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The sodium pump and other mechanisms of thermogenesis in selected tissues

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Whole-body heat production is a consequence of several biochemical events. Specific sites of heat production have been isolated, some being greater contributors to whole-body oxygen consumption than others. Tissues of the splanchnic bed (the gastrointestinal tract and liver) make up only 4–6% of whole-body mass, but account for 40% of total ATP utilization. Skeletal muscle accounts for a much larger proportion of whole-body mass (50%), but accounts for only 20% of whole-body ATP use. Two major biochemical events contributing to energy use are Na^+,K^+-ATPase (*EC* 3.6.1.3) activity and protein turnover. Discussion of these two processes will dominate the present paper but reference will be made to other energy-consuming processes within selected tissues.

First, a major contributor to cellular energetics is the activity of the enzyme Na^+,K^+-ATPase, which extrudes three Na^+ from the cell and moves two K^+ into the cell against their respective concentration gradients at the cost of one high-energy phosphate bond (Balaban *et al.* 1980). The actions of this enzyme are responsible for the active transport of substrates, maintenance of ionic homeostasis, membrane potential and cell multiplication (Rossier *et al.* 1987; Huntington & McBride, 1988).

TECHNIQUES FOR MEASURING THE ENERGETIC COST OF Na^+,K^+-ATPase

The sodium pump is specifically inhibited by the cardiac glycoside ouabain (10^{-4} M; Glynn, 1964), such that the proportion of oxygen consumption due to this process may be measured. Cells or tissue slices are incubated in vitro in media patterned after blood plasma (Early *et al.* 1988*b*). O_2 consumption is determined polarographically using a Clark-style electrode in an incubation chamber. On determination of total O_2 consumption, ouabain may be added to determine ouabain-sensitive and -insensitive respiration (McBride & Milligan, 1984). Saddlier & De Luise (1986), using an indirect comparison, correlated in vitro Na^+,K^+-ATPase activity with both in vitro muscle and in vivo whole-body O_2 consumption showing that the Na^+-pump is a significant contributor to whole-body heat production.

Conversely, Summers *et al.* (1989) measured both the in vitro and in vivo energy expenditures in the ovine parotid gland. This in vivo technique involves the vascular

isolation of the gland and subsequent arterial administration of ouabain. O_2 consumption rate was determined by the arterio-venous O_2 concentration differences multiplied by blood flow-rate through the gland. In this experiment, in vivo results were substantially higher than those for in vitro preparations, indicating that the in vitro technique may only give minimal estimates. A second in vivo technique has recently been reported by Swaminathan *et al.* (1989), in which the contribution of the Na^+-pump to whole-body O_2 consumption was measured in guinea-pigs via intraperitoneal injection of ouabain. Using this method and an Eadie-Hofstee plot of whole-body O_2 consumption *v.* ouabain dose, it was estimated that the Na^+-pump accounted for a minimum 28% of whole-body O_2 consumption.

For the determination of the energetic cost of the Na^+-pump in the gastrointestinal tract, biopsies were taken through rumen cannulas (Kelly *et al.* 1989) or intestinal cannulas (McBride & Milligan, 1984, 1985*a*) or acquired at slaughter (McBride & Early, 1989). Viability and morphology of intestinal tissues were determined with histological preparations and microscopy techniques (McBride & Milligan, 1984, 1985*a*).

Liver metabolism has been studied via three different methods. Samples were excised as biopsies in vivo (McBride & Milligan, 1985*a*), or taken immediately following slaughter (either slices or isolated hepatocytes; McBride & Early, 1989; McBride *et al.* 1989), or through perfusion of the liver with collagenase resulting in free hepatocytes (McBride & Milligan, 1985*b*,*c*). Viability and morphology were determined with trypan-blue staining and electron microscopy (McBride & Milligan, 1985*c*).

Skeletal muscle presents a more complex problem in that it is difficult to obtain samples of muscle fibres which have not been damaged through excision. Wijayasinghe *et al.* (1984) developed a technique which permitted the excision of the external intercostal muscle (EIC) with intact whole muscle bundles which are tied at the tendons at each end. This sample is then mounted on a support which is placed in the O_2 electrode chamber. This method allows for the muscle to be incubated at resting length with a constant tension.

