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Forum

Optimum efficiency of energy transformation and the evolution of catabolic pathways

INTRODUCTION

Evolutionary success (fitness) depends on optimization of phenotypic traits that can be measured as survival, fecundity and generation time (Sibly & Calow 1986). Optimization of energy transformation in metabolism relates fundamentally to these aspects of fitness. Two important performance measures of energy transformation are: (1) generation of power output as required to meet functional demands; and (2) efficiency of resource utilization.

Survival, fecundity and generation time are optimized when energy is limited, by shifting the emphasis from maximization of effective power output (*power strategy* or *P* strategy) to efficient and economical resource utilization (*economy strategy* or *E* strategy; Gnaiger 1987). This 'ergodynamic' power/economy concept (erg=work) emphasizes the direct connection between measures of fitness and the adaptive traits of selecting high-power or high-efficiency pathways under different environmental constraints and physiological demands.

Many invertebrate species tolerate long-term environmental anoxia by reducing metabolic heat flux (Gnaiger 1983; Hand & Gnaiger 1988). Under such *passive anoxia* the low-power high-efficiency pathways are employed with production of succinate, propionate and acetate. In contrast, the high-power low-efficiency lactate and opine pathways are optimized for physiological, *active anoxia*. The power/economy dichotomy is analogous to the distinction between *r* and *K* selection in habitats which are characterized by low and high population densities in relation to the resources (Gnaiger 1983).

The negative correlation between power and efficiency in active and passive anoxia is a direct contradiction to the claim of coevolution of power and efficiency (Watt 1986). In view of the experimental failure of this claim, Watt (1992) proposes a 'simpler explanation' for pathway choice at limiting oxygen. However, this is inapplicable under anoxic conditions and does not address selection for low efficiency in the high-power lactate pathways. This high power/low efficiency relation is an experimental fact. It rejects the net-fitness measures proposed by Watt (1986) on quantitative grounds, regardless of any particular theory of metabolic flux control.

The ergodynamic concept on the trade-off between metabolic power and efficiency (Stucki 1980; Gnaiger 1987; Westerhoff & Van Dam 1987) is criticized by Watt (1986, 1992). Here I clarify several misunderstandings:

1. Watt claims that flux/force linearity is generally restricted to conditions near equilibrium. In experimental practice, however, linear flux/force relations are observed far beyond equilibrium at levels of complexity ranging from gas reactions (Prigogine, Outer & Herbo 1948) to oxidative phosphorylation (Fig. 1).

2. Watt claims that the power/efficiency trade-off is based on near-equilibrium linearity between fluxes and forces. In fact non-linearity is an integral part of the description of power and economy strategy (Gnaiger 1987, p. 24).

3. Watt (1986) refers to concentration changes or ATP/ADP ratios instead of chemical forces, consequently drawing wrong conclusions on flux–force relations.

4. Watts fails to distinguish flux ('rate'; chemical change per unit of time) and power (Gibbs energy change per unit of time), unacceptable in disputing the power/efficiency trade-off.

5. Watt discusses individual forces, such as glucosyl potential, when coupled input and output force pairs are at issue. The lack of a relation between oxygen flux and catabolic input force at the onset of insect flight is not surprising: it is the very nature of fluxes in coupled processes not to respond to a single (input or output) force.

6. Watt confuses efficiency and ATP stoichiometry. When properly analysed, the high-power energetics of aerobic insect flight — Watt's counter-example — turns out to provide additional support for the power/efficiency trade-off.

The dispute on kinetics *vs* non-equilibrium thermodynamics is not new (Wilson & Westerhoff 1982). Further conceptual developments aim at bridging the gap between thermodynamics and kinetics to improve our understanding of the energetics of metabolic pathways as relevant for evolutionary biology in general and evolutionary energetics in particular (Gnaiger 1987, 1989a; Westerhoff & Van Dam 1987).

