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In vitro Na^+,K^+-ATPase (*EC* 3.6.1.3)-dependent respiration and protein synthesis in skeletal muscle of pigs fed at three dietary protein levels

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1. Eighteen pigs were offered diets containing 130, 170 or 210 g protein/kg with three barrows and three gilts per diet from 20 to 60 kg live weight. Oxygen consumption, Na^+, K^+-ATPase (*EC* 3.6.1.3)-dependent and -independent respiration and protein synthesis were measured in vitro in intercostal and sartorius muscle preparations from these pigs.

2. Increasing dietary protein concentration increased ($P < 0.01$) daily gain and dissectible muscle in carcass.

3. O_2 consumption and Na^+, K^+-ATPase-dependent respiration of the intercostal and sartorius muscles increased linearly ($P < 0.01$) with increase in dietary protein concentration. The requirement for the support of the transport of Na^+ and K^+ across the cell membrane in these muscles, on average, accounted for 22–25% of the O_2 consumption.

4. Synthesis rate (mg/g per d) of protein in the sartorius muscle increased ($P < 0.05$) from 3.05 to 5.07 and increased ($P < 0.1$) from 2.57 to 4.06 in the intercostal muscle as dietary protein increased from 130 to 210 g/kg diet.

5. Regression of Na^+,K^+-ATPase-dependent respiration against protein synthesis in each of intercostal and sartorius muscles showed a linear relation, an attestation of a close link between productive processes and auxiliary energy expenditure.

Although the effect of dietary protein on live weight gain, feed efficiency and body composition of pigs is well known (Luce *et al.* 1976; Tyler *et al.* 1983), there is little information on how dietary protein affects the rate of protein synthesis, muscle respiration and other cellular processes. Increased nutrient supply will involve an elevation of cellular transport processes, many of which require the expenditure of energy (MacRae & Lobley, 1986). One of these processes is Na^+,K^+-ATPase (*EC* 3.6.1.3)-dependent respiration (Milligan, 1971). The Na^+,K^+-pump participates in the maintenance of cellular ionic homeostasis involving the conversion of 1 ATP to 1 ADP for every 3 Na^+ extruded from the cell and 2 K^+ pumped into the cell (Mandel & Balaban, 1981). The support of Na^+,K^+-ATPase accounts for 20% or more of the total in vitro energy expenditure in a variety of tissues (Milligan & McBride, 1985). The activity of the Na^+,K^+-pump may be influenced by the concentration of extracellular amino acids available for transport across the plasma membrane and their eventual use in cellular metabolism. Since dietary protein concentration influences the concentration of amino acids in the plasma and tissues, Na^+,K^+-ATPase activity may be influenced and the activity may be related to the rate of protein synthesis.

There are auxiliary expenditures of energy associated with, but not necessarily directly expended in, protein synthesis (Reeds *et al.* 1985); a part of this energy is used in support of Na^+,K^+ transport. Quantification of the contribution of this energy-consuming process to energy expenditure is important in the energetics of growth. We, therefore, sought to obtain estimates of the magnitude of the energy costs of active Na^+,K^+ transport and protein synthesis in pigs offered 130, 170 and 210 g protein/kg diet from 20 to 60 kg live weight. A part of this work has been the subject of a preliminary communication (Adeola *et al.* 1987).

EXPERIMENTAL

Materials

Aquasol, a scintillation fluid, and L-[ring-2,6-^{3}H (N)] phenylalanine (44·1 Ci/mmol) were purchased from New England Nuclear Corporation, Boston, Massachusetts. Hydrochloric acid, perchloric acid and a biological oxygen monitor (Yellow Springs Instruments (YSI), Model 53) were purchased from Fisher Scientific, Whitby, Ontario. Delta 300 6890 liquid scintillation counting system was supplied by Searle Analytic Incorporated, Oakville, Ontario; high-performance liquid chromatograph and Millex-gs filter were supplied by Waters Chromatography, Toronto, Ontario and norleucine and amino acid standards by Pierce Chemical Co., Rockford, Illinois. Medium 199 with Earle's salts, 0·6 mM-L-glutamine and 25 mM-hydroxy-ethylpiperazine ethane sulphonic acid (HEPES), bovine serum albumin (BSA), sodium bicarbonate, ouabain and phenylalanine were purchased from Sigma Chemical Company, St Louis, Missouri.

Animals and diets

Eighteen Yorkshire–Landrace crossbred barrows and gilts (sex ratio, 1:1) of 20·7 (SD 1·63) kg live weight were housed in individual pens (1 m × 1·6 m) where a temperature of approximately 25° and a 12 h light (06.00 to 18.00 hours) – 12 h dark cycle were maintained. Three barrows and three gilts were randomly allocated to each of three diets (Table 1). Maize was hammer-milled (4·8-mm screen), incorporated into complete diets and pelleted (4·8 mm). The pigs were offered feed *ad lib.* with free access to water. Feed intake and live weight were monitored every week, and twice a week as the pigs approached 60 kg. The pigs were slaughtered at 60·4 (SD 6·35) kg by captive bolt pistol and exsanguinated. Sartorius muscle and a section of intercostal muscle were excised immediately and washed in Medium 199 with Earle's salts, 0·6 mM-L-glutamine 25 mM-HEPES, 20 g BSA/l and 4 mM-$NaHCO_3$ (M199). Each carcass was eviscerated, split longitudinally into two halves and chilled at approximately 4° for about 24 h after which the loin and ham from one half were physically separated into bone, muscle and fat (subcutaneous and intermuscular).