INFLUENCE OF LEVEL OF INTAKE ON Na^+,K^+-ATPase ACTIVITY

The influence of various physiological changes on Na^+-pump activity is summarized in Table 1. Intake has been shown to markedly affect intestinal and hepatic O_2 consumption and associated Na^+,K^+-ATPase activity (McBride & Milligan, 1985*a*,*b*). Fasting causes a depression in in vitro respiration in the duodenum (McBride & Milligan, 1985*a*) which may be a response to depressed blood flow to, and O_2 flux across, the portal-drained viscera (Eisemann & Nienaber, 1989). Animals fed to maintenance or twice maintenance energy intakes exhibit higher total and ouabain-dependent respiration than fasted animals (McBride & Milligan, 1985*a*). Not surprisingly, increased intake is also associated with gut hypertrophy (Fell *et al.* 1972; Fell & Weekes, 1975). An increase in intestinal mass is also observed with the increased intakes occurring during lactation (Williamson, 1986). McBride & Milligan (1984) described elevations in in vitro O_2 and Na^+,K^+-ATPase-dependent respiration in intestinal mucosa from lactating dairy cows. This is also related to the increased blood flow and O_2 flux across the portal-drained viscera during lactation (Huntington, 1984; Huntington & McBride, 1988). Increased feed intake, or an endocrine shift enhancing growth factor or other mitogenic activity, may produce a cascade involving Na^+. Higher rates of absorption are expected

Table 1. *The effect of various physiological phenomena on Na^+,K^+-ATPase (EC 3.6.1.3) selected tissues*

Species	Phenomenon	Effect	Reference
Skeletal muscle			
Rat myotubes	T_3, T_4	↑	Brodie & Sampson (1988)
Mice	Obesity	↓	Lin *et al.* (1979)
Pig	Cold	↑	Herpin *et al.* (1987)
	Increased diet protein	↑	Adeola *et al.* (1989)
Sheep	T_3, T_4	↑	McBride & Early (1989)
	Insulin	NC	Early *et al.* (1988*b*)
	Lactation	↑	Gregg & Milligan (1982*c*)
	Increasing age	↓	Gregg & Milligan (1982*c*)
Liver			
Mice	Obesity	↓	Hughes & York (1983)
Rats	T_3	↑	Ismail-Beigi & Edelman (1970)
Sheep	Insulin	↑	Jessop (1988)
	Thyroidectomy	↓	Gregg & Milligan (1987)
	T_3	↑	Gregg & Milligan (1987)
	Lactation	↑	McBride & Milligan (1985*b*)
	Increasing age	↓	McBride & Milligan (1985*b*)

↑, Increased; ↓, decreased; NC, no change; T_3, triiodothyronine; T_4, thyroxine.

to be concurrent with higher concentrations of intracellular Na^+. In an attempt to maintain ionic homeostasis, two mechanisms may be enhanced: Na^+,K^+-ATPase and the Na^+,H^+-antiport system. Increased activity of the former will facilitate absorption of nutrients while an elevated rate of nutrient absorption will elevate cytosolic pH enhancing the environment for RNA and DNA synthesis. This will, in turn, increase the numbers of transport enzymes and aid in mucosal cell proliferation, further enhancing the capacity for absorption. This increased cellular metabolism may be a consequence of altered endocrine status, thereby altering the metabolic signals being interpreted by the plasma membrane; however, the serum mitogen(s) responsible for the control of intestinal hypertrophy remain to be discerned.

Hepatic activity of Na^+,K^+-ATPase is subject to changes similar to those occurring in the gut during conditions of increased intake. Starved sheep had lower O_2 consumptions associated with Na^+,K^+-transport than control animals while lactating ewes fed at high levels of intake had higher Na^+,K^+-ATPase-dependent respiration compared with control animals (McBride & Milligan, 1985*b*). The changes in Na^+,K^+-ATPase activity may be a consequence of the altered nutrient supply as influenced by blood flow and substrate concentration (Eisemann & Nienaber, 1989), or a local response to an endocrine change associated with starvation or lactation.

HORMONAL EFFECTS ON Na^+,K^+-ATPASE ACTIVITY

Hormonal effects on energy metabolism are often reflected in their perturbations of biochemical events, but the responses may be tissue specific. This is the case for Na^+,K^+-ATPase activity, which may be regulated by two specific mechanisms; either

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through induction of Na^+,K^+-ATPase gene expression or via post-translational modification of already existing units through mediation of plasma membrane receptors and second messengers (Rossier *et al.* 1987). The influence of hormonal status on Na^+-pump activity is also shown in Table 1.

Thyroid hormones produce an elevation in whole-body heat production (Kennedy *et al.* 1986), organ metabolism (Ismail-Beigi & Edelman, 1970) and cellular energetics. By artificially elevating plasma triiodothyronine (T_3), free T_3 and thyroxine (T_4), McBride & Early (1989) increased the activity of Na^+,K^+-ATPase by 33% in hepatocytes isolated from sheep. Herpin *et al.* (1987) and Gregg & Milligan (1982*a*) found similar elevations in Na^+-pump activity in muscle preparations from cold-exposed animals, a condition associated with increased thyroid status (Kennedy *et al.* 1986). Furthermore, depressed thyroid status during heat stress results in depressed Na^+-pump-dependent respiration in fetal placenta (McBride *et al.* 1987*a*).

