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Review

Energy metabolism reactions in ruminant muscle: responses to age, nutrition and hormonal status*

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Summary — Energy expenditure in muscle comprises reactions related to intermediary metabolism and those of posture and activity. The metabolic reactions respond to a wide range of nutritional and hormonal stimuli and are often apparently co-ordinated; in magnitude, however, their contribution to energy requirements can be minor compared with locomotion and posture. Metabolic reactions include protein turnover, ion transport and substrate cycles. In young ruminants muscle protein synthesis responds to intake but effects on energy expenditure are less pronounced; the situation with the adult is unclear. The involvement of insulin in ruminants may differ from that in monogastrics but effects are observed with thyroid hormones. Ruminant muscle may have a higher energy requirement for Na^+, K^+ transport which responds in proportion to total oxygen uptake to alterations in intake. Thyroid hormone treatment and, probably, the catecholamines enhance both Na^+, K^+ and Ca^{2+} transport. Muscle has fewer substrate cycles than liver and each may contribute only 1–3% toward oxygen consumption. Several are sensitive to insulin, but larger responses are observed with thyroxine and epinephrine and under stress conditions, therefore, may account for significant proportions of heat increment. Energy costs of standing may be considerable and posture movements may change with diet quality and quantity. Locomotory activity may mask changes in the contribution of metabolic reactions in response to different stimuli.

Approximately 80% of energy costs for muscle *in vivo* are accounted for by protein turnover (20–25%), ion transport (25–30%), substrate cycling (5–8%) and standing (30%). Better integration of experiments *in vivo* and *in vitro* is required to improve the quantification and resolve data anomalies.

skeletal muscle / ruminant / energy expenditure / protein turnover / substrate cycle

Résumé — Métabolisme énergétique au niveau du muscle chez le ruminant : influence de l'âge, de l'état nutritionnel et de l'état hormonal. *Les dépenses énergétiques du muscle correspondent aux réactions biochimiques du métabolisme intermédiaire ou sont liées à la posture et à l'activité physique de l'animal. Les réactions métaboliques répondent à une grande variété de stimuli nutritionnels et hormonaux et sont souvent apparemment coordonnées; cependant, leur part dans les besoins énergétiques du muscle peut être faible comparée à celles de la locomotion et de la posture. Les réactions métaboliques comprennent le renouvellement des protéines, les transports ioniques et les cycles de substrats. Chez les jeunes ruminants la synthèse protéique musculaire est fortement influencée par le niveau d'alimentation mais les effets de ce dernier sur la dépense énergétique sont moins marqués. Chez l'adulte, la situation n'est pas claire. Le rôle de l'insuline chez les ruminants peut être différent de celui observé chez les monogastriques mais les hormones thyroïdiennes exercent une influence certaine. Le muscle du ruminant peut avoir un besoin en énergie élevé pour le transport de Na^+, K^+ qui varie proportionnellement à la consommation totale d'oxygène*

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en fonction du niveau d'alimentation. Le traitement par les hormones thyroïdiennes et les catécholamines stimule le transport de Na^+, K^+ et Ca^{2+}. Le muscle est le siège de moins de cycles de substrats que le foie et chacun contribue pour 1 à 3% seulement à la consommation d'oxygène. Plusieurs cycles de substrats sont sensibles à l'insuline mais des réponses plus importantes sont obtenues avec la thyroxine, l'épinéphrine et dans les conditions de stress; c'est pourquoi ils peuvent représenter une part importante de l'accroissement de la production de chaleur.

muscle squelettique / ruminant / dépense énergétique / turnover des protéines / cycle de substrats

INTRODUCTION

Total energy expenditure in animals is the sum of the contributions by individual tissues; these differ in metabolic activity per unit mass as identified from studies *in vitro* on cell respiration (Barcroft, 1946). This has been confirmed for ruminant tissues both *in vitro* (*eg* Gregg and Milligan, 1982a; McBride and Milligan, 1985a, 1985b) and *in vivo* (*eg* Bell *et al,* 1974; Webster *et al,* 1975; Oddy *et al,* 1984, Huntington *et al,* 1988). Thus liver and the gastrointestinal tract together comprise approximately 10% of the body weight of ruminant animals but account for nearly 50% of total oxygen consumption (Huntington *et al,* 1988; Huntington, 1990). In contrast, the skeletal musculature of sheep (approximately 30% of body weight) contributes a minor proportion (0.08–0.16) to whole body energy expenditure when animals are in confined laboratory conditions (table I).

There are many energy requiring processes which are common to all cells *eg*, those associated with protein turnover and ion movements. There are others which are specific to individual organs or tissues, *eg* ureogenesis in liver, reabsorption from kidney tubules, contractile activity in smooth and skeletal muscle. In particular the dynamics of muscle metabolism must be assessed against 2 major functions: the provision of mobility and the maintenance of the largest mobile store of reserve protein within the animal. Such functions are basic to all animals but the activities of specific reactions may differ between species and even within the same individual depending on prevailing physiological requirements.

Four major processes will be considered in detail:

— protein turnover;

— ion transport;

— substrate (futile) cycles;

— effects of posture and activity.

There is a considerable body of literature on energy requiring processes in rodent muscle but data for the farm species and man are more limited. It is recognised that real differences may exist between rodents and ruminants in the distribution of energetically expensive reactions in muscle but in order to provide a wide enough base data are included, where appropriate, from laboratory as well as commercial species.