O_2 consumption and Na^+,K^+-ATPase-dependent respiration

Excised muscles were pinned onto a petri dish at resting length in M199 and gassed continuously with O_2 and CO_2 (95:5, v/v). With the aid of a dissecting microscope, duplicate muscle samples about 10 to 20 mm long and less than 0·5 mm thick were prepared, mounted on plastic grids and transferred to a YSI O_2 electrode chamber containing 4·5 ml M199. O_2 consumption rates were measured polarographically at 37° for 10 min using a YSI model 53 biological O_2 monitor after which 100 μl ouabain, a specific inhibitor of Na^+,K^+-ATPase (Glynn, 1964; Albers *et al.* 1968), was added to give a final ouabain concentration of 0·3 mM, and O_2 consumption was measured for another 10–15 min. Ouabain concentration of 1 μM or greater has been shown to give maximum inhibition of respiration (Gregg & Milligan, 1982*a*). The difference between the initial O_2 consumption and the O_2 consumption of the ouabain-treated sample was termed Na^+,K^+-ATPase-dependent respiration. Percentage inhibition was calculated using the ratio of this difference to the initial O_2 consumption.

Measurement of protein synthesis rate

Sartorius and intercostal muscles were prepared in duplicate, as described above and placed in 25 ml Erlenmeyer flasks containing 4 ml M199 with 2·8 mM-phenylalanine, 1 μCi L-[ring-2,6-^{3}H (N)]phenylalanine/ml and 4 μg insulin/ml (Fulks *et al.* 1975). The samples were incubated in a shaking water bath at 37° for 1 h. At the end of the incubation, the muscle

Table 1. *Ingredient and nutrient composition (g/kg) of diets*

Dietary protein (g/kg)...	130	170	210
Ingredients			
Maize	890	788·5	685·5
Soya-bean meal	75·5	177	280
Calcium phosphate	13	13	13
Limestone	10	10	10
Salt*	5	5	5
Vitamin–mineral premix†	6·5	6·5	6·5
Nutrients‡			
Dry matter	881	879	882
Protein	130	168	205
Ether extract	22·5	24·8	27·8
Calcium	6·8	7·6	8·4
Phosphorus	6·6	7·5	8·0
Digestible energy (MJ/kg)§	14·71	13·78	14·65
Arginine	6·4	10·7	12·8
Histidine	2·9	4·1	4·8
Isoleucine	5·4	7·3	9·4
Leucine	14·4	16·1	18·9
Lysine	4·4	7·5	10·1
Phenylalanine	6·2	8·4	9·0
Threonine	4·1	6·1	6·9
Tyrosine	6·2	7·5	8·8
Valine	8·9	12·9	10·4

* Cobalt-iodized salt with 40 mg cobalt/kg; 70 mg iodine/kg and 990 g sodium chloride/kg.

† Vitamin–mineral premix supplied the following/kg diet: 1·14 mg vitamin A, 17·5 μg vitamin D, 13·5 mg vitamin E, 2 mg menadione sodium bisulphite, 4 mg riboflavin, 20 mg niacin, 10 mg D-calcium pantothenate, 20 μg vitamin B12, 100 mg choline chloride, 60 mg manganese, 70 mg iron, 10 mg copper, 100 mg zinc, 100 μg selenium.

‡ Analysed values on as fed basis.

§ Measured in a digestion trial as described by Adeola *et al.* (1986).

preparations were blotted, frozen in liquid nitrogen and stored at −18° until subsequent analysis. Muscle samples were thawed, blotted, weighed and homogenized in cold $HClO_4$ (20 ml/l) to precipitate the protein. After centrifugation, the protein pellets were washed in methanol twice and hydrolyzed in constant boiling 6 M-HCl at 110° for 24 h. The hydrolysates were freeze-dried, reconstituted in 0·1 M-HCl containing norleucine as an internal standard and passed through 0·22 μm Millex-gs filter unit. A portion of the filtrate was analysed for phenylalanine by high-performance liquid chromatography as described by Bidlingmeyer *et al.* (1984). Radioactivity in the filtrate was measured in a Delta 300 liquid scintillation counting system with an efficiency of approximately 32% using Aquasol as the scintillation fluid. In a previous work, by collecting fractions of a muscle protein hydrolysate it was shown that the radioactivity measured was due only to phenylalanine (Early *et al.* 1988).

Fractional synthesis rate (K_s, %/d) was calculated as described by Smith *et al.* (1983):

$$K_s = (S_B/S_M)(2400/t),$$

where S_B is the specific activity of protein-bound phenylalanine (disintegrations/min (dpm) per μmol), S_M is the specific activity of phenylalanine in the blank medium (dpm/μmol) and t is the incubation time (h).

Table 2. *Performance and carcass composition of pigs offered 130, 170 or 210 g protein/kg diet*

Dietary protein (g/kg)...	130	170	210	SD
Average daily gain* (kg)	0·59	0·83	0·89	0·13
Average daily feed intake (kg)	1·81	2·13	2·04	0·45
Gain/feed* (kg/kg)	0·33	0·39	0·44	0·06
Daily protein intake* (kg)	0·245	0·338	0·417	0·036
Dissectible fat in ham and loin† (kg)	3·04	2·46	2·23	0·43
Dissectible muscle in ham and loin* ‡ (kg)	5·20	6·63	6·55	0·41
Dissectible bone in ham and loin (kg)	1·48	1·53	1·47	0·19

* Linear increase ($P < 0·01$).
† Linear decrease ($P < 0·01$).
‡ Quadratic effect ($P < 0·01$).

