ENERGY SUPPLY COMPONENTS

J. exp. Biol. **115**, 149–164 (1985)
Printed in Great Britain

FUELS AND PATHWAYS AS DESIGNED SYSTEMS FOR SUPPORT OF MUSCLE WORK

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SUMMARY

Muscle in all animals relies upon four potential sources of energy: ATP hydrolysis, phosphagen hydrolysis, fermentations or oxidative metabolism. Although the relative contributions of different fuels varies greatly in different organisms, in none is there a simple reliance on stored ATP. Muscle work therefore requires a balance between rates of utilization and formation of ATP, a provision supplied by one of the three remaining fuels and metabolic pathways. Useful endogenous fuels must be storable at high level, and rapidly mobilizable with minimal perturbation of [ATP] and with minimal end-product effects on pH, charge or osmotic balance. In addition to displaying these properties, good exogenous fuels must be transferable at high rates between depot sites and muscle; actual flux rates of exogenous fuels depend upon respective ATP yields and are lowest for fuels which most amplify the yield of ATP per mol substrate oxidized. Subtrate flux rates must be matched with O_2 flux rates and with rates of endogenous substrate mobilization in order that the right energy-yielding pathways are activated at the right times. Of various control possibilities, an effective competition for ADP (and possibly Pi) seems at this time to be the dominant strategy for assuring integration of aerobic and anaerobic ATP-yielding pathways.

INTRODUCTION

Nothing is new, it is often said, except arrangement. And so it may be with the way animals adjust muscle energy metabolism according to specific locomotory needs. All animals are able to harness at least four sources of utilizable energy for the support of muscle work: ATP hydrolysis *per se*, phosphagen hydrolysis, anaerobic fermentations and oxidative metabolism. But all animals do not arrange and use all these sources in the same way. By close inspection of innovative ways that the same source of energy can be used, this paper aims to clarify: (i) mechanisms which ensure that the right energy source is activated at the appropriate time at the correct rate, and (ii) the properties of fuels which place limits or constraints on different energy-yielding processes.

Key words: Anaerobic metabolism, oxidative metabolism, metabolic control.

ATP AS UTILIZABLE ENERGY

The immediate source of energy tapped by most endergonic processes in the cell is, of course, that released by hydrolysis of ATP. In most tissues, the rates of such processes are low because most metabolic pathways operate rather sluggishly. However, muscle is exceptional: during transition from rest to work, its rate of ATP cycling or turnover (in flux units defined as $\mu mol\,g^{-1}\,min^{-1}$) can increase by orders of magnitude, rather than by small percentage changes. In metabolic terms the first step in this transition involves activation of actomyosin (ATPase)-mediated hydrolysis of ATP, with maximum flux rates in man estimated at nearly 400 units (Table 1). To maintain such high rates of work using exclusively endogenous ATP would require large storage depots in muscle, a requirement nature chooses not to meet, presumably because it would generally disrupt both the rates and regulation of metabolism (Atkinson, 1977). Fast-twitch or white type muscle (WM) usually contains only about 5–8 $\mu mol\,g^{-1}$ ATP while slow-twitch oxidative or red muscle (RM) usually contains even less (Guppy, Hulbert & Hochachka, 1979; Howald, Von Glutz & Billeter, 1978). If only these endogenous ATP supplies were available, they obviously would be very quickly exhausted during high work rates. Instead, as is well known, ATP levels are remarkably refractory to increased flux in a variety of species (see Gadian *et al.* 1981; Sutton, Jones & Toews, 1981; Sahlin, Palmskog & Hultman, 1978; Driedzic & Hochachka, 1976). The only way high ATPase rates can be sustained with minimal change in ATP level is through balanced ATP replenishment. At highest power output, the required replenishment of ATP is provided by phosphagen hydrolysis.

PHOSPHAGEN METABOLISM

Criteria for 'good' phosphagen-type fuels

Skeletal muscle working at near maximal rates can be viewed as a temporary and fairly isolated system (Petrofsky *et al.* 1981). To match the need for ATP at such

Table 1. *Estimated maximum power output for skeletal muscle (of man) utilizing different substrates and metabolic pathways**

Process	Power output (μmol ATP g^{-1} wet weight min^{-1})
Aerobic metabolism	
fatty acid oxidation	20·4
glycogen oxidation	30·0
range for mammals	27–227†
Anaerobic metabolism	
glycogen fermentation	60·0
CrP and ATP hydrolysis	96–360

* From McGilvery (1975) with modification.

† Calculated for species 0·007–254 kg in weight, assuming 90 % of maximum O_2 uptake is due to skeletal muscles, from Hochachka, Runciman & Baudinette (1985).

times, a minimum set of criteria must be met for a phosphagen to be a useful endogenous fuel.