LINEAR FLUX/FORCE RELATIONS EXPERIMENTALLY OBSERVED FAR FROM EQUILIBRIUM

Limits of linearity

Ergodynamic flux/force relations are phenomenological (independent of mechanism). Linearity is theoretically confined to the near-equilibrium range at Gibbs forces (molar reaction Gibbs energies) less exergonic than $-RT$ or $-2{\cdot}5\,\mathrm{kJ\ mol^{-1}}$, as deduced from gas kinetics (Prigogine *et al.* 1948; Gnaiger

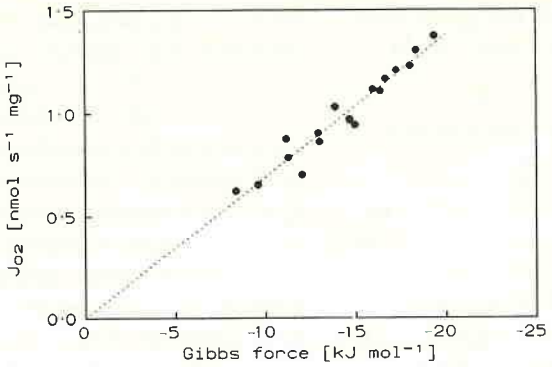

Fig. 1. Linear flux–force relation in oxidative phosphorylation far beyond the near-equilibrium range. The slope is the conductivity, L (equation 5), incorporating the ATP/O_2 stoichiometry. Oxygen flux, J_{O_2} (nmol O_2 s^{-1} mg^{-1} protein), *vs* net Gibbs force, $\Delta_k G_{\infty ATP}+\Delta_p G_{ATP}$ (kJ mol^{-1} ATP) (equation 6), in mitochondria isolated from rat liver, measured in the CYCLOBIOS Oxygraph at varying concentrations of ADP and P_i, by G. Méndez (G. Méndez, T. Haller and E. Gnaiger, manuscript in preparation).

1989a). Near-equilibrium conditions are not generally important in bioenergetics. Linearity, however, is frequently obeyed far beyond the near-equilibrium range. Therefore, linear relations far from equilibrium are a matter of experimental test and are outside the scope of theory of conventional non-equilibrium thermodynamics (Katchalsky & Curran 1967; Stucki 1980; Gnaiger 1989a).

Elementary reactions vs *pathways*

It is meaningless to compare the limiting value of RT (Watt 1992) with the Gibbs force of a pathway. RT is relevant exclusively for single elementary reactions. The sequence of elementary reactions is the mechanism of an enzyme-catalysed reaction which must be known for rigorous application of kinetics. The sequence of enzyme-catalysed reactions and transport systems, in turn, constitutes a pathway. With more than 10 enzyme-catalysed reactions in the high-efficiency glycolytic pathways, the net force of a pathway (Table 1) is distributed over a large number of elementary reactions, each with linearity up to or beyond $-RT$.

Linearity in mitochondrial oxidative phosphorylation

Studies of oxidative phosphorylation provide the most important biological examples of linearity far from equilibrium (Fig. 1; Stucki 1980; see also Berry *et al.* 1989). The underlying adenylate and phosphate control can be described on the basis of generalized flux/pressure relations (Gnaiger 1989a). Linearity is observed at apparent net forces up to -20 kJ mol^{-1} ATP (Fig. 1), more exergonic than the corresponding value of -11 kJ mol^{-1} ATP in high-efficiency anoxic catabolism (Gnaiger 1987). The effective forces in oxidative phosphorylation and anoxic glycogen catabolism are comparable (Table 1).

Contrary to an 'internal obedience of oxidative phosphorylation to near-equilibrium rules' (Watt 1992), quantitative information on chemical potentials in mitochondrial respiration rules out a near-equilibrium explanation of linearity in chemosmotic ATP production (Fig. 1; see also Westerhoff & Van Dam 1987; Gnaiger 1989a). Oxidative phosphorylation is a vectorial process directed across the mitochondrial membrane. Scalar metabolism (glycolysis) lacks orientation in space. Three general errors are contained in the claim by Watt (1992) that linear relations are appropriate when analysing vectorial oxidative phosphorylation, but are 'completely invalid' in glycolysis. First, the succinate pathway is not merely a scalar process: anoxic mitochondrial electron transport is coupled to ATP production in the reversed succinate dehydrogenase reaction (Saz 1981; Hochachka & Somero 1984). Second, non-equilibrium thermodynamics is a phenomenological theory of flux/force relations independent of the mechanism of chemical energy transformation. The assumption that linearity is extended in vectorial flux compared to chemical reactions is based on the misleading interpretation of Fick's First Law as a flux/force relation. This is a classical textbook error (reviewed by Gnaiger 1989a). Flux/force relations must be replaced by generalized flux/pressure relations (Gnaiger 1989a; Gnaiger & Jacobus 1989). Third, Watt (1992) ignores that the linear range is expanded several times in enzyme-catalysed reactions, due to linear approximation (Westerhoff & Van Dam 1987). Importantly, any linear or