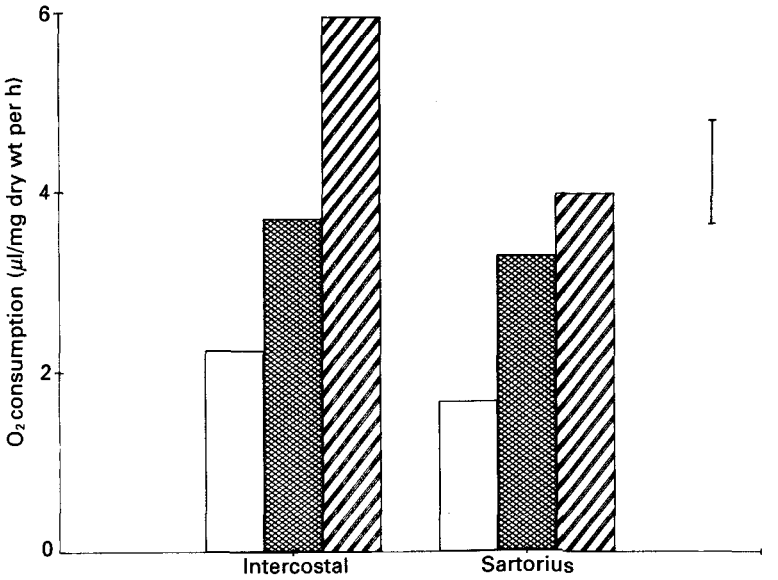

Fig. 1. Muscle oxygen consumption (μl O_2/mg dry weight per h) in intercostal and sartorius muscles of pigs offered 130 (□), 170 (⊠) or 210 (▨) g protein/kg diet. Values are means of six observations. The pooled standard deviation was 1·17 and is represented by a vertical bar.

Statistical methods

Statistical analyses were by analysis of variance procedures and regression analysis; treatment means were compared by linear and quadratic contrasts (Snedecor & Cochran, 1980).

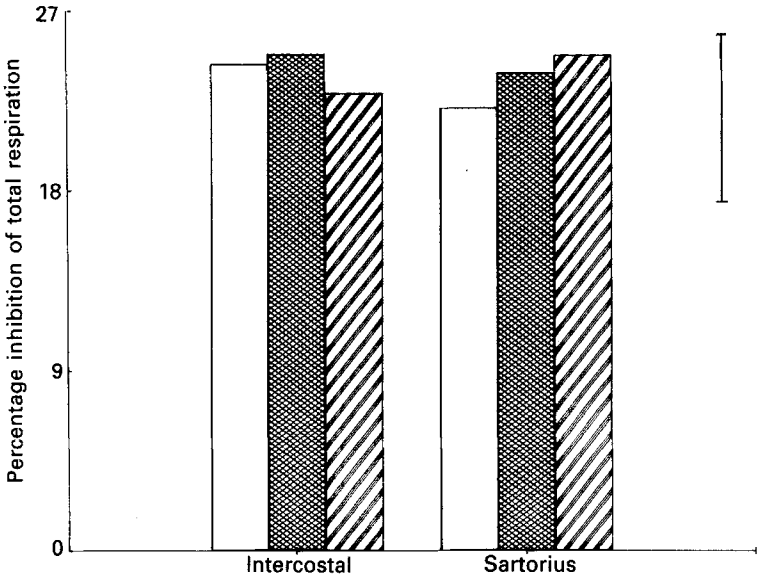

Fig. 2. Percentage inhibition of total respiration by ouabain in intercostal and sartorius muscles of pigs offered 130 (□), 170 (⊠) or 210 (▨) g protein/kg diet. Values are means of six observations. The pooled standard deviation was 6·68 and is represented by a vertical bar.

RESULTS

Body-weight, feed intake and dissectible tissues of ham and loin

The rate of change in body-weight increased linearly ($P < 0.01$) from 0·59 kg/d for pigs offered the diet containing 130 g protein/kg to 0·89 kg/d for pigs offered 210 g protein/kg (Table 2). Pigs that received the two higher protein diets tended to consume more than those that received 130 g protein/kg; the efficiency with which feed was converted into body-weight gain increased linearly ($P < 0.01$) with increasing dietary protein concentration. Subcutaneous and intermuscular fat dissected out of the ham and loin decreased ($P < 0.01$) with increasing dietary protein level, while a quadratic effect ($P < 0.01$) was observed with dissectible ham and loin muscle.

O_2 consumption and Na^+,K^+-ATPase-dependent respiration

O_2 consumption rates (μl O_2/mg per h) of intercostal and sartorius muscle preparations are shown in Fig. 1. In the intercostal muscle, there was a linear increase ($P < 0.01$) in muscle O_2 consumption as dietary protein concentration increased. The increase in total O_2 consumption of the intercostal muscle was about 64% in pigs that received 170 compared with 130 g protein/kg and 60% in pigs that were offered 210 compared with 170 g protein/kg. There also was a linear increase in total O_2 consumption rate of the sartorius muscle preparations as dietary protein concentration increased. At the highest dietary protein concentration, total O_2 consumption rate was higher ($P < 0.05$) in the intercostal muscle than the sartorius muscle.

Fig. 2 shows the proportion of respiration inhibited by ouabain. In the intercostal muscle, this ranged from 22·8% in pigs that received the 210 g protein/kg diet to 24·8% in pigs that received the 170 g protein/kg diet. In the sartorius muscle, the proportions of total O_2 consumption inhibited by ouabain were 22·1, 23·8 and 24·8% for pigs offered 130, 170 and 210 g protein/kg diet respectively.