Phosphagens must be storable at high concentrations

All currently known phosphagens are substituted phosphoguanidinium compounds. The two best studied systems are phosphocreatine (PCr) and phosphoarginine (PArg), [formed from creatine (Cr) and arginine (Arg) by creatine phosphokinase (CPK) and arginine phosphokinase (APK) respectively]. Creatine itself is formed from methionine, glycine and arginine; in man, this occurs in liver and pancreas, while in some species the first step occurs in the kidney and the second in the liver (Walker, 1979). Creatine is accumulated in skeletal muscles, myocardium and brain, but to my knowledge mechanisms controlling storage amounts are unknown, although feedback repression of synthesis in the liver may limit total availability at these sites (Walker, 1979). Nevertheless, by usual standards, the total pool sizes of PCr + Cr are high (usually about $30\,\mu mol\,g^{-1}$ in WM and somewhat lower in RM, heart and brain). On short- and long-term basis, the amount of PCr available for burst work seems to be controlled by hypertrophy, not by concentration adjustments (Holloszy & Booth, 1976). This is not the case for PArg. It too can be stored at high concentrations (up to $50\,\mu mol\,g^{-1}$ in some species) but in this case there seems to be a reasonable correlation between burst work capacities and the amount of PArg stored (De Zwaan, 1983).

Phosphagens must be rapidly mobilizable

For a phosphagen to be a useful ATP source, its ~P group must be transferable to ADP at high rates and at appropriate times. In the case of PCr, this capacity is based upon (i) very high activities of the cytosolic isozyme of CPK and (ii) appropriate kinetic properties (Hochachka, Dobson & Mommsen, 1983*a*). Within any species, CPK activities are highest in WM, lower in RM, myocardium and brain; comparisons between species indicate that the most CPK occurs in animals capable of high burst speeds, sluggish species having lower activities. A similar relationship appears to hold for PArg kinases (De Zwaan, 1983). These adjustments in enzyme amount per gram muscle would, in themselves, favour high rates of ATP formation from phosphagen when and where needed, a process further facilitated by the kinetic properties of these enzymes.

During highest intensity muscle work, the direction of net flux is

$$PCr + ADP \longrightarrow ATP + Cr. \qquad (1)$$

Even though CPK keeps this reaction close to equilibrium at all times, it is instructive that for the human muscle cytosolic isozyme of CPK, the K_d values for PCr are 72 and $32\,mmol\,l^{-1}$ for the binary and ternary complexes respectively, while for ADP they are 0·2 and $0{\cdot}06\,mmol\,l^{-1}$ (Jacobs & Kuby, 1980). This implies that under most *in vivo* concentrations PCr is not saturating and the enzyme is maximally responsive to

changes in [PCr]. On the other hand, its affinity for ADP is relatively high, making it very competitive for ADP. At least during early phases of muscle work, rising [ADP] drives the CPK reaction to the right (Gadian *et al.* 1981), but it is not known with certainty for later stages of work whether or not CPK functions under ADP saturation. Yet, in many invertebrate muscles there is no doubt that during hard work ADP levels gradually rise high enough to saturate APK fully (Baldwin & Hochachka, 1985). From this point on its catalytic activity would be largely maintained by PArg supplies or by metabolite modulators including reaction products (England & Baldwin, 1983). Thus both enzyme content and enzyme kinetic adaptations of CPK and APK favour high rates of ~P transfer to ADP. However, because phosphagen supplies are non-saturating *and* diminish during bursts of work, these rates must decline rapidly with time.

Phosphagen mobilization should rarely perturb ATP pools

Another characteristic of a useful phosphagen is that, except at extremes, its utilization should proceed with minimal effect on ATP levels. In this regard, compounds such as PCr can be viewed as being fitted for the job because the equilibrium constant (K_{eq}) for ATP formation by the CPK reaction is large; at pH 7·0, the K_{eq} for reaction (1) above is about 2×10^9 (Dawson, Gadian & Wilkie, 1978; Gadian *et al.* 1981). As a result PCr can be almost completely converted with no change in ATP concentrations (Table 2). It would not do to use another compound (such as nucleotide triphosphate, NTP), with a K_{eq} for hydrolysis similar to that of ATP because the concentration ratio of ATP/ADP would change along with that of NTP/NDP. With this kind of phosphagen, muscle metabolic transitions would sustain much larger fluctuations in ATP levels; using up three-quarters of such a hypothetical ~P donor, for example, would cause a 75% decline in ATP concentration. That is why compounds such as PCr, rather than NTPs, have evolved as muscles phosphagens and why the seemingly extreme equilibria of their ~P group transfer reactions are, in this role, advantageous.

