lower (14%) [37] in hypertensives male offspring and this has been confirmed in a preliminary examination on T-lymphocytes (normotensive off-
spring: 31,000/cell; age- and weight-matched controls: 34,500/cell).

Our working hypothesis today is therefore that in offspring of hypertensives the cellular adaptation to BP-rising external factors (e.g. the sodium intake, the low intake of potassium and magnesium, alcohol) might be disturbed because, at least during treatment with digitalis and diuretics (hyperkalemia), the red cell (2H)-ouabain binding sites in man [38], not, however, with full compensation in many cases [39].

We have made several follow-ups of essential hypertensives offspring [32, 40] and in our last [41] 5-year follow-up on 239, subjects aged 18–65 years (mean age at screening 35 years), 38 (16%) developed sustained hypertension (DBP supine 10 min x 3 ≥ 100–105 mm Hg). In the age group 40–49, 33% among both sexes developed hypertension and were given treatment; this is at least four times more common than in the whole population of Malmö [42, 43]. The prognostic factors for hypertension are BP, heart rate and serum gamma glutamyl transferase (GGT) [42–44], also an objective marker for the long-term alcohol intake.

Diabetes II and Overweight

There is a considerable correlation between overweight and diabetes II, a condition now recognized to be caused by a relative insulin resistance in most tissues [45]. So far it seems that the most specific metabolic abnormality is a chronically decreased amount of glucose transport protein in the intracellular phase of adipocytes and probably also muscle cells [46]. In areas, where the calory intake is high but low in sodium and high in potassium (e.g. Amazon Indians [47] and Greenland Eskimos), diabetes II does not exist despite some overweight, at least in Greenland. Also in short-term experiments, severe deficiency of potassium will induce glucose intolerance in man [48] or diabetes in rats [49], which is completely ameliorated in the potassium-repleted state. In a recent study Norgård et al. [50] showed on rats that in the striated muscle the (2H)-ouabain binding decreased during severe depletion of potassium and returned quickly to normal after repletion of potassium. This finding is in line with a decreased intracellular potassium and hence among other things ATP. However, during the mild depletion of potassium in humans, i.e. during treatment with digitalis and diuretics, at least the red cell (2H)-ouabain binding seems to increase as a sign of adaptation [38], but whole body potassium decreases [51].

Several studies have shown a curbed function of the sodium-potassium pump in erythrocytes from severely overweight patients [52, 53]. Differences between races seem also to exist [54].

In a recent examination we have found that in T-lymphocytes from middle-aged males with mild diabetes II the (2H)-ouabain binding sites were significantly higher than in controls (10%) [55]. The dissociation constants (Scatchard plot) were equal which shows that the receptor (Na/K ATPase) belongs to the same class in both groups. In a small group (n=11) of normoglycaemic offspring of patients with diabetes II the T-lymphocytes showed less (2H)-ouabain binding, however, not significantly, despite a significantly lower plasma potassium (3.8 mmol/l versus 4.1 mmol/l in controls).

In our ongoing preventive medicine screening in Malmö we have also seen a clear significant correlation between glucose intolerance and alcohol consumption or GGT not only related to overweight [56]. These findings tend to show a mixed adaptation to the external diabetogenic factors, e.g. alcohol, calory intake, high salt intake, low potassium intake. Future follow-up studies will probably show other factors to be important too, but the four mentioned above are overwhelmingly enough to start with!

Discussion

There is a considerable overlap between essential hypertension, diabetes II and overweight in our populations [22] and it has to be considered that some very common life-style factors, e.g. high intake of alcohol, a high sodium/potassium ratio in food and a relatively low intake of magnesium and phosphate, however slow acting, are responsible. It is more and more evident that sodium restriction together with potassium supplementation or a high intake of fresh food is effective in the treatment of hypertension [57] in diabetes II [47, 58] and overweight [59]. Clearly, a substantial number of patients have got their diabetes II because of the administration of diabetogenic diuretics [22], a treatment which in my opinion should be abandoned and replaced at once with potassium-sparing diuretics or other drugs.