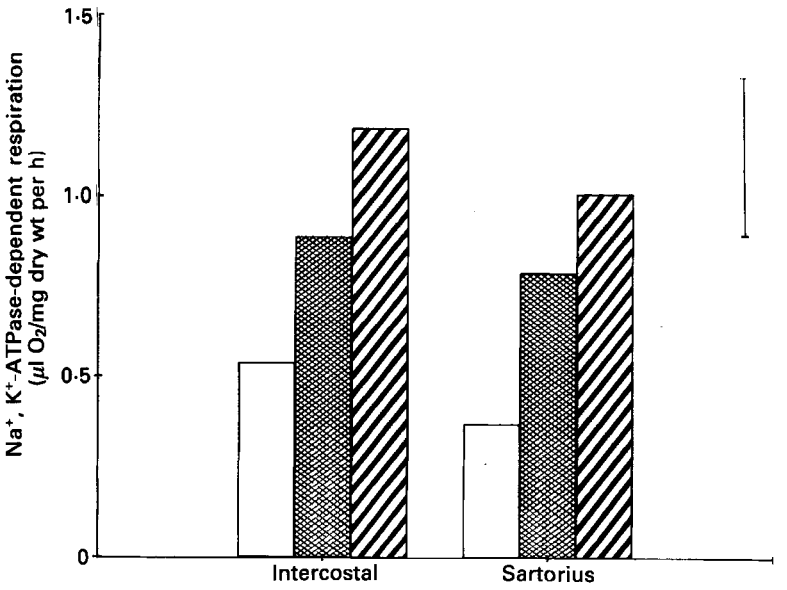

Fig. 3. Na^+,K^+-ATPase-dependent respiration (μl O_2/mg dry weight per h) in intercostal and sartorius muscles of pigs offered 130 (□), 170 (⊠) or 210 (▨) g protein/kg diet. Values are means of six observations. The pooled standard deviation was 0·44 and is represented by a vertical bar.

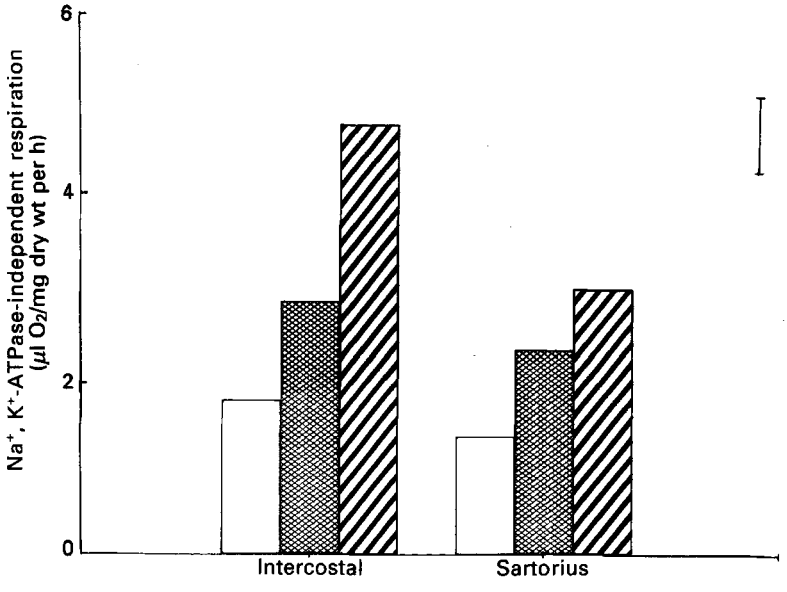

Fig. 4. Na^+,K^+-ATPase-independent respiration (μl O_2/mg dry weight per h) in intercostal and sartorius muscles of pigs offered 130 (□), 170 (⊠) or 210 (▨) g protein/kg diet. Values are means of six observations. The pooled standard deviation was 0·84 and is represented by a vertical bar.

Table 3. *Fractional and absolute protein synthesis rates in vitro*

Muscle	Dietary protein level (g/kg)	Fractional synthesis rate (%/d)	Synthesis rate*† (mg/g wet muscle per d)
Intercostal	130	1·91	2·57
	170	2·91	4·18
	210	2·71	4·06
Sartorius	130	2·20	3·05
	170	2·54	4·58
	210	2·91	5·07
SD		1·01	1·45
n		6	6

* Linear response ($P < 0·1$).
† Linear response for sartorius muscle ($P < 0·05$).

The amount of O_2 estimated to have been consumed in support of the ouabain-inhibitable portion of total O_2 consumption in intercostal and sartorius muscles was influenced ($P < 0·01$) by dietary protein concentration (Fig. 3). The Na^+,K^+-ATPase-dependent respiration of the intercostal muscle increased linearly ($P < 0·01$) by about 64% when dietary protein was increased from 130 to 170 g/diet and by about 33% when dietary protein was increased from 170 to 210 g/diet. In the sartorius muscle, the Na^+,K^+-ATPase-dependent respiration (μl O_2/mg per h) was 0·37 for pigs offered 130 g protein/kg diet; the value for pigs offered 170 g protein/kg diet was 0·79, a 113% increase; and 1·01 for pigs offered 210 g protein/kg diet, a 28% increase over pigs offered 170 g protein/kg diet.

The Na^+,K^+-ATPase-independent respiration, the residual portion of total O_2 consumption used in support of other cell functions that was unaccounted for by the Na^+,K^+-ATPase activity, also increased linearly ($P < 0·01$) in both muscles as dietary protein concentration increased (Fig. 4). The Na^+,K^+-ATPase-independent respiration (μl O_2/mg per h) of the intercostal muscle of pigs offered the lowest protein diet was 1·72 while that of the pigs offered the highest protein diet was 4·78. The corresponding values for the sartorius muscle were 1·32 and 2·96. At the highest protein level, the Na^+,K^+-ATPase-independent respiration of the intercostal muscle was greater ($P < 0·05$) than that of the sartorius muscle.

Protein synthesis rates

The fractional and absolute protein synthesis rates are presented in Table 3. Fractional synthesis rates (%/d) increased ($P < 0·01$) from 1·91 in the intercostal muscle of pigs offered the lowest protein diet to 2·81 in the intercostal muscle of pigs offered the two higher protein diets. Sartorius muscle preparation from pigs offered the 210 g protein/kg diet had a 14% increase in fractional synthesis rate over the sartorius muscle preparation from pigs given the 170 g protein/kg diet, which in turn had about a 15% increase in fractional synthesis rate over the sartorius muscle preparation from pigs offered the lowest protein diet. Protein synthesis rate (mg/g per d) was about 60% greater ($P < 0·05$) in the intercostal muscles of pigs offered the high protein diet compared with the lowest protein diet. In the sartorius muscle, the increase in protein synthesis over the pigs offered the lowest protein diet was about 66%.