Table 1. ATP-coupling stoichiometry (ATP/glycosyl-unit) *vs* ergodynamic efficiency (force efficiency of ATP production, $-\Delta G_{ATP}/\Delta_k G_{\infty ATP}$; equation 7) in aerobic and anoxic catabolism of glycogen. Power strategy, P, and economy strategy, E, are related to low and high efficiency at varying stoichiometry (after Gnaiger 1987)

Pathway	State	Stoichiometry ATP/Glyc	Efficiency $-\Delta_p G/\Delta_k G$	Strategy
Aerobic	Rest	37·0	0·80	*E*
Aerobic	Active	37·0	0·66	*P*
Lactate	Active	3·0	0·63	*P*
Succinate	Passive	4·71	0·74	*E*
Propionate+acetate	Passive	6·33	0·79	*E*

non-linear increase of flux with exergonic force results in a constraint of power at high efficiency.

Far from equilibrium states vs *covariation of power and efficiency*

In ergodynamics the distance from equilibrium is not assumed but quantitatively measured by the Gibbs forces of reactions and pathways. Therefore, it is paradoxical to interpret (Watt 1992) 'irreversible' as a synonym for 'near equilibrium' here; and it is misleading to argue that the concept on the power/efficiency trade-off is based on the 'assumption that these pathways operate near equilibrium'. To operate near equilibrium, maximization of efficiency would be required, pushing coupled processes towards ergodynamic equilibrium (see below). Covariation of power and efficiency (Watt 1986), therefore, would result in such near-equilibrium conditions in active metabolic states. However, efficiency is not maximized but optimized, in accordance with the ergodynamic explanation. Watt (1992) avoids to draw this important conclusion.

HIGH POWER–LOW EFFICIENCY: AN EVOLUTIONARY PARADOX?

Inverse relation in contrast to covariation of power and efficiency under anoxia

Leeches (*Hirudo medicinalis*; Zebe, Wiemann & Wilps 1981) and oligochaetes (*Lumbriculus variegatus*; Putzer 1985) produce succinate, propionate and acetate under passive anoxia at high efficiency (up to 80%), while the lactate pathway is *kinetically* suppressed. When stimulated under strict anoxia, these animals show bursts of activity over short periods of time. The corresponding high metabolic power is generated by the low-efficiency (60–65%) lactate pathway. The succinate pathway continues operating during anoxic burst activity, slowly even when the option for aerobic respiration is removed.

Coevolution of high power and high efficiency (Watt 1986) leads to the postulate that the high-power lactate pathway should operate at high efficiency. The contrary is observed (Gnaiger 1983, 1987; Table 1). Why are the high-efficiency pathways not employed under active anoxia when ATP demand is highest? More than twice the ATP could be obtained per unit of glycogen (Table 1). Is there a 'simple' explanation for this apparent high power–low efficiency paradox?

According to Watt (1992) the high-efficiency pathways simply cannot run in parallel with high aerobic respiration. If true, this does not explain (1) why the efficiency in the lactate pathway is low. Nor does it explain (2) the low capacity for metabolic power of these pathways under anoxia when the complete lack of oxygen prevents any aerobic respiration. Anoxia occurs either locally while aerobic respiration proceeds in spatially separate regions, or in fully anoxic animals. (3) Watt's proposed explanation is not even true under aerobic and hypoxic conditions. In anaerobic succinate formation the citric acid cycle operates simultaneously in the forward and reversed direction. Each succinate generated from the dismutation of malate via citrate provides reducing equivalents for a five-fold faster operation of the reversed malate–fumarate–succinate sequence (Gnaiger 1977). Under progressive hypoxia, redox balance is maintained by a gradual shift from oxygen to fumarate as the terminal electron acceptor. This shift does not impose any restrictions upon aerobic respiration beyond those set by limiting oxygen conditions. Other, similarly inadequate explanations of the restricted power of the succinate–propionate–acetate pathways (Zandee, Holwerda & de Zwaan 1980) have been discussed by Gnaiger (1983). High efficiency and corresponding ergodynamic inhibition remain the simplest answer.

Why selection for low efficiency?

Watt (1992) emphasizes the need for evolutionary performance measures, yet his discussion of pathway choice does not resolve the apparent evolutionary paradox of selection for low efficiency in high-power pathways. The ATP-coupling stoichiometry is a result of pathway evolution (Atkinson 1977). Therefore, the stoichiometry and low efficiency of the lactate pathway cannot be treated as a casual occurrence that escaped the mechanism of natural selection. The claim that glycolytic flux is not constrained by high efficiency under *in vivo* conditions suggests under-utilized scope for high efficiency. The low efficiency of the lactate pathway — common among many phyla and retained by natural selection over millions of generations — would remain a distressing contradiction to the evolutionary paradigm of adaptation. High ATP efficiency in the succinate pathway is adaptive; is the lower efficiency of the lactate pathway maladaptive?