Comparisons in vitro *and* in vivo

An additional concern with comparative data is the difference in the rates and controls of processes *in vivo* and *in vitro.* The rate of protein synthesis, for example, is lower with isolated muscle than *in vivo* (*eg* Palmer *et al,* 1981; Preedy and Garlick,

Table I. Contribution of muscle energy expenditure *in vivo* to whole body oxygen consumption in sheep. [a] Based on CO_2 exchange; [b] Assumes whole body energy expenditure. Data based on hind limb studies corrected as follows. The contribution of muscle to leg energy expenditure assumed in same proportion as contribution to weight (62%; Oddy *et al,* 1984). Leg muscle assumed representative of total musculature, which comprises 28% body weight (Palsson and Verges, 1952).

Growing lambs	0.14–0.19	Harris *et al,* 1989
Adult	0.12–0.15	Bird *et al,* 1981[a]
Adult	0.21	Pethick *et al,* 1987[b]
Adult	0.12	Teleni *et al,* 1986[a,b]
Adult	0.17	Oddy *et al,* 1984
Adult pregnant	0.14	Oddy *et al,* 1984
Adult lactating	0.12	Oddy *et al,* 1984

1983) although the magnitude of these differences varies (*cf* Palmer *et al,* 1981; Early *et al,* 1988b). Energy comparisons are more difficult as different muscles are used for ruminant studies *in vitro* (sternomandibularis or external intercostal) and *in vivo* (total or lower hind leg musculature). These preparations differ considerably in oxygen consumption per unit mass with those *in vivo* generally lower (table II). This may result in part from the contribution of bone, adipose tissue and skin in the arteriovenous preparations used *in vivo*, although both skin and bone are metabolically active as judged from rates of protein synthesis (*eg* Lobley *et al,* 1980; Attaix *et al,* 1988). In addition the different fibre-type composition of the muscles (Suzuki, 1971) leads to variations in metabolic rate, vascularity and protein synthesis (Lewis *et al,* 1984). Moreover, removal of the muscle from the body may result in dissociation of metabolic integration and uncoupled phosphorylation. Therefore comparisons from disparate data sources may not necessarily be valid.

PROTEIN TURNOVER

The term protein turnover encompasses the concomitant synthesis and breakdown of cell proteins (Waterlow *et al,* 1978). Both the anabolic and catabolic functions are extremely active and each may involve energy costs.

Protein breakdown

Earlier assumptions that intracellular proteolysis involves little or no energy cost now need to be revised. Gronostajski *et al* (1985) suggested that the maintenance of the acid pH of lysosomes requires ATP and the binding to protein of ubiquitin, which signals that protein degradation can proceed, also involves ATP (Ciechanover *et al,* 1984). In reticulocytes the inhibition of intracellular proteolysis effected a saving equivalent to 1 ATP per peptide bond hydrolysed (Siems *et al,* 1984; Rapoport *et al,* 1985). Recently Driscoll and Goldberg (1989) demonstrated that the proteasome (a multicatalytic endoproteinase complex) is activated 4–12-fold by ATP. Energy-independent proteolysis has, however, also been demonstrated in chick muscle (Wainberg *et al,* 1989). The ability of cells to use both energy-dependent and -independent systems to cleave peptide bonds means that "fixed" costs cannot be ascribed to proteolysis (see Summers *et al,* 1986); unfortunately our current know-

Table II. Comparison of rates of oxygen consumption (mmol O_2/h/kg wet wt) by ruminant muscle *in vitro* and *in vivo*. [1] Assumes 23% dry matter; [2] Included contribution from bone, skin and adipose tissue metabolism; some calculations based on CO_2 exchanges.

In vitro [1]		
Sheep		
2 weeks	55.1	Gregg and Milligan, 1982[c]
2-5 yr	37.1	Gregg and Milligan, 1982[c]
Cold	31.7	Gregg and Milligan, 1982[a]
Warm	21.5	Gregg and Milligan, 1982[a]
Control + T_4	23.3	McBride, 1986
Control + insulin	25.5	Early *et al*, 1988[b]
Adult	12.3	Barcroft, 1946
Cattle		
2–3 weeks	33.6	Gregg and Milligan, 1982[b]
7 months	28.2	Gregg and Milligan, 1982[b]
In vivo [2]		
Sheep		
Sw	13.8	Oddy and Lindsay, 1986
5–9 months	15.2	Harris *et al*, 1989
Adult	15.4	Bird *et al*, 1981
Adult	8.4–19.1	Oddy *et al*, 1984

ledge is insufficient to estimate the range of energy requirements in skeletal muscle from either laboratory or farm species.

Protein synthesis

In contrast to protein degradation there is an extensive literature which covers both whole body and muscle protein synthesis in chickens (*eg* MacDonald and Swick, 1981; Muramatsu *et al*, 1987), pigs (*eg* Garlick *et al*, 1976; Simon *et al*, 1978; Reeds *et al*, 1980; Sève *et al*, 1984), sheep (*eg* Buttery *et al*, 1975; Arnal *et al*, 1976; Davis *et al*, 1981; Pell and Bates, 1987; Attaix *et al*, 1988; Harris *et al*, 1989), goats (Muramatsu *et al*, 1988) and cattle (*eg*, Eisemann *et al*, 1986a, 1986b; Lobley *et al*, 1980, 1987). In all cases whole body protein synthesis exceeds protein intake by 2–4-fold (see Reeds and Lobley, 1980) and even at fasting total synthesis rates of 18 g/d/kg$^{0.75}$ have been reported (see Lobley, 1988). Correlations were soon established between rates of whole body protein synthesis and heat production for adults of many species at maintenance intake (*eg* Garlick, 1980; Webster, 1981); with the release of 20–25 kJ of heat for every g protein synthesis. This general relationship was also maintained in nutritional trials with young growing pigs (Reeds *et al*, 1980) and lambs (Harris *et al*, 1989) as well as with fattening cattle (Lobley *et al*, 1987); these experiments encompassed fasting animals and those at 3 x maintenance (see Lobley, 1988). Such correlations led to the suggestion that protein synthesis comprised a larger proportion of heat production than could be considered on purely theoretical grounds (Webster, 1981). Estimation of the costs associated directly or indirectly with protein synthesis have been based on either the stoichiometric or empiral approach.