The most important risk-factor in the world today besides undernutrition, is without doubt alcohol, which in Sweden at least is responsible for 33% of all premature deaths in the middle age [44]. Alcohol is also, even in moderate amounts (i.e. as indicated by GGT), clearly correlated positively to BP [42], glucose intolerance [56], body weight [60], atherogenic lipids [61] and some can-
cer forms [62]. The fact that occlusive arteriosclerotic diseases are less common the further south one goes in Europe [63] may indicate some protective factors in these countries, i.e. a high intake of potassium and other protective minerals, and lower intake of sodium. The food preparation (e.g. steaming, oil boiling, barbeque) seems here to be an important factor because salt-water boiling as mostly used in Northern Europe without doubt causes a considerable loss of potassium, magnesium, phosphate and other important minerals [64]. Maybe also the fact that wine contains at least 15–25 mmol K+/l together with some magnesium and phosphate can explain some of the lower occurrence of arteriosclerotic diseases seen in the mostly wine-consuming countries [63].

Many factors – positive and negative – relevant to the degenerative vascular diseases seen in our part of the world are not known yet, but we need now to start prevention of the most fatal or crippling disorders such as stroke, CHD and cancer. For the persistence of the normal cell, the proper food and liquids are our best weapons.

Besides being human sapiens we are also animals!

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Contribution of anaerobic energy expenditure to whole body thermogenesis
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Abstract
Heat production serves as the standard measurement for the determination of energy expenditure and efficiency in animals. Estimations of metabolic heat production have traditionally focused on gas exchange (oxygen uptake and carbon dioxide production) although direct heat measurements may include an anaerobic component particularly when carbohydrate is oxidized. Stoichiometric interpretations of the ratio of carbon dioxide production to oxygen uptake suggest that both anaerobic and aerobic heat production and, by inference, all energy expenditure – can be accounted for with a measurement of oxygen uptake as 21.1 kJ per liter of oxygen. This manuscript incorporates contemporary bioenergetic interpretations of anaerobic and aerobic ATP turnover to promote the independence of these disparate types of metabolic energy transfer: each has different reactants and products, uses dissimilar enzymes, involves different types of biochemical reactions, takes place in separate cellular compartments, exploits different types of gradients and ultimately each operates with distinct efficiency. The 21.1 kJ per liter of oxygen for carbohydrate oxidation includes a small anaerobic heat component as part of anaerobic energy transfer. Faster rates of ATP turnover that exceed mitochondrial respiration and that are supported by rapid glycolytic phosphorylation with lactate production result in heat production that is independent of oxygen uptake. Simultaneous direct and indirect calorimetry has revealed that this anaerobic heat does not disappear when lactate is later oxidized and so oxygen uptake does not adequately measure anaerobic efficiency or energy expenditure (as was suggested by the “oxygen debt” hypothesis). An estimate of anaerobic energy transfer supplements the measurement of oxygen uptake and may improve the interpretation of whole-body energy expenditure.

Background
"...(animals) take up oxygen and complex compounds made by plants, discharge these compounds largely in the form of car-bonic acid (CO₂)and water as the products of combustion and partly as simpler reduced products, thus consuming a certain quantity of chemical potential energy, and generate thereby heat and mechanical energy" (H.L.F. Helmholtz, 1821-1894)

Measurements of heat loss and oxygen uptake are the two major methods for determining energy expenditure although they do not always provide equivalent results at equivalent time points [1-4]. The focus on oxygen uptake follows from the extensive involvement of mitochondria in ATP re-synthesis accompanied by concomitant heat production [5-8]. Sites of ATP hydrolysis (e.g. contracting muscle) represent another source of energy transfer and
heat exchange. Non-steady state periods of rapid growth and development, disease, arousal from torpor, heavy/severe exercise and hypoxia, however, offer proof of how tenuous the relationship between heat loss and oxygen uptake can be [1,3,4,9-11]. In isolated mammalian cells, for example, the accelerated production of lactate has been shown to make a substantial contribution to heat production beyond mitochondrial (aerobic) involvement [12]. If heat serves as the standard measure of energy expenditure then anaerobic energy transfer, specifically rapid glycolysis and glycogenolysis with lactate production (i.e., rapid anaerobic ATP re-synthesis) has the potential to make significant contributions to cellular energy expenditure.