The concept of the power/efficiency trade-off resolves this paradox. Several errors in the critique of this concept (Watt 1992) are discussed below in a sequence of increasing complexity of the underlying thermodynamic parameters (see also Gnaiger 1989b, 1990).

FAILURE OF DISTINCTION BETWEEN FORCE AND CONCENTRATION

Chemical potential and Gibbs force

The force driving a chemical reaction (Gibbs force) is calculated as the molar Gibbs energy change of reaction (kJ mol^{-1}). To quantify the Gibbs force, the chemical potentials of substrates and products must be calculated. The chemical potential of a substance

B is the sum of the standard chemical potential, μ^{o}_{B}, and the logarithm of the activity of B:

$$\mu_B=\mu^{o}_{B}+RT\ln a_B \qquad \text{eqn 1}$$

A chemical reaction must be defined in terms of the stoichiometric numbers of all chemical species *i*, ν_i, positive for products and negative for substrates. The Gibbs force is the sum of the products $\nu_i\mu_i$ (Alberty & Daniels 1980):

$$\Delta_k G_{Glyc}=\sum_i \nu_i\mu_i \qquad \text{eqn 2}$$

The Gibbs force of glycogen respiration, $\Delta_k G_{Glyc}$, relates to a catabolic half-reaction, excluding net ATP formation (subscript k for catabolic). The subscript Glyc indicates that the reaction stoichiometry is defined in terms of unit glycogen ($\nu_{Glyc}=-1$), whence the stoichiometric numbers for oxygen and CO_2 are -6 and 6. $\Delta_k G_{Glyc}$ is $-2880\,kJ\ mol^{-1}$ glycosyl-unit under cellular conditions (Gnaiger 1983).

Watt (1992) alluded to the 'improper' symbol ΔG. Following Westerhoff & Van Dam (1987), Watt (1992) uses indeed an improper symbol and terminology which fails to appreciate the fundamental distinction between extensive and intensive quantities, ΔG and $\Delta_r G$, respectively. ΔG is an extensive quantity (unit: J), the difference of the Gibbs energies of a system at two different states, or of reactants and products. $\Delta_r G$ is the molar Gibbs energy of reaction r, appropriately called the Gibbs force (unit: $J\ mol^{-1}$; see Gnaiger 1989b, 1990). It is the partial molar derivative of the Gibbs energy per extent of reaction, $\Delta_r G=\delta G/\delta\xi$ (Mills *et al.* 1988). The driving force of the reaction is not the 'free energy difference between reactants and products' (Watt 1992), but the sum over all stoichiometric potentials, $\nu_i\mu_i$, of reactants and products (equation 2). Thus, the partial molar derivative of Gibbs energy is calculated as a difference (hence the Δ in $\Delta_r G$), that is the sum of positive (for products) and negative (for substrates) stoichiometric numbers times chemical potentials.

Watt (1986) explained the molar Gibbs energy of the catabolic reaction as the (molar) 'free energy difference between one glycosyl residue and the carbon product in question' (compare Watt 1992). This is not merely improper but wrong. The chemical potentials must be multiplied by the corresponding stoichiometric numbers (equation 2). [The stochiometric numbers are missing in equation 1b of Watt (1992).] Stoichiometric numbers of aerobic and anoxic carbon products are 1·7 to 6 per unit glycosyl residue. Furthermore, the chemical potential of all reactants and products (water, protons, etc.) must be included in the calculation (equation 2). Unfamiliarity with the classical thermodynamic definition of molar Gibbs energies of reaction leads to a wrong expression of efficiency (equation A1 in Watt 1986, p. 649), application of which would violate the Second Law of thermodynamics. Watt's equation A1 is a misquotation, unrelated to metabolic efficiency (Gnaiger 1983).

In dilute aqueous solution, concentrations can be used instead of activities (equation 1). Cellular glycogen does not behave as an ideal solute. Hence changes in concentration may influence the activity to a minor extent. Even when assuming that activity changes proportionately to concentration, a drop to 10% of the initial trehalose or glycogen activity amounts to a change of the chemical potential by $RT\ \ln(10/100)=-6\,kJ\ mol^{-1}$ glycosyl-unit. This renders the catabolic Gibbs force of $-2880\,kJ\ mol^{-1}$ less exergonic by merely 0·2%! Hence, despite a drop in substrate levels, the driving force is nearly constant during prolonged insect flight, sustaining a steady power requirement of muscular activity.