Stoichiometric approach

It is generally accepted that, for mammalian tissues, protein synthesis requires 4 high-energy phosphate bonds per peptide unit (but see Summers *et al,* 1986). Two of these are involved in the activation of amino acids to the aminoacyl-tRNA and the other 2 are needed as GTP in attachment and translocation on the ribosome (see Buttery and Boorman, 1976). Yields of ATP per unit of metabolisable energy depend on the nutrient source; the values for glucose and acetate are 13.2 and 11.4 mol ATP/MJ ME, respectively (Baldwin, 1968; Lobley, 1986). Based on an average molecular weight of peptide amino acid of 110, minimum theoretical costs are 2.8–3.2 kJ per g protein synthesis. In practice a wider range of values has been used and often the figure of 4.5 kJ per g, as proposed by Webster (1981), is adopted. The rationale for this higher cost is that ancillary processes such as transport of amino acids into the cell, synthesis of RNA and DNA species, activation of ribosomal proteins and membrane-associated GTP hydrolysis are all involved in the synthesis of polypeptide bonds; these all require energy.

In practice the rates and energy requirements of these ancillary processes are poorly defined. Rates of amino acid transport into rat muscle *in vivo* have been reported by Banos *et al* (1973) to be between 16 (threonine) and 0.2 (aspartate) mol/min/g tissue. The energy costs of such transport have not been clarified; movements across the cell membrane are associated with concomitant transport of ions but the action of the membrane ATPase involves 3 sodium and 2 potassium ions per ATP hydrolysed (Mandel and Balaban, 1981). Based on the assumption that all amino acids are actively transported and that 2 molecules can be transferred per ATP hydrolysed then total transport in rat muscle (8.7 μmol amino acids•h^{-1}•g^{-1}; from Banos *et al,* 1973) would require 4.4 μmol ATP. For similar rats muscle protein synthesis rates of 6 μmol•h^{-1}•g^{-1} have been reported (from Lewis *et al,* 1984); therefore amino acid transport might require an additional ATP per peptide bond synthesised.

Syntheses of the nucleic acid species also require energy (*eg* Sims *et al,* 1983) but the magnitude of macromolecule turnover and the associated thermal costs are still undefined, although regulation of ribosome number may be controlled primarily through alterations in breakdown rather than alterations in synthesis (Ashford and Pain, 1986).

Empirical approach

Attempts have been made to measure directly the costs associated with protein synthesis by use of the inhibitor of protein synthesis, cycloheximide which blocks peptide-bond formation. In isolated muscle from laboratory species the degree of inhibition in oxygen consumption varied from 0.14–0.23 (Reeds *et al,* 1985; McBride, 1986; Early *et al,* 1988b); while in 10-day chicks heat production declined by 0.33 in association with a 0.87 suppression of protein synthesis (Aoyagi *et al,* 1988). Aoyagi *et al* (1988) calculated that the energy cost of protein synthesis was 5.4 kJ/g in the chick whereas the data of Early *et al* (1988b) on isolated sheep intercostal muscle preparations implies a value of 7.2 kJ/g (calculations based on a calorific value for oxygen of 21 J/ml O_2). Both values are higher than those adopted traditionally from stoichiometric considerations (*vide supra*) but the empirical technique may involve inhibition of other processes such as ion transport and nucleic acid turnover. Suggestions that the ATP costs of peptide

bond formation may not be constant (see Summers *et al,* 1986) or that formation of amino-acyl t-RNA may not be rate-limiting and reformation of the free amino acid may occur with consequent energy penalties may also account in part for the differences in values reported.

Muscle protein synthesis

Age

The reduction in basal metabolic rate (expressed per unit metabolic body weight; $kg^{0.75}$) which occurs as ruminants mature (Blaxter, 1962) is matched by declines in both muscle oxygen consumption (Gregg and Milligan, 1982b, c) and muscle protein synthesis (Arnal *et al,* 1976; Lobley *et al,* 1980; Oddy *et al,* 1987; Attaix *et al,* 1988). Direct measurements of changes in muscle energy expenditure and protein synthesis with age are not available, however, and comparisons between isolated studies may be confounded by environmental and behavioural factors.

Nutrition

Level of nutrition has a marked effect on protein synthesis in both the whole body (*eg* Lobley *et al,* 1987; Oddy *et al,* 1987; Harris *et al,* 1989) and in muscle (Oddy *et al,* 1987; Boisclair *et al,* 1988; Harris *et al,* 1989). Between fasting and *ad libitum* intake milk fed lambs increased the fractional rate of protein synthesis across the hind leg from 0.071–0.115/d (Oddy *et al,* 1987). In growing ruminants increases in feed intake from 0.6 to 1.8 x maintenance caused corresponding increases in hind limb protein synthesis from 2.3 to 5.2 g/h in cattle (Boisclair *et al,* 1988) and from 0.2 to 0.59 g/h in lambs (Harris *et al,* 1989). For mature sheep, however, deprivation of feed for 40 h did not effect either hind-limb or whole body protein metabolism (Teleni *et al,* 1986) but fasting for 3 days (Pell *et al,* 1986) caused a marked decline in both whole body (–28%) and hindquarter (– 50%) protein synthesis. The period of food withdrawal may therefore be critical, but similar contradictions have been observed for adult man where lack of response to overnight fast in whole body protein synthesis (*eg* Young *et al,* 1987; Melville *et al,* 1989) contrasts with changes in forearm (muscle) protein synthesis when intake is reduced (Cheng *et al,* 1985, 1987). Age-related differences have also been observed in rodents. In mature rats responses in muscle protein synthesis to either 12–42-h fasts or insulin are negligible (Baillie *et al,* 1988), but are quite marked in young animals (Garlick *et al,* 1983).