Glycolysis as a form of fermentation has been a part of life for an estimated three billion years [13]. It has been observed that anaerobic glycolysis and oxygen uptake often behave in a reciprocal manner. Pasteur, for example, demonstrated that glucose utilization in yeast was more rapid when oxygen was absent [14]. It was subsequently hypothesized that alterations in aerobic respiration influence glycolytic rate. Crabtree [15] described the suppression of oxygen uptake when an abundance of glucose was provided to tumor cells. More recently it has been shown that this "Crabtree Effect" is not the result of altered respiratory function, but rather an induction of the glycolytic enzymes during cellular proliferation as lactate dehydrogenase (LDH) increased 10-fold and appeared to influence the subsequent routing of NAD+ to the cytoplasm and away from immediate mitochondrial respiration [16]. As it pertains to cellular metabolism then, a distinct trade-off between anaerobic and aerobic metabolic pathways can be seen; high rates of mitochondrial ATP re-synthesis have the potential to suppress anaerobic glycolysis and, conversely, rapid glycolytic ATP re-synthesis can suppress aerobic respiration. In an experiment with yeast, the relative contributions of anaerobic and aerobic processes to total ATP re-synthesis were genetically modified by increasing the glycolytic enzyme, phosphofructokinase (PFK). This modification resulted in yeast with enhanced anaerobic ATP re-synthesis – accompanied by a 36% lower oxygen uptake – but unchanged total ATP turnover compared to normal aerobic-respiring yeast [17]. It appears then, at least in single cell-types, anaerobic ATP re-synthesis has the potential to promote a discrepancy between energy expenditure (heat loss) and oxygen uptake. The question that remains is whether similar discrepancies are seen at the level of the whole-animal.

This review contains four sections. The first briefly describes thermodynamic and bioenergetics interpretations of energy transfer. The second section describes the traditional (stoichiometry and gas exchange) and contemporary (bioenergetic) interpretations behind metabolic heat production. The third section describes energy transfer as lactate production and lactate removal. In the fourth section examples are provided that suggest how an estimate of anaerobic energy transfer along with a separate measure of oxygen uptake may better influence the interpretation of whole-body efficiency and energy expenditure.

Energy transfer
The first law of thermodynamics states that energy can not be created or destroyed but can and does change form. The second law describes how energy is transferred from one form to another. For example heat, as an expression of energy, always flows in one direction – from hot to cold. Other ways of stating this are that energy flows "downhill" or, from a state of lower entropy to one of higher entropy. Entropy represents energy that is not available to perform work so that simply put, energy transfer is inefficient. Inefficiency also appears in the form of heat production that is usually discarded into the environment. In the late 1800’s Josiah Gibbs acknowledged the importance of entropy and enthalpy in his explanations of chemical energy transfer. The Gibbs free energy is recognized as energy that is available to perform work at constant temperature and pressure and is the usual thermodynamic parameter for identifying spontaneity of chemical reactions. Thermodynamics was further developed in the context of a closed system where heat but not matter was exchanged with the environment (e.g. test tube reactions). This description was also applied to living cells. It is of interest that A.V. Hill shared the 1922 Nobel Prize in part for his recognition that muscle cells were not heat-to-mechanical motion converters as modeled by the steam engine, but could rather be understood as chemo-mechanical converters.

Biological energy transfer or bioenergetics is accurately described in the context of an open system where matter and energy are continuously exchanged between a cell and its immediate environment [18-20]. In open or in closed systems the Gibbs free energy 'drives' biochemical and chemical reactions, respectively. Closed systems have specific starting and ending points for the Gibbs free energy change during energy transfer. In an open system however, the Gibbs free energy availability may change as the rate of energy transfer and the ratio of product to reactant varies during the exchange (e.g., as the distance from equilibrium is altered) [2]. Within cells, heat and entropy production are the continuous result of energy transfer during ATP hydrolysis and re-synthesis, collectively known as ATP turnover. ATP undergoes hydrolysis to "fuel" a variety of cellular functions such as muscle contraction, the sodium-potassium pump within cell membranes and coupling to endergonic reactions. Aerobic and anaerobic metabolisms serve to re-synthesize ATP.
Entropy production can not be directly measured. Heat loss can be quantified with direct calorimetry as a measure of energy expenditure (transient heat storage is not described here so that heat loss is equated with heat production). Heat production also can be estimated with measures of oxygen uptake and carbon dioxide as indirect calorimetry.

**Gas exchange and energy expenditure**

Lavoisier first described both biological respiration and combustion in terms of their equivalence of gas exchange and heat production. At the end of the nineteenth century experiments by Eduard Pfüger and others compared direct measurements of heat production with indirect measures of gas exchange. Pfüger utilized a stoichiometric analysis to uncover the relationship between the chemical compositions of different foodstuff and their oxidation. From this data, for example, glucose oxidation is described as,

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 \rightarrow 6 \text{CO}_2 + 6 \text{H}_2\text{O}
\]

The stoichiometric ratio of CO₂:O₂ as measured from the mouth became known as the respiratory exchange ratio (RER) and serves as a valuable means of interpreting substrate utilization and heat production. When all-carbohydrate diet is being oxidized the RER is 1.00 (6 CO₂: 6 O₂) and one liter of oxygen uptake estimates heat production at 19.6 kilojoules (1 l O₂ = 19.6 kJ). When fatty acids are the principal substrate oxidized, the RER is 0.70 (palm oil oxidation = 16 CO₂: 23 O₂) and one liter of oxygen uptake estimates heat production at 19.6 kilojoules (1 l O₂ = 19.6 kJ).

The higher RER for carbohydrate oxidation has been interpreted to mean that fat oxidation requires more oxygen and results in less heat production than carbohydrate oxidation [21]; this does not signify that carbohydrate is the more efficient fuel source. Only 2-carbon intermediates (acetyl CoA) can enter into the Krebs cycle for complete aerobic oxidation and the product of anaerobic carbohydrate breakdown – pyruvate – must undergo de-carboxylation (i.e., carbon dioxide production) by the enzyme pyruvate dehydrogenase (PDH) before it can be oxidized aerobically. In comparison, fat is broken down by mitochondrial beta-oxidation enzymes into 2-carbon intermediates; no de-carboxylation takes place prior to entrance into the Krebs cycle. Per volume of ATP re-synthesized aerobically then, the complete oxidation of glucose and glycogen has additional relative carbon dioxide production, not less relative oxygen uptake, as compared to fat oxidation. The conversion of one liter of carbon dioxide into an estimate of heat production for glucose and fat oxidation reveals larger discrepancy in energy expenditure at 21.1 and 27.6 kJ, respectively [22]. If oxygen uptake better represents energy expenditure than carbon dioxide production, then it must be concluded that the ratio of CO₂/O₂ provides a poor explanation of energy transfer efficiency.

Heat measurements that are independent of carbon dioxide production reveal a strong linear relationship between oxygen uptake and the enthalpy of combustion of many organic compounds [22-24]. The calorimetric to respiratory (CR) ratio is similar for both combustion and respiration at ~460 kJ·mol O₂⁻¹ (± 5%) because enthalpy production per electron equivalent approximates -115 kJ·mol O₂⁻¹ regardless of the carbon source (a carbon atom has four valences so that four electrons represent ~460 kJ·mol O₂⁻¹ ± 5%). In this regard, differences in heat production per unit of oxygen among fat and carbohydrate oxidation are better interpreted by bioenergetic explanations of energy transfer as opposed to gas exchange stoichiometry.

Of the ~36 total ATP re-synthesized by complete glucose oxidation, 2 come from glycolysis (~6% of the total) and 34 come from mitochondrial substrate level phosphorylation [25]. The anaerobic 1.5 kJ increase in heat production per oxygen equivalent when carbohydrate is oxidized compared to fat (at 21.1 kJ vs. 19.6 kJ) may be better attributed to the small but requisite energy transfer production of heat and entropy during anaerobic substrate level phosphorylation [25]. The anaerobic 1.5 kJ increase represents ~7% of the total heat production of complete glucose oxidation and is similar to the ~6% anaerobic ATP re-synthesis (2 of 36 ATP); like all energy transfer, glycolytic ATP re-synthesis (phosphorylation) is inefficient.

**Bioenergetics and energy expenditure**

Glycolytic phosphorylation and mitochondrial respiration represent separate and distinct acts of energy transfer. Glycolysis and glycogenolysis take place in the cytoplasm of cells, within and around the contractile apparatus of muscles for example. Glycolysis and glycogenolysis require multiple enzymes that catalyze proton and electron transfer. Moreover, glycolytic phosphorylation takes place where the useful energy within glucose and glycogen is converted to ATP. These reactions can be summarized as a series of phosphate transfers, phosphate shifts, isomerizations, dehydrations and aldol cleavages [26]. The inefficiency of glycolytic substrate level ATP re-synthesis is a result of heat and entropy production.

In comparison, the mitochondria are distinct double-membrane cellular organelles; these membranes create an effective compartment that is separated from the cellular cytoplasm. Within these membranes are a collection of further enzymes that continue to strip protons and electrons from substrate. Protons and electrons are subsequently delivered by carriers (e.g., NAD⁺) to the electron transport chain (ETC). Energy transfer in the aerobic re-
synthesis of ATP is not directly related to enzymatic glycolytic phosphorylation. Instead, reduction of reduced carriers by oxygen is used to create a gradient of protons. Using the inner membrane as a barrier, protons are pumped to one side; the subsequent gradient of protons creates an uphill-downhill energy transfer scenario whereby specific membrane portals known as mitochondrial ATPases allow protons to pass through. The energy of this downhill flow is exploited to re-synthesize ATP [26]. Mitochondrial heat production has been traced largely to the flow of protons down this gradient [6].

Contemporary bioenergetic interpretations of anaerobic and aerobic metabolism recognize the energy transfer independence of anaerobic and aerobic ATP re-synthesis; each has different reactants and products, uses dissimilar enzymes, involves different types of biochemical reactions, takes place in separate cellular compartments, exploits different types of gradients and, ultimately, each operates with different efficiency [27]. Thus, the heat and entropy production of anaerobic metabolic energy transfer can not possibly be represented by mitochondrial respiration (or vice-versa for that matter). Dissimilar energy transfer formats and operational efficiency must both be kept soundly in mind when interpreting energy expenditure. Nonetheless, glycolytic phosphorylation can proceed aerobically whereby pyruvate is immediately and directly routed for mitochondrial respiration (within the Krebs cycle). When the rate of glycolytic phosphorylation (with 2 ATP; 1.5 kJ per l O₂) matches the rate of mitochondrial respiration (with 34 ATP; 19.6 kJ per l O₂) then the anaerobic and aerobic components of glucose and glycogen oxidation can be added together to interpret the collective ATP turnover with the energy expenditure conversion, 21.1 kJ per liter of O₂ (~36 ATP).

**Lactate production**

Anaerobic glycolysis and glycogenolysis can proceed by the rapid reduction of pyruvate to form lactate (i.e., exceeding mitochondrial respiratory rates and regardless of oxygen availability). In an open system the rate of energy transfer and alterations in the product to reactant ratio can promote greater inefficiency [2,28]. When rapid glycolytic ATP re-synthesis exceeds mitochondrial rates, lactate and heat production ensues and a measure of oxygen uptake no longer accurately reflects the rate or the amount of ATP re-synthesis that takes place. Recall that the calorimetric to respiratory (CR) ratio during respiration is -460 kJ·mol O₂⁻¹ (± 5%). In cultured mammalian cells however, the ratio of heat production to oxygen uptake was found to vary from -490 to -800 kJ·mol O₂⁻¹ or more [12]. Gnaiger and Kemp found that the ~30 kJ to ~340 kJ·mol O₂⁻¹ increase was best related to the increase in lactate formation and presumably an increase in the anaerobic energy expenditure contribution to total ATP re-synthesis [12,17].

Lactate production in resting fully oxygenated cells is readily apparent [12,16,29,30]. In addition to providing ATP, rapid glycolytic phosphorylation has been suggested to maintain the redox potential within mammalian cells [31], to protect cells against oxidative stress [32], to promote the formation of biosynthetic precursors in growing cells [33] and as a mechanism of control in cellular growth [34]. Whatever its role, rapid glycolytic ATP re-synthesis with lactate production is associated with heat and entropy production and by definition inefficiency and energy expenditure. It appears that the most important step for heat production during rapid rates of glycolysis and glycogenolysis is the reduction of pyruvate to lactate at ~63 to ~80 kJ per mol of lactate (dependent on the immediate internal and external environments) [12,35]. This energy expenditure is irreversible.

**Lactate removal**

Removal of lactate involves conversion back to pyruvate. Pyruvate, in turn, can be converted into a variety of compounds that may include glucose within the liver (Cori Cycle), glycogen within cells (gluconeogenesis) or alanine (an amino acid). It is presumed that the ATP turnover that is required for these conversions comes from mitochondrial energy transfer (as 19.6 kJ per l O₂) [22].

Lactate can also be removed via the complete aerobic oxidation of pyruvate [36]. The application of energy conservation as expressed in Hess’s law (reactions that start and end with the same reactants and products produce the same amount of enthalpy regardless of path) led to the idea that anaerobic energy expenditure during exercise could be measured via subsequent oxygen uptake during the recovery from exercise, as part of the so-called "oxygen debt" [37]. This hypothesis proposes that all ATP re-synthesized via glycolytic phosphorylation is included in the net aerobic ATP yield when pyruvate undergoes subsequent aerobic oxidation (36 ATP; 21.1 kJ per l O₂), even if it passes transientsly through lactate. Gaesser and Brooks argued that the many fates of lactate and pyruvate removal in addition to complete aerobic oxidation indicate that the oxygen debt does not adequately represent anaerobic glycolytic energy expenditure [38]. Moreover, both aerobic and anaerobic biochemical reactions are often held far-from-equilibrium as part of an open system and this occurs at an irreversible expense [2,18,19].

Strict application of Hess’s law to the in vitro exothermic reaction of pyruvate to lactate requires that the reverse reaction should consume an equivalent amount of heat. While this is true within closed systems it should not be the case within an in vivo open system. It is in the heat loss
(calorimetric) to oxygen uptake (respiratory) ratio (kJ·mol O2⁻¹) that this is most clearly revealed. We found that in cell preparations and cardiac muscle fibers that respire on externally supplied pyruvate or lactate, there is equivalent heat production when expressed per mol of oxygen uptake [20]. That is, heat is not consumed when lactate is converted back to pyruvate; the reaction is not thermodynamically reversible, energy transfer during mitochondrial respiration does not represent energy transfer in the form of rapid or accelerated anaerobic glycolytic ATP re-synthesis with lactate formation. It is therefore ironic that for most of the 20th century muscle cells were known to be chemo-mechanical converters as part of an open system yet energy transfer, as described by the oxygen debt hypothesis, continued to be explained from a traditional thermodynamic closed system standpoint.

**Application and interpretations**

Indirect calorimetry is a much simpler procedure than direct calorimetry accounting for its continued popularity in estimating biological heat production. When anaerobic energy expenditure contributions are large, however, whole-body energy expenditure may be significantly underestimated (figure 1). It is unfortunate that no valid measure of anaerobic heat production is available – this appears to be another reason for the hesitation to include an anaerobic component as separate from an oxygen-only interpretation of energy expenditure. The problem lies in the inherent difficulties of the collection of anaerobic metabolites from within active cells. Moreover, there are stores of ATP and phosphocreatine (PC) contained within muscle tissue that are utilized during heavy to severe exercise as anaerobic energy transfer but that are re-synthesized aerobically during the recovery from exercise as excess post-exercise oxygen consumption (EPOC). Thus one part of this ATP/PC turnover is anaerobic, the other is aerobic [38-40]. In fact, heat measurements taken during brief intense exercise have revealed anaerobic metabolism to be more efficient than aerobic metabolism [27]. Such a finding must be considered carefully however as the heat loss during the oxygen deficit portion of exercise contains separate proportions of rapid glycolytic phosphorylation (that represents full ATP turnover) and stored ATP/PC usage (but not ATP/PC re-synthesis) [40].

There are non-invasive methodologies that estimate only the anaerobic substrate level phosphorylation component of anaerobic energy expenditure (i.e., glycolysis and glycogenolysis without the ATP/PC stores). One such estimate suggests that every millimole of blood lactate above resting levels equals an energy expenditure of 3 milliliters of oxygen uptake per kilogram of body weight [41]. For example, a 65 kg woman with a resting blood lactate level of 1.1 mmol engages in a 400 meter sprint to exhaustion. Peak lactate levels for her sprint are 12.1 mmol so that the change in blood lactate is 11.0 mmol, resulting in an anaerobic energy expenditure contribution of ~45 kJ (~11 kcals).

Because blood lactate concentrations provide at best an approximate description of muscle lactate levels and glycolytic ATP re-synthesis, it is clear that more research is needed to obtain a valid estimate of anaerobic energy expenditure. Excerpts from the interpretation of whole-body thermogenesis? Below are a few examples where anaerobic energy expenditure contributions may be sufficiently large that their inclusion may improve current interpretations of whole-body energy expenditure.

**Exercise energy expenditure**

It has been concluded from exercise oxygen uptake-only measurements that a one-set circuit weight training regimen consisting of 8 exercises was 15 kcals short of meeting the energy expenditure criteria for a healthy lifestyle in men (i.e., 150-200 kcals per exercise session) [45]. However, these criteria would appear to have been met if an estimate of rapid glycolytic ATP re-synthesis were included with the exercise oxygen uptake measurements. Depending on the size of the exercising muscle mass, my students and I have found blood lactate contributions to a single bout of weight training exercise (i.e., 1 set) to range from 3 to 12 kcals per exercise; a minimal contribution of 3 kcal per exercise would result in an increase in energy expenditure of almost 25 kcal for this weight training circuit. The use of both an anaerobic estimate and an aerobic measure of energy expenditure would provide support for regular circuit weight training as an effective method of obtaining a healthy lifestyle in men. The anaerobic energy expenditure component needs to be large to make a significant contribution to total energy expenditure and this is best seen during brief heavy to severe exercise (total energy expenditure includes exercise anaerobic and aerobic energy expenditure and an acute measure of EPOC) (figure 1).

The effect of anaerobic energy expenditure on total energy expenditure can be seen in the observation that exercise duration and intensity in reptiles and humans have been shown to affect EPOC size [46-48]. It may be inferred that anaerobic and aerobic energy expenditure interact to promote a larger EPOC. In sprinting mice however, EPOC has been found to be independent of either exercise duration or intensity [49]. Mice are very aerobic and may have a limited anaerobic energy expenditure contribution to...
sprinting, explaining why EPOC volumes are limited in sprinting mice. Unfortunately anaerobic energy expenditure was not estimated in the mouse study. It is of interest to speculate whether, if energy transfer as rapid glycolytic ATP re-synthesis had originally been considered separate from oxygen uptake, the concept of oxygen debt would have been recognized as an interaction between aerobic and anaerobic energy expenditure (metabolism) rather than being interpreted as "repayment on a loan."

**Exercise economy**

Exercise economy is traditionally defined as the oxygen uptake required to perform a bout of work at a given rate (e.g., a specific running or cycling pace). During steady state light to moderate intensity exercise, oxygen uptake remains level and provides a sufficient measure of economy. However oxygen uptake steadily increases as heavy to severe steady-state work continues (with ultimate exhaustion) and this has been termed the "slow oxygen
uptake component” [50]. This phenomenon remains, for the most part, unexplained yet it is thought that motor unit recruitment patterns may be altered resulting in "additional energy expenditure” [50]. The term "slow oxygen uptake component" implies an aerobic-only approach because the anaerobic glycolytic component is a well known part of heavy to severe exercise. Bioenergetic interpretations might suggest that "additional energy expenditure" is the result of the further dissipation of Gibbs free energy under cellular conditions where both anaerobic and aerobic energy expenditure contributions are changing [2,28].

Ramp-type stress tests, unlike steady-state exercise, utilize a continually increasing power output until the test is terminated at exhaustion (figure 2). At low to moderate workloads, oxygen uptake and power output are linear for both slow (e.g., 15 Watts·min⁻¹) and fast (e.g., 60 Watts·min⁻¹) ramping tests. As the exercise intensity becomes "heavy to severe", the oxygen uptake to Watts ratio increases for the slow ramp test (top line). The opposite is true for the fast ramp test to exhaustion where the oxygen uptake to Watt ratio decreases (bottom line). Notice that the peak Watts are significantly different but the VO₂ maximum for the two tests is similar [51, 52]. Contributions of both anaerobic and aerobic energy transfer may explain these apparently disparate phenomena as described in the text.

Figure 2
Continuously increasing ramp exercise tests to exhaustion. Resting oxygen uptake is seen until the start of exercise (vertical line). At low to moderate work rates the oxygen uptake to Watts ratio is similar and linear for both slow (e.g., 15 Watts·min⁻¹) and fast (e.g., 60 Watts·min⁻¹) ramping tests. As the exercise intensity becomes "heavy to severe", the oxygen uptake to Watts ratio increases for the slow ramp test (top line). The opposite is true for the fast ramp test to exhaustion where the oxygen uptake to Watt ratio decreases (bottom line). Notice that the peak Watts are significantly different but the VO₂ maximum for the two tests is similar [51, 52]. Contributions of both anaerobic and aerobic energy transfer may explain these apparently disparate phenomena as described in the text.

Ramp-type stress tests, unlike steady-state exercise, utilize a continually increasing power output until the test is terminated at exhaustion (figure 2). At low to moderate workloads, oxygen uptake and power output are linear for slow and fast ramp testing, but this is not seen at heavy to severe workloads [51, 52]. Slow ramps to exhaustion have gradual increases in power output so that the test can be lengthy, lasting many minutes. Toward the end of a slow ramping test, the ratio of oxygen uptake to power output begins to increase so that exercise oxygen uptake appears to contain a "slow oxygen uptake component"; a larger relative aerobic versus anaerobic energy expenditure component is found with slow ramping [52]. On the other hand, fast ramping utilizes rapidly increasing power outputs that promote fatigue quickly, resulting in brief test lengths. Toward the end of a fast ramping test to exhaustion the ratio of oxygen uptake to power output may decrease, the traditional interpretation being that this promotes larger relative anaerobic energy expenditure. An alternative explanation is that the decrease in the rate of oxygen uptake is caused by a faster rate of rapid glycolytic phosphorylation that results in a larger relative anaerobic energy expenditure contribution; that is, a whole-body "Crabtree effect" where a non-linear component to "additional energy expenditure" in the form of anaerobic energy transfer is found [53]. Measures of economy for all types of exercise testing would be improved by an estimate of anaerobic energy expenditure.

Exothermic to endothermic transition
Mammals are avid consumers of oxygen and well known producers of heat. Mammalian cellular membranes have been shown to leak ions at a rate that is several-fold greater than those in reptiles; the result is an obligatory increase in ion pumping to maintain the electro-chemical membrane potential [54]. Stevens [55] has suggested that stimulation of the sodium pump was an important evolutionary development toward endothermy. Brisk activity of the sodium pump necessitates a rapid rate of ATP re-synthesis. If this is true then it is important to recognize that in some cells lactate with presumed heat production is better correlated with sodium and potassium pumping than is oxygen uptake [29]. The removal of lactate as provided by mitochondrial ATP re-synthesis further contributes to heat production (e.g., Cori cycle, gluconeogenesis, aerobic oxidation). Because resting lactate turnover in endotherms is as much as 1,500-fold higher than in a similar sized ectotherm, the potential for extensive anaerobic ATP re-synthesis needs to be considered as part of basal whole-body thermogenesis in mammals [56]. It seems logical to conclude that most mammalian energy expenditure does come from aerobic metabolism but the evolution of a metabolic acceleration with concomitant heat production comes from both anaerobic and aerobic pathways. The relative contributions of each pathway to whole-body thermogenesis are not known.

Arousal from torpor
Tucker [11] has shown that heat production in hibernating mice as estimated by oxygen uptake does not account for all of the temperature increases when mice arouse from their metabolic torpor. It is possible therefore that heat production can be accounted for in full when anaer-
obic energy expenditure is considered as an addition to oxidation-only measurements. Arousal from torpor often induces intense shivering that promotes rapid glycogen degradation accompanied by lactate production and perhaps, like heavy to severe exercise, additional heat production above oxygen uptake-only estimates [57]. The addition of an anaerobic-heat component to whole-body oxygen uptake would appear beneficial to thermogenesis during arousal. Lactate may later be re-converted back to glycogen, a process that may be fueled by mitochondrial fat oxidation to conserve glycogen stores. Such a "turtle cycle" of lactate turnover that is, rapid glycogenolysis (lactate appearance) coupled to gluconeogenesis (lactate disappearance to form glycogen) would be of importance to an obese hibernator who undergoes multiple arousal periods over the course of a winter and has limited access to carbohydrate but has substantial body fat reserves.

Synopsis

Metabolic energy transfer takes place in part as the oxidation of carbohydrate that includes an anaerobic (glycolysis) and aerobic (mitochondrial) component. Rapid glycolytic ATP re-synthesis with lactate production can exceed mitochondrial rates and under these conditions the efficiency of anaerobic energy transfer can not be interpreted using gas exchange stoichiometry. When rapid glycolytic ATP re-synthesis with concomitant heat production is extensive, the anaerobic contribution to energy expenditure can be significant both in cells and in whole-animals. The interpretation of efficiency and energy expenditure may be improved if a separate estimate of anaerobic ATP turnover is provided along with a measure of oxygen uptake.

References

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