Inadequacy of ATP/ADP ratios

ATP/ADP ratios provide insufficient information in the context of flux/force relations. The Gibbs force of phosphorylation of ADP to ATP, $\Delta_p G_{ATP}$, is calculated on the same basis as equation 2 or by an equivalent, more convenient equation:

$$\Delta_p G_{ATP}=\Delta_p G_{ATP}'+RT\ \ln\left(\frac{[ATP]}{[ADP]\,[P_i]}\right) \qquad \text{eqn 3}$$

[ATP], [ADP] and [P_i] are the dilute concentrations of adenosine triphosphate, adenosine diphosphate and inorganic phosphate. $\Delta_p G_{ATP}'$ is the standard Gibbs force at specified pH, magnesium activity, temperature and unit activity of ATP, ADP and P_i (Alberty & Daniels 1980).

$\Delta_p G_{ATP}'$ depends strongly on proton activity at physiological pH, which changes when acid anoxic end products accumulate. Inorganic phosphate controls the Gibbs force of ATP production in aerobic tissue (Kushmerick 1985). $\Delta_p G_{ATP}$ ranges from 44 to $72\,kJ\ mol^{-1}$ ATP *in vivo* (Dawson, Gadian & Wilkie 1978; Gnaiger 1983; Meyer, Brown & Kushmerick 1985). The effects of pH and P_i are obscured by reference to ATP/ADP ratios or energy charge (Atkinson 1977). The actual catabolic and phosphorylation Gibbs forces must be quantified to obtain ergodynamic and corresponding evolutionary performance measures.

CONFUSION OF FLUX (RATE) AND POWER

Watt (1986, pp. 634, 636, 637; 1992) defines power erroneously as metabolic flux ('rate'), which is the chemical change per unit of time. Metabolic power is Gibbs energy change (J) per unit of time (s) (Gnaiger 1989b, 1990). For comparison, mechanical power output is work per unit of time, or velocity times mechanical force. Maximum velocities change dramatically as the mechanical force changes. On a biomass basis, metabolic power ($J\ s^{-1}\ g^{-1}=W\ g^{-1}$) is

metabolic flux (mol s^{-1} g^{-1}) times Gibbs force (J mol^{-1}). Phosphorylation power output, ${}_pP$, is ATP flux (${}_pJ_{ATP}$, ATP production) times force (see equation 3):

$${}_pP = {}_pJ_{ATP}\,\Delta_pG_{ATP} \qquad \text{eqn 4}$$

When the important distinction between metabolic flux anmd power is not made, a misunderstanding of the power/efficiency trade-off is inevitable. Efficiency is the output/input power ratio, distinguished from the ATP/glycogen flux ratio (see below).

FLUX MUST BE RELATED TO THE NET FORCE

The reduced energy flux in anoxic *Mytilus* (Shick, Widdows & Gnaiger 1988) has, according to Watt (1992) 'no proportional drop in glucosyl potential'. Similarly, he argues that the catabolic force, Δ_kG_{Glyc} (his $-X_G$) does not show a 'massive increase' during the transition between rest and flight, to drive increased flow in insect muscle. Such criteria would apply to a decoupled flux, yet catabolic and ATP fluxes are coupled. With proper evaluation of net Gibbs energy transformations in coupled ATP production, the economy strategy in anoxic *Mytilus* provides an important example for the concurrence of low power and high efficiency and the corresponding flux/force relations (Gnaiger 1983). Conversely, flux and net force are increased at constant catabolic force by a decrease of Δ_pG_{ATP} at the onset of insect flight. Kinetic control does not confound, but is complementary to, the effect of force on flux. Despite the fact that kinetic control is dominating, maximum power of flight is possible only very far from equilibrium, that is at low ergodynamic efficiency.