Growth hormone (GH) is also a potent regulator of metabolism in selected tissues (McBride *et al.* 1988). In steers treated with bovine somatotropin (bST), McBride *et al.* (1989) found an elevation in total (39%) and ouabain-sensitive (68%) hepatic respiration but no effect on skeletal muscle energetics. In dairy cows, McBride *et al.* (1987*b*) found no change in skeletal muscle energetics when they were treated with bST, which is consistent with the increased energetic efficiency of milk synthesis (Bauman *et al.* 1985) given that the maintenance energy expenditures on Na^+,K^+-ATPase remains unchanged but milk production capacity of the animal increases. These findings corroborate the concept that the elevation in heat production associated with bST-treated cows (Tyrrell *et al.* 1982) is due to tissues other than skeletal muscle. The other major sites involved are probably the mammary gland and adipose tissue.

Recent work by Early *et al.* (1988*a*,*b*, 1989*b*) has indicated that ruminant skeletal muscle is less sensitive to the actions of insulin than that of non-ruminants. No differences in the absolute rate or proportional energetic costs of the Na^+-pump were found in insulin-treated sheep (Early *et al.* 1988*b*). This is significant in that Na^+,K^+-ATPase action influences the transport of amino acids (protein synthesis) and other nutrients such as glucose into the cell, and that Na^+,K^+-transport in the non-ruminant is known to be insulin-sensitive (Moore, 1983).

Evolutionary development of mammalian homeothermy can be traced through the activity of Na^+,K^+-ATPase. When comparing lizards (*Amphibolurus vitticeps*; the bearded dragon lizard) and rats of comparable metabolic body size, weight and preferred body temperature, Else & Hulbert (1987) showed that the absolute rate of O_2 consumption and Na^+,K^+-dependent respiration in the liver, kidney and brain from the rat were substantially higher than the respective values from the lizard. This was in agreement with previous work which indicated a 4–9-fold increase in Na^+,K^+-dependent respiration in the same tissues between mice and lizards (Hulbert & Else, 1981). Indeed, in both the kidney and liver, the rat had a higher passive diffusion of ^{42}K from the tissue, this being an indication that mammals may have leakier membranes than reptiles, thus the added energy demand to support higher Na^+,K^+-ATPase activity (Else & Hulbert, 1987). Consequently, this leads to a higher level of heat production and, thus, more ability for thermoregulatory control.

Depressed heat production in tissues of genetically obese animals *(ob/ob)* is paralleled by reductions in Na^+-pump activity. Hughes & York (1983), found a 65% depression in hepatocyte Na^+,K^+-ATPase activity in genetically obese mice *v.* lean mice. Similarly,

Table 2. *Contribution of Na^+,K^+-ATPase (EC 3.6.1.3) to whole tissue energy expenditure in selected tissues*

Tissue	Species	Proportion of tissue total oxygen consumption (%)	Reference
Skeletal muscle			
	Pigs	17·6–25·2	Herpin *et al.* (1987)
		22·1–24·8	Adeola *et al.* (1989)
	Sheep	23	Early *et al.* (1988*b*)
		22	McBride & Early (1987)
		17·7–26·3	McBride & Early (1989)
		28·7–36·3	Gregg & Milligan (1982*a*,*c*)
	Cold sheep	45	Gregg & Milligan (1982*a*)
	Calves	39·2–41·6	Gregg & Milligan (1982*b*)
	Steers	9·0–10·5	McBride *et al.* (1989)
	Dairy cows	18·0–22·9	McBride *et al.* (1987*b*)
Liver			
	Fetal hepatocytes	23–31	Vatnick *et al.* (1989)
	Lambs	47·8–51·0	McBride & Milligan (1985*c*)
	Sheep	36·5	McBride & Milligan (1985*b*)
	Starved sheep	17·8	McBride & Milligan (1985*b*)
	Lactating sheep	45	McBride & Milligan (1985*b*)
	Sheep	28·6–35·7	McBride & Early (1989)
	Steers	16·2–21·1	McBride *et al.* (1989)
	Human	35	Baldwin & Smith (1974)
Gastrointestinal tract			
Rumen	Steers	16·9–19·3	Kelly *et al.* (1989)
Duodenum	Starved sheep	28·6	McBride & Milligan (1985*a*)
	Fed sheep	48·1–61·3	McBride & Milligan (1985*a*)
	Cows	34·9	McBride & Milligan (1984)
	Lactating cows	53·8–55·0	McBride & Milligan (1984)
Jejunum	Steers	26·1–26·2	McBride *et al.* (1989)
Whole-body			
	Sheep	20	Gill *et al.* (1989)
	Guinea-pig	28–39	Swaminathan *et al.* (1989)

Lin *et al.* (1979) showed that obese mice had much lower whole-body heat production and [^{3}H]ouabain binding to liver and hind-limb muscles compared with lean mice, thus, indicative of lower Na^+,K^+-ATPase receptor sites. It would appear that genetic obesity may be a consequence of reduced activity of thermogenicly important biochemical transactions. The Na^+-pump may be one of the biochemical reactions reduced as a consequence of obesity.