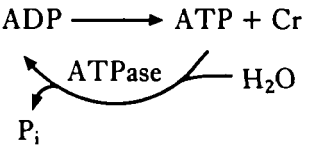
Phosphagens should not generate metabolically deleterious end products

None of the end products of phosphagen mobilization appear to be deleterious, with one exception: arginine (formed from PArg), which may have relatively non-specific, but deleterious effects on enzymes. This led Somero and his students (Bowlus &

Table 2. *Illustrative calculations showing that large changes in phosphagen concentrations need not lead to any change in [ATP] for the CPK reaction at equilibrium*

Muscle state	Metabolite concentration ($mmol\,l^{-1}$) PCr	Cr	ATP	ADP	K_{eq}
Resting	30	3	6	0·003	2×10^9
Maximum work rates	3	30	6	0·300	2×10^9

Somero, 1983) to suggest that an important function of the octopine dehydrogenase reaction in molluscan muscles is to serve as a sink for arginine. Thus, even here, the end product, directly phosphagen-derived, does not seem to be a problem. However, CPK or APK *in vivo* are coupled to myosin ATPase:



(2)

In a closed system the end products accumulating are the guanidinio compound plus P_i. One reason why the former is not deleterious is because it is involved in no other important metabolic sequences; its main metabolic fate is reconversion to the phosphagen form. (Again, arginine may be an exception, see Hochachka *et al.* 1983*b*.) P_i, however, is another matter. It is a highly reactive metabolite involved in numerous enzyme reactions in intermediary metabolism. Just as in the case of ATP, where unduly high concentrations are avoided because of generalized perturbing effects, the need rigorously to control the accumulation of P_i may place stringent limits upon how much phosphagen can be stored as an ATP buffer.

Phosphagen mobilization should not cause osmotic, ionic or charge disturbances

When fully depleting the phosphagen supply the CPK reaction might be expected to lead to ionic and charge perturbations. For example, in the resting state, 30 mmol l^{-1} PCr^{2-} would require similar amounts of a divalent counterion. Cr is of course uncharged and its accumulation in burst work would appear to leave the cell with an anion gap. This problem is alleviated by CPK coupling to ATPase, since in terms of charge, P_i^{2-} is equivalent to PCr^{2-}; moreover, the former is a better buffer at near-neutral pH values than all known phosphagens. So the stoichiometry not only avoids serious osmotic or charge imbalances, but simultaneously minimizes perturbing effects of any $[H^+]$ changes.

A good phosphagen can take on ancillary roles during aerobic metabolism

The value of phosphagens as fuels would be greatly amplified if they were also usable in aerobic metabolism. For PCr, at least three such aerobic functions have been suggested; namely, in the shuttling of P_i between sites of ATP formation and utilization (Bessman & Geiger, 1981), in the preferential channelling of ATP to SR-bound ATPase (Levitskii *et al.* 1977), and in the facilitated diffusion of ATP (Meyer, Sweeney & Kushmerick, 1984).

In summary, then, two potential advantages can arise from using phosphagens in support of burst muscle work: *high power output and effective buffering of ATP levels*. We can assert with confidence that the first is more important than the second, because

numerous invertebrates (England & Baldwin, 1983) and at least one teleost (G. P. Dobson, P. W. Hochachka & T. P. Mommsen, in preparation) are known to sustain very large drops in [ATP] during intense muscle work. Thus *maintenance of [ATP] during intense muscle work is neither universal nor absolutely necessary*. On the other hand, in all systems we know about the power output of muscles working on phosphagen as fuel is higher than that obtainable from any other pathways.

Phosphagens, however, show some critical limitations, the most obvious being the 30–50 mmol l^{-1} maximum storage level (placing a modest ceiling on the total amount of muscle work supportable by this fuel). Again, we can make this assertion with confidence, since muscles in many animals store *less*, but none are known which store more, than this amount. While my analysis cannot specify the exact basis for limits to phosphagen storage, the most likely reasons seem to be either: (i) that phosphagens are strongly anionic (and if levels were too high they would begin to create undesirable effects), or (ii) that the amount of phosphagen-derived P_i could lead to significant metabolic perturbations. Whatever the underlying reasons, the modest limit put on storage amount means that phosphagen-based work is only possible for very short times (5–10 s in mammals, for example); any further muscle work therefore requires a back-up pathway for ATP replenishment.

FERMENTATIVE METABOLISM

Under O_2-limiting conditions, anaerobic glycogenolysis or glycolysis appears to be the main 'back-up' mechanism for ATP replenishment after phosphagen supplies are depleted. In invertebrates, the potentials also exist for energetically more efficient fermentations pathways, such as glycogen → succinate or glycogen → propionate, as well as others, with or without coupling to aspartate → succinate (see Hochachka, 1980).