Flux/force relations in coupled reactions

The relation between chemical flux (reaction velocity, ${}_rJ_B$, expressed in terms of substance B) and Gibbs force (Δ_rG_B) is described by the phenomenological equation:

$${}_rJ_B = -L\,\Delta_rG_B \qquad \text{eqn 5}$$

where L is the conductivity (phenomenological coefficient). If L is practically constant over the observed range of the force, the flux/force relation is linear. The effects of the coupled and opposing catabolic and phosphorylation forces on ATP production must be considered simultaneously (Fig. 1). The strongly negative catabolic force shifts the equilibrium of the coupled process into the direction of ATP synthesis, resulting in a negative net force. The amount of ATP produced per glycosyl-unit is the 'gear ratio' (ATP/Glyc), the fraction of Δ_kG_{Glyc} disposable to compensate and surmount the positive Δ_pG_{ATP}. Δ_kG_{Glyc} divided by the ATP/Glyc ratio is the normalized catabolic force, $\Delta_kG_{\infty ATP}$ (kJ mol^{-1} ATP turnover), acting opposite to the output force of ATP production. In aerobic respiration, $\Delta_kG_{\infty ATP}$ equals $-2880/37 = -78$ kJ mol^{-1} ATP turnover. The flux of ATP production, ${}_pJ_{ATP}$ (mol ATP s^{-1} g^{-1}), is related to the net force, $\Delta_kG_{\infty ATP} + \Delta_pG_{ATP}$:

$${}_pJ_{ATP} = -L\left(\frac{\Delta_kG_{Glyc}}{ATP/Glyc} + \Delta_pG_{ATP}\right) = -L\,(\Delta_kG_{\infty ATP} + \Delta_pG_{ATP}) \qquad \text{eqn 6}$$

Therefore, a change of flux at constant catabolic force is no argument against flux/force relations. Indeed, the phosphorylation potential or phosphorylation force is an eminently important variable contributing to changes of the net force and to the control of flux in skeletal muscle (Kushmerick 1985; Gnaiger 1987; di Prampero 1989).

Near-equilibrium conditions in vivo*?*

All forces, Δ_rG, are zero at physico-chemical equilibrium, whence the net flows are zero. Low values of Δ_pG_{ATP} and Δ_pG_{Glyc} (near physico-chemical equilibrium) are irrelevant in living systems. Near ergodynamic equilibrium these coupled input and output forces are very large *in vivo* (Gnaiger 1987). The catabolic–phosphorylation net force (equation 6) is low at high efficiency and is zero at maximum efficiency. Maximum efficiency and maximum flux are mutually exclusive.

Degree of coupling

Uncoupling is a metabolic control mechanism, lowering the efficiency and increasing the catabolic flux. The corresponding increase of power at low efficiency upon uncoupling is a well-established direct test of the power/efficiency trade-off. To account for uncoupling in equation 6 requires multiplication of $\Delta_kG_{\infty ATP}$ by a coefficient, $d \leq 1{\cdot}0$, the degree of coupling (Kedem & Caplan 1965). d equals 1·0 in fully coupled processes, and equation 6 is applicable as written. Full coupling at a fixed ATP stoichiometry characterizes several anoxic pathways with substrate-level phosphorylation and yields a close approximation for aerobic respiration (Gnaiger 1989a; see also Lemasters 1984). d equals zero in a completely uncoupled process. Upon uncoupling, ATP flux is disconnected from the catabolic force, which is multiplied by $d = 0$ and effectively removed from equation 6.

Interpretation of muscle phosphagens and adenylate kinase as '*de*coupling devices' (Watt 1992) is a misunderstanding of the concept of ergodynamic coupling. Production or utilization of phosphagens are coupled ergobolic processes (Gnaiger 1987; di Prampero 1989). Generally, ergobolic flux, ${}_eJ$, is the flux of ATP equivalents (including ATP and phosphagens), either the output of catabolism during energy restoration, or the input of muscular burst

activity (Gnaiger 1983, 1987). Moreover, decoupling is misunderstood when viewing the 'evolution of storage reserves' as a process 'whose adaptive purpose is to decouple catabolism from anabolism' (Watt 1992). Synthesis of storage reserves is an anabolic reaction which requires ATP (e.g. for glucosidic or peptide bonds) and is thus linked to catabolic ATP regeneration.

Anabolism and catabolism are conductance matched when catabolic ATP supply is balanced by anabolic ATP demand. Such conductance matching, defined by the ratio of catabolic and anabolic conductivities (Gnaiger 1987, p. 30), is central to the analysis of two-compartmental optimum efficiency. It is wrong that the power/economy concept depends on any other interpretation of catabolic—anabolic coupling (contra Watt 1992).