The Na^+-pump, then, is a major contributor to organ and whole-body O_2 consumption. In the gastrointestinal tract, liver and skeletal muscle, the activity of Na^+,K^+-ATPase accounts for 16·9–61·3%, 17·8–51·0% and 17·6–45% of tissue O_2 consumption, respectively. Estimates of its contribution to whole-body O_2 consumption range from 20% in sheep (Gill *et al.* 1989) to 28–39% in guinea-pigs (Swaminathan *et al.* 1989) (see Table 2). It is, therefore, an important contributor to heat production in both small and large mammals.

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A second major component of energy expenditure in cells is that associated with protein turnover. This process includes the synthesis and degradation of intracellular proteins, the latter process exerting a direct demand for energy not linked to that required for protein synthesis (Hershko, 1988; Summers *et al.* 1988). Presently, the energetic cost of protein synthesis is assumed to be 5 mol ATP per mol peptide bond formed (Millward *et al.* 1976; Lobley, 1986), with 1 mol ATP ascribed to the transport of an amino acid across the plasma membrane and 4 mol ATP responsible for the actual synthetic processes (Gill *et al.* 1989). This equates to 4·5 kJ/g assuming that 1 mol ATP requires 85 kJ metabolizable energy (Waterlow *et al.* 1978). Heat production is closely related to protein deposition, implying that protein deposition is energetically inefficient (Reeds *et al.* 1980). Estimates of the contribution of protein synthesis to whole-body heat production range from 12 to 25% (Summers *et al.* 1988; Harris *et al.* 1989).

PROTEIN SYNTHESIS

Estimates of the energetic cost of protein synthesis in specific tissues have been acquired via two distinct methods. Isolation of biopsies and their subsequent incubation in an in vitro medium allows specific inhibition of various biochemical events. Cycloheximide (10^{-4} M), a specific inhibitor of cytosolic protein synthesis at the elongation phase (Siems *et al.* 1984), can be added to the medium after determination of uninhibited respiration in a Clark-style electrode system. The decline in respiration rate of that tissue gives an indication of the O_2 cost attributable to that specific process (McBride & Early, 1989). While rapid changes in protein synthesis are present in vitro, Garlick *et al.* (1983) showed that the same changes occur with equal rapidity in vivo indicating that the in vitro system is a valid model of the in vivo reality (Reeds & Palmer, 1986).

A second method involves the isolation and perfusion of the specific organ system (e.g. hind-limb preparation) and measurement of labelled amino acid while accounting for the oxidation of the labelled amino acid and the average percentage content of amino acid within the tissue and assuming a constant stoichiometry for ATP use for protein synthesis (Garlick *et al.* 1980; Lobley *et al.* 1980; Reeds *et al.* 1987).

Estimates of the energetic cost of protein synthesis in tissues exist in the literature and range from 2 to 30% (Summers *et al.* 1988) of tissue ATP utilization (Table 3). This disparity in values probably reflects both procedural differences coupled with the biological response to physiological state of the animal. The most commonly studied tissues include skeletal muscle, liver and gastrointestinal tissues. Protein synthesis is affected by a variety of physiological conditions imposed by lactation (Vincent & Lindsay, 1985), cold temperatures (Thompson *et al.* 1987), endocrine manipulation (Vernon, 1989), age (Attaix *et al.* 1986), diet composition and level of intake (Garlick *et al.* 1985; Boisclair *et al.* 1987; Jepson *et al.* 1988) and sepsis (Hasselgren *et al.* 1986) (Tables 2 and 3).

Skeletal muscle protein synthesis accounts for approximately 14–33% of whole-body protein synthesis in different species (Buttery, 1984). Various estimates of the energetic cost of protein synthesis in muscle are shown in Table 3. The very low estimate of Gregg & Milligan (1982*a*) is in contrast to all other estimates (15–25%) and is probably reflective of the low rates of protein synthesis of this isolated muscle preparation. In vitro protein synthesis estimates using the EIC prepared with attached tendons and mounted

Table 3. *Contribution of protein synthesis to energy expenditure (%) in selected tissues*