Criteria for 'good' fermentable fuels

Since the high power output supported by anaerobic glycolysis (second only to phosphagen hydrolysis) also exceeds a critical threshold (Petrofsky *et al.* 1981), muscle in this state is still a relatively isolated system. As with phosphagens, this sets conditions and limits which must be met in order for a compound to be a useful fermentable fuel for muscle work.

Fermentable fuels must be storable at high and adjustable concentrations

This criterion is well met by muscle glycogen, which in vertebrate WM can be stored at about 100 μmol glucosyl unit g^{-1}; a level which can be adjusted according to need. In invertebrates with higher relative dependence on this pathway, muscle glycogen levels may constitute a significant fraction of the tissue weight. To increase efficiency of storage, some muscles resort to specific glycogen-membrane associations or to larger intracellular granules (Hochachka, 1980). Some gastropods store galactogens along with glycogen but the former do not appear to be used for muscle work (De Zwaan, 1983). In many marine invertebrates, the free amino acid pool is

large and several of these (aspartate and the branched chain amino acids in particular) can be used at low rates to supplement glycolysis (Collicutt & Hochachka, 1977).

Fermentable fuels should amplify the molar yield of ATP

This property is particularly well met by glycogen which yields 3 mol ATP/glucosyl unit. Thus, the complete fermentation of $100\,\mu mol\,g^{-1}$ generates at least $300\,\mu mol$ ATP, and the total work possible on this fuel is about 10 times that supportable by PCr (because the latter is stored at only $30\,\mu mol\,g^{-1}$ and only generates maximally the same amount of ATP). The amplification in the aspartate → propionate path is only two-fold, as it is in the glucose → lactate path; that is one reason why glycogen is a better fuel for anaerobic muscle work than either aspartate or glucose.

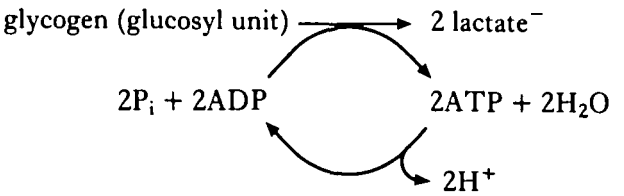
Fermentable fuels should be rapidly mobilizable to high and sustainable rates

Current evidence indicates that the main reason why sequences such as aspartate → propionate or branched chain amino acids → volatile fatty acids are not much utilized to support anaerobic muscle work is because they do not supply sufficient power (see De Zwaan, 1983). In contrast, the high power output of anaerobic glycolysis is probably the chief reason why this pathway is almost universally used in support of anaerobic work (Table 1). The 'flare up' characteristics of muscle glycolysis are based upon high catalytic activities of glycolytic enzymes and upon the occurrence, at virtually every step in the glycogen → lactate conversion, of a muscle-specific isozyme form or forms. The control properties of glycolysis are largely determined by the catalytic and regulatory properties of the muscle isozymes of phosphorylase, phosphofructokinase (PFK) and pyruvate kinase (PK), although other isozymes may also play a role (Hochachka *et al.* 1983*a*). Stimulation of muscle glycolysis is triggered by a variety of signals (hormones, ions and metabolites), although the mechanisms which ensure that the pathway is appropriately phased in with other ATP-generating pathways is frequently under-emphasized. Nevertheless, during anaerobic work it is often observed that phosphagen hydrolysis usually precedes full glycolytic activation; PCr utilization can, for example, approach completion before glycolysis is phased in. This is because glycolysis is competing with CPK as a source of ATP (APK in invertebrate systems). Three enzymes are involved: CPK, phosphoglycerate kinase (PGK) and pyruvate kinase (PK). These enzymes are present in the $10^{-4}\,mol\,l^{-1}$ range (Lebherz, Petell, Shackelford & Sardo, 1982), while free [ADP] in resting muscle occurs in the $10^{-6}\,mol\,l^{-1}$ range (McGilvery, 1975). During early phases of work, both the high activity of CPK and its affinity for ADP ensure that this pathway dominates (see above). On the other hand, the kinetic properties of PGK and PK are suitably tailored for back-up function. In the case of PK, for example, the K_d for phosphoenolpyruvate (PEP) is drastically lowered from a non-physiological range to values between 0·01 and $1\,mmol\,l^{-1}$ on addition of ADP. PEP has the same effect on binding of ADP (i.e. the binding of one substrate leads to about an order-of-magnitude increase in enzyme affinity for the co-substrate, Dann & Britton, 1978). This means that as anaerobic work continues and [ADP] rises, PK becomes ever more competitive and serves to complete the coupling of myosin ATPase and glycolysis (see Hochachka *et al.*

1983*a*). Although the rate of work supportable by this system is related in some complex way to the catalytic potentials of the enzyme pathway *per se* (Emmett & Hochachka, 1981), it is clear that the enzyme content of a pathway cannot be elevated without limit because of potentially debilitating effects of the end products. It is the need to minimize such problems that defines another criterion of useful fermentable fuels.