Onsager reciprocity

L in equation 6 is a straight coefficient (conductivity), expressing the dependence of ATP flux on the Gibbs force of ATP production. A cross-coefficient (Kedem & Caplan 1965; Westerhoff & Van Dam 1987) relates the ATP flux to the catabolic force. When the catabolic force per glycosyl-unit is concerned, the cross coefficient is (from equation 6, inserting the degree of coupling, d):

$$L\frac{d}{\text{ATP/Glyc}}$$

Watt (1992) claims that cross-coefficients 'do not exist'. Without a non-zero positive cross-coefficient, ATP production from ADP cannot occur in the living cell, or it would violate the Second Law of thermodynamics. The phosphorylation force, Δ_pG_{ATP}, is positive, whence the uncoupled flux would be negative, running backwards in the direction of spontaneous ATP hydrolysis. The existence of cross-coefficients between the input and output fluxes and forces is imperative when the output flux is driven against an endergonic (positive) Gibbs force. Importantly, cross-coefficients and straight conductivities do not have to be constant to 'exist'.

Dependence of the catabolic flux on the phosphorylation force is described by a second cross-coefficient. The equivalence of the two cross-coefficients is referred to as 'Onsager reciprocity' which does not generally hold far from equilibrium (Kedem & Caplan 1965). However, a far from equilibrium state is an insufficient condition for the absence of reciprocity. Watt (1992) fails to recognize that a fixed stoichiometry and tight coupling (see above) necessarily yield Onsager reciprocity (Gnaiger 1983, 1987).

Uncoupling leads to a high catabolic flux and high-power input at a low efficiency. The degree of coupling, ATP/Glyc stoichiometry, and efficiency must be clearly distinguished.

CONFUSION OF EFFICIENCY AND STOICHIOMETRY

No information on efficiency can be deduced from stoichiometry alone (Table 1). Following an inadequate but unfortunately common terminology among biochemists, Watt (1986) equates stoichiometry and efficiency. According to Watt (1992), the extremely high metabolic power observed in insect flight at the high aerobic ATP/Glyc stoichiometry presents a counter-example against the trade-off between power and efficiency. Efficiency, however, requires consideration of input and output forces in addition to the ATP/Glyc stoichiometry. In fact, the efficiency of aerobic ATP production in activated tissue is low and optimum for power strategy (Gnaiger 1987). Consequently, insect flight does not contradict but fully supports the concept of the power/efficiency trade-off: power output is high while the output force, Δ_pG_{ATP}, and efficiency are low.

Definition of efficiency

The distinction between stoichiometry and efficiency is critically important for an understanding of optimum efficiency and adaptation. Efficiency of coupled catabolic ATP production, ${}_k f$, is the output/input Gibbs force ratio, normalized for the ATP/Glyc stoichiometry (compare equation 6):

$${}_k f = -\frac{\Delta_pG_{ATP}}{\Delta_kG_{Glyc}/(ATP/Glyc)} = -\frac{\Delta_pG_{ATP}}{\Delta_kG_{\infty ATP}} \qquad \text{eqn 7}$$

For fully coupled processes ($d=1$), the power efficiency, ${}_k\eta$ (negative power input divided by power output, expressed in units $W\,W^{-1}$; Kedem & Caplan, 1965), is equivalent to the Gibbs force efficiency, ${}_k f$. In isolated mitochondria, a fixed ATP/O_2 stoichiometry (full coupling) is obtained by subtracting the maintenance level from the total input (Gnaiger 1989a).

Aerobic ATP stoichiometry is high but efficiency may be low

The efficiency in the high-power mode of aerobic respiration is nearly as low as the efficiency in the high-power lactate pathway (approximately 0·66; Table 1). While the efficiencies are equal, the respective ATP stoichiometries differ by more than an order of magnitude (37 and 3 mol ATP/mol glycosyl-unit; Table 1). This tight regulation of efficiency, despite variation in stoichiometry, indicates the functional importance of optimizing efficiency for power strategy in tissues which undergo dramatic transitions between rest and work.

During insect flight, ATP and phosphoarginine levels decrease (Schneider, Wiesner & Grieshaber 1989) and the output force drops. Thereby, aerobic respiration is switched from economy strategy and high efficiency of ATP production during rest, to

power strategy and low efficiency during flight. Similarly, the aerobic efficiency is low in the high-power mode of skeletal muscle and increases to a maximum at decreasing power (di Prampero 1989). Thereby, aerobic respiration spans the same range of efficiencies as the anoxic pathways (0·6 to >0·8; Table 1).