For ruminants the relationship between muscle protein synthesis and energy expenditure in response to intake is confused. In young cattle Boisclair *et al* (1988) observed similar changes in both parameters such that, if a value of 5.4 kJ/g protein synthesis (Aoyagi *et al,* 1988) is applied, 25% of hind limb energy expenditure was attributable to protein synthesis. In contrast, Harris *et al* (1989) found in growing lambs that, unlike protein synthesis, hind leg oxygen uptake did not increase with intake; in consequence the contribution of protein metabolism to energy expenditure altered from 12 to 31% between 0.6 and 1.8 x maintenance. In young lambs plasma CO_2 production across the hind-limb increased by 16% (from Oddy *et al,* 1987) between fasting and supra-maintenance, compared with 62% in protein fractional synthesis rate; again the contribution of synthesis to energy expenditure apparently increased from 22 to 30% with intake. While effects of intake on adult ruminant hindquater oxygen uptake have generally

been small, even when metabolisable energy intake has been varied 3-fold (Bird *et al,* 1981; Oddy *et al,* 1984; Teleni *et al,* 1986), changes of 35% in CO_2 production were assumed in the calculations of Pell *et al* (1986) and these approximated to the changes in synthesis.

Hormonal effects

In ruminants the involvement of insulin in protein dynamics is controversial. Thus while insulin infusion will increase protein gain in fed pigs (Fuller *et al,* 1977) no effect is observed in lambs (Sumner and Weekes, 1983). In fasted lambs exogenous hormone appears to reduce both muscle protein breakdown and synthesis (Oddy *et al.,* 1987); the latter observation is in contrast to findings in the fasted rat (Garlick *et al,* 1983; Reeds *et al,* 1985). In the experiments of Oddy *et al* (1987) exogenous hormone administration had no effect on protein synthesis in milk fed lambs but endogenous insulin concentrations may have been maximal for effects on protein dynamics in these animals (see Jepson *et al,* 1988). In growing sheep infused with insulin plus glucose the entry rate of phenylalanine across the hind limb was stimulated (17 *vs* 9 μmol/h/$kg^{0.75}$) although incorporation into gastrocnemius muscle protein and *in vitro* analysis of synthesis rates in external intercostal muscle were not significantly different from controls (Early *et al,* 1988a, b). Neither muscle oxygen uptake *in vitro* nor the proportion inhibited by cycloheximide were altered by insulin administration. Differences in both age and circulating concentrations of insulin existed between the studies of Oddy *et al* (1987) and Early *et al* (1988a) and may account in part for the apparently contradictory responses.

Isolated muscles analysed *in vitro* from sheep made hyperthyroid by injections of 10 mg/d thyroxine did show increased protein synthesis (+ 35%; McBride, 1986) and this also represented an increased proportion of oxygen uptake (23 *vs* 18%, as assessed by cycloheximide inhibition). The proportion of cycloheximide-inhibited respiration also increased, albeit non-significantly, in incubated intercostal muscles from lactating cows treated with b-somatotropin compared with control tissue; muscle oxygen consumption also increased (0.53 *vs* 0.40 μl $O_2 \cdot mg^{-1} \cdot h^{-1}$; McBride *et al,* 1987). Administration of b-somatotropin also increased protein fractional synthesis rate of muscle in growing steers (Eisemann *et al,* 1989) and lambs (Pell and Bates, 1987). Epinephrine increases both whole body and muscle oxygen consumption (Lundholm and Svedmyr, 1965). In contrast the catecholamine depressed protein synthesis and increased amino acid oxidation in the hind limb of lambs (Oddy *et al,* 1987), observations compatible with the reduced rates of protein incorporation in muscles incubated with epinephrine (Wool, 1960). These actions of epinephrine probably also involve interactions between thyroid hormones and insulin status (*vide infra*).

Summary

Despite the apparent close association between rates of protein synthesis and heat production from a variety of nutritional trials it is obvious from experiments in which individual hormones have been varied that the contribution made by protein metabolism to muscle energy expenditure can increase or decrease in response to prevailing physiological or pharmacological conditions. Perhaps the most perplexing question at present relates to the apparent contradiction between the large increases in protein synthesis which accompany ac-

company extra intake in ruminants and the lack of any simple relationship with insulin (as is the case for growing monogastric animals). Possibly this indicates that regulation of protein synthesis in ruminants may involve a greater role for other hormones or growth factors.

Based on stoichiometric values and the use of inhibitors with incubated muscles the contribution of protein synthesis to energy expenditure in muscle under normal conditions would appear to be 15–25%. Extra costs associated with protein breakdown cannot at present be quantified although the value is unlikely to exceed 5–10%.

ION TRANSPORT

Movements of ions into, out of and across the cell represent a major metabolic function and are involved in transport of substrates, regulation of hormone action (Rosenthal *et al,* 1988) and the control of muscle contraction.

Na^+, K^+ transport

Most attention has focussed on the Na^+, K^+-ATPase of the membrane where the movement of every 3 mol of Na^+ out of and 2 mol of K^+ into the cell involves the hydrolysis of one mol of ATP (Mandel and Balaban, 1981). The process is blocked by the cardiac glycoside ouabain and this has enabled the contribution to cell respiration to be studied under a wide range of conditions, but exclusively *in vitro* (*eg* Whittam, 1961; Ismail-Beigi and Edelman, 1971; Gregg and Milligan, 1982a, b, c).