Tissue	Species	Proportion of tissue total oxygen consumption (%)	Reference
Skeletal muscle			
	Rabbits	22·6	Reeds *et al.* (1985)
		14·6	Reeds *et al.* (1987)
	Chicks	19·8	Summers *et al.* (1988)
	Baby pigs	18·4–21·8	B. W. McBride (unpublished results)
	Pigs	17·2	Reeds *et al.* (1985)
		19·1–20·7 (in vivo)	Reeds *et al.* (1987)
	Lambs	12–30	Harris *et al.* (1989)
	Sheep	14	Early *et al.* (1988*b*)
		27	McBride & Early (1987)
		17·5–22·6	McBride & Early (1989)
	Calves	2·0–3·3	Gregg & Milligan (1982*a*)
	Dairy cows	14·8–18·3	McBride *et al.* (1987*b*)
Liver			
	Rats	2·6–5·4	Ismail-Beigi *et al.* (1976)
		15·1	Reeds *et al.* (1985)
	Rabbits	26·5	Reeds *et al.* (1987)
	Sheep	15·5–24·4	McBride & Early (1989)
		23–26	McBride & Early (1987)
Gastrointestinal tract			
Rumen	Steers	15–25	J. M. Kelly, B. W. McBride & L. P. Milligan (unpublished results)

at resting length on grids are considerably higher and the energy costs thereof reflect these higher rates of protein synthesis (Early *et al.* 1988*b*).

Contributions of hepatic protein synthesis to hepatic O_2 consumption are available in the literature for a variety of species (Table 3) and are affected by physiological manipulation. Similarly, the gastrointestinal tract contributes substantially to whole-body O_2 utilization (Huntington & McBride, 1988), and even though it only amounts to 4–6% of the total body-weight of cattle, it accounts for 40% of whole-body protein synthesis (Lobley *et al.* 1980). The relatively high fractional synthesis rates (FSR) in the ruminant gut range from 10 to 78%/d (Schaeffer *et al.* 1986; McBride *et al.* 1989). The energetic cost of protein synthesis has been estimated at 20·2% of total O_2 consumption in ovine duodenal mucosa (Huntington & McBride, 1988), which is similar to the estimate of J. M. Kelly, B. W. McBride and L. P. Milligan (unpublished results) for rumen epithelium (15–20%).

HORMONAL EFFECTS ON PROTEIN SYNTHESIS

Endocrine manipulation of cellular metabolism has been shown to affect the rate of protein synthesis in different tissues (Tables 4 and 5).

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Table 4. *Effect of various physiological phenomena on protein synthesis in skeletal muscle from different species*

Species	Phenomenon	Effect	Reference
Dwarf mouse	GH	↑	Bates & Holder (1988)
	Thyroxine	↑	Bates & Holder (1988)
Normal mouse	GH	NC	Bates & Holder (1988)
	Thyroxine	↑	Bates & Holder (1988)
Rats	Free amino acids	↑	Garlick *et al.* (1985)
	Increasing protein	↑	Jepson *et al.* (1988)
	0–18 h post feed	↑	Garlick *et al.* (1973)
	24 h + post feed	↓	Garlick *et al.* (1973)
	Fast	↓	Preedy & Sugden (1989)
	Fast + hypoxia	↓	Preedy & Sugden (1989)
	Cold temperature	↑	Millward *et al.* (1985)
	Obesity	↓	Reeds *et al.* (1982)
	Tumour necrosis factor	↓	Charters & Grimble (1989)
	Sepsis	↓	Hasselgren *et al.* (1986)
	Corticosterone	↓	Odedra *et al.* (1983)
	Insulin	↑	Stirewalt & Low (1983); Garlick *et al.* (1985); Jepson *et al.* (1988)
	T_3	↑	Carter *et al.* (1982); Jepson *et al.* (1989)
	Thyroidectomy	↓	Brown & Millward (1983)
	Thyroidectomy + T_3	NC	Brown & Millward (1983)
Pigs	Thyroxine	↑	Skjaerlund *et al.* (1988)
Lambs	Increased intake	↑	Harris *et al.* (1989)
	Fasted	↓	Oddy *et al.* (1987)
	GH	↑ or NC	Pell & Bates (1987); Crompton & Lomax (1989)
	Estradiol	↓ or NC	Hunter *et al.* (1987)
	Insulin	↓	Oddy *et al.* (1987)
	Age	↓	Davis *et al.* (1981); Oddy *et al.* (1987); Attaix *et al.* (1988)
	Trenbolone acetate	↓	Sinnett-Smith *et al.* (1983)
	Zeranol	↓	Sinnett-Smith *et al.* (1983)
Sheep	Added concentrate	↑	Bryant & Smith (1982)
	T_3	↑	McBride & Early (1989)
	Insulin	NC	Early *et al.* (1988*b*)
Cultured ovine muscle cells	IGF-I	↑	Harper *et al.* (1987)
	EGF	↑	Harper *et al.* (1987)
	Bovine GH	NC	Harper *et al.* (1987)
Goats	Age	↓	Muramatsu *et al.* (1988)
Steers	Underfed	↓	Boisclair *et al.* (1987)
	GH treatment	NC	McBride *et al.* (1989)

↑, Increased; ↓, decreased; NC, no change; T_3, triiodothyronine; GH, growth hormone; IGF-I, insulin-like growth factor I; EGF, epidermal growth factor.

