Muscle function and nutrition

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Wasting of muscle and a negative nitrogen balance are obvious effects of malnutrition and have led to the use of anthropometric measurements and nitrogen balance for assessing the extent of malnutrition. A positive nitrogen balance and an increase in limb muscle circumference are believed to be indices of the beneficial effects of nutritional support. In experiments with growing rats and young children nitrogen retention and growth are recognised to be the desirable effects of optimal nutritional intake. This concept has been applied to malnourished adult humans (non-growing) who have been considered to be potentially able to "regrow" the lost tissue.

While it is true that patients receiving long term (more than six months) home total parenteral nutrition gain body weight and nitrogen over many months and years of observation, this process is not seen during shorter (less than 40 days) nutritional intervention given in hospital.¹ Despite adequate intakes of nitrogen and calories little or no increase in total body nitrogen is seen in a variety of patients receiving total parenteral nutrition in hospital over several weeks.²⁻⁷ Despite the absent or very modest gain in nitrogen nutritional support does seem to improve outcome by reducing complications and mortality after a period of support so short that body composition is hardly changed. Young *et al* showed that although amino acids and amino acids plus calories both resulted in equivalent sparing of body nitrogen, amino acids plus calories was associated with quicker wound healing and fewer complications.³

Thus the outcome and body composition data suggest that the reversal of the adverse effects of malnutrition is not based on improvement of the traditional variables of nutrition, such as gain in body nitrogen, or a demonstrable increase in muscle mass or plasma proteins.⁸ This discrepancy is further supported by the observation that global clinical assessment is at least equivalent to, and in some respects better than, individual objective traditional measurements of nutritional state in predicting outcome.⁹

On the basis of the foregoing evidence, there are grounds for suspecting that functional abnormalities in adults may not be the result of simple loss of lean tissue and may recover before such lean tissue is regained.

One of the major organ systems of the human body is the musculoskeletal system, and it is therefore important to determine the effect of malnutrition on the musculoskeletal system. Previous studies of muscle function have been largely related to the examination of fatigue, myopathy, and endocrine-metabolic abnormalities.¹⁰⁻¹¹ This review will discuss the effect of nutrition on skeletal muscle function in relation to the effects of feeding and fasting in normal subjects, in patients with critical illness, and also in a rat model of malnutrition.

Techniques for investigating the effect of nutrition on muscle function

MUSCLE FUNCTION TESTS

The contraction-relaxation characteristics and endurance properties of the adductor pollicis muscle in man and the gastrocnemius muscle in rats have been studied.¹²⁻¹³

Human studies

Supramaximal ulnar nerve stimulation was performed with square wave pulses for 60-70 microseconds (and surface EMG recordings made), at frequencies increasing from 10 Hz to 100 Hz, for one to two seconds at a time, and the force of contraction recorded. Then the maximal rate of muscle relaxation was noted after stimulation at 30 Hz for two to three seconds. Finally, the adductor pollicis muscle was stimulated continuously at 20 Hz for 30 seconds, and the degree of fatigue, judged by a fall in force of contraction with time, was noted over this period. Recently, the clinical protocol has been shortened to a sequence of 10, 20, and 50 Hz stimuli only, and the relaxation rate is observed during a one to two seconds 20 Hz stimulation. The $F_{10}:F_{20}$ and $F_{10}:F_{50}$ ratios were found to give the same sorts of results as the $F_{10}:F_{100}$ ratio.

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Animal studies

Animals anaesthetised with barbiturates had the gastrocnemius and soleus muscles freed, keeping their blood supply intact and isolating their common nerve supply, the sciatic nerve. The body and hind limbs of the animal were immersed in modified Liley's solution kept at 37°C. The same measurements were made as those made in the human studies but at frequencies from 0.5 to 200 Hz. The effects of stimulating the sciatic nerve on the contraction characteristics of the gastrocnemius and soleus muscles were noted.

MUSCLE BIOPSY STUDIES

Human studies

Muscle biopsy specimens were obtained from the gastrocnemius in morbidly obese subjects, firstly from those on a weight maintaining diet and then after two weeks from those on a 400 kcal/day diet.¹⁴ These were taken immediately after the muscle function tests reported here had been performed.

Animal studies

The biopsy specimens were taken from the contralateral gastrocnemius at the time of the muscle function tests.

BIOPSY MEASUREMENTS

Biopsy specimens from animals and humans were analysed for total water content; total sodium, potassium, chloride, calcium, phosphate and magnesium content (and the intracellular concentrations of these chemicals were also calculated); activities of phosphofructokinase (PFK), succinate dehydrogenase (SDH), and β hydroxyacyl Co-A dehydrogenase (ACDH); concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate (CP); and pyruvate and lactate values. Histochemical treatment for myosin ATPase and sodium and potassium ATPase and electron microscopic examination were also performed to look for changes in fibre type. Details of these studies have been published elsewhere.^{13 14}

The muscle electrolytes were measured using a modification of the methods described by Graham *et al*¹⁵ for human muscle. Muscle chloride content was determined by a modification of the method of Schales and Schales.¹⁶ The sodium, potassium, calcium and magnesium contents of the muscle biopsy specimen were determined by atomic absorption spectrophotometry. Muscle phosphate was determined by a colorimetric method.¹⁷

The determination of extracellular and intracellular water was based on the chloride method.¹⁸ Chloride is freely diffusible across the skeletal muscle fibre membrane at rest and is distributed

according to the Nernst equation.^{19 20} Assuming a constant membrane potential of 85 mV, the $Cl_E:Cl_I$ ratio calculated from the Nernst equation is 24:1. If the total water and chloride of the muscle tissue and the extracellular concentration of the chloride (obtained by correcting the plasma chloride concentration for a Donnan factor and a factor for plasma water²¹) are known extracellular and intracellular electrolyte concentrations can be calculated.

As the validity of this calculation depends on the assumption that the muscle membrane potential was 85 mV, we also measured the membrane potential directly using intracellular electrodes in control rats, those fasted for five days, and those fed a hypocaloric diet for 21 days. The measurements were made in the muscles of living anaesthetised rats, as described by Charlton *et al*²² in 10–20 muscle fibres in the first and second layers of the muscle, using a microelectrode filled with potassium chloride of 5–10 megohms resistance and low (<5 mV) tip potential. The exposed muscles were immersed in modified Liley's solution at 37°C during measurement. The results showed that hypocaloric feeding resulted in only minimal changes to the membrane potential, such that there was no appreciable effect on the calculated value of intracellular electrolytes. The details of this process have been published elsewhere.¹³

OBJECTIVE MEASUREMENTS OF NUTRITIONAL STATE

Nutritional state is a term used to denote lean body mass, as determined by anthropometric measurements, determination of serum protein (albumin and transferrin), measurement of total body nitrogen, and total body potassium, creatinine-height index, and observations of delayed cutaneous hypersensitivity (DCH) to recall antigens.

Studies of muscle function in models of pure malnutrition in man

DEFINITION OF MALNUTRITION

Intake of a diet sufficient to meet or exceed individual needs will keep the composition and function of otherwise healthy subjects within the normal range. This equilibrium is disturbed by three processes: decreased intake; increased requirements; and changed use, all of which prevent nutrients from being used for tissue repair. When the above mentioned states of disequilibrium occur then loss of body tissue ensues. Not all body constituents, however, are lost at the same rate. Proteins are the least dispensable and most important of the body components; hence the adverse effects of insufficient food intake are reduced by compensatory mechanisms that protect body pro-

teins at the expense of body fat. Even though starvation or undernutrition result in a loss of both fat and protein, the loss of protein is minimised by reducing the need to use it as a source of energy.²³ This is effected by mobilising fat and enhancing fat oxidation as the principal source of energy. Unfortunately, protein wasting continues and rapidly accelerates after fat stores have been consumed.

Losses of body fat and protein result in a fall in body weight, reduced fat fold thickness, and reduced muscle bulk. Reduced muscle bulk will be seen as thin arms and legs and a reduced excretion of creatinine in the urine. Weight loss may be masked by concomitant retention of fluid.^{24 25} Lack of nutrients will ultimately reduce weight, skin fold thickness, and arm muscle circumference, all of which have been advocated for assessing the nutritional state – and that goes for everybody.

Unfortunately, body wasting occurs as the result of several factors not related to the nutritional disequilibrium described above – for example, inactivity will reduce muscle protein synthesis and cause wasting.²⁶ Trauma and cancer likewise induce wasting and loss of lean tissue. Thus malnutrition defined on the basis of lean body mass or factors based on it (such as body potassium and nitrogen), will not distinguish those instances caused by a true lack of nutrients from those caused by other forms of injury and illness. Furthermore, such a definition does not make allowance for body and especially muscle function being reduced independently of wasting and change in body composition.

Because of these factors, we have defined muscle malnutrition as the presence of abnormalities that are seen when nutrients are withdrawn and which are correctable by refeeding. Such changes occur independently of body composition. More importantly, after hypocaloric feeding the ultrastructure of muscle has been observed to change, showing fibre atrophy at a time when total body composition was still normal.^{14 27}

In clinical terms such a definition is relevant because it alerts the clinician to an abnormality that can be positively and acutely influenced by refeeding rather than being seen as an untreatable one.

EFFECT OF FASTING AND REFEEDING OR REDUCED NUTRIENT INTAKE ON MUSCLE FUNCTION

Fasting in the morbidly obese

The object of this study was to observe the effect of pure fasting in obese but otherwise normal subjects. In six morbidly obese subjects²⁷ muscle function was measured before and after two weeks of a 400 kcal/day diet, and again after an additional two weeks of fasting. After two weeks of refeeding these measurements were repeated.

Refeeding patients with severe malnutrition caused by anorexia nervosa

Six severely depleted patients with primary anorexia nervosa, with a mean loss of 23% of body weight, were studied before and after four and eight weeks of refeeding.²⁸ Muscle function tests and objective measurements of nutritional state were performed at these intervals.

MUSCLE FUNCTION TESTS

There were important changes in three variables of muscle function in malnourished humans and animals compared with those of normal controls (Fig. 1). In both humans and animals hypocaloric dieting and fasting and the malnutrition associated with anorexia nervosa had a uniform effect, irrespective of species or clinical setting.

Change in force-frequency curve

In normal humans and unstarved obese patients there was a rise in the force of muscle contraction as the frequency of stimulation increased from 10 Hz to 100 Hz. The maximum force was attained at 50 Hz. Expressed as a percentage of this maximum attained, the force developed at 10 Hz was 29% (Fig. 1a).

Hypocalorically fed and fasted humans and untreated patients with anorexia had a decrease in the increment of the force at higher stimulation frequencies, with maintenance of force at lower frequencies. Thus the ratio or percentage of the latter, in relation to the maximum attainable (which was less), increased in these instances. The force at 10 Hz was about 48% of the maximum in these malnourished states. This value was significantly higher than that of controls (Fig. 1a).

Change in muscle relaxation rate

In normal human subjects the muscle relaxation rate at 30 Hz was 9.6% force loss/10 milliseconds.

The relaxation rate was significantly slower with fasting (7.6% force loss/10 milliseconds) and in untreated anorectic patients (6.6% force loss/10 milliseconds).

Fatigue

In controls the muscle scarcely lost power when stimulated continuously for 30 seconds (3.5% force loss/30 seconds).

In contrast, there was a significant increase in muscle fatiguability after fasting (13.7% force loss/30 seconds) and in the untreated anorectic patient (18.6% force loss/30 seconds).