DISCUSSION

As expected, increases in dietary protein concentration increased daily gain, feed efficiency and lean deposition, and decreased fat accretion. Skeletal muscle O_2 consumption (μl O_2/mg per h) increased with increasing dietary protein concentration. The increased O_2 consumption would not be unexpected since higher nutrient intake will have associated with it increased absorption, transport and metabolism of the nutrients, and perhaps also a contribution from substrate cycles, all of which increase energy expenditure (Reeds *et al.* 1985). The rates of O_2 consumption measured for porcine intercostal muscle preparations were similar to those measured with sternomandibularis muscle for sheep (Gregg & Milligan, 1982*a*) and cattle (Gregg & Milligan, 1982*b*) and porcine intercostal muscle (Herpin *et al.* 1987).

Exposing the muscle preparations to 0·3 mM-ouabain led to approximately 23% inhibition of total O_2 consumption which lies within the range of 18 to 25% reported for pigs (Herpin *et al.* 1987), sheep (McBride, 1986) and cattle (McBride *et al.* 1987). However this value appears to be considerably lower than those reported previously for skeletal muscle from rats (Asano *et al.* 1976), mice, sheep and cattle (Gregg & Milligan, 1980, 1982*a*,*b*). Values in the literature for the proportion of O_2 consumption required for the support of the Na^+,K^+-ATPase reaction varies from 3 to 42% due to either technical problems or major species differences (Reeds *et al.* 1985). Muscle bundle preparations that are trimmed lead to K^+ leakage and Na^+ influx. This could alter the activity of the Na^+,K^+-pump in an attempt to maintain intra- and extracellular ion concentrations and this may in part explain the wide variation reported in the literature. Milligan & Summers (1986) observed that the most recent measurements of liver biopsies and intercostal muscle preparations from their laboratory, incubated in a medium more complex than the previously used minimal media, indicate that the proportion of total muscle O_2 consumption required for support of Na^+,K^+ transport may be 20–25% rather than the previous higher values of greater than 35%. This more complex medium was used in the experiment reported here.

Increased nutrient supply will involve an elevation of cellular transport processes requiring the expenditure of energy, of which the most widely studied is that catalysed by Na^+,K^+-ATPase (MacRae & Lobley, 1986). The level of feed intake of animals has been shown to influence the magnitude of cellular O_2 consumption in support of Na^+ and K^+ transport (Milligan & McBride, 1985). The observed increase in Na^+,K^+-ATPase-dependent respiration in the present experiment provides evidence that dietary protein concentration has an effect on this transport process. The increase in the Na^+,K^+-ATPase-dependent component of total O_2 consumption as a result of increasing the dietary protein concentration from 130 to 210 g protein/kg diet accounted for 18% of the increased total O_2 consumption in the intercostal muscle. The corresponding value for the sartorius muscle was 28%.

The Na^+,K^+-ATPase-independent respiration, which would comprise the O_2 consumption required for oxidative phosphorylation to support all endergonic cellular processes other than Na^+ and K^+ transport, also increased with increasing dietary protein concentration. One of the components of this portion of total respiration, protein synthesis, also increased as the dietary protein concentration increased (Table 3). Protein synthesis was, thus, one of the factors responsible for the observed changes in the Na^+,K^+-ATPase-independent respiration. Webster (1980) suggested that elevated metabolic rates in animals receiving high intakes may be related to higher rates of protein synthesis. The results reported here are consistent with this notion.

The linear increase in total O_2 consumption per unit muscle weight with increasing dietary protein resulted from increases in both the Na^+,K^+-ATPase-dependent and

-independent components of respiration. The elevation of Na^+,K^+-ATPase activity appears to be an important event in both hypertrophic and hyperplastic growth (Milligan & McBride, 1985) and dietary protein has been reported to affect hypertrophic growth of muscle in pullets (Timson *et al.* 1983) and pigs (Staun, 1972). High protein meals stimulate insulin release because the resultant increase in plasma amino acids is a potent insulin secretagogue. The Na^+,K^+-pump is known to be stimulated by insulin (Moore, 1983) and many amino acids are transported by Na^+-dependent mechanisms. It is therefore possible that the increased amino acid supply resulting from giving the higher levels of dietary protein leads to an elevation of the activity of Na^+,K^+-ATPase, and a concommitant rise in protein synthesis.

The fractional synthesis rates (Table 3) are in good agreement with those reported by Nicholas *et al.* (1977) for rabbits 1·3–2·2%/d) in vitro; Lobley *et al.* (1980) for cattle (0·8–2·0%/d) as determined by constant infusion of [^{3}H]tyrosine in vivo; Gregg & Milligan (1982*b*) for cattle (0·7–1·5%/d) in vitro; and Smith *et al.* (1983) for rabbits (1·91–2·85%/d) as determined in vitro. Mulvaney *et al.* (1985) reported fractional synthesis rates of protein in porcine skeletal muscle ranging from 4·8 to 6·0%/d, which is higher than that obtained in the present study. The difference may relate to the different methods of measurement adopted and the class of pigs used in the two experiments. Mulvaney *et al.* (1985) employed a 6 h continuous infusion and used intact male pigs weighing 22 or 45 kg. The increase in fractional synthesis rate with increasing dietary protein concentration is consistent with previous reports by Smith *et al.* (1982) and Emery *et al.* (1983) for rat skeletal muscle, and Sampson *et al.* (1986) for rat mammary tissue. The effects of changes in the diet on protein synthesis are complex (Young, 1985); however, Millward *et al.* (1975) observed that synthesis varied with protein intake and that differences in synthesis best accounted for changes in growth rate.

Protein synthesis was increased in the sartorius muscle by increasing dietary protein from 170 to 210 g/kg, but in the intercostal muscle, protein synthesis was similar for these two dietary protein levels. The growth rates of sartorius and intercostal muscles relative to total carcass muscle, calculated by an allometric growth equation, are 1·15 and 1·02 respectively (Richmond & Berg, 1982). Intercostal muscle, with its lower relative growth rate, may have reached its highest rate of protein synthesis at 170 g protein/kg diet; whereas the sartorius muscle, possibly because of its higher relative growth rate, was still able to respond to dietary protein higher than 170 g/kg for support of increased protein synthesis.

The absolute synthesis rates of protein were about 58 and 66% greater in the intercostal and sartorius muscles, respectively, of pigs offered the 210 g protein/kg diet than those offered the 130 g protein/kg diet. N retention is closely related to heat production because protein synthesis and N retention are closely related (Reeds *et al.* 1985), therefore it can be deduced that O_2 consumption will be correlated to protein synthesis. The regressions of O_2 consumption (ml O_2/g per d) on protein synthesis (mg/g per d) in the intercostal and sartorius muscles were examined and are shown in Figs 5 and 6 respectively. Respiration in each muscle in the absence of protein synthesis is represented by the intercepts. The slopes stand for total O_2 consumption, by each muscle, per unit weight of protein synthesized. Also, Na^+,K^+-ATPase-dependent respiration (ml O_2/g per d) was regressed on protein synthesis in intercostal and sartorius muscles (Figs 7 and 8 respectively). The ratio of the two slopes described in Figs 5 and 7 for intercostal muscle (0·24) is the contribution to total energy expenditure of the energy expended in support of transmembrane movement of Na^+ and K^+. The same ratio from Figs 6 and 8 for the sartorius muscle is 0·25. This may be part of the auxiliary energy expenditure, unaccounted for by Reeds *et al.* (1985), which is connected but not directly associated with protein synthesis. The Na^+,K^+-ATPase-dependent respiration was correlated to protein synthesis rate (Figs 7 and 8) which supports the assumption of a close association between rate of protein synthesis and Na^+,K^+-ATPase

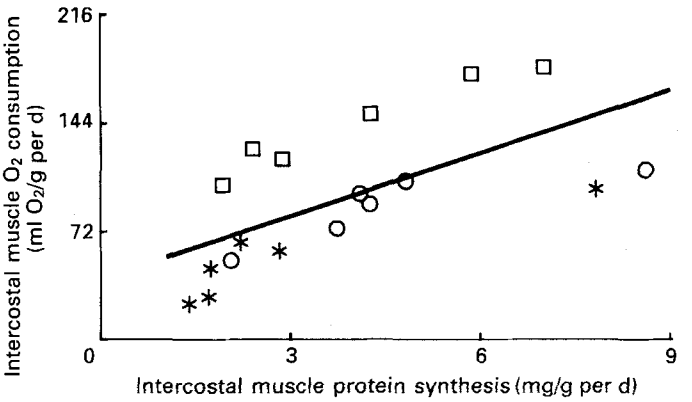

Fig. 5. Relation between intercostal muscle oxygen consumption (ml O_2/g per d) and protein synthesis (mg/g per d) in pigs offered (*) 130, (○) 170, or (□) 210 g protein/kg diet. The regression equation was:

$$O_2 \text{ consumption} = 45{\cdot}2\,(\text{SE } 17{\cdot}88) + 12{\cdot}9\,(\text{SE } 4{\cdot}04)\,\text{protein synthesis}$$

(residual SD 36·74, n 18, r 0·625).

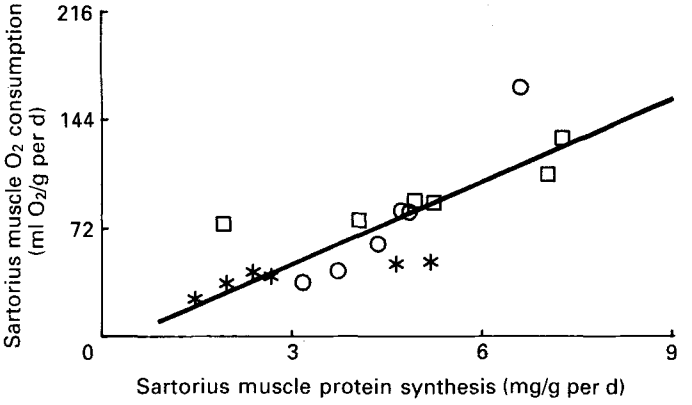

Fig. 6. Relation between sartorius muscle oxygen consumption (ml O_2/g per d) and protein synthesis (mg/g per d) in pigs offered (*) 130, (○) 170, or (□) 210 g protein/kg diet. The regression equation was:

$$O_2 \text{ consumption} = -0{\cdot}3\,(\text{SE } 14{\cdot}49) + 17\,(\text{SE } 3{\cdot}18)\,\text{protein synthesis}$$

(residual SD 22·77, n 18, r 0·801).

activity and would indicate that productive processes are closely linked with auxiliary expenditures of energy.

The difference in O_2 consumption and in Na^+,K^+-ATPase-independent respiration between intercostal and sartorius muscles is not immediately explicable. This difference may be connected with the activity and function (intercostal is respiratory and sartorius is postural) and preparation of each muscle for measurement of these variables. About 65% of the muscle fibres in the intercostal muscle are of white fibre type, whereas muscles of the proximal hind-limb do not usually contain more than 50% white type fibres. This difference could influence O_2 consumption and protein synthesis.

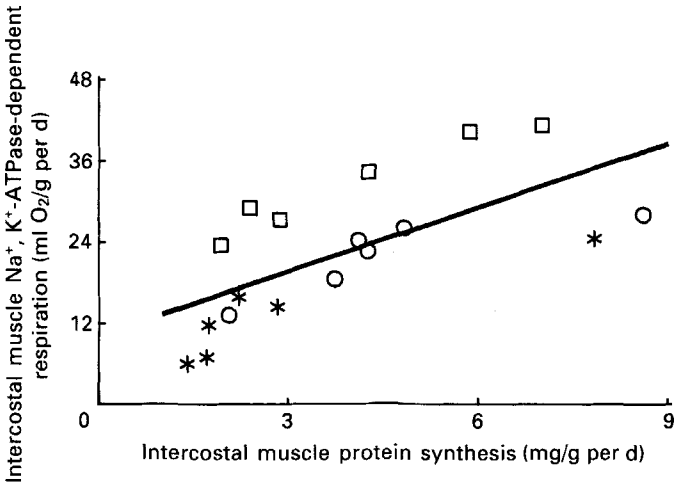

Fig. 7. Relation between intercostal muscle Na^+,K^+-ATPase-dependent respiration (ml O_2/g per d) and protein synthesis (mg/g per d) in pigs offered (*) 130, (○) 170, or (□) 210 g protein/kg diet. The regression equation was:

Na^+,K^+-ATPase-dependent respiration = 10·7 (SE 3·82) + 3·1 (SE 0·86) protein synthesis

(residual SD 7·8, *n* 18, *r* 0·666).

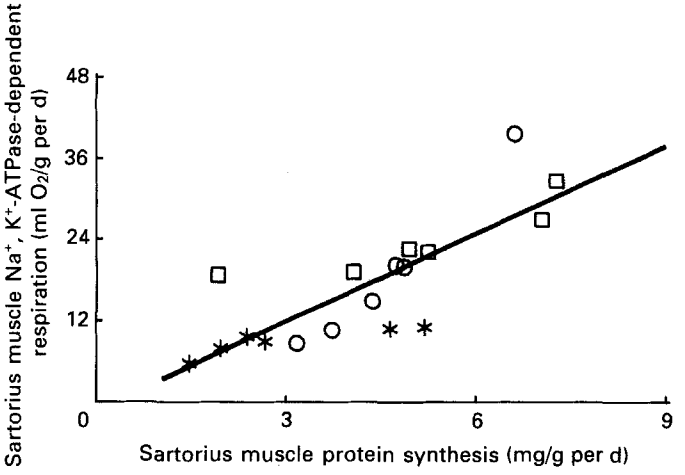

Fig. 8. Relation between sartorius muscle Na^+,K^+-ATPase-dependent respiration (ml O_2/g per d) and protein synthesis (mg/g per d) in pigs offered (*) 130, (○) 170, or (□) 210 g protein/kg diet. The regression equation was:

Na^+,K^+-ATPase-dependent respiration = −0·8 (SE 3·65) + 4·3 (SE 0·8) protein synthesis

(residual SD 5·73, *n* 18, *r* 0·799).

Because of the nature of the investigation, it was decided that the pigs should have a similar weight at the end of the experiment. This approach made it difficult to separate the effects of dietary treatment from those of chronological age. The differences in chronological age at similar body-weights resulted from the imposed dietary treatment. The available evidence however lends weight to the importance of Na^+,K^+-ATPase activity as

contributing to cellular energy expenditure in muscle. The variables in relation to dietary treatment discussed are at best approximations since they were derived from in vitro procedures. However, in vitro procedures are less costly and provide relative information that is indicative of processes in vivo.

In conclusion therefore, the requirement for the transport of Na^+ and K^+ across the plasma membrane of cells in the muscle of pigs accounts for 22–25% of the O_2 consumption of this tissue in vitro. Furthermore, O_2 consumption in support of Na^+,K^+ transport is influenced by dietary protein concentration and dietary protein concentration-induced increase in Na^+,K^+-ATPase activity is closely associated with increased protein synthesis rate.

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REFERENCES

Adeola, O., Young, L. G., McBride, B. & Ball, R. O. (1987). Na^+,K^+-ATPase-dependent respiration in skeletal muscle of pigs offered different dietary protein levels. *Canadian Federation of Biological Societies.* **30**, 132.

Adeola, O., Young, L. G., McMillan, E. G. & Moran, E. T. Jr (1986). Comparative protein and energy value of OAC Wintri triticale and corn for pigs. *Journal of Animal Science.* **63**, 1854–1861.

Albers, R. W., Kovala, G. J. & Siegel, G. J. (1968). Studies on the interaction of ouabain and other active steroids with sodium-potassium-activated adenosine triphosphatase. *Molecular Pharmacology* **4**, 324–336.

Asano, Y., Liberman, J. Q. & Edelman, I. S. (1976). Thyroid thermogenesis: relationship between Na^+-dependent respiration and Na^+ and K^+-adenosine triphosphatase in rat skeletal muscle. *Journal of Clinical Investigation* **57**, 368–379.

Bidlingmeyer, B. A., Cohen, S. A. & Tarvin, T. L. (1984). Rapid analysis of amino acids using precolumn derivatization. *Journal of Chromatography* **336**, 93–104.

Early, R. J., McBride, B. W. & Ball, R. O. (1988). Phenylalanine metabolism in sheep infused with glucose plus insulin. II. Effects on in vivo and in vitro protein synthesis and related energy expenditures. *Canadian Journal of Animal Science* **68**, 721–730

Emery, P. W., Rothwell, N. J. & Stock, M. J. (1983). Protein synthesis in liver, skeletal muscle and brown adipose tissue of rats fed protein deficient diet. *Bioscience Reports* **3**, 569–575.

Fulks, R. M., Li, J. B. & Goldberg, A. L. (1975). Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. *Journal of Biological Chemistry* **250**, 290–298.

Glynn, I. M. (1964). The action of cardiac glycosides on ion movements. *Pharmacological Reviews* **16**, 381–408.

Gregg, V. A. & Milligan, L. P. (1980). Inhibition by ouabain of the oxygen consumption of mouse soleus and diaphragm muscles. *General Pharmacology* **11**, 323–325.

Gregg, V. A. & Milligan, L. P. (1982*a*). Role of Na^+,K^+-ATPase in muscular energy expenditure of warm- and cold-exposed sheep. *Canadian Journal of Animal Science* **62**, 123–132.

Gregg, V. A. & Milligan, L. P,. (1982*b*). In vitro energy costs of Na^+,K^+-ATPase activity and protein synthesis in muscles from calves differing in age and breed. *British Journal of Nutrition* **48**, 65–71.

Herpin, P. R., McBride, B. W. & Bayley, H. S. (1987). Effect of cold exposure on energy metabolism in the young pig. *Canadian Journal of Physiology and Pharmacology* **65**, 236–245.

Lobley, G. E., Milne, V., Lovie, J. M., Reeds, P. J. & Pennie, K. (1980). Whole body and tissue protein synthesis in cattle. *British Journal of Nutrition* **43**, 491–501.

Luce, W. G., Johnson, R. K. & Walters, L. E. (1976). Effects of levels of crude protein on performance of growing boars. *Journal of Animal Science* **42**, 1207–1210.

McBride, B. W. (1986). Cellular energy expenditure on Na^+,K^+ transport and protein synthesis in hyperthyroid sheep. *Journal of Dairy Science.* **69**, Suppl. 1, P 309.

McBride, B. W., Burton, J. H. & Macleod, G. K. (1987). Skeletal muscle energy expenditure associated with Na^+,K^+ transport and protein synthesis in somatotropin-treated lactating cows. *Journal of Dairy Science* **70**, Suppl. 1, P215.

MacRae, J. C. & Lobley, G. E. (1986). Interactions between energy and protein. In *Control of Digestion and Metabolism in Ruminants*, pp. 367–385 [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Englewood Cliffs, NJ: Prentice Hall.

Mandel, L. T. & Balaban, R. S. (1981). Stochiometry and coupling of active transport to oxidative metabolism in epithelial tissues. *American Journal of Physiology* **240**, F357–F371.
Milligan, L. P. (1971). Energetic efficiency and metabolic transformations. *Federation Proceedings* **30**, 1454–1458.
Milligan, L. P. & McBride, B. W. (1985). Energy costs of ion pumping by animal tissues. *Journal of Nutrition* **114**, 1374–1382.
Milligan, L. P. & Summers, M. (1986). The biological basis of maintenance and its relevance to assessing response to nutrients. *Proceedings of the Nutrition Society* **45**, 185–193.
Millward, D. J., Garlick, P. J., Stewart, R. J. C., Nnanyelugo, D. O. & Waterlow, J. C. (1975). Skeletal muscle growth and protein turnover. *Biochemical Journal* **150**, 235–243.
Moore, R. D. (1983). Effects of insulin upon ion transport. *Biochimica et Biophysica Acta* **737**, 1–49.
Mulvaney, D. R., Merkel, R. A. & Bergen, W. G. (1985). Skeletal muscle protein turnover in male young pigs. *Journal of Nutrition* **115**, 1057–1064.
Nicholas, G. A., Lobley, G. E. & Harris, C. I. (1977). Use of constant infusion technique for measuring rates of protein synthesis in the New Zealand White rabbits. *British Journal of Nutrition* **38**, 1–17.
Reeds, P. J., Fuller, M. F. & Nicholson, B. A. (1985). Metabolic basis of energy expenditure with particular reference to protein. In *Substrate and Energy Metabolism in Man*, pp. 46–57 [J. S. Garrow and W. Halliday, editors]. London: John Libbey & Co.
Richmond, R. J. & Berg, R. T. (1982). Relative growth patterns of individual muscles in the pig. *Canadian Journal of Animal Science* **62**, 575–586.
Sampson, D. A., Hunsaker, H. A. & Jansen, G. R. (1986). Dietary protein quality, protein quantity and food intake: effects on lactation and on protein synthesis and tissue composition in mammary tissue and liver in rats. *Journal of Nutrition* **116**, 365–375.
Smith, C. K., Durschlag, R. P. & Layman, D. K. (1982). Response of skeletal muscle protein synthesis and breakdown to levels of dietary protein and fat during growth in weanling rats. *Journal of Nutrition* **112**, 255–262.
Smith, R. H., Palmer, R. M. & Reeds, P. J. (1983). Protein synthesis in isolated rabbit forelimb muscles. *Biochemical Journal* **213**, 153–161.
Snedecor, G. W. & Cochran, W. G. (1980). *Statistical Methods*. Ames: Iowa State University Press.
Staun, H. (1972). The nutritional and genetic influence on number and size of muscle fibers and their response to carcass quality in pigs. *World Review of Animal Production* **8**(3), 18–26.
Timson, B. R., Chi, M. S. & Bowlin, B. K. (1983). The effect of reduced dietary protein on the anterior latissimus dorsi muscle fibers in the single comb white leghorn pullet. *Poultry Science* **62**, 2230–2233.
Tyler, R. W., Luce, W. G., Johnson, R. K., Maxwell, C. V., Hintz, R. L. & Walters, L. E. (1983). The effects of level of crude protein on performance of growing boars. *Journal of Animal Science* **57**, 364–372.
Webster, A. J. F. (1980). Energy cost of digestion and metabolism in the gut. In *Digestive Physiology and Metabolism in Ruminants*, pp. 469–484 [Y. Ruckebusch and P. Thivend, editors]. Lancaster: MTP Press.
Young, V. R. (1985). Muscle protein accretion. *Journal of Animal Science* **61**, Suppl. 2, 39–56.

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