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Table 5. *Effect of various physiological phenomena on hepatic protein synthesis in different mammalian species*

Species	Phenomenon	Effect	Reference
Dwarf mouse	Growth hormone	↑	Bates & Holder (1988)
	Thyroxine	↑	Bates & Holder (1988)
Mouse	Growth hormone	↑	Bates & Holder (1988)
	Thyroxine	↑	Bates & Holder (1988)
Rat	Obesity/18 d age	NC	Reeds *et al.* (1982)
	Obesity/25 d age	↑	Reeds *et al.* (1982)
	0–24 h post feed	↑	Garlick *et al.* (1973)
	24 h + post feed	↓	Garlick *et al.* (1973)
Lambs	Estradiol	↓	Hunter *et al.* (1987)
	Age	↓	Attaix *et al.* (1986); Hunter *et al.* (1987)
Sheep	Thyroxine	↑	McBride & Early (1989)
Steer	Growth hormone	↑	McBride *et al.* (1989)

↑, Increased; ↓, decreased; NC, no change.

Thyroidectomy decreases the FSR of skeletal muscle in rats (Brown & Millward, 1983) whereas T_3 treatment of mice or rats increases rates of protein synthesis (Carter *et al.* 1982; Bates & Holder, 1988; Jepson *et al.* 1988) (see Table 4). The study of Jepson *et al.* (1988) indicated a significant linear relationship between the rate of muscle protein synthesis and T_3 treatment. Similarly, McBride & Early (1989) found a linear relationship between free-T_3 in plasma and the fractional rate of muscle protein synthesis in sheep. This is in agreement with Brown & Millward (1983) who indicated that supplementation of thyroidectomized rats with exogenous T_3 returned protein synthesis levels to control values. Gregg & Milligan (1982*a*,*b*) found increased rates of protein synthesis in skeletal muscle of cold-exposed sheep, a condition which is associated with elevated thyroid status (McBride *et al.* 1985; Herpin *et al.* 1987). It has been suggested that the increase in protein synthesis is due to an increase in the concentration of RNA within the cell (Brown & Millward, 1983; Reeds, 1987; Jepson *et al.* 1988). Hyperthyroidism is associated with elevated FSR in skeletal muscle (Buttery, 1983). In order to continue protein synthesis at this high rate, a concomitant increase in energy usage would be expected since the large amounts of energy are required for the synthesis of peptide bonds (Gill *et al.* 1989).

McBride & Early (1989) stated that protein synthesis increased in hepatocytes from hyperthyroid sheep (see Table 5). Thyroid hormones have been shown to significantly enhance heat production (Ismail-Beigi & Edelman, 1970) and, perhaps through an elevation in RNA synthesis, increase the FSR in skeletal muscle. This may also be the case in liver metabolism. Indeed, increased energy utilization in support of protein synthesis in thyroxine-stimulated animals may be due to increased activity in support of processes such as Na^+,K^+-ATPase action (McBride & Early, 1989).

The effects of insulin on muscle protein synthesis are also well documented. Indeed, it has been called the most important factor regulating skeletal muscle protein balance in

simple-stomached animals (Goldberg *et al.* 1980). Insulin has been implicated in enhanced uptake of amino acids (Buttery, 1983) as evidenced through elevated α-(methyl)aminoisobutyric acid uptake through system A via an enhanced V_{max}, which is independent of *de novo* protein synthesis (Guma *et al.* 1988). In rodents, pigs and humans there appears to be an enhancement of the protein synthetic rate by insulin induction (Stirewalt & Low, 1983; Garlick *et al.* 1985; Jepson *et al.* 1988). In ruminants, the effect of insulin on protein synthesis is less consistent. Insulin has been shown to depress (Oddy *et al.* 1987), have no effect (Early *et al.* 1988*a*,*b*, 1989*b*) or increase protein synthesis in ruminants (Buttery, 1983). However, insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF) have both been implicated in raising the level of embryonic ovine skeletal muscle protein synthesis (Harper *et al.* 1987). Insulin secretion causes a subsequent increase in prostaglandin release (Reeds & Palmer, 1986). Prostaglandin F_{2a}, and also its major metabolic precursor arachidonic acid, has been implicated in the stimulating muscle protein synthesis (Reeds & Palmer, 1986).