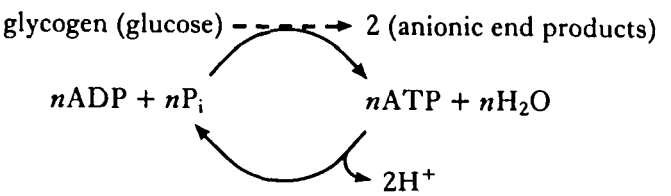
Fermentable fuels should not generate metabolically deleterious end products

As in phosphagen hydrolysis, the end products concerned are not strictly those formed by glycolysis *per se*, but from the glycolysis-myosin ATPase couple. In this system, the net reaction and the end products are:



(3)

At steady state, two end products accumulate: lactate anions (formed by glycolysis *per se*) and protons (formed mainly in ATP hydrolysis). Exactly the same proton stoichiometry prevails for other fermentation pathways in animals, the main difference being that n (the number of mol ATP per mol glucosyl unit) varies from 2 (in glucose fermentation) to about 7 in the glycogen → propionate path:



(4)

Thus, during anaerobic work two classes of end products accumulate: organic anions (commonly lactate, octopine, alanopine and strombine) and protons. In principle, both could have deleterious effects. However, compounds such as lactate (which accumulate in mammalian muscle to 20–40 μmol $^{-1}$g) can rise to 200 mmol l^{-1} in species such as the turtle. So apart from modest osmotic and cation gap problems they present few difficulties for metabolism *per se* (Hochachka, 1983). Protons, however, are another matter. Many workers consider H^+ to be a critical limiting factor in anaerobic systems, so it is important to place this metabolic intermediate in context.

In the first place, it is usually overlooked just how much H^+ is cycled through normoxic metabolism, with no net accumulation or depletion. A simple calculation can show that in a 70-kg man (with a resting metabolic rate of about 700 mmol O_2 h^{-1}) over 100 mol of protons are produced per day. The main sink for this huge quantity of H^+ is oxidative metabolism, and a close balance between rates of H^+ production and

removal ensures stable pH. Although it is well known that the balance of this system breaks down during anaerobiosis, it is usually also overlooked that *pathways such as glycogen → lactate represent net H^+-consuming reactions at metabolically relevant pH values*. At pH 7·4, for example, this pathway consumes 0·4 H^+/glucosyl unit, while the pathway glycogen → propionate at this pH consumes an order of magnitude more H^+/glucosyl unit (Hochachka & Mommsen, 1983), indicating that both H^+ stoichiometry and ATP yield are pathway specific. In both cases, it is the coupling with ATPase that leads to net H^+ production. This important insight emphasizes that the metabolically relevant relationship is between H^+ and ATP, not between H^+ and glucosyl units. For the (glucose → lactate)-ATPase couple, 1 μmol of ATP is cycled/μmol H^+ produced, whereas for the (glycogen → lactate)-ATPase couple, 1·5 μmol of ATP are cycled through/μmol H^+ generated. In succinate fermentation, 2 μmol ATP are cycled/μmol H^+, whereas in glycogen → propionate, up to 3·5 μmol ATP are cycled/μmol H^+ formed (Hochachka, 1983). The strategy of maximizing the amount of ATP turned over per mol of H^+ produced in muscles working anaerobically *at its limit would mean a balance between H^+ formed and H^+ consumed during ATP cycling, as in aerobic metabolism* (Vaghy, 1979).

This could be achieved by alcohol fermentations, but such pathways do not occur in muscle for they generate their own set of problems. Nevertheless, it is clear that Nature can, and has, invented anaerobic ATP-yielding pathways which, as in oxidative metabolism, fully consume the H^+ released during ATP hydrolysis. Why, then, do only a few animals employ such mechanisms? One possibility is that protons *per se, may play important, but thus far largely unrecognized, roles during O_2-limiting periods*.

From current data, we have considered four such roles for H^+ production: (i) the creation of conditions which are more favourable for unloading available O_2 (i.e. the Bohr shift); (ii) the facilitation of phosophagen hydrolysis; (iii) the establishment of a pH optimum for glycolysis, or at least for allowing its catalytic or regulatory potentials to be expressed; and (iv) the facilitation of lactate efflux (Hochachka & Mommsen, 1983). Even if H^+ favours some or all of these related functions, its production cannot proceed indefinitely. That may explain why glycogen is stored in muscle far below possible levels; in some species (Hochachka, 1980), it may be stored at over 1 mol l^{-1} in liver (where the main end product of glycogen metabolism is glucose, not lactate and H^+). That is also an important reason why a number of properties – such as (i) stored [glycogen], (ii) catalytic capacities of glycolytic enzymes, (iii) myosin [ATPase] and (iv) muscle buffering power – all co-adapt. Upward adjustments in any one of the parameters usually means adjustments in them all. Yet even the best adapted glycolytic systems tend to be critically time-limited, which is why sustained muscle work at any reasonable rates must be supported by aerobic, ATP-replenishing pathways.

OXIDATIVE METABOLISM

On transition from rest to maximum work in mammals, the metabolic rates of the muscles involved increase by an order of magnitude or more. The necessary ATP is

derived from the complete oxidation of carbohydrates, fats and proteins or amino acids. Interestingly, most of the enzymes of β-oxidation, the Krebs cycle, and the electron transfer system do not appear to occur in isozymic form, so the dependence upon muscle-specific activating signals is even greater than in anaerobic metabolism. Another distinguishing feature of oxidative metabolism is that the complete combustion of most substrates takes place in at least two separate compartments, the cytosol and the mitochondria. This introduces problems of transport of (i) carbon substrate *per se*, (ii) reducing equivalents and (iii) adenylates *per se* – all of which may influence flux rates (Holloszy & Coyle, 1984; Tager *et al.* 1983). Because no single fuel feeds oxidative metabolism and because aerobically working muscles remain perfused, relatively open systems, the criteria for useful substrates depend upon whether the fuel is stored in muscle (endogenous) or in other depots (exogenous).

Criteria for endogenous fuels of aerobic muscle metabolism

Storage quantities must be adjustable and high

The main endogenous fuels in mammalian muscles are glycogen and triglycerides. Both pools are expandable during short-term training or in long-term adaptation (Holloszy & Booth, 1976). In salmon and squid, for example, expendable proteins are dominant energy sources during migration, although there is uncertainty as to pathways of mobilization of the resultant amino acids (Mommsen, French & Hochachka, 1980). The amino acid pool is also utilizable for aerobic metabolism in many invertebrates (Storey & Storey, 1983). Interestingly, levels of endogenous fuels in oxidative fibre need not be as high as in glycolytic fibre because of higher molar yield of ATP.

Fuels for aerobic metabolism markedly amplify molar ATP yields

The most striking difference observed in comparing phosphagens and glycogen in anaerobic ATP generation with fuels for aerobic metabolism is the immense increase in yield of ATP/mol of the starting substrate. For triglyceride (calculated for glycerol +3 palmitate), free fatty acids (calculated for palmitate), glycogen, glucose, proline and lactate, the respective molar yields of ATP are 403, 129, 38, 36, 21 and 18, assuming complete combustion; these values are 18–400 times higher than the molar yield of ATP from phosphagen hydrolysis, and 6–130 times the yield from anaerobic glycogenolysis. The advantages for sustained muscle work are self-evident.

Good fuels for aerobic metabolism should not generate undesirable end products

As pointed out by Atkinson & Camien (1982), the main end products of triglyceride and glycogen oxidation are molecular CO_2 and H_2O, while oxidations of proteins, amino acids and carboxylates yield CO_2, H_2O and HCO_3^- as well as NH_4^+. The latter two create metabolic problems which are overcome by urea synthesis and excretion. CO_2, on the other hand, can be removed at the lungs or gills while H_2O is essentially harmless. Two other metabolic end products of concern are alanine and IMP. The former may accumulate during augmentation of Krebs cycle intermediates, but the

levels are normally too modest to present any serious problems (see Hochachka & Somero, 1984). Also, under these conditions in mammalian muscle, AMP may be deaminated to IMP leading to adenylate depletion *via* the sequence:

$$\begin{aligned} ATP + H_2O &\longrightarrow ADP + P_i \\ 2ADP &\longrightarrow ATP + AMP \\ AMP &\longrightarrow IMP + NH_3 \xrightarrow{H^+} NH_4^+ \end{aligned} \qquad (5)$$

However, not all animals have this system. In many molluscs and crustaceans, for example, AMP deaminase is absent and [ATP] may drop drastically (below 1 μmol g^{-1}) with a concomitant rise in [ADP] and [AMP] *but with no net change in the adenylate pool* (England & Baldwin, 1983). In exhausted trout, muscle [ATP] is similarly allowed to drop (to as low as 0·5 μmol g^{-1}), but in this case, because AMP deaminase is present, there is also a large reduction in the adenylate pool (G. P. Dobson, P. W. Hochachka & T. P. Mommsen, in preparation). This implies that the work obtainable from ATP hydrolysis remains adequate at quite widely varying adenylate levels, with or without AMP deaminase function, with or without 'defending' [ATP] at a threshold of 2–3 μmol g^{-1}, with or without ADP and AMP accumulation (Dawson *et al.* 1978).

What then are the functions of AMP deaminase in species which possess it? The most likely answer is that by supplying IMP for adenylsuccinate synthetase, AMP deaminase sets the stage for fumarate formation from asparate and, thus, for augmenting Krebs cycle intermediates when they are needed. This would explain the occurrence of this enzyme in vertebrate muscles, as well as its absence in invertebrates, where proline and glutamate are abundant and serve anapleurotic roles (De Zwaan, 1983; Storey & Storey, 1983). It also explains why IMP, in this view, a potentially adaptive end product of aerobic metabolism, may have arisen.

Criteria for useful exogenous fuels of aerobic muscle metabolism

Good exogenous fuels must be storable at sites other than working muscles

This obvious need for glucose by vertebrates is met by liver glycogen depots; for FFA, by adipose fat. When lactate and amino acids are used as fuels they are mobilized mainly from glycogen and protein stores in non-working muscles (see below).

Exogenous fuels should be able to sustain high flux rates

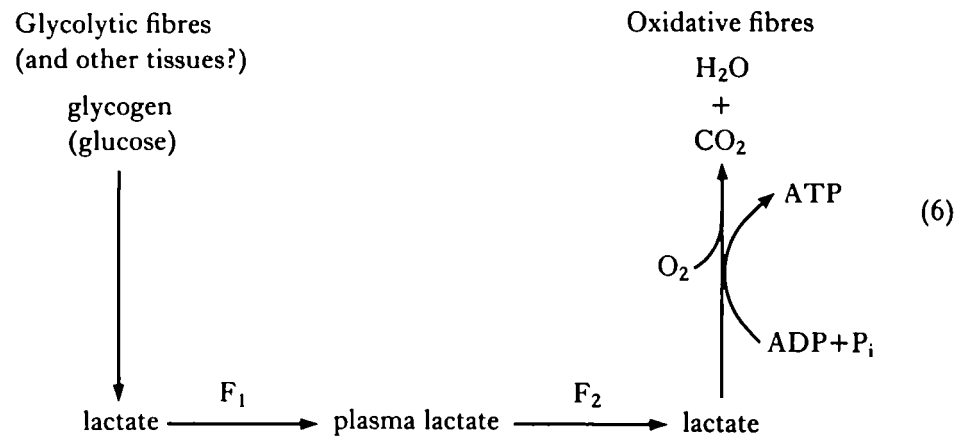
Because aerobically-working muscles are perfused, a critical feature of any exogenous substrate is that it must be capable of sustaining adequate flux rates. In units defined as μmol g^{-1} min^{-1}, *the flux rates required depend upon the molar yield of ATP*. Thus an ATP turnover rate in human muscle of 20 μmol g^{-1} min^{-1} could be supported by a palmitate flux of 0·15 units, a glucose flux of about 0·6 units, a lactate flux of about 1·2 units and an O_2 flux of about 3·3 units. The highest flux is required by

O_2, which of course is an important reason why its transfer from lungs or gills to muscle mitochondria is facilitated by haemoglobins (or their analogues) and by muscle myoglobins. On the other hand, the more energetically efficient a fuel is (in terms of ATP/substrate burned) the less rapidly it need be fluxed from depot site to working muscle. That is why enzymes (e.g. acylCoA synthetases) mobilizing efficient fuels can occur at lower activities per gram muscle than those mobilizing relatively inefficient fuels or those operating in anaerobic metabolism.

Flux of exogenous fuels should be adjustable according to needs of working muscle

In general, uptake at the working muscle varies with delivery rate (perfusion) and with plasma [substrate], so this feature requires provision for plasma [substrate] varying with working needs. Plasma glucose often does not satisfy this provision at low work rates, but may do so at high ones (Jones *et al.* 1980). Plasma FFAs on the other hand increase in availability during modest exercise making them a quantitatively important fuel (Maughan, Williams, Campbell & Hepburn, 1978); in man, however, their contribution decreases during heavy exercise (Jones *et al.* 1980).

Although lactate has been emphasized as a muscle fuel from time to time (Stainsby & Welch, 1966; Issekutz, Shaw & Issekutz, 1976), all but a handful of workers have overlooked the magnitude of its contribution. In most recent analyses (see Hochachka, Runciman & Baudinette, 1985), it is supposed that lactate is formed in sites such as fast-twitch glycolytic (FG) fibres at about the same rates as it is utilized in more oxidative type fibres, and that, at the upper limit, plasma lactate replacement rates equal oxidation rates in aerobically working muscles:



(6)

In this model, F_1 (lactate flux from sites of formation) equals F_2 (lactate flux from the plasma compartment to aerobically working muscle fibres). In rats, as much as 50% of the carbon flux to CO_2 (during maximum sustained muscle work or at high lactate levels) accords with this model, while in dogs and tammar wallabies, the maximum value is 20–30%. These rates are only high by the standards of aerobic pathways. Because of the energetic inefficiency of anaerobic glycolysis, the latter (left arm of above model) therefore hardly affects total ATP turnover. In wallabies, for example,

anaerobic glycogenolysis in FG fibres (adequate to account for the maximum flux of lactate to aerobically working muscles) would contribute only 1–2 % to the total ATP cycling rate. These rates of anaerobic metabolism during aerobic exercise are similar to those found by others (Taylor *et al.* 1981; Brooks, Donovan & White, 1984) and lead to an interesting conclusion, namely that during sustained exercise *the function of anaerobic glycogenolysis is less to make ATP at production sites than it is to mobilize glycogen* (via *lactate*) *as a fuel for aerobically working muscles* (Hochachka *et al.* 1985). If, in a 70-kg man with 20 kg of muscle, 10 kg were used for steady work rates, while 10 kg were used intermittently, it is easy to show that enough glycogen is available (100 μmol g^{-1}) to sustain observed lactate turnover rates of 500 μmol kg^{-1} min^{-1} for about 3 h, while with training the work time could increase (due to higher amounts of stored glycogen). Hence, the supplies of precursor are adequate to meet a significant energy need, which can be used to augment ATP generated by other fuels.

The use of lactate as a carbohydrate fuel appears to confer at least three advantages. First, it conserves endogenous glycogen in aerobic fibres. Secondly, the high activities in FG fibres of isozymes which are kinetically suited for lactate generation and, in more oxidative type fibres, of lactate oxidase isozymes ensures high substrate flux capacities. Thirdly, lactate fluxes can be geared to energy needs: by the F_2 flux (uptake by muscle) which is dependent upon plasma [lactate] and can vary over ranges at least as large as the scope for aerobic metabolism, and by the F_1 flux (generation of lactate) which varies with the energy needs of aerobically-working muscles (i.e. varying with $\dot{V}_{O_2}$). These relationships suggest that the greater the running speed, the higher the steady state plasma [lactate], the fluxes of lactate to aerobically working muscles, and the $\dot{V}_{O_2}$. In fact, during heavy exercise in man, lactate may be favoured as a substrate while the use of plasma FFAs seems actually to slow down (Jones *et al.* 1980), an organization presumably selected because of the higher power output achievable by lactate metabolism.

INTEGRATING AEROBIC AND GLYCOLYTIC PATHWAYS

It is widely observed *in vivo* that as exercise intensity increases, V_{O_2} rises to a maximum level at which time further muscle work depends upon anaerobic glycolysis. The final question thus arises of how this integration of aerobic and glycolytic pathways is achieved. Although many regulatory signals may be involved (see Hochachka & Somero, 1984), the main mechanisms seem to revolve around acceptor control of muscle mitochondrial function. According to current evidence (e.g. Chance *et al.* 1981), mitochondria in non-working muscle are in state 4: flux rates through the electron transfer system (ETS) are low because of limiting [ADP] or [P_i]. During muscle work, mitochondrial respiration and phosphorylation rates increase (the mitochondria are said to enter state 3) as [ADP] and/or [P_i] increase. While the overall response *in vitro* is hyperbolic, at K_m substrate levels respiration increases directly with [ADP] or [P_i] (Jacobus, Moreadith & Vandegaer, 1982), which is presumably also why work rates vary in a linear fashion with [Pi]/[PCr], quantified *in vivo* (Chance *et al.* 1981). Aside from being consistent with recent

studies of isolated mitochondria (Jacobus *et al.* 1982), these *in vivo* studies also satisfactorily explain the advantage, with endurance training, of increasing the capacities of the electron transfer system (ETS) and oxidative phosphorylation (Davies, Packer & Brooks, 1981): for any given submaximal work rate in trained muscle, flux through the ETS per gram will be lower, because the amount of ETS per gram is up to two-fold higher. Muscle mitochondria from endurance-trained individuals, therefore, almost necessarily operate at lower [ADP]. Secondly, because *in vivo* operation occurs at lower [ADP] ranges and because the maximum capacity for state 3 flux is elevated, the overall metabolic scope for activity is elegantly expanded. Finally, when the state 3 capacity is surpassed in either trained or untrained muscle, [ADP] and [Pi] rise even further, creating conditions favouring glycolytic competition for both. This ensures in all species, irrespective of speed capacity or size (Seeherman, Taylor, Maloiy & Armstrong, 1981), a phasing in of anaerobic glycolysis at the appropriate time: when the ATP replenishing capacity of oxidative phosphorylation is being surpassed.

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