Refeeding the starved obese patient and the anorectic resulted in the disappearance of muscle function abnormalities within two and four weeks, respectively.^{14 28}

Fig. 1 Contraction characteristics of human adductor pollicis and rat gastrocnemius muscles under conditions of nutritional deprivation. Open bars=reported normals. Wide diagonal hatched=obese patients after two weeks of fasting. Shaded=anorectic patients at baseline. Close diagonal hatched=rats after 21 days of hypocaloric feeding. MRR=maximal relaxation rate. * $p<0.05$, ** $p<0.025$, *** $p<0.01$, **** $p<0.001$. (After JPEN 1985; 9: 415–21.)

CORRELATION OF OBJECTIVE MEASUREMENTS OF BODY COMPOSITION WITH MUSCLE FUNCTION

The fasted obese human did not lose significant amounts of total body potassium or total body nitrogen, nor was there a significant change in creatinine-height index at a time when abnormalities of muscle function were obvious (Table 1). Con-

versely, during refeeding these subjects rapidly lost muscle fatigability and regained normal contraction-relaxation characteristics at a time when there was no significant increase in lean body components or in body weight.

In the anorectic patient gross muscle fatigue and abnormal function were present when first seen. At

Table 1 Changes in standard variables of nutritional assessment during period of abnormal muscle function

	Total body nitrogen (kg)	Total body potassium (g)	Creatinine-height index (%)	Albumin (g/dl)	Total iron binding capacity (μg/dl)
Obesity study:					
Normal (predicted ⁷¹)	1.52 (0.05)	96 (14)	100	3.5–5.0	250–400
Baseline	1.56 (0.02)	129 (8)	135 (7)	3.9 (0.2)	268 (21)
	(n=4)	(n=4)	(n=6)	(n=6)	(n=6)
Fasted	1.38 (0.05)	111 (11)	115 (8)	3.9 (0.1)	245 (8)
	[−11.5%]	[−14.0%]	[−14.8]		
Anorexia study:					
Predicted ⁷¹	1.70 (0.08)				
	(n=5)				
Baseline	1.21 (0.11)	61 (7)	49.7 (7.4)	4.0 (0.1)	283 (49)
	(n=5)	(n=5)	(n=5)	(n=6)	(n=6)
Four weeks refeed	1.33 (0.10)*	73 (8)*	64.9 (3.4)	4.4 (0.3)	333 (63)
	[+9.9%]	[+19.7%]	[+30.6]		
Eight weeks refeed	1.37 (0.10)*	81 (7)*	67.0 (7.7)	4.4 (0.3)	384 (76)
	[+13.2%]	[+32.8%]	[+34.7]		

* $p<0.05$ compared with baseline value. None of the other changes are significant. (From JPEN 1985; 9: 415–21.)

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that time these patients had normal plasma proteins. Initially they had pronounced loss of lean body mass and of total body nitrogen and potassium. When refed, muscle fatigue disappeared within four weeks, and all functional abnormalities were restored by eight weeks of refeeding, at which time the creatinine-height index was still very low at 67% and total body nitrogen had risen by only 13%. Interestingly, the total body potassium had risen at four weeks by only 19.6% and at eight weeks by 32.7% in patients who, based on their creatinine-height indices, had lost 50% of their muscle mass. Thus the total body potassium was still well below the normal expected for their height (Table 1). Body fat, which is regarded as "average" for a woman at 24%,²⁹ rose from 14.0 (SE 0.5%) at baseline to 17.1 (SE 1.0%) at eight weeks.

Despite an incomplete return to normal body composition clinically these patients had restored their ability to exercise with normal muscle function.

Studies of muscle function in models of malnutrition in the rat

To determine whether these abnormalities could be reproduced in animals by hypocaloric feeding and reversed by refeeding a rat model was studied. The effects of short and long term changes were also measured, and the muscle under study was biopsied.

Muscle function tests and muscle biopsies were performed in rats initially weighing about 250 g (eight weeks old). They were studied as follows:

- (i) Nine control rats eating purina laboratory chow unrestrainedly, eight rats fasted for two days, and five rats fasted for five days.
- (ii) Six control rats eating purina laboratory chow unrestrainedly and six hypocalorically fed animals given only 25% of the food eaten by its pair fed control.

MUSCLE FUNCTION TESTS

There were significant changes in three variables of muscle function in experimental animals compared with those of normal controls (Fig. 1). Hypocaloric dieting and fasting had the same effect as in the human.

Change in force-frequency curve

In normal rats there was a rise in the force of muscle contraction as the frequency of stimulation increased from 10 Hz to 100 Hz. The maximum force was attained at 100 Hz. Expressed as a percentage of this maximum attained, the force developed at 10 Hz was 29% (Fig. 1a).

Hypocaloric dieting and fasting in rats resulted in a decrease in the increment of force being exerted at

higher stimulation frequencies, with maintenance of force at lower stimulation frequencies. Thus the ratio or percentage of the latter, in relation to the maximum attainable, which was less, increased in these instances. In malnourished rats it was 40% of the maximum and was significantly higher than that of controls (Fig. 1a).

Change in muscle relaxation rate

In normal rats the muscle relaxation rate at 100 Hz was 36.3% force loss/10 milliseconds (Fig. 1b).

In rats five days of fasting slowed the relaxation to 19.3% force loss/10 milliseconds and 21 days of hypocaloric dieting to 10.8% force loss/10 milliseconds (Fig. 1b).

Fatigue

When stimulated continuously for 30 seconds the control rat lost 20.8% force/five seconds (Fig. 1c).

Fasted rats (five days) and hypocalorically fed animals showed double the loss of force on sustained stimulus (45% force loss/five seconds) (Fig. 1c).

EFFECT OF REFEEDING ON MUSCLE FUNCTION OF THE RAT

Rats weighing 250 g were allocated to three groups, one control and two pair, fed 25% of the control intake. After three weeks the control and one hypocaloric group were studied, as described above. The third hypocaloric group was refed freely for two weeks and studied. As indicated above the force at 10 Hz expressed as a percentage of that at 100 Hz was higher in the hypocaloric group and the relaxation rate slower. On refeeding, these changes were reversed and the refed group was not different from the control group.³⁰

CHANGES IN THE MUSCLE BIOPSY ON FASTING OR HYPOCALORIC FEEDING

Fibre type

In obese patients fasting resulted in type II fibre atrophy. In animals two to five days' fasting resulted in an increase of slow twitch oxidative fibres (type I), but prolonged hypocaloric dieting resulted in the appearance of fibres depleted in both myosin and sodium and potassium ATPase, a fibre type not normally seen (Atwood, Russell, and Jeejeebhoy, unpublished data). Despite these very different fibre type patterns the muscle function abnormalities and fatigability were similar to those seen in nutritional deprivation. In human studies specific Z band degeneration was also noted, with preservation of A and I bands (Fig. 2).

Electrolyte abnormalities

The most striking finding during hypocaloric dieting

Fig. 2 Biopsy specimen of human gastrocnemius muscle after two weeks of hypocaloric dieting showing atrophic fibres with degeneration of Z bands but preservation of I and especially A bands. (From *Am J Clin Nutr* 1984; 39: 503-15.)

and fasting in both humans and rats was an increase in the total water content, mainly extracellular, and an increase in the intracellular concentration of muscle calcium in both human and rat studies (Fig. 3). In contrast, total potassium, magnesium, chloride and phosphate concentrations remained normal.

Metabolite abnormalities

In both rats and humans the phosphocreatine:ATP ratio fell while the ATP value remained normal. Muscle lactate rose in hypocalorically fed rats. As muscle pH is closely linked to lactate activity³¹ a fall in pH is likely. The formula of Sahlin *et al*³¹ predicted that the pH had fallen from 7.19 to 6.97. Supporting our findings are studies by Jacobs *et al* (Fig. 4).³²

Muscle enzyme content

PFK fell with short term fasting while SDH and

Fig. 3 Concentrations of intracellular calcium in human and rat gastrocnemii after nutritional deprivation. * $p < 0.05$, ** $p < 0.001$. (From *JPEN* 1985; 9: 415-21.)

ACDH remained normal or rose, suggesting a change to more oxidative fibres with early starvation. Prolonged hypocaloric feeding, however, was followed by a reduction in PFK and SDH in humans and rats, and ACDH in the rat (Fig. 5).

Effect of non-nutritional factors on muscle function

AGE AND SEX

There is a weak ($r^2 = 0.09$) but significant ($p < 0.001$) correlation between age and the ratio of the force at 10 Hz with that at 50 Hz ($F_{10}:F_{50}$). The maximal relaxation rate did not correlate with age.³³

EFFECT OF RENAL FAILURE, CHRONIC OBSTRUCTIVE LUNG DISEASE, STEROID ADMINISTRATION AND MAJOR SURGICAL PROCEDURES

Neither renal failure per se, nor peritoneal dialysis, nor haemodialysis changed the $F_{10}:F_{50}$ ratio or the maximal relaxation rate.³⁴ The same was true of chronic obstructive lung disease.³⁵ Steroid administration for Crohn's disease in doses of 20 mg/day for at least three weeks also did not change the $F_{10}:F_{50}$ ratio or the maximal relaxation rate from the control

Fig. 4 Ratios of ATP:ADP and lactate:pyruvate in rat model of nutritional deprivation. * $p<0.05$, ** $p<0.01$. (From JPEN 1985; 9: 415–21.)

Fig. 5 Enzyme concentrations of human and rat gastrocnemius muscles after hypocaloric diets. * $p<0.05$, ** $p<0.02$, *** $p<0.001$. (From JPEN 1985; 9: 415–21.)

value.³³ Patients studied in the recovery room immediately after a laparotomy or aortic surgery also had normal $F_{10}:F_{50}$, $F_{10}:F_{20}$ ratios and maximal relaxation rate.³³

EFFECT OF MAJOR TRAUMA WITHOUT SEPSIS

Major trauma was associated with a slight fall in maximal relaxation rate when studied in the emergency room. Next day and a week later the $F_{10}:F_{50}$ and $F_{10}:F_{20}$ ratios and maximal relaxation rate were normal despite a severely negative nitrogen balance not compensated for by an increased nutrient intake or special support.³³

EFFECT OF SEPSIS

Severe sepsis in patients who were eating well before the episode or those receiving parenteral nutrition when studied (Stoner index $>10^{36}$) was associated with a noticeable but slight rise in $F_{10}:F_{50}$ ratio but a normal maximal relaxation rate. The changes were considerably less than those seen in patients with a caloric intake less than 90% of their measured resting metabolic rate. These subjects also had an appreciably slower maximal relaxation rate.³³

EFFECT OF REDUCED CALORIE INTAKE AND NUTRITIONAL SUPPORT IN PATIENTS WITH SEPSIS AND GASTROINTESTINAL DISEASE

Protocol of study

Patients admitted to a general surgical service were studied before and sequentially at weekly intervals during their treatment with parenteral nutrition. They were assessed for sepsis score,³⁶ muscle function, dietary and parenteral nutrient intake, metabolic rate by indirect calorimetry, and objective nutritional assessment, as defined above.

SENSITIVITY AND SPECIFICITY OF MUSCLE FUNCTION IN DETECTING REDUCED CALORIE INTAKE

Patients, irrespective of their clinical state, were divided at the time of admission into those not malabsorbing and taking calories $\geq 90\%$ of their resting metabolic rate, and those with less than that intake and with malabsorption. A receiver operator characteristic (ROC) curve was drawn to determine the best sensitivity and specificity of the $F_{10}:F_{50}$ ratio, $F_{10}:F_{20}$ ratio, maximal relaxation rate, and maximal force at 50 Hz in separating these two states of nutrition. The $F_{10}:F_{50}$ separated these two states at a ratio of 0.4 (F_{10} above 40% of F_{50}) with a sensitivity of 0.83 and a specificity of 0.98. The maximal relaxation rate likewise separated these groups at a value of 10% of maximum force lost/10 milliseconds with a sensitivity of 0.80 and a specificity of 0.93%. During this study several healthy women fed

hypocaloric diets were also studied. They had been on a self administered diet, which was not accurately adhered to for variable periods of time. When controls were added to the patient data the sensitivity and specificity remained unchanged, but when data from such dieters were added the sensitivity fell to 0.6 for both the $F_{10}:F_{50}$ ratio and maximal relaxation rate without affecting specificity. In short, periodic dieting does not affect muscle function to the same degree as poor intake with malabsorption. Even under these circumstances, however, the specificity remains high.³³

EFFECT OF PARENTERAL NUTRITION

Parenteral nutrition appreciably reduced the $F_{10}:F_{50}$ and $F_{10}:F_{20}$ ratios and increased maximal relaxation rate within two to three weeks. There was also an appreciable rise in the force of muscular contraction with nutritional support without an increase in the arm muscle circumference, triceps skin fold thickness, or the serum albumin concentration. Thus nutrition increased the force of contraction without increasing the muscle mass or albumin concentrations.

There was a linear fall in $F_{10}:F_{50}$ ratio and a rise in maximal relaxation rate and force of contraction with increased duration of parenteral nutrition. Thus there seems to be a progressive effect of nutritional support with time.

CHANGES IN BODY COMPOSITION AND MUSCLE FUNCTION BY REDUCING NUTRIENT INTAKE

These studies indicate that there is a lack of relation between changes in total body nitrogen or potassium (measures of lean body mass) and muscle function. It was possible to improve muscle force and other variables in patients without affecting muscle mass, fat stores, and albumin concentration. It is clear that the loss or restoration of lean body mass is not essential for the occurrence of corresponding changes in muscle contraction-relaxation characteristics, force, and endurance properties. Our findings are consistent with the somewhat similar data concerning morbidity and mortality mentioned earlier. Thus there is a need for questioning whether the proof of malnutrition should depend on changes in lean body mass, and conversely, whether the restoration of lean body mass and improved nitrogen balance constitute the gold standards for good nutritional support. Our findings indicate that the failure to restore body nitrogen, observed in earlier studies, does not negate the fact that such support may have restored function.

The findings of the changes in the muscle biopsy specimens in patients and animals suggest that skeletal muscle function may be changed because of

factors affecting the energy state of the muscle, which affects both the contraction-relaxation characteristics of muscle and inhibits calcium efflux across the sarcolemma. Calcium efflux may promote muscle proteolysis and injury.

Clinical importance of changed muscle function

FATIGUE AT 30 HZ STIMULATION (HUMAN) AND 100 HZ (ANIMALS)

There is an inability to sustain tetanic force when compared with the control for 30 seconds (human) and five seconds (animal) at a tension which is close to 86% of the maximum in the human¹⁴ and 100% in animals.¹³ This fatigue in the human studies is at low frequency stimulation and has been shown, in similar studies with the adductor pollicis by Bigland-Ritchie,³⁷ to be caused by muscular factors and not by a failure of nerve conduction.

Even though the animal muscles fatigued at 100 Hz, at which frequency of stimulation nerve conduction may also be affected, it has been shown that an identical pattern of fatigue occurs in curarised rat extensor digitorum longus,³⁸ which is comparable (type II fibres) with the gastrocnemius seen in our data.¹³ Studies by Luttgau³⁹ have shown that unless the muscle contracts, continued stimulation does not affect the action potential during tetanus. Hence it would be reasonable to conclude that in human and animal studies there is enhanced muscular fatigue in the underfed human and rat, which can be reversed by refeeding.^{12 27 28}

SLOW RELAXATION RATE

Of greater interest is that in the above mentioned studies the relaxation rate is slowed when measured after brief tetanus of only one to two seconds. One possibility is that the type II fibre atrophy that has been seen¹⁴ could reduce the relaxation rate because the remaining type I fibres have a slower relaxation rate. Contradicting this explanation, however, are the preliminary observations by Whittaker *et al* that slow relaxation was seen in the malnourished soleus muscle which is mainly composed of type I fibres.³⁰

There are other mechanisms causing a slow relaxation rate. From as early as 1915 a slow relaxation rate has been shown to be associated with fatigue.⁴⁰⁻⁴⁸ Indeed Esau *et al*⁴⁶ have proposed a simple clinical test of diaphragmatic fatigue based on measurement of relaxation rate. As relaxation rate depends on the rate at which myosin cross bridges dissolve following binding with ATP⁴⁷ it is likely to be affected by factors related to the ATP hydrolysis or binding to actomyosin. The exact mechanisms that slow relaxation are debatable, and the proposed causes are given below:

1 Reduced energy release on ATP binding is related to a fall in the free energy change for ATP hydrolysis.⁴⁸

2 Raised ADP values reduce detachment rate and inhibit the calcium ATPase activity for pumping calcium into the sarcoplasmic reticulum (lateral cisternae).⁴⁹

3 ATP production is reduced.⁵⁰

RATIO OF LOW FREQUENCY TO HIGH FREQUENCY FORCE

The low:high frequency force ratio rises in the malnourished muscle mainly because, while it maintains the level of force developed at low frequency stimulation, there is a reduction of the increment in the force produced at high frequencies.^{13 27} Previous studies compared the force developed at 10 Hz with that at 100 Hz, and it may be argued that the loss of force at high frequencies could be due to failure of nerve or end plate conduction. Our recent studies in humans, however, show that the ratio of the force at 10 Hz (F10) to that at 20 Hz (F20), F10:F20, is also appreciably increased in malnourished subjects and restored by refeeding.³³ What is the importance of this shift? Previous studies have shown that with fatigue the relaxation rate slows, and thus the muscle tetanises to an almost maximal extent at a lower frequency, and also that increasing the frequency does not result in a further rise in tension. In other studies this change has been shown to be associated with a reduced heat production in the adductor pollicis and reduced ATP turnover in mouse soleus.^{42 51} Furthermore, during isometric contraction the energy cost of generating an isometric force increases linearly with the force-time integral.^{52 53} Thus the lower total maximal force generated and reduced thermogenesis can be interpreted as an inability to receive or use the necessarily greater rate of energy required for this purpose.

Thus all the electrophysiological findings, particularly the slow relaxation, which is independent of the mechanical factors of the experiment,⁵⁴ suggest a reduction of the ability of the muscle to sustain a maximal change in the enthalpy (heat+work done) of the system. Why does this happen in the malnourished patient or animal?

To understand the possible ways in which the muscle in a malnourished creature may not be able to use energy at the same rate as in a normally nourished one it is necessary to look at the following factors: (i) availability of substrate; (ii) activity of enzyme pathways; (iii) changes in the pH; (iv) changes in free energy change due to ATP hydrolysis; (v) changes in calcium kinetics.

SUBSTRATE AVAILABILITY

Glycogen stores were not decreased in the obese patients fed a 400 kcal diet, even though the relaxation rate was slowed and the F10:Fmax (ratio of the force at 10 Hz stimulation to that at 100 Hz stimulation) had significantly increased.²⁷ While glycogen stores were reduced by 50% in the rat studies, there was sufficient glycogen to provide energy for the duration of the applied stimulus – two seconds. Fatty acids are the other source of energy. Muscle contracting above 30% of maximal, however, is ischaemic.⁵⁵ Hence fatty acid oxidation cannot supply energy at the time of a stimulus of 20 Hz or more.

ENZYME ACTIVITY RELATED TO ENERGY METABOLISM

The muscle fibres derive energy from glycolysis or oxidative metabolism, depending on the fibre type. For a short duration of stimulation, however, the immediate source of energy is creatinine phosphate and then glycolysis. Later oxidative recovery occurs, but it is not a factor when the muscle is contracting at more than 30% of its maximal force because of its ischaemia.⁵⁵

In our experimental system limitation of glycolysis could account partially for the fatigue after a five second stimulus and perhaps even for relaxation slowing after a one to two second stimulus for the following reasons. While data for the gastrocnemius itself are not available we can use data derived from a muscle of similar fibre composition (mainly type II) in the rat. It has been shown the maximum heat production during isometric contraction of the extensor digitorum longus is 43.9 mcal/g at 27°C.⁵⁶ Furthermore, the heat rate increased in a linear fashion with increase in force. The amount of ATP required for hydrolysis to meet this energy need per second depends on the free energy change for ATP hydrolysis (ΔG):

$$\Delta G = \Delta G_0 + 2.8 \ln[\text{ADP}]/[\text{ATP}] \times [\text{P}_i]$$

At 37°C and pH 7.0 the $\Delta G_0 = -37.4$.⁵⁷

A pH of 7.0 was chosen, because the malnourished muscle was shown to have this pH by NMR.³² The next part of the equation requires the measurement of the free ADP:ATP ratio, which cannot be done directly from experimental data. As the creatine kinase reaction is in equilibrium, however, the free ADP can be calculated from the creatine phosphate and creatine measurements as follows:

or, as this is in equilibrium,

$$-\Delta G_o = RT \ln \frac{[ATP][Cr]}{[CrP][ADP][H^+]}$$

$$\begin{aligned} \text{As } \Delta G_o &= -RT \ln K, \text{ therefore,} \\ RT \ln K &= RT \ln \frac{[ATP][Cr]}{[CrP][ADP][H^+]} \\ [ADP] &= \frac{[ATP][Cr]}{[CrP][H^+]} K. \dots \dots (2) \end{aligned}$$

$$\begin{aligned} \text{Free energy change for ATP hydrolysis} &= \\ \Delta G_o + 2.58 \ln \frac{[ADP][Pi]}{[ATP]} &= \\ \Delta G_o + 2.58 \ln \frac{[Cr][Pi]}{[CrP][H^+]} K \end{aligned}$$

With pH=7.0, $[H^+]=10^{-7}$, and as creatine phosphate concentration in malnourished muscle was 0.009 M/l, the free creatine was 0.0016 M/l, $[Pi]=0.01103$, and $k=2 \times 10^9$, (unpublished results).¹³

Therefore:

$$\begin{aligned} \Delta G &= -37.4 + 5.9 \log_{10}(0.0016 \times 0.01103 / 0.009 \times \\ &\quad 2 \times 10^9 \times 10^{-7}) \\ &= -66.95 \text{ KJ/mol} \\ &= -0.06695 \text{ mJ/micromol (mJ=millijoules)} \end{aligned}$$

As the maximum heat production of extensor digitorum longus is 43.9 mcal/g/second = $43.9 \times 4.18 = 183.5$ mJ/g/second, then the rate of ATP hydrolysis required for this heat production is $0.184 / 0.0669 = 2.7$ micromol/second. With a possible Q_{10} of 1.8⁵¹ the ATP hydrolysis at 37°C would be 5.0 micromol/second.

The creatine phosphate content of malnourished muscle was at its lowest in the 21 day hypocalorically fed rat – 45.8 micromol/g dry weight or 9.1 micromol/g wet weight (recalculated from reference¹³). Hence this rate of ATP hydrolysis can be maintained for less than two seconds before creatine phosphate stores run out. Thus it would be necessary for ATP to be formed from glycolysis during a contractions lasting in excess of two seconds. In the hypocaloric rat¹³ the maximum activity of 6-phosphofructokinase (PFK), which is a non-equilibrium enzyme limiting the rate of glycolysis, was 58.6 micromol/minute/g, or about 1 micromol/g/second. This will enable the synthesis of 3 micromol of ATP/g/second, which is about 3/4.9 or 61% of the maximal needs, and thus it would be calculated that after the first one to two seconds, the rate of energy derived from glycolysis would result in a fall in force of $100 - 61 = 39\%$ of the maximum force. Interestingly, the loss of force in hypocaloric rats was 44% of the maximum attained, which is within 5% of the calculated loss expected for the enzyme activity.

Similarly, the predicted glycolysis rate in control rats should result in the regeneration of 3.85 micromol/second/g muscle or enough to meet $3.85 / 4.9 = 78\%$ of requirements for maximal force. This in turn would be expected to result in a fall of force of about 22%. This calculated value is 1% lower than the measured fatigue of 20.8%.¹³ Finally, when total

force at 200 Hz is plotted against the PFK activity there is a linear correlation ($p < 0.01$). Similarly, fatigue and the F10:F100 ratio are negatively correlated in a highly significant manner ($p < 0.01$) (Fig. 6). Thus it seems that reduced PFK activity may be related to the reduced force. Despite this relation between expected and observed "fatigue" it is unclear how the lower glycolytic activity would be recognised by the contractile machinery and translated into a fall in force. In our studies the ATP value was normal in malnourished rats, but the measurements were made in unstimulated muscle. Even if the concentration fell a little after stimulation it is unlikely that it would fall below that required to saturate myofibrillar ATPase (k_m 0.1 mM). This problem is not unique to fatigue in malnutrition, and the same observations and questions have arisen in connection with fatigue induced by exercise.⁵⁹ Hence other factors to be discussed below may contribute to the slow relaxation and fatigue seen in malnutrition.

The above calculations are for a muscle with type II fibres. The heat production for a comparable slow twitch muscle, however, such as the soleus in the rat, is only 4 mcal/g/second at maximum tension, which would result in only about 0.5 micromol of ATP being hydrolysed, per second/g.⁵⁶ The difference between fast and slow twitch muscle is not due to a difference in the maximum force generated, because heat production, corrected for force, was 17.9 and 3.0 mcal/g/kg force/cm² for extensor digi-

Fig. 6 Correlation of (i) ratio of force at 10 Hz to that at 100 Hz as % (F10:F100) (●) and (ii) fatigue (% force loss per five seconds) (○) with PFK activity (μmol/g/minute). Both negative correlations are significant ($p < 0.01$).

torum longus and the soleus of the rat, respectively, even when corrected for fibre length.⁵⁶ Under these circumstances there is enough creatine phosphate activity for a six second stimulus.

In preliminary studies, however, Whittaker *et al*³⁰ showed that the relaxation of even the soleus muscle was still slow in malnourished rats and recovered with refeeding. Clearly, the slower relaxation of the soleus cannot be due to limitation of glycolytic energy and must therefore be due to other factors to be discussed below.

CHANGES IN MUSCLE pH

The lactate activities were higher in the hypocalorically fed rats. As there is a good correlation between the lactate activities and pH in muscle,³¹ these findings suggest that hypocalorically fed animals may have a low muscle pH. A lower pH may influence the glycolytic pathway through the inhibition of PFK.⁵⁹ Furthermore, a fall in pH may decrease the release of calcium by the sarcoplasmic reticulum and thus influence contractility,⁶⁰ or even have a direct effect on muscle.⁶¹ Nevertheless, low muscle pH could not be the only factor influencing muscle relaxation, as significant slowing of the relaxation was seen even after two and five day fasts when lactate activities were not significantly raised in the fasted animals.¹³

FREE ENERGY FOR ATP HYDROLYSIS

This was calculated for the control, two day and five day fasted animals from data¹³ and unpublished observations (Table 2).

The data show good correlation between the relaxation rate and delta G, whereas there is no such correlation with fatigue or the F10:F100 ratio. The correlation coefficient is 0.968. Dawson *et al*⁴⁸ found a linear correlation between the relaxation rate and delta G expressed in the same way as ours. They believed that the slow relaxation rate was due to a fall in the free energy change for ATP hydrolysis during fatigue. On the other hand, Hultman *et al* did not find this correlation.⁵⁰ Their measurement of

the delta G was based on total ADP measured biochemically. As the total ADP is not necessarily representative of the free ADP their results are difficult to interpret. Our results, while consistent with those of Dawson *et al*,⁴⁸ are nevertheless inconclusive as the calculations were done by amalgamating data from two different studies.

What is the importance of the relation of relaxation rate to free energy change for ATP hydrolysis? Muscle relaxation is caused by reuptake of calcium into the lateral cisternae of the sarcoplasmic reticulum.

The total force available to drive Ca²⁺ into the endoplasmic reticulum during relaxation is:

$$-(dG/dE)_{\text{total}} = -(dG/dE)_{\text{ATP}} - n (dG/dE)_{\text{Ca}} \quad \dots \dots \dots (1).$$

The work required to pump a mole of calcium into the sarcoplasmic reticulum is:

$$(dG/dE)_{\text{Ca}} = 2.58 \ln [\text{free Ca}^{2+}]_{\text{in}} / [\text{free Ca}^{2+}]_{\text{out}} \text{ KJ mol}^{-1}. \quad \dots (2)$$

As the hydrolysis of 1 mol of ATP results in the uptake of 2 mol of Ca²⁺, therefore n=2.

Substituting (2) in (1), when $-(dG/dE)_{\text{total}}=0$ and no calcium is pumped then:

$$(dG/dE)_{\text{ATP}} = 5.98 \ln [\text{free Ca}^{2+}]_{\text{in}} / [\text{free Ca}^{2+}]_{\text{out}} \\ \text{Rearranging the equation,} \\ [\text{free Ca}^{2+}]_{\text{in}} / [\text{free Ca}^{2+}]_{\text{out}} = e^{(dG/dE)_{\text{ATP}}/5.9} \quad \dots \dots \dots (3)$$

Hence small changes in dG/dE of ATP (change in the free energy change of ATP hydrolysis) may have profound effects on the regulation of free cytosolic calcium.^{50 64}

CHANGES IN CALCIUM KINETICS

In both human and the two animal studies^{13 14 30} we noticed that the malnourished muscle has an appreciably higher intracellular calcium concentration, compared with that of controls. The rise in cell calcium concentration means that there is a net positive balance of calcium across the sarcolemma. It is likely that this increase is largely due to an

Table 2 Free energy change for ATP hydrolysis (delta G)

Status	F10:F100 ratio	Fatigue (% force loss in 5 seconds)	Relaxation* (1/t ms ⁻¹)*	Delta G KJ/M
Control	26.6	20.8	5.0	-71.30
Two day fast	35.8	35.0	3.16	-68.30
Five day fast	38.3	41.7	2.67	-68.80
21 day hypocaloric	39.5	44.5	1.49	-66.90

*69:3/t_{1/2}

increase in the mitochondrial calcium for the following reasons:

1 From the above equation a reduction in the free energy change for ATP hydrolysis will only result in a fall in the sarcoplasmic free calcium. Assuming that the free calcium is in equilibrium with the bound calcium, this will result in a fall in sarcoplasmic calcium concentration. Hence the increase is unlikely to be in the sarcoplasmic reticulum (lateral cisternae).

2 The reduced free energy change for ATP hydrolysis means that the cytosolic uptake will be reduced, resulting in a rise in free cytosolic calcium. This will result in an increase of mitochondrial calcium influx.⁶³

3 The steep electrochemical gradient for calcium across the sarcolemma makes the entry of calcium into the cell a passive process.⁶⁴ Hence calcium efflux has to occur to maintain homeostasis. The mechanisms for efflux are the CaMgATPase system⁶⁵ and sodium exchange.⁶⁶ More relevant to our observations is that Jones *et al*⁶⁷ showed that muscle fatigue, induced by stimulating in a hypoxic or normoxic medium, was followed by release of lactate dehydrogenase. If not stimulated, the hypoxic muscle did not release lactate dehydrogenase to any appreciable extent. Thus muscle fatigue was shown to result in muscle enzyme release. In a subsequent study⁶⁸ using the hypoxia model they showed that there was Z band degeneration associated with the enzyme release and that both could be prevented by excluding external calcium from the medium. Finally, they found that fatigued muscle had twice the amount of intracellular calcium when incubated in medium containing calcium.⁶⁹ It is interesting to note that malnourished muscle also showed Z band degeneration with an increase in intracellular calcium.¹⁴ In the studies cited above it was shown that, unlike cardiac muscle, inhibiting energy metabolism by cyanide or iodoacetate also acts synergistically with fatigue to enhance lactate dehydrogenase release. Thus there is evidence to suggest that fatigue associated with a reduction in available energy may prevent calcium efflux and induce a positive calcium balance in the muscle cell.

The postulated mechanism would be as follows: the calcium entering the cell has to be removed by a process of efflux. There are two processes of efflux from muscle – sodium and calcium exchange and $\text{Ca}^{2+} + \text{Mg}^{2+} \text{ATPase}$ Ca^{2+} transporter. The first, while not linked to ATP hydrolysis, is stimulated by it. The second is linked to ATP hydrolysis, much as it is in the sarcoplasmic reticulum. Thus it is possible that the CaMgATPase system may be inhibited by the fall in free energy change for ATP hydrolysis. Furthermore, this process is activated by calmodulin

calcium and magnesium,⁷⁰ and malnutrition may change calmodulin concentrations.

Proposed central hypothesis

Based on the above analysis, we propose the following central hypothesis:

1 Reduction in food intake depresses muscle glycolytic enzyme activity, thus reducing the availability of energy from glycolysis during contraction. The limited rate of glycolysis reduces the total force and thus the high frequency response of muscle.

2 When there is an imbalance between the ability to generate ATP via the glycolytic pathway and energy needs for muscle contraction, then creatine phosphate activity is used and the ratio of creatine phosphate:creatine falls. As the creatine kinase reaction is in equilibrium this is associated with a lower ADP:ATP ratio and a rise in free phosphorus. Hence as the CR and Pi rise and CrP falls, the log of the right side of this equation will become less negative or more positive, and thus the overall free energy change will become less negative, and because it is a negative quantity, will be reduced. This reduction may in turn slow the relaxation rate.

3 The fall in force and the slow relaxation rate are similar to the changes seen in fatigue induced by exercise. Based on the observations of Jones, Jackson, and Edwards^{67–69} on experimental fatigue associated with calcium accumulation and Z band degeneration, together with the fact that both these phenomena are seen in malnourished muscle, it is postulated that similar mechanisms may apply to malnourished muscle and further injure muscle. Accumulation of calcium could explain the lag in recovery of muscle function noted for some time after the start of nutritional support, when malnutrition is severe and prolonged.

In conclusion, while many interesting avenues need to be confirmed and explored, there is sufficient evidence to suggest that in the adult human the adverse functional effects of malnutrition on muscle function cannot be equated to, nor quantitated by, a simple loss of lean body mass. Furthermore, restoration of muscle function cannot be equated to “regrowth” of lean body mass assessed by a gain in body nitrogen or the attainment of a positive nitrogen balance. The functional effects of malnutrition and changes in cellular electrolytes at a time of functional impairment, and especially the role of calcium in modulating nutritional effects and protecting muscle from “outstripping” its energy supplies need further study.

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