GH effects on protein synthesis seem to be modulated by the maturity of the animal as well as the physiological state of the animal. Treatment of steers with GH increased FSR and the energetic cost associated with hepatic protein synthesis (McBride *et al.* 1989). In skeletal muscle, Bates & Holder (1988) found that mice containing a dwarfism gene responded to GH with an increased protein synthetic rate but this was not evident in normal mice. Growing lambs also had larger FSR than control animals during short-term (Crompton & Lomax, 1989) or long-term (Pell & Bates, 1987) administration. In fully mature, lactating Holstein cattle, though, no change in the FSR or the O_2 consumption associated with skeletal protein synthesis (14·8–18·3% of total O_2 consumption) was evident (McBride *et al.* 1987*b*) and this was also the case for skeletal muscle of GH-treated steers during the final stages of growth (McBride *et al.* 1989). This was corroborated by the work of Early *et al.* (1989*a*) who found no change in the FSR of the intercostal, sartorius or semitendinosus muscles in GH-treated steers and also found no change in either protein accretion or degradation rates. In contrast to Pell & Bates (1987), these results suggest that protein synthesis rates may differ between chronic and acute GH treatment, and also that the effects of GH may predominate during the initial phases of administration. The acute stimulatory effects of GH on protein synthesis may be due to the subsequent actions of IGF-I (Harper *et al.* 1987).

PROTEIN DEGRADATION

Cellular proteolysis is mainly performed by two distinct processes: the ATP-dependent ubiquitin pathway and the lysosomal proteolytic pathway (Hershko & Ciechanover, 1982). Protein degradation allows for the removal of incorrectly synthesized proteins and allows for the maintenance of enzymic and structural integrity within the cell (Summers *et al.* 1988).

In the former system, ATP is required for the conjugation of ubiquitin, a seventy-six-residue polypeptide, to proteins (Finley & Varshavsky, 1985). Evidence for the existence of this system was shown in rabbit muscle and liver cells by Fagan *et al.* (1987) who also concluded that it is probably present in all mammalian cells. Lysosomal degradation, although the most dominant system of protein degradation (Mayer & Doherty, 1986), appears to be primarily non-selective (Hershko, 1988) and does not have a direct requirement for ATP. Rather, ATP utilization could involve the maintenance of the

large H^+ concentration within the lysosome itself through the action of H^+-pumps, as well as the formation of the autophagosome.

Direct estimates of the energetic cost of protein degradation acquired from cells of agricultural species are not evident in the literature. There are, however, estimates of the fractional degradation rates of various tissues under different physiological states. Also, recent experimentation indicates that myofibrillar and total protein in muscle may be differentially degraded (Kadowaki *et al.* 1989). These authors indicated that during starvation and streptozotocin-induced diabetes, myofibrillar proteins were preferentially degraded, but during protein deficiency, non-myofibrillar protein degradation was selectively depressed.

Fasting causes a net release of branched-chain amino acids from the hind limb of cattle indicating elevated protein degradation (Early *et al.* 1987). Although protein synthesis in skeletal muscle may be elevated during lactation, protein degradation may also be elevated to a larger extent, thus muscle wasting may be evident during lactation (Vincent & Lindsay, 1985) implying a larger requirement for energy for protein turnover in skeletal muscle during lactation. This is, however, in contrast to the work of Harris *et al.* (1981), who indicated via measurement of 3-methylhistidine excretion that protein degradation in the lactating cow is less during lactation.

Hyperthyroidism is associated with increased proteolysis (Buttery, 1983) since fractional growth rates of muscle may be decreased with T_3 treatment (Carter *et al.* 1982) even though FSR are increased (McBride & Early, 1989). Indeed, on stimulation with T_3 in thyroidectomized animals, Brown *et al.* (1981) found a 2·2-fold increase in the rate of skeletal muscle protein degradation, and Skjaerlund *et al.* (1988) found a 33% increase in in vitro protein degradation in similarly stimulated pigs.

Insulin, probably through an increase in the release of prostaglandin E_2, causes increased rates of protein degradation in fasted rabbits (Palmer *et al.* 1985) under physiological concentrations. This is in opposition to much of the previous literature which indicated that protein degradation was inhibited by insulin, but as pointed out by Reeds & Palmer (1986), most of the previous studies had used 'pathological' levels of insulin in their investigation. Insulin probably encourages more, rather than less, heat production in skeletal muscle due to enhanced protein degradation as well as enhanced protein synthesis.

Work of Eisemann *et al.* (1986, 1989), using urinary 3-methylhistidine as an index of myofibrillar protein degradation, has indicated that GH does not affect protein degradation rates. This is corroborated by the work of Early *et al.* (1989*a*).

In animal metabolism, protein turnover is, then, a major energetic drain, particularly in skeletal muscle (Reeds, 1987) and in tissues of the splanchnic bed (Huntington & McBride, 1988). Manipulation of the rates of synthesis and degradation and their associated energy costs may provide a means of achieving a more efficient animal. Certainly the effects of physiological (lactation, age, endocrine status) and environmental states (cold temperatures) alter these rates, and the use of a partitioning agent such as GH provides an opportunity to direct nutrients to more productive processes and, hence, lessen the energetic load of specific, non-productive tissues. Protein turnover is responsible for a significant amount of O_2 consumption and, thus, ATP utilization; however, the energetics of protein degradation remain less well defined and consequently require further study to elucidate its quantitative role in energy metabolism.

A third important contributor to animal metabolism can be found in substrate cycles.

These have been viewed as 'futile' cycles, but it has been debated whether the generation of heat in the maintenance of homeothermy is futile. Any biochemical process in which a substrate goes through a complete turn, at the expense of energy may be termed a substrate cycle. The major substrate cycles in the glycolytic pathway include glucose to glycogen, glucose to glucose 6-phosphate, fructose 6-phosphate to fructose 1,6-bisphosphate, phospho-enol pyruvate to pyruvate to oxaloacetate and acetyl-CoA to acetate (Newsholme, 1987; Newsholme & Stanley, 1987). In fatty acid metabolism, free fatty acids and glycerol cycle through triacylglycerol at the expense of 8 mol ATP per turn (Milligan, 1971). Although individually these cycles do not constitute a large energetic demand on the animal, in concert their demand for energy can be substantial (Summers *et al.* 1988). Similar to the Na^+-pump and protein turnover, these cycles are subject to manipulation by various physiological phenomena. For example, Brooks *et al.* (1982, 1983) and Challiss *et al.* (1984*a*,*b*) demonstrated that catecholamines increase their activity six to thirteen times, while Dunshea & Bell (1989) showed that lactating goats had a 6-fold increase in fatty acid–triacylglycerol cycling. Quantitatively, because of the high concentration of glycolytic–gluconeogenic machinery in the liver and the requirement of the liver to respond to large short-term changes in nutrient supply (Summers *et al.* 1988), substrate cycles may contribute up to 23% of hepatic energy expenditure (Rabkin & Blum, 1985), but probably contribute substantially less in skeletal muscle and the gastrointestinal tract (Summers *et al.* 1988).

Other energy-consuming processes include the turnover of nucleic acids, Ca^{2+}-ATPase (*EC* 3.6.1.32) activity (especially in muscle), the Na^+,H^+-antiport (maintenance of intracellular pH and ionic homeostasis), glutamate–glutamine and aspartate–asparagine cycling, phospholipid turnover, urea biosynthesis and the Cori cycle. These processes are generally of smaller magnitude in whole-body energy expenditures; however, within individual tissues each cycle may become more predominant, whilst in other tissues they may be of less importance.

It is evident that both the operation of the Na^+-pump and protein turnover represent substantial energetic costs to the animal. Within different tissues, the activity of these processes varies widely in the maintenance of cellular homeostasis and integrity, depending on the metabolic role of that tissue. For very metabolically-active tissues such as the liver, and in particular, the gastrointestinal tract, the Na^+-pump and protein turnover account for a minimum 14% of whole-body energy expenditure (Gill *et al.* 1989).

The energetic cost of protein turnover appears to be relatively stable and accounts for approximately 20% of ATP utilization on both an organ and whole-body basis. Gill *et al.* (1989) presented a very thorough study asking questions concerning the stoichiometry of protein synthesis and degradation. Because there exist several estimates of the ATP cost of protein synthesis and degradation (3–5 and 0–2 mol ATP per peptide bond synthesized or degraded, respectively) in the scientific literature, Gill *et al.* (1989), in their model of ovine energy metabolism, showed that by increasing the number of mol ATP per peptide bond synthesized from four to five or the ATP cost of protein degradation from 1 to 2 mol ATP per peptide bond degraded, the percentage of whole-body energy expenditure on protein turnover was augmented by only 4 and 3·3 percentage units respectively.

In real terms, a dichotomy exists since metabolic control leads to ensured function and thermal independence but at the cost of 'nutritional efficiency' (energy output–energy input). Growth and rate thereof increase the activity of energy-consuming processes

(during growth and in support of growth (homeostasis)). A possibility for advancing animal energetic efficiency would be to down-regulate the energetically costly events within supportive tissues, yet maintain adequate function to support growth. For example, a depression in the rate of cell turnover or reduction in Na^+-pump activity within the gastrointestinal tract with the maintenance of sufficient absorption may improve efficiency. The challenge which exists is to further reduce metabolic heat production and still maintain metabolic control within the animal.

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