The changes in heat loss or oxygen uptake induced by ouabain for muscle tissues have been very variable. Several groups have claimed that 20–50% of aerobic respiration is required for maintenance of Na^+, K^+ gradients while others have suggested that values of 6–12% are more realistic (table III). The reasons for these differences are controversial; preparations *in vitro* are dependent on minimising tissue damage and cut surfaces, establishing appropriate incubation media and ensuring that oxidative phosphorylation remains tightly coupled (for discussion, see Chinet *et al,* 1977; Gregg and Milligan, 1982b; Chinet, 1990). Species differences may also exist. Fortunately, many of the studies conducted with ruminant muscle are based on response analyses so that effects of nutritional, endocrinological and physiological stimuli will have qualitative importance even if some revision of their quantitative nature needs to be applied.

Age

As with protein synthesis, Na^+,K^+-ATPase activity declines as the animal matures (Gregg and Milligan, 1982a, c) such that it remains a relatively constant fraction of cell energy expenditure. This degree of association between cell respiration and key processes such as protein synthesis and ion transport has been extended into the concept of linked mechanisms and integrated cell responses (Webster, 1981).

Intake and hormonal effects

Direct studies on the effects of intake on Na^+, K^+-ATPase have not been reported. In lactation, however, intake is increased considerably and was doubled between the dry and lactating state for ewes offered chaffed oaten hay by Oddy *et al* (1984). This was accompanied by a 35% increase in hind leg oxygen consumption, an almost similar increase to the oxygen uptake observed for muscles *in vitro* between lactat-

Table III. Na^+, K^+-ATPase contribution to muscle energy expenditure *in vitro*. All values based on use of cardiac glycoside ouabain. By O_2 consumption except for * by microcalorimetry.

Muscle	*% Contribution*	
Rat soleus	6*	Chinet *et al*, 1977
Mouse soleus	7*	Biron *et al*, 1979
Rat diaphragm	12	Ismail-Beigi and Edelman, 1971
Sheep intercostal	18	McBride, 1986
Mouse soleus	19	Gregg and Milligan, 1980
Hamster diaphragm	23	Horwitz and Eaton, 1977
Sheep intercostal	23	Early *et al*, 1988b
Lamb intercostal	31	McBride and Early, 1987
Rat pectoralis	34	Guernsey and Stevens, 1977
Sheep sternomandibularis	37	Gregg and Milligan, 1982b
Cattle sternomandibularis	41	Gregg and Milligan, 1982c
Rat diaphragm	41–51	Asano *et al*, 1976

ing and dry ewes by Gregg and Milligan (1982a, c). In this latter study, however, ouabain-sensitive respiration remained a fixed proportion of muscle oxygen uptake. Similarly, Early *et al* (1988b) reported that insulin altered neither the contribution (23%) of Na^+, K^+-ATPase nor the total oxygen consumption in isolated sheep muscle.

Alterations in the relative activity of ion-transport do occur however under conditions of stress. Muscle from sheep maintained in the cold (1 °C) has a greater (50%) oxygen consumption than that from animals housed at 25 °C and 70% of the extra uptake is attributable to Na^+, K^+-ATPase activity (Gregg and Milligan, 1982a). In consequence the proportion of energy expenditure attributable to the process increased from 29 to 45%. Cold stress is associated with changes in thyroid hormone and catecholamine status (Christopherson *et al*, 1978) and in hyperthyroid sheep (given exogenous T_4) there was a 50% stimulation of ouabain-sensitive respiration (McBride, 1986) although muscle oxygen uptake was similar to that in control animals. Curiously there were concomitant increases in muscle protein synthesis as a result of T_4 administration (McBride, 1986) such that the two processes together accounted for 49% of tissue respiration compared with only 36% for control animals. This result may be misleading as ouabain, in a manner analogous to cycloheximide, may alter other processes including protein synthesis by direct or indirect means. If the observations are additive, however, then other thermogenic processes must decline in the presence of T_4.

Calcium

Ca^{2+} movements are vital for the execution of muscle contraction but even in resting muscle 7% of energy expenditure may be attributed to the activity of the sarcoplasmic reticulum Ca^{2+}-ATPase (Hasselbach and Oetliker, 1983). Translocation of Ca^{2+} across other muscle membranes such as

the T-tubules, sarcolemma (Michalak *et al,* 1984) and mitochondria (Mickelson and Marsh, 1980) may account for a further 13% (cited by Summers *et al,* 1986).

Again, thyroid status influences Ca^{2+} transport and in hyperthyroid animals increases in total enzyme and maximal activity occur for tonic muscles (Kim *et al,* 1982) whereas in the hypothyroid state increases in the coupling of Ca^{2+} transport across the sarcolemma counteract the effect of lowered ATPase activity (Simonides and Van Hardeveld, 1985). Current estimates of the contribution of Ca^{2+} movements to muscle oxygen consumption are approximately 10% (Summers *et al,* 1986; Chinet, 1990).

General

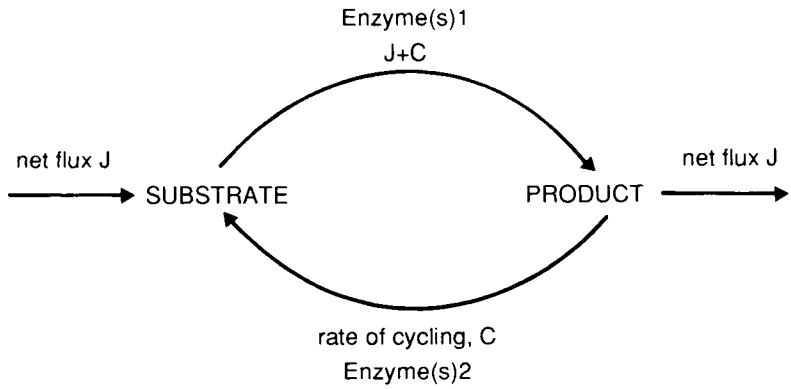
Based on recent studies with ruminant muscle preparations *in vitro*, cell bioenergetics include a value of 18–23% for Na^+, K^+-ATPase which, together with 10% for Ca^{2+} transport, would give an ion-transport contribution of approximately 30% to energy expenditure. These values may increase under conditions of severe stress and in particular where thyroid hormone status is elevated.

SUBSTRATE CYCLES

Reversible reactions which are catalysed by 2 different enzymes (or enzyme complexes) and involve the expenditure of energy are termed substrate cycles; the old terminology of "futile cycles" has now fallen into disuse. The simultaneous synthesis and breakdown of metabolites under the control of separately regulated enzymes (fig 1) affords several advantages for cellular mechanisms. Such reactions contribute to heat production required to maintain homeothermy and provide a flexible response system in situations where increased energy dissipation is necessary, *eg* non-shivering thermogenesis. In addition, accumulation of products which may exert either harmful or regulatory effects on other cellular processes can be avoided through the action of substrate cycles. Furthermore, because the rate of cycling can be large compared with the flow of substrates through a metabolic sequence small changes in the rate of the forward or back reaction can induce considerable changes in net flux. The system is thus very sensitive to regulators and in consequence substrate cycles are invariably found as the key reactions of metabolic sequences and under hormonal control (Newsholme and Crabtree, 1976); this may constitute their primary role.

The list of possible substrate cycles is extensive (Katz and Rognstad, 1976; Newsholme and Crabtree, 1976; Rabkin and Blum, 1985) but in most cases the contribution of any individual reaction to cell energy requirements is small. The most notable exception to this is protein turnover (which simultaneously involves synthesis and breakdown under the action of different enzyme complexes). Nevertheless in liver other substrate cycles may contribute 19–28% of ATP hydrolysis (Rabkin and Blum, 1985). There are considerable technical difficulties associated with measurement of such cycles in that often dual tracers are required to obtain true values (Katz and Rognstad, 1976; Challis *et al,* 1984b; Crabtree and Lobley, 1988); in consequence many earlier values may need to be reassessed (*eg* Clark *et al,* 1973).

Fewer substrate cycles exist in muscle (fig 1) and quantitative data are limited, especially for the larger species. Thus for the triglyceride: fatty acid cycle no values are available, although considerable changes



Substrate Product	Enzyme(s) 1 Enzyme(s) 2	ATP costs of cycling
1. glycogen glucose 1-phosphate	phosphorylase glucose -1- phosphate uridyltransferase + glycogen synthase	1
2. triacylglycerol fatty acid	triacylglycerol lipase fatty acyl CoA synthetase + esterification enzyme complex	8
3. fructose 6-phosphate fructose 1,6-bisphosphate	phosphofructokinase fructose 1,6-bisphosphatase	1
4. acetate acetyl CoA	acetyl CoA synthetase acetyl CoA hydrolase	2

Fig 1. Substrate cycles in skeletal muscle. Each turn of the cycle causes hydrolysis of ATP.

in the rates of reesterification/deesterification occur during the lactation curve in dairy goats (Dunshea and Bell, 1989). While much of this activity may be in white adipose tissue (Brooks *et al,* 1982) the potential for this cycle does exist in muscle.

Acetate–acetyl CoA cycle

Acetate is a major fuel for ruminant muscle (Jarrett *et al,* 1976) and enters both anabolic and catabolic sequences as acetyl-CoA. The CoA ester is however a potent regulator of many reactions and in situations where aceyl-CoA cannot effectively enter an anabolic or catabolic sequence the ability to effect hydrolysis to acetate provides an important safety measure. The hydrolase enzyme involved has been shown to be especially active in the cytoplasm of rat hepatocytes (Rabkin and Blum, 1985; Jessop *et al,* 1986) but additional observations *in vivo* have been

made in sheep muscle (Pethick *et al,* 1981) and refined and extended by Crabtree *et al* (1987). Rates of muscle acetyl-CoA hydrolysis were equivalent to 0.4 mmol ATP•kg^{-1}•h^{-1}, equivalent to 2% of muscle energy expenditure (Harris *et al,* 1989). Under the experimental conditions adopted by Crabtree *et al* (1987), *ie* mature wethers fed to maintenance on grass pellets, doubling the acetate supply to the animal did not produce a significant stimulation of this substrate cycle in muscle.

The studies of Pethick *et al* (1981) and Crabtree *et al* (1987) provide the only data to date on substrate cycles in ruminant muscle and for further information it is necessary to use studies on other species, usually rodents.

Glycogen/glucose-1-phosphate (G/GIP) cycle

This cycle regulates the synthesis and breakdown of glycogen, the major internal fuel store in muscle and sensitive hormonal regulation is essential especially for phasic muscles. Under basal conditions GIP production exceeds glycogen synthesis and net flux (respectively 0.74, 0.54 and –0.20 glucosyl equivalents•g^{-1}•h^{-1}) in isolated rat epitrochlearis muscle (Challiss *et al,* 1987). The direction of net flux is reversed through the addition of insulin; this is achieved by a decrease in glycogen breakdown and an increase in synthesis (0.38 and 0.97 glucosyl equivalents/g/h respectively). If applied to the *in vivo* state this would suggest that even in the fed condition cycling still occurs. The measured rates of glycogenolysis represent < 1% of the maximal activity of phosphorylase present in muscle and the low expression of potential cycle activity under basal or normal physiological conditions is typical for the majority of substrate cycles. For the G/GIP cycle the simultaneous stimulation of glycogen synthetase and inhibition of phosphorylase by insulin is associated, in part at least, by changes in the activity of protein phosphatases-1 and 2 which regulate the phosphorylation status of the two enzymes (Tung *et al,* 1985).

Epinephrine can induce marked changes (x 15) in rates of glycogen breakdown but the major effects are observed only under conditions (x 100) in excess of normal unstressed physiological catecholamine concentration (≈ 1 nM; Buhler *et al,* 1978). Responses at lower (10 nM) epinephrine concentrations can be induced, however, if thyroid hormones are present (Challiss *et al,* 1987) and this is consistent with the general hypothesis that muscle sensitivity to catecholamines is enhanced by triiodothyronine (Williams and Lefkowitz, 1983). Indeed the greater control appears exerted through regulation of breakdown (*ie* phosphorylase activity), as the post-exercise increase in muscle glycogen synthesis observed even in the fasted condition for man (Hermansen and Vaage, 1977) and rat (Davis *et al,* 1986) may be associated more with reduced glycogenolysis rathen than a stimulation of glycogen synthase (Challiss *et al,* 1987). In consequence this cycle is unlikely to contribute to the increase in oxygen consumption reported for the post-exercise condition (Gleeson *et al,* 1982; Gaesser and Brooks, 1984).

As it is the reverse reaction, *ie* glycogen synthesis, which provides the thermic response of this cycle obviously in situations such as stress or exercise the extra heat production will not include an additional contribution through the activity of glycogen synthase. Under basal or normal physiological conditions the contribution to muscle oxygen consumption will be 1–1.5% (from Challiss *et al,* 1987). This minor role in oxygen consumption should not detract from the importance in regulation of the internal fuel source of glycogen which will be

important for other thermogenic reactions. Such an example is in starvation where muscle glycogen synthesis is enhanced (Challiss *et al,* 1987).

Fructose 6-phosphate/fructose-1,6-bisphosphate (F6P/F16bP)

This is perhaps the most studied substrate cycle in muscle and it occupies a key role in glycolysis and gluconeogenesis. Regulation of phosphofructokinase in glycolytic flux is well established but the activity of fructose bisphosphatase is also acutely sensitive to hormonal regulation in a similar manner to the G/GIP cycle. This is to be expected as they are components of the same glycogenolytic sequence.

Most of the available information comes from rat muscle studies *in vitro* and the rates of both the forward and reverse reactions are increased (x 2) by insulin (Challiss *et al,* 1984a). Epinephrine (10 nM) is a major regulator of the fructose biphosphatase and can increase the reverse reaction x 3 without a corresponding increase in glycolytic rate (Challiss *et al,* 1984a, b). During starvation the rate of formation of F16BP is unaltered while conversion back to F6P is reduced to one-third (Challiss *et al,* 1985b).

Control of cycling again involves a major interaction, between epinephrine and thyroid status. Muscles from hyperthyroid rats incubated in the presence of insulin do not show changes in either the glycolytic or cycle rate compared with control rats but in the presence of 10 nM epinephrine the reverse reaction doubles (Challiss *et al,* 1985a). Similar effects are found with muscles from acutely exercised (90 min) rats when the cycle rate increased from 0.20 to 0.53 $\mu mol \cdot h^{-1} \cdot g^{-1}$. The experiment (Challiss *et al,* 1985a) included an incubation period for the muscles *in vitro* so that increases in cycle rate may have been even greater immediately post-exercise.

Unlike the G/GIP cycle, therefore, F6P/F16bP does contribute to post-exercise oxygen consumption. The rates of cycling reported in the literature range from 0.2–0.7 μmol/h/g, under basal conditions (with insulin, present), and this would represent 0.2–1.0% of oxygen consumption.

In stress conditions the contribution may rise to 2–3% of total energy expenditure but would represent a much greater proportion of the heat increment associated with the trauma. This is shown most dramatically in pigs which suffer from the malignant hyperthermia syndrome (Berman *et al,* 1970) and where either stress (*eg* transport to market) or halothane anaesthesia induce a rapid increase in body temperature, muscle rigor and often death. Most of the extra thermogenesis is muscle-derived and involves the F6P/F16bP cycle. In non-stressed animals the rate of cycling varies from 0.3–3.0 $mmol \cdot h^{-1} \cdot kg^{-1}$ muscle and would account for 0.3–3.0% of tissue ATP hydrolysis (from Clark *et al,* 1973). Non sensitive pigs exhibit no change in cycle rate during halothane administration but sensitive animals increase arteriovenous blood temperatures by 1.5° and increase the rate of cycling 10-fold (Clark *et al,* 1973). This stimulation of the reverse reaction may be underestimated by as much as 4-fold due to the single isotope method used (see Katz and Rognstad, 1976) and, based on a blood flow of 70 ml $min^{-1} \cdot kg^{-1}$ muscle for the anaesthetised animal, the contribution to heat increment by the cycle may be 7–30%. This is an extreme case but indicates the potential for heat production which exists for substrate cycles and may be invoked under stress conditions.

Regulation of the F6P/F16bP cycle may involve another substrate cycle, fructose 6-phosphate:fructose 2,6-biphosphate(F6P:F26bP). This sytem is active in liver (see

van Schaftingen, 1987) and may provide the oscillations which allow communication (and thus control) between F16bP and phosphofructokinase (see Crabtree and Newsholme, 1985). In rat muscle, insulin and epinephrine increase the concentration of F26bP, but the potential contribution to control of phosphofructokinase is approximately 10% that of F16bP (see van Schaftingen, 1987). This additional control cycle may therefore be of less importance in muscle compared with liver but as yet this has not been determined for ruminant species.

Summary

It must be remembered that both protein and lipid turnover represent substrate cycles as defined and both make substancial contributions to energy metabolism in the whole animal and muscle. For the other known substrate cycles, while it is difficult to extrapolate studies from rodents and preparations *in vitro* to ruminant muscle *in vivo,* probably a value of 5–8% can be ascribed, on the basis of current knowledge, as their contribution to heat production under normal conditions.

POSTURE AND ACTIVITY

Increases in physical activity elevate both whole animal and muscle energy expenditure. Exercise, even at moderate levels, can stimulate oxygen uptake by the hind limbs of sheep and cattle by 2–10-fold (*eg* Bird *et al,* 1981; Pethick *et al,* 1987) and this is accompanied by increases in blood flow. Most measurements of hind-limb metabolic activity referred to in this review have been derived, however, from animals which have been restrained to the standing or lying condition plus minor movements. Nevertheless, even these postural changes can be energetically expensive and the costs of standing in cattle have been variously reported as 4.6 to 17 $J \cdot min^{-1} \cdot kg^{-1}$ body weight (Forbes *et al,* 1927; Hall and Brody, 1933; Ku-Vera *et al,* 1989). A recent systematic study conducted by Brockway (1987; unpublished observations) involving 20 growing steers and heifers and encompassing several thousand 30-min measurements during respiration chamber confinement yielded a value of 25 $J \cdot min^{-1} \cdot kg^{-1}$ live weight.

The importance of activity to overall (and muscle) heat production is illustrated in figure 2. Calorimetric studies often yield data which show considerable daily inter- and intra-animal variation. The data in figure 2 are from 2 animals of similar breed, age and weight, fed the same quantity of ration and measured at the same time. Total daily heat production differed by 8 MJ, but when adjusted for the difference in time spent standing (7 *vs* 14 h), "corrected" heat productions were very similar. Removel of postural effects from both whole animal and muscle oxygen uptake measurements will be essential for assessment of changes due to metabolic activity.

Diet quality and quantity can influence activity patterns (Wenk and Van Es, 1976) and in the study of Oddy *et al* (1984) marked differences in hind leg oxygen consumption for non-pregnant, non-lactating ewes were observed depending on whether the animals were fed lucerne or oaten hay (2.4 *vs* 1.2 $MJ \cdot d^{-1}$ per total muscle respectively); these may involve metabolic effects but more likely are postural differences.

The value of 25 $J \cdot min^{-1} \cdot kg^{-1}$ live weight may not be applicable to all farm species (*eg* pigs, 132 J/min/kg; Hornicke, 1970; sheep, 8 J/min/kg; Hall and Brody, 1933; 37 J/min/kg, Brockway *et al,* 1969) but if all this expenditure is attribuable to muscle

Fig 2. Effect of standing on energy expenditure in cattle. Two Hereford x Friesian heifers (A, 570 kg; B, 590 kg) offered same diet quantity (1.8 x maintenance). Observed heat production corrected for standing time (25 J/kg body wt/min; Brockway, 1987). Data from Mollison and Brockway (unpublished).

then the oxygen uptake would be 3–6 ml O_2/min/kg muscle for the standing ruminant. In the experiment of Harris *et al* (1989) the lambs stood for ≈ 50–70% of the experimental period and therefore 30–50% of hind-limb oxygen consumption could be attributed to posture requirements even for animals restricted within a metabolism cage-respiration hood system.

Summary

Energy requirements for cellular processes can be relatively minor compared with effects of posture and activity *in vivo*. Intake or exogenous hormone treatment may alter the activity status and mask or confound the effects of such manipulations on metabolic energy expenditure. Furthermore, although allocation of 30–50% towards muscle oxygen requirement *in vivo* may be appropriate, such additions should not be applied in the situation *in vitro* where usually muscles are either relaxed or maintained at resting length.

CONCLUSIONS

Current knowledge would suggest that only 40–60% of energy requirements for skeletal muscles can be attributed to the metabolic demands of protein turnover, ion-transport and substrate cycles (fig 3). The separation of these 4 components is to some extent arbitrary in that considerable interactions between the processes exist. Transport of amino acids, with associated ion movements, interacts with protein synthesis; increased metabolite flow required during exercise also involves stimulation of ion-transport; catabolism of amino acids during prolonged, strenuous activity will alter rates of protein turnover; cation movements, especially Ca^{2+}, are crucial to metabolic regulation, including control of certain substrate cycles. Because of these interactions it is possible for under- (or over-) estimation of energy costs to have been made both *in vivo* and *in vitro* for each process. A considerable portion of oxygen consumption measured *in vivo* may be a consequence of postural

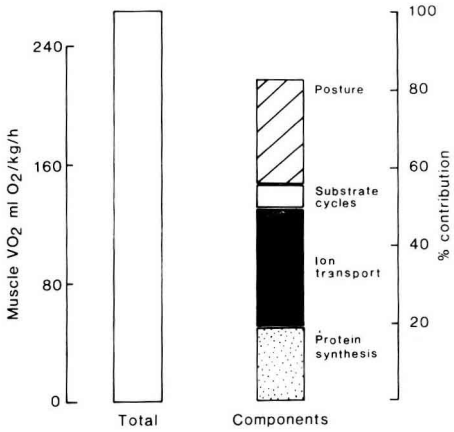


Fig 3. Contribution of specific processes to sheep hindquarter energy expenditure *in vivo*. Data based on total oxygen uptake across hindleg muscle of 35-kg lambs at maintenance intake (from Harris *et al*, 1989).

status but this will have a smaller value *in vitro*. The proportional contribution of protein synthesis to oxygen requirements *in vivo* and *in vitro* are similar, which suggests that other processes may occur *in vitro* to "replace" that for activity in the whole animal. Certain studies, *eg* ion transport and substrate cycles, have been largely or wholly restricted to incubated muscles and there is an urgent need for these to be extended to the whole animal. By sensible integration of studies *in vivo* and *in vitro* the current anomalies in the balance-sheet for muscle energy expenditure can be eliminated.

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