Osmotic diarrhoea and skeletal muscle protein synthesis in vivo

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Abstract

The pathogenic nature of the wasting seen in diarrhoea is unknown. This study measured protein synthesis in an established model of diarrhoea using lactose for seven days. Comparisons were also made with data obtained from rats fed an identical diet in which lactose was replaced by isocaloric glucose ad libitum (that is, the control diet). To account for diarrhoea induced anorexia, a third group of rats were included, which were fed identical amounts of the control diet as the rats with diarrhoea inducing diet. Comparisons of the diarrhoea induced group with rats fed the control diet ad libitum showed that diarrhoea caused a significant reduction in body weights. Type I and type II muscles showed significant reductions in protein, RNA, and DNA contents, as well as a fall in the derived parameters, RNA/DNA, protein/DNA, and RNA/protein. Fractional rates of protein synthesis (k_p) were also reduced. However, synthesis rates of type I and II muscles relative to RNA (k_{RNA}) were unchanged in these muscles in diarrhoea induced rats compared with ad libitum fed controls. In the jejunum there was an increase in the RNA/DNA ratio, and reductions in k_p and k_{RNA} . Comparisons were also made between rats with diarrhoea and rats pair fed the control diet. There were no changes in total muscle protein, RNA or DNA contents. This suggests that an important feature of body wasting in diarrhoea is the element of anorexia, which induces severe metabolic changes. The comparison between rats with diarrhoea and the pair fed group showed that histological features of the plantaris were not overtly changed, though diarrhoea caused significant reductions in RNA/DNA, protein/DNA, k_p , and k_{RNA} . Similar changes were seen for the soleus; though the reduction in k_p failed to attain statistical significance. In the jejunum a comparison of diarrhoea induced rats with pair fed controls, showed increases in the ratios of RNA/DNA and protein/DNA.

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Keywords: muscle, type I muscle, type II muscle, jejunum, protein synthesis.

Osmotic diarrhoea is a common consequence of small intestinal disease, for example, infections or toxins such as ethanol.¹⁻³ In rotavirus induced diarrhoea there is shortening of villi

with enterocyte loss and establishment of a relatively young cell population with monosaccharide and disaccharide malabsorption and reduced sodium potassium ATPase activity leading to faeces with low sodium concentrations.⁴⁻⁷ Diarrhoea can be life threatening in the malnourished and the very young and there is also an increased risk of infection.^{7,8} Diarrhoea also causes tissue wasting and enhanced nitrogen excretion as well as reduced rates of growth,^{6,9} which may be contributory mechanisms in the aetiology of impaired immune function.¹⁰ In addition, skeletal muscle is an important contributor to whole body protein metabolism, namely about 40% of tissue mass and about 25% of whole body synthesis.¹¹ It is therefore possible that the perturbed nitrogen homeostasis commonly seen in diarrhoea (reviewed by Booth⁶) may in part result from defects in skeletal muscle protein retention. However, the biochemical effects of diarrhoea on skeletal muscle biochemistry are generally unknown. The changes in growth and perturbed nitrogen excretion implicate a role for changed protein turnover, and thus compensatory adjustments in protein synthesis or degradation, or both.¹¹ In this study we investigated the contribution of protein synthesis and studied different muscle types (type I and type II fibre predominant skeletal muscles and small intestine). Measurements were carried out in vivo using what is arguably the most reliable method for measuring protein synthesis in small laboratory animals, which effectively considers precursor pools.^{12,13} The diarrhoea was induced by a method that has also been described in this journal.⁷

Methods

Treatment of animals

Osmotic diarrhoea was induced in male Wistar rats 4 weeks of age (approximately 60 g body weight; Charles Rivers, Margate, Kent, England) with lactose.¹⁴ Table I gives the composition of the diet. The rats were housed in a humidified, temperature controlled environment on a 12 h light/12 h dark cycle. The rats were put in wire bottomed cages to minimise coprophagy, and there was no bedding available as additional source of calories. While every effort was made to ensure rats received the correct amount of calories, the study design did not influence how the nutrients were metabolised. The groups were:

Group (1) rats were fed ad libitum on a nutritionally complete liquid diet. The diet was identical to that used for treating groups (2)

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TABLE 1 Feeding regimens

	Content (g)	Total kcal	Total kJ	kJ/ml
<i>Control diet, fed ad libitum to group (1) or pair fed to group (3)</i>				
Water	800.0	0.0	0.0	0.0
Vitafood	121.0	533.6	2232.6	2.401
Glucose	85.0	318.8	1333.7	1.434
Casein	10.0	40.0	167.4	0.18
Orovite	3.8	14.3	59.6	0.064
Total	1019.8	906.6	3793.3	4.079
<i>Lactose diet fed ad libitum to group (2)</i>				
Water	800.0	0.0	0.0	0.0
Vitafood	121.0	533.6	2232.6	2.401
Lactose	85.0	318.8	1333.7	1.434
Casein	10.0	40.0	167.4	0.18
Orovite	3.8	14.3	59.6	0.064
Total	1019.8	906.7	3793.3	4.079

Caloric values were calculated assuming carbohydrate=3.75 kcal/g, protein=4.0 kcal, fat=9.0 kcal/g, and 4.18 kJ was assumed to be equal to 1 kcal. The total measured volume of both diets was 930 ml. The groups were as follows: (1) control, ad libitum; (2) diarrhoea; (3) control, pair fed. Orovite contains sucrose, hence its low contribution to overall caloric intake.

and (3), albeit differences in the proportion of calories provided by lactose in group (2).

Group (2) rats were fed ad libitum the liquid diet containing lactose. The treatment regimen induced diarrhoea. The presence of diarrhoea was distinctly conspicuous; the floors of the cages were soiled and the stools liquified. The anal areas were wet and the fur around the anus of these rats was damp. These effects were not seen in control rats.

Group (3) rats were subjected to control feeding and were given identical amounts of the diet consumed by group (2) though lactose was replaced by isocaloric glucose (that is, a pair feeding regimen).

Method of pair feeding

Before the study began all rats were ranked in order of body weight and divided into the above groups. The method of pair feeding entailed measuring the volume of liquid diet consumed by each rat in group (2) over a 24 hour period. Using this figure an identical amount of control liquid diet was given to the counter matched rat in group (3). The amount of diet consumed by the rats in group (2) were recorded daily and the pair feeding principle applied to rats in group (3) on a daily basis.

The model of inducing diarrhoea has been described previously¹⁴ as well as in this journal.⁷ The basis of the model is the limited ability of intestinal tissue to metabolise lactose and indeed some other sugars. For example diarrhoea can also be induced with fructose.¹⁵ The impaired ability of the small intestine to digest the lactose raises intraluminal osmolarity. To compensate for this, water traverses into the lumen of the intestine, to facilitate an equilibrium of osmolarity in the intracellular and extracellular partitions. The increased water content in the lumen causes watery stools. However, the physiological processes are not entirely this simple as the lactose will be subjected to bacterial fermentation giving rise to short chain fatty acids, methane, hydrogen, carbon dioxide, and lactate.¹⁶ The histological changes in the small bowel mucosa are similar to those seen in young subjects with persistent diarrhoea or

gastroenteritis. An exceptionally important feature of our study, however, is the inclusion of a group of pair fed rats (group (3)), to take account of the anorexia or reduced dietary intake. Sadly, numerous studies on the pathophysiology of infectious and non-infectious diarrhoea have failed to consider this aspect and this study is novel in this respect.

Measurement of protein synthesis

After one week of treatment, rates of protein synthesis were measured with a flooding dose of L-[4³H]phenylalanine (injected at a dose of 150 mmol/l, 1 ml/100 g body weight, intravenously) to label the intramuscular and extracellular free amino acid pools.¹⁷ After death the hind legs were stripped free of skin and quickly placed in an ice and water slurry. The whole small intestinal length was also dissected, rapidly cooled in ice cold saline (0.15 mol/l NaCl), and its length measured, by suspending one end with a weight (3.2 gram). Whole jejunal segments (15 cm taken from the centre of the small intestine) were then rapidly dissected out and flushed with ice cold saline, blotted, weighed, and frozen in liquid nitrogen. The soleus (type I fibre predominant) and plantaris (type II fibre predominant) muscles were dissected, blotted, weighed, and plunged into liquid nitrogen. The time between injection of the isotope and immersion of the legs in the ice water slurry, or intestine into saline, was accurately timed (that is, about 11 and 12 minutes for the muscle and intestine). All tissues were stored at a temperature of -70°C until processing.

All ensuing steps of tissue processing were kept between 0-4°C. Tissues were homogenised with an ultrasonic tissue homogeniser (Polytron, Kinematica, Philip Harris Scientific, London, UK) and processed for determination of protein bound and free phenylalanine specific radioactivities.¹⁷ Fractional rates of protein synthesis (defined as the percentage of tissue protein renewed each day, that is, %/day) were calculated from the formula: $k_s = (S_b \times 100) / (S_i \times t)$; where 'S_b' is the specific radioactivity of phenylalanine in tissue protein (dpm/nmol); 'S_i' is the specific radioactivity of free phenylalanine in acid soluble fractions of muscle or intestinal homogenates (dpm/nmol); 't' is the period between injection of the isotope and immersion of the legs into the ice water mixture or intestine in ice cold saline (in days). Both S_i and S_b were assayed according to the method of Garlick *et al.*¹⁷ The formula assumes that the free amino acid at the site of protein synthesis (the amino acyl tRNA) was represented by the free amino acid in the intracellular pools. Other assays were routine procedures for protein,¹⁸ RNA,¹⁹ and DNA.²¹

Methodological considerations

In these studies we were mindful of the fact that a large number of the metabolic studies on muscle are carried out in isolated systems, or have used inappropriate methodology

to measure protein synthesis. We were also mindful of the fact that any in vitro phenomena must be transposed to the intact mammalian system. These facets have recently been the subject of much controversy and have been reviewed by Garlick *et al.*,¹² Rennie *et al.*,¹³ and Preedy *et al.*²² In this study, we carried out protein synthesis studies in young growing rats using what is arguably the most reliable technique for measuring protein synthesis in laboratory animals in vivo to date. The flooding dose technique effectively considers that any reliable protein synthesis data have to measure the specific radioactivity of the precursor at the site of protein synthesis.^{11, 12} Theoretically this is the aminoacyl tRNA (S_{tRNA}). However, the practicalities of amino-acyl tRNA measurements are such that their determinations are difficult to facilitate on a routine basis. In the flooding dose technique, the large amount of phenylalanine floods all endogenous free amino acid pools, such that all tissue free phenylalanine specific radioactivities (S_i) in the different pools attain similar values.^{12, 17} Free phenylalanine specific radioactivities are also similar to the values in the extracellular pools (S_p).¹⁷ Thus $S_{\text{tRNA}} \equiv S_i \equiv S_p$.¹⁷ Measurements of acid supernatants of tissue homogenates are therefore sufficient for extracting free radio-labelled phenylalanine for subsequently calculating rates of protein synthesis.¹⁷ However, we also measured k_s by substituting S_i with S_p in the formula for calculating synthesis rates (effectively considering the possibility the S_{tRNA} values were better represented by the free amino acid in extracellular pools) and identical qualitative conclusions were obtained (not shown for brevity). It is possible that differences in k_s and k_{RNA} resulted from changes in precursor enrichment – that is, changed S_i or S_p values. In the type I and type II muscles, however, S_i values were not significantly different in any experimental group. Similarly S_p was only changed by the diarrhoea treatment regimen by 5% (not shown for brevity) and therefore cannot be responsible for the differences in k_s or k_{RNA} .

Histological assessment

Tissue samples were placed on cork discs, secured with cryostable adhesive, snap frozen in isopentane (maintained at -196°C for one minute), and stored at -70°C . Serial sections of 10 μm thickness were cut and stained with haematoxylin and eosin and for myosin-ATPase at pH 9.5^{23–25} to allow for fibre typing. The haematoxylin and eosin and ATPase sections were examined qualitatively (having coded test and control samples so that assessment was made 'blind') then histomorphometric measurements of skeletal muscle fibre diameters were made by placing the ATPase sections on the stage of a Zeiss IIRS microscope (Carl Zeiss (Oberkochen), Welwyn Garden City, Herts, UK) to which was attached an Ikegami CCD camera

(Ikegami Electronics (Europe) GmbH UK Branch, Chertsey, Surrey, UK). Captured images were digitised and fed to a Colourmorph interactive image analysis system (Perceptive Instruments, Haverhill, Suffolk, UK) running on an Elonex 386S microcomputer with a NEC 'Multisync plus' monitor at a resolution of 640×480 pixels. The equipment used enabled the optical image of the histological section examined down the microscope to be visualised on the monitor screen. A mouse was used to interactively demarcate the lesser fibre diameter of each fibre.

Statistics

Data are presented as the mean (SEM) of five to six findings in each group. Differences between means were assessed using an analysis of variance, followed by Student's *t* test incorporating the pooled estimate of variance. NS, not significant; $p > 0.05$.

Results

Body and tissue weights and gut lengths

The data in Table II show that in comparison to the ad libitum glucose fed rats (group (1)), group (2) rats showed a significant reduction in final body weights (-14% , $p < 0.001$) and concomitant reductions in the weights of the soleus and plantaris (by 22 and 29% respectively). However, there was no significant change in jejunal weights. The mean lengths of the jejunum in groups (1), (2), and (3) were 99.1 (2.8), 111.2 (2.5), 94.9 (2.5) cm respectively. Compared with the rats in group

TABLE II Body and tissue weights in experimental diarrhoea

		% Change from group (1)	p Value
Body weights (g)			
Initial			
(1) Control, ad libitum	59.3 (1.1)		
(2) Diarrhoea	59.7 (1.4)	+1	NS
(3) Control, pair fed	59.4 (1.2)	0	NS
(2) versus (3), +1%, NS			
Final			
(1) Control, ad libitum	92 (2)		
(2) Diarrhoea	79 (1)	-14	<0.001
(3) Control, pair fed	74 (2)	-20	<0.001
(2) versus (3), +6%, $p < 0.05$			
Tissue wet weights (mg)			
Soleus (type I)			
(1) Control, ad libitum	74 (3)		
(2) Diarrhoea	58 (3)	-22	<0.001
(3) Control, pair fed	61 (3)	-18	<0.005
(2) versus (3), -5%, NS			
Plantaris (type II)			
(1) Control, ad libitum	136 (4)		
(2) Diarrhoea	96 (4)	-29	<0.001
(3) Control, pair fed	100 (5)	-26	<0.001
(2) versus (3), -3%, NS			
Jejunum			
(1) Control, ad libitum	769 (25)		
(2) Diarrhoea	716 (40)	-7	NS
(3) Control, pair fed	680 (29)	-12	<0.05
(2) versus (3), +5%, NS			

Male Wistar rats were either fed the glucose or lactose containing diet ad libitum (groups (1) and (2), respectively). To compensate for the anorexia, rats of group (3) were pair fed. Rats were killed after one week and pairs of soleus and plantaris muscles and 15 cm of jejunum taken for analysis. Differences between groups (2) and (3) are shown under each set of data and expressed as a percentage of group (3). Data shown as mean (SEM).

TABLE III Protein RNA and DNA contents in experimental diarrhoea

		% Change from group (1)	p Value
Tissue protein content (mg)			
<i>Soleus (type I)</i>			
(1) Control, ad libitum	13 (1)		
(2) Diarrhoea	9 (1)	-31	<0.001
(3) Control, pair fed	10 (1)	-23	<0.025
(2) versus (3), -11%, NS			
<i>Plantaris (type II)</i>			
(1) Control, ad libitum	24 (1)		
(2) Diarrhoea	14 (2)	-42	<0.001
(3) Control, pair fed	15 (1)	-38	<0.001
(2) versus (3), -7%, NS			
<i>Jejunum</i>			
(1) Control, ad libitum	35 (1)		
(2) Diarrhoea	33 (2)	-6	NS
(3) Control, pair fed	31 (1)	-11	<0.05
(2) versus (3), +6%, NS			
Tissue RNA content (mg)			
<i>Soleus (type I)</i>			
(1) Control, ad libitum	0.16 (0.01)		
(2) Diarrhoea	0.10 (0.01)	-38	<0.001
(3) Control, pair fed	0.11 (0.01)	-31	<0.001
(2) versus (3), -10%, NS			
<i>Plantaris (type II)</i>			
(1) Control, ad libitum	0.25 (0.01)		
(2) Diarrhoea	0.13 (0.02)	-48	<0.001
(3) Control, pair fed	0.15 (0.01)	-40	<0.001
(2) versus (3), -15%, NS			
<i>Jejunum</i>			
(1) Control, ad libitum	7.3 (0.2)		
(2) Diarrhoea	7.4 (0.3)	+1	NS
(3) Control, pair fed	6.5 (0.3)	-11	NS
(2) versus (3), +12%, NS			
Tissue DNA content (mg)			
<i>Plantaris (type II)</i>			
(1) Control, ad libitum	0.49 (0.02)		
(2) Diarrhoea	0.32 (0.03)	-35	<0.001
(3) Control, pair fed	0.31 (0.01)	-37	<0.001
(2) versus (3), +3%, NS			
<i>Jejunum</i>			
(1) Control, ad libitum	2.41 (0.07)		
(2) Diarrhoea	2.19 (0.13)	-9	NS
(3) Control, pair fed	2.23 (0.10)	-7	NS
(2) versus (3), -2%, NS			

For experimental details see methods section and legend to Table II. There was insufficient sample for soleus DNA measurement. Data shown as mean (SEM).

(1) the length of the gut in rats of group (2) achieved significance $p < 0.001$.

Biochemical findings: comparison of group (1) and (2)

Table III shows that protein, RNA, and DNA contents were reduced in the soleus and plantaris of rats in group (2) when compared with ad libitum fed rats in group (1); (reductions of between 31–48%, $p < 0.001$). Derived parameters (Table IV) were also changed in group (2) compared with group (1): in the soleus and plantaris this included the RNA/protein ratio (-16% and -10%, $p < 0.005$ and $p < 0.05$, respectively). The plantaris RNA/DNA and protein/DNA were also reduced by 23% and 12% respectively ($p < 0.001$ and $p < 0.025$, respectively). In the jejunum, there was a small increase in the RNA/DNA ratio (+10%, $p < 0.05$, see Table IV). There was insufficient material for determination of DNA in the soleus.

Rats in group (2) had lower rates of soleus and plantaris muscle protein synthesis (by 16–17%, $p < 0.025$ in both instances, see Table V) when compared with rats in group (1). This was not reflected by a reduced translation index (k_{RNA}) in the soleus and plantaris suggesting that part of the change in protein

synthesis in the rats resulted from transcription – that is, reductions in total tissue RNA. A considerable reduction in k_{RNA} was seen in the jejunum (-26%, $p < 0.025$).

Biochemical findings: comparison of group (2) and (3)

A comparison of group (2) and (3) showed a significant reduction in the following variables: plantaris RNA/DNA (-12%, $p < 0.01$, cell size (protein/DNA ratio, -11%, $p < 0.05$), k_s (-19%, $p < 0.025$), k_{RNA} (-23%, $p < 0.01$). Similar qualitative changes were seen for the soleus, though many of the variables failed to attain statistical significance, except the soleus k_{RNA} (-20%, $p < 0.005$). In the jejunum the following differences were seen when comparing groups (2) and (3): RNA/DNA (+13%, $p < 0.005$) and protein/DNA (+7%, $p < 0.05$).

A comparison of groups (1) and (3) showed that k_s values were reduced in jejunum but not in soleus or plantaris. The reason for this differential sensitivity is not known.

Relative sensitivity of type I and II muscles

The type II fibre predominant plantaris was more sensitive compared with the type I fibre predominant soleus and this was also confirmed by inspecting soleus/plantaris ratios – that is, type I/type II ratios for tissue weights, protein contents, and RNA contents for group (1) were 0.55 (0.02), 0.52 (0.03), and 0.63 (0.05),

TABLE IV Derived parameters

		% Change from group (1)	p Value
RNA/protein (mg/g)			
<i>Soleus (type I)</i>			
(1) Control, ad libitum	13.0 (0.6)		
(2) Diarrhoea	10.9 (0.6)	-16	<0.005
(3) Control, pair fed	11.1 (0.4)	-15	<0.005
(2) versus (3), -2%, NS			
<i>Plantaris (type II)</i>			
(1) Control, ad libitum	10.5 (0.3)		
(2) Diarrhoea	9.4 (0.5)	-10	<0.05
(3) Control, pair fed	9.6 (0.4)	-9	NS
(2) versus (3), -2%, NS			
<i>Jejunum</i>			
(1) Control, ad libitum	210.1 (8.0)		
(2) Diarrhoea	225.4 (5.8)	+7	NS
(3) Control, pair fed	209.2 (4.9)	-1	NS
(2) versus (3), +7%, NS			
RNA/DNA (mg/mg)			
<i>Plantaris (type II)</i>			
(1) Control, ad libitum	0.52 (0.02)		
(2) Diarrhoea	0.40 (0.02)	-23	<0.001
(3) Control, pair fed	0.46 (0.03)	-11	<0.01
(2) versus (3), -12%, $p < 0.01$			
<i>Jejunum</i>			
(1) Control, ad libitum	3.06 (0.12)		
(2) Diarrhoea	3.38 (0.07)	+10	<0.05
(3) Control, pair fed	2.94 (0.16)	-4	NS
(2) versus (3), +13%, $p < 0.005$			
Protein/DNA (mg/mg)			
<i>Plantaris (type II)</i>			
(1) Control, ad libitum	49.2 (0.89)		
(2) Diarrhoea	43.2 (2.11)	-12	<0.025
(3) Control, pair fed	48.0 (1.94)	-2	NS
(2) versus (3), -11%, $p < 0.05$			
<i>Jejunum</i>			
(1) Control, ad libitum	14.6 (0.18)		
(2) Diarrhoea	15.0 (0.44)	+3	NS
(3) Control, pair fed	14.0 (0.58)	-4	NS
(2) versus (3), +7%, $p < 0.05$			

The RNA/DNA ratio value is equivalent to the amount of synthetic material per cell, protein/DNA represents cell size, and RNA/protein represents synthetic capacity. For other details see method section and legend to Table II. Data shown as mean (SEM).

Fibre diameter distribution in plantaris muscles from diarrhoea and control, pair fed rats. Plantaris muscles from rats in groups (2) and (3) (diarrhoea and control, pair fed rats, respectively) were subjected to quantitative histomorphometry to measure the diameters of type I and type II fibres. The frequency distribution of lesser fibre diameters in groups (2) and (3) were not significantly different for any of the ranges. This generally reaffirms the quantitative compositional analysis of plantaris muscles from groups (2) and (3). A detailed histological description of the qualitative features of the plantaris muscles in these two groups is also given in the Results section. It is necessary to point out that a comparison of compositional parameters in plantaris muscles from groups (1) and (2) (control, ad libitum and control, pair fed rats, respectively) did indeed show considerable differences, but these changes were largely as a result of the anorexia.

respectively. In the rats with diarrhoea (group (2)) the following type I/type II ratios were obtained for weight: 0.61 (0.03) ($p < 0.025$); protein content 0.71 (0.09) ($p < 0.01$), and RNA content 0.84 (0.14) ($p < 0.025$).

Histological examination

Because of the practical constraints of limited resources we were unable to compare groups (1) and (2). Histological examinations, however, were confined to a comparison between groups (2) and (3). The light microscopy of the skeletal muscle samples showed no qualitative differences between lactose fed rats and pair fed control rats. Specifically, there was no abnormality of muscle fibre sarcoplasm and the mosaic of fibre types seemed normal for the type of muscle. There was no evidence of inflammation or necrosis or excess of fibrous or adipose tissue. Histomorphometric analysis of ATPase stained sections gave the frequency distribution of lesser fibre diameters shown in

TABLE V Fractional rates of protein synthesis

		% Change from group (1)	p Value
Rates of protein synthesis in diarrhoea (k_p ; %/day)			
<i>Soleus (type I)</i>			
(1) Control, ad libitum	15.1 (1.1)		
(2) Diarrhoea	12.5 (0.7)	-17	<0.025
(3) Control, pair fed	14.6 (0.9)	-3	NS
(2) versus (3), -17%, NS			
<i>Plantaris (type II)</i>			
(1) Control, ad libitum	14.1 (0.8)		
(2) Diarrhoea	11.9 (0.9)	-16	<0.025
(3) Control, pair fed	14.2 (0.5)	+1	NS
(2) versus (3), -19%, $p < 0.025$			
<i>Jejunum</i>			
(1) Control, ad libitum	164.4 (29.1)		
(2) Diarrhoea	128.9 (8.2)	-22	<0.05
(3) Control, pair fed	117.9 (7.0)	-28	<0.025
(2) versus (3), +8%, NS			
RNA activities (k_{RNA} ; mg protein/day/mg RNA)			
<i>Soleus (type I)</i>			
(1) Control, ad libitum	11.2 (0.6)		
(2) Diarrhoea	10.8 (0.5)	-4	NS
(3) Control, pair fed	13.0 (0.8)	+16	<0.025
(2) versus (3), -20%, NS			
<i>Plantaris (type II)</i>			
(1) Control, ad libitum	13.4 (0.6)		
(2) Diarrhoea	12.4 (0.8)	-7	NS
(3) Control, pair fed	15.2 (0.6)	+13	<0.05
(2) versus (3), -23%, $p < 0.01$			
<i>Jejunum</i>			
(1) Control, ad libitum	7.7 (1.2)		
(2) Diarrhoea	5.7 (0.4)	-26	<0.025
(3) Control, pair fed	5.6 (0.2)	-27	<0.025
(2) versus (3), -5%, NS			

For details see methods section and legend to Table II. Data shown as mean (SEM).

the Figure. These analyses showed no significant change in muscle fibre diameters in the treated group from the pair fed group. Effectively, the quantitative morphometry confirm the biochemical findings showing that a substantial component of the biological changes in muscles during diarrhoea was caused by the anorexia.

Discussion

Infectious diarrhoea is a common cause of secondary lactose intolerance²⁶ and repeated episodes lead to reduced rates of growth and increased mortality and morbidity.⁶ However, the biochemical mechanisms for changed growth rates remain obscure. The objective of this study was to find out (a) if osmotic diarrhoea in itself was inducing metabolic changes in muscle and (b) the mechanisms responsible for any changes in muscle protein content. With regard to (b), the concept of protein turnover implicates changes in tissue protein mass as resulting from changes in protein synthesis or protein degradation, or both.¹¹ Although we did not measure the contribution of degradative pathways (because there are no reliable methods for determining this process in muscle *in vivo*²⁷), we considered the problem by measuring rates of protein synthesis with the flooding dose technique and quantifying muscle RNA to show whether transcription was also modulated by diarrhoea. Two important points emerged from the results, the first was that diarrhoeal processes lead to metabolic changes in a tissue system far removed from the primary site of insult, namely skeletal muscle. These included loss of muscle weights, protein, RNA, and DNA contents, and reduced rates of synthesis. Secondly, reduc-

tions in protein synthesis still occur when controlled feeding has been considered. However, when paired feeding had been taken into consideration (comparison of groups 2 and 3) the compositional changes were moderate. This was confirmed by the histological examinations.

Other studies have similarly shown body weight reductions in diarrhoