The biological basis of maintenance and its relevance to assessing responses to nutrients

By L. P. MILLIGAN* and M. SUMMERS, *Department of Animal Science, University of Alberta, Edmonton, Canada T6G 2P5*

Animals exhibit a first priority, or threshold, energy requirement that must be met from dietary intake before there will be a yield of products. When fasted, rested and held in a thermoneutral environment, animals exhibit a basal level of energy expenditure that is supported by oxidation of substrates, principally lipids and amino acids, mobilized from body tissues. It is widely presumed that the maintenance energy requirement is the dietary metabolizable energy (ME) required to meet this first-call basal metabolic rate plus the energy needed to support minimal activity and urinary energy excretion (Agricultural Research Council, 1980); that is maintenance ME provides for energy balance under confined conditions. Questions that immediately arise are: what are the metabolic components of maintenance energy expenditure, what are their quantitative roles, is energy balance achieved by simply meeting basal energy expenditure and do the metabolic components of basal metabolism change with level of intake? These questions will be addressed in the present paper.

Components of basal energy expenditure

Basal energy expenditure has been categorized into costs for service functions necessary for the whole organism and costs necessary for existence of individual cells and tissues (Baldwin & Smith, 1974; Baldwin *et al.* 1980). The service functions were considered to account for 36–50% of basal energy expenditure and unlikely to be amenable to external influence (Table 1). The cell and tissue costs were estimated to account for 40–56% of basal energy expenditure. However, it was recognized that at the level of specific metabolic events, there would be considerable overlap between the categories (Baldwin & Smith, 1974). For example, most of the energy cost of neuronal activity and of kidney work actually entails energy expenditure on ion transport. Baldwin *et al.* (1980) appear not to have included the same expenditure in both categories (Table 1).

The estimates of Baldwin *et al.* (1980) involved a number of assumptions and extrapolations, and will, therefore, doubtlessly be subject to considerable refinement. Perhaps the suggested (Table 1) expenditure of 30–40% of basal energy for ion transport is generous on the basis of only sodium and potassium ion transport. Indeed, we have found (McBride & Milligan, 1985*a*) that the proportion of total oxygen uptake required to support Na^+,K^+ transport by sheep hepatocytes

*Present address: Department of Animal and Poultry Science, University of Guelph, Guelph, Canada N1G 2W1.

Table 1. *Energy expenditures in several major maintenance functions (from Baldwin* et al. 1980)

Function	% Basal energy expenditure
Service functions	
Kidney work	6–7
Heart work	9–11
Respiration	6–7
Nervous functions	10–15
Liver functions	5–10
Total	36–50
Cell maintenance	
Protein resynthesis	9–12
Lipid resynthesis	2–4
Ion transport	30–40
Total	40–56

was reduced to 23% on fasting. Furthermore, our most recent measurements for liver biopsies and intercostal muscle preparations (Gregg & Milligan, 1986) incubated in a complex medium including insulin and levels of amino acids as found in plasma, indicate that the proportion of total O_2 uptake of these tissues required for support of Na^+,K^+ transport may be 20–25% rather than the higher values (greater than 35%) we measured previously using minimal media. The value provided by Baldwin *et al.* (1980) could also include the cost of active calcium ion transport. Ca^{2+} is actively transported across a variety of cell membranes including the plasma membrane, the sarcoplasmic reticulum and the mitochondrion. Hasselbach & Oetliker (1983) estimated that 7% of resting skeletal muscle energy expenditure was for Ca^{2+} transport into the sarcoplasmic reticulum; it is unlikely that costs of sequestration into the endoplasmic reticulum to control cytoplasmic concentrations in other tissues would be greater than in muscle. Siems *et al.* (1984) estimated that 2·6% of the O_2 uptake of reticulocytes was to support Ca^{2+} transport. It is likely, therefore, that Ca^{2+} transport would account for less than 10% of resting energy expenditure.

Estimates of energy expenditure on protein synthesis (Table 1) are, obviously, directly dependent on the high-energy phosphate (ATP) costs assumed for amino acid incorporation into a growing peptide chain. A popular value has been the hydrolysis of five pyrophosphate bonds during the incorporation of each amino acid, which includes an allowance of one ATP for the active transport of amino acids across cell membranes. The assumptions implicit in arriving at this estimate were discussed by Reeds *et al.* (1985) and Millward *et al.* (1976); they pointed out reasons why this may be a conservative estimate. Co-transport of an amino acid entails movement of one Na^+ down its transmembrane gradient and then the gradient is actively regenerated by the action of Na^+,K^+-ATPase which catalyses movement of three Na^+ for each ATP hydrolysed. Calculation of one ATP for transport of each amino acid used in protein synthesis, therefore, may be an

overestimate. Furthermore, it is fascinating that in studies assessing energy use by reticulocytes to support protein synthesis, the intact cells appeared to expend only three ATP per amino acid incorporated into globin (Siems *et al.* 1984). Two independent methods for determining the ATP requirements for globin synthesis yielded similar results. One must view current estimates of energy costs of protein synthesis as being tentative.

The energy cost of muscle tone and posture was not included by Baldwin *et al.* (1980) in their consideration of basal energy expenditure. However, Webster (1978) summarized results indicating that the energy costs of standing and of changing position were only approximately 1% of the total energy expenditure of a confined, fed steer. Omission of the energy cost of muscle tone and posture from estimates for basal metabolism may not have been serious.

Substrate cycles resulting in the hydrolysis of ATP (Newsholme & Crabtree, 1976) seem to have engendered nearly as much interest in their 'futility' as in their role in metabolic control. Challis *et al.* (1984) recently measured that cycling between fructose-6-phosphate and fructose-1,6-biphosphate accounted for less than 1% of the energy expenditure of rat epitrochlearis muscle in vitro. Other steps of potential cycling in glycolysis had also previously been considered to be of minor energy cost (Katz & Rognstad, 1976; Baldwin *et al.* 1980). However, substrate cycling, including steps of glycolysis, triglyceride turnover and the Cori cycle, was considered to account for approximately 7·5% of ATP turnover in the young pig (Reeds *et al.* 1985). The recent report of Rabkin & Blum (1985) indicated that substrate cycles for steps of glycolysis plus the acetyl-CoA–acetate pair accounted for up to 26% of ATP expenditure by isolated hepatocytes and thus further necessitates consideration of the quantitative energetic role of substrate cycles. Perhaps for some tissues, particularly those that must support a high capacity of pathways in both overall forward and reverse directions, substrate cycles are a marked energy cost in vivo. The possibility of a substantial energy cost for acetyl-CoA–acetate cycling may be particularly pertinent to ruminants in view of their emphasis on acetate metabolism and the very high endogenous entry rates for acetate reported by Bergman & Wolff (1971).

Nutritional history and physiological state influence the relative body proportions of metabolically-intense abdominal organs (e.g. tissues of the intestinal tract, pancreas, liver, kidneys) (Canas *et al.* 1982; Koong *et al.* 1982; Baldwin & Bywater, 1984) and fasting metabolic rates appear to vary in parallel with changes in these proportions. It was presumed that the metabolic rate of these organs is always high and, therefore, when they constitute an increased portion of body-weight, overall metabolic rate per unit body size is increased.

There are other energy-related metabolic occurrences that still need to be considered as to their quantitative importance in basal metabolism and, as a result, maintenance. There is increasing research emphasis on energy utilization during intracellular protein degradation. The ATP-requiring attachment of ubiquitin to a protein is the first step of one route of intracellular degradation (Ciechanover *et al.* 1984). However, this would appear to entail expenditure of only a very few

pyrophosphate bonds per protein molecule. Intracellular proteolysis also occurs in lysosomes. Gronostajski *et al.* (1985) suggested that lysosomal degradation may require ATP expenditure to establish and maintain the internal acid pH of these organelles. These suggestions do not point to a very substantial energy cost for intracellular protein degradation. However, the recent reports of Siems *et al.* (1984) and Rapoport *et al.* (1985) in which they specifically inhibited intracellular proteolysis, indicated that in reticulocytes there was one ATP expended per peptide bond hydrolysed and that protein degradation accounted for 15% of total energy expenditure. Protein degradation has not previously been assigned an energy cost when considering in vivo protein turnover but it seemingly should be. Unfortunately, the tracer-based estimates of protein turnover reported to date do not reveal the portion of degradation that occurs intracellularly, which obviously poses a serious limitation to arriving at an estimate of cost.

RNA degradation in reticulocytes is also energy-dependent (Park & Morgan, 1984) but specific details of the mechanism have not been elucidated, nor is there information as to the rate of RNA turnover.

The role of membrane phospholipid turnover also needs to be considered in the energy metabolism of the whole organism (Reeds *et al.* 1985). Phospholipids undergo rapid energy-requiring synthesis and breakdown (Berridge, 1984), a substantial part of which may be involved in metabolic regulation. Although Reimann *et al.* (1981) concluded that 60% of the ATP expenditure of erythrocytes was for turnover of polyphosphoinositides, the erythrocyte is a very restricted cell type in a metabolic sense. More recently, Verhoeven *et al.* (1985) concluded that although there is marked enhancement of phosphotidylinositol metabolism and of protein phosphorylation during secretion by platelets, the amount of energy expended on these was minor in relation to total energy use.

Endo- and exocytosis provide a means of this movement of large molecules into and across the plasma membrane and of modulating hormone responsiveness of cells by changing receptor availability. Energy appears to be required both for endocytosis, as exemplified by fibroblasts (Pearse & Bretscher, 1981), and for exocytosis, as exemplified by an amoeba (Oates *et al.* 1982). There is also movement of intracellular proteins across the mitochondrial (e.g. F_1-ATPase) and nuclear (e.g. ribosomal protein, RNA) membranes, such movement being energy-linked (Gasser *et al.* 1982; Purello *et al.* 1983; Reid, 1984; Tsurugi & Ogata, 1984). Clearly, the foregoing movements in and across membranes are biologically important, but one would expect that if only one or a few pyrophosphate bond equivalents were expended per vesicle or per large molecule moved, then the overall energy cost to the cell would be minor.

Relation of maintenance and basal energy expenditure

As noted earlier, the maintenance energy requirement is presumed to be the ME intake required to meet basal energy expenditure plus limited activity. However, achievement of energy balance requires intake of an amount of ME that is approximately 40% greater than the basal energy expenditure (Agricultural

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Research Council, 1980). This unexpectedly high level of ME is needed even though it is presumed that the exogenous nutrients simply offset the mobilization and oxidation of endogenous substrates (lipids and proteins). Indeed, Blaxter (1962) demonstrated that the effectiveness with which exogenous, continuously supplied, energy sources are used for maintenance reflects their expected yield of ATP during intermediary metabolism. Thus, since the effectiveness of conservation of energy in a biologically useful form (ATP) is lower during oxidation of volatile fatty acids than during oxidation of long-chain fatty acids (Milligan, 1971; Baldwin *et al.* 1980), about 4–12% more ME would be required as volatile fatty acids than as stearate to meet the same metabolic energy requirement. Furthermore, although the ruminant at maintenance does not require net mobilization of endogenous substrates to support its energy expenditure, McKay (1974) estimated that, for maintenance-fed sheep 12 h after eating, 23% of their heat production resulted from oxidation of plasma long-chain fatty acids. These fatty acids would have been synthesized in the animal largely from acetate, a process which entails loss, as heat, of 43% as much energy as is conserved in the resultant fatty acids. Therefore, oxidation of endogenously synthesized long-chain fatty acids to meet 23% of energy expenditure at maintenance would require an elevation of ME intake amounting to 10% more than fasting heat production to support the energy loss during synthesis of these fatty acids.

An animal at maintenance must expend energy on the activities of food intake and digestion; these costs would not occur during the fasting state imposed for measurement of basal energy expenditure. It was concluded by Webster (1978, 1980, 1984) that the combined costs of ingestion and digestion of feeds account for 24–41% of the increased heat production at energy balance over that during fasting.

Although some of the discrepancy between basal energy expenditure and ME required for maintenance can be accounted for, a disturbingly large proportion of the difference is still unexplained. As noted previously, major energy expenditures in the resting state appear to be for protein synthesis and for ion transport. Reeds & Fuller (1983) reviewed evidence that during fasting, protein synthesis in mammals is less than when fed. Indeed, they derived positive linear relations for both protein synthesis and degradation over a range of intake extending from fasting to three times that yielding energy balance. For the present discussion it is important to note that the findings summarized by Reeds & Fuller (1983) indicate approximately a doubling of protein synthesis at energy balance over that during fasting. This increase, in turn, would cause the ME required to achieve energy balance to increase about 10–15% over basal energy expenditure. McBride & Milligan (1985*a*,*b*) found that in vitro energy expenditure on Na^+,K^+ transport by hepatocytes and duodenal epithelium is positively responsive to intake. Indeed, O_2 uptakes in support of Na^+,K^+ transport at an intake to provide for energy balance were elevated 1·82-fold for duodenal epithelium and 2·65-fold for hepatocytes over expenditures during fasting. Thus, even if Na^+,K^+ transport (excluding nervous and kidney work) only accounted for 20% of basal energy expenditure, the increase

elicited by feed intake would make a very substantial contribution to the ME above basal expenditure that is required to achieve maintenance. It is evident, then, that maintenance energy expenditure is not simply a matter of meeting the processes of minimal activity plus basal expenditure utilizing exogenous nutrients; there are at least quantitative, and perhaps qualitative, metabolic differences between basal and maintenance states.

Maintenance functions during positive energy balance

In nutritional practice, as noted previously, animals are given feed to meet their maintenance requirement and any additional intake is thought to be available for product formation. Indeed, this is (perhaps with the exception of wool growth) the general pattern of animal response. However, there is now information available that gives cause to question the validity of thinking that energy expenditure at energy balance occurs as an unchanged entity at higher intake levels.

It was noted earlier in this review that the costs of two of the principal metabolic components of basal energy expenditure, Na^+,K^+ transport and protein turnover, were accentuated by the intake of feed to achieve energy balance. In fact, these costs are further accentuated by intake above energy balance! The positive, linear relations of rates of whole-body protein synthesis and breakdown (Reeds & Fuller, 1983) extended from fasting to intake levels of three times that for maintenance. Further, Reeds *et al.* (1985) summarized results showing that in proceeding from negative energy balance to a level of intake yielding a positive energy balance and protein deposition of 6 g/kg body-weight per d in growing pigs, whole-body protein synthesis increased at twice the rate of protein deposition. That is, protein turnover quite clearly increased with intakes above maintenance. Furthermore, it appears that the enhancement of protein turnover is specifically more responsive to increased intake of dietary protein than of non-protein dietary energy (Reeds & Fuller, 1983). In our laboratory we have shown not only marked increases in tissue energy expenditure for Na^+,K^+ transport from fasting to maintenance intake, but when sheep were given an intake of twice maintenance, there was an additional 37% increase in aerobic energy expenditure for Na^+,K^+ transport by duodenal mucosa (McBride & Milligan, 1985*b*). We have also shown that tissue energy expenditure for Na^+,K^+ transport is markedly influenced by physiological state, being elevated in young as compared with older animals, in lactating *v.* dry females and by exposure to a cold environment (Milligan & McBride, 1985). The increases in aerobic energy expenditure by muscle tended to parallel increased total respiration, while the increases for liver and intestinal epithelium were the result of both increased total tissue metabolism and an increased proportion of respiration being used to support Na^+,K^+ transport.

The increased non-fermentative heat production in the digestive tract on feed consumption by sheep held below energy balance was 32 kJ/MJ ME consumed and increased spectacularly to 156 kJ/MJ ME consumed above energy balance, amounting to nearly one-sixth of the ME intake (Webster, 1980). The mechanism underlying this massive elevation in heat production was not studied, but it was

thought to be related to the high rate of turnover of cells and protein of the gut epithelium. In view of our measurements (McBride & Milligan, 1985*b*) of increased intestinal epithelial energy expenditure on Na^+,K^+ transport with increased intake, we expect that this is possibly one of the components. In fact, the suggestions of both enhanced cell growth and Na^+,K^+ transport may very well be complementary because elevated Na^+,K^+ transport is a very early response of a number of cultured mammalian cells when exposed to mitogens (Quastel & Kaplan, 1968; Kaplan, 1978) and is seemingly necessary to resultant increased cell proliferation.

Apparently several of the metabolic occurrences that comprise the usual concept of maintenance energy expenditure are of a greater magnitude above energy balance than at energy equilibrium. The idea that some of the energy intake above maintenance is used to support energy-expending processes that are not directly part of the pathways from precursors absorbed to products synthesized is actually becoming more widely accepted (Armstrong & Blaxter, 1984; Reeds *et al.* 1985). On pragmatic grounds, Armstrong & Blaxter (1984) proposed that increased energy expenditures that occur when the plane of nutrition is increased to achieve production are caused by the productive state and, therefore, should be charged against the productive process. (Extension of this argument would lead to the suggestion that all dietary energy costs are incurred because of, and are chargeable to, the productive purpose for which farm animals are managed. Therefore there may not be any great value in distinguishing maintenance at all.) However, an outcome of the approach suggested by Armstrong & Blaxter (1984) could be that the nutrition research community might regard the total incremental energy cost of production as a functionally single entity rather than the net outcome of a number of processes. It would be regretable if such a singular concept obscured the need for quantitative identification and measurement of the specific changes in support metabolism that accompany production. As noted by Reeds *et al.* (1985), there is a pressing need to know how tightly increases in productive processes are coupled to increases in ancillary energy expenditures. Can accompanying support energy expenditures differ between individuals, or with metabolic state?

The efficiency of formation of products by animals will differ with the metabolic 'distance' of the precursors used for synthesis from the product being formed (Milligan, 1971). However, animals appear not to differ from one another (or basically even from bacteria!) in the pathways utilized for conversion of precursors to products. Therefore, differences in the efficiency of formation of products may very well entail variation in the extent to which there is energy use for dissipating conversions above maintenance. It is unlikely that the control of these ancillary expenditures rests entirely in greater intake of feed. Rather, as is emerging for the relation of protein turnover and dietary protein level (Reeds *et al.* 1985), control will possibly be found to be related to specific dietary components. Therefore, there is a very real need to attain quantitative measurement of the occurrence of ancillary energy expenditures as related to the components of the diet. This will indeed be a most important component of assessing the responses of animals to nutrients but will be difficult to attain. However, it appears to be one of the keys to

understanding variation in the energetic efficiency of animal production.

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