ERGODYNAMIC AND KINETIC CONTROL ARE COMPLEMENTARY

Complementary to kinetic allosteric control, the net force and efficiency exert control over metabolic flux and power. Substituting efficiency (equation 7) and power output (equation 3) into equation 6 yields:

$$_{p}P = L\ \Delta_{k}G_{\infty ATP}{}^{2}{}_{k}f(1-{}_{k}f) \qquad \text{eqn 8}$$

This shows the two-fold effect of efficiency on power output (Kedem & Caplan 1965; Gnaiger 1987). At low efficiencies, power output increases with increasing $_{k}f<0{\cdot}5$ (here covariation of power and efficiency occurs!). However, at physiologically relevant efficiencies >0·5 the decreasing term $1-{}_{k}f$ dominates and power output declines with increasing efficiency (ergodynamic inhibition; Gnaiger 1987). The optimum efficiency for maximum power output is 0·5 in a fully coupled one-compartmental linear energy converter. Importantly, higher catabolic efficiencies are predicted for power strategy in two-compartmental catabolic-anabolic energy conversion, in accordance with observation (Gnaiger 1987; Table 1).

Sigmoidal flux/force relations of enzyme-catalysed reactions (Westerhoff & Van Dam 1987) can amplify the ergodynamic inhibition at high efficiency. Chemical flux is less constrained by efficiency only for specific patterns of non-linearity. Such patterns have not been adequately analysed. The present controversy might thus stimulate an interesting line of future research.

Kinetic modulation of the phenomenological coefficient, L, is complementary to the effect of the forces on the flux. The corresponding non-linear flux/force relations are an integral part of the ergodynamic model on optimum efficiency and 'optimized power output of a non-linear system' (Gnaiger 1987, p. 24). The examples on the kinetic mechanisms in transitions from rest to high power of flight (Watt 1992) can be extended to incorporate transitions to extreme low-power economy states (Hand & Gnaiger 1988). Such modes of regulation are important for fast metabolic control, e.g. the role of pH as a direct mechanism of inducing metabolic arrest under anoxia (Hand & Gnaiger 1988).

METABOLIC POWER AND EFFICIENCY — INTERDISCIPLINARY APPROACHES

A vague terminology intrinsic to scientific specialization generates conflict when applied in an interdisciplinary context. Watt (1986, 1992) confuses ATP stoichiometry and efficiency, flux and power, concentration and force. This is unacceptable and reenforces the artificial borders between scientific disciplines such as thermodynamics, kinetics and evolutionary ecology. However, more interdisciplinary investigations are required, combining biophysics, and chemical and molecular biology, to appreciate the extent of synergistic kinetic and ergodynamic control.

Protein heterozygosity is an important genetic mechanism for optimization of pathway conductivity, since it is recognized that 'a chain of intermediate fitnesses can result in superior fitness' (Mitton & Grant 1984). Similarly, intermediate as opposed to maximized efficiencies of catabolic ATP production and anabolic ATP utilization result in a more economically regulated energy chain. Therefore, metabolic pathways have evolved to operate in a range of catabolic efficiences above 60% (optimum in two-compartmental energy conversion), well below the maximum limit set by the Second Law of thermodynamics (Gnaiger 1987).

The overall efficiency of an energy chain is the product of the compartmental efficiencies. Likewise, the efficiencies of trophic levels are multiplied and thus additional trophic levels diminish the ecosystem production efficiency in the food chain. Catabolic and anabolic compartmental efficiencies do not represent the only constraints on gross growth efficiencies. Maintenance processes such as protein turnover bear on efficiency such that more efficient heterozygotes show higher phenotypic growth rates (Hawkins, Bayne & Day 1986). More information is required on the possible effect of heterozygosity in optimizing internal, catabolic and anabolic energy transformations.

The maximum power/efficiency trade-off is a general ergodynamic theorem. It is the exact magnitude of optimum efficiency with changes as a function of non-linearities, degree of coupling, the number of serial and parallel energetic compartments, and fitness parameters in the ecological context.

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SHORT REPLY TO GNAIGNER'S ARTICLE

Gnaiger (1992) does not effectively defend his claim of an 'ergodynamic' metabolic power/efficiency trade-off against criticism (Watt 1992), but instead attacks the critique — inaccurately. Full response is in preparation; exemplary inaccuracies are corrected here.

Gnaiger charges Watt (1986, 1992) with definitional errors, e.g. 'equating stoichiometry with efficiency'. Not so; read the papers carefully.

Gnaiger claims that Watt's (1986, p. 649) equation A1 omitted reaction stoichiometry. It did not. 'ΔG_C' in A1 symbolizes aggregate free energy of carbon product(s), *including their stoichiometry*; '$\Sigma n_{iC} \Delta G_{iC}$' ($n \equiv$stoichiometric coefficient, $_{iC} \equiv i^{th}$ carbon product) can substitute for 'ΔG_C'. A stoichiometrically detailed example calculation, which Gnaiger ignores, follows A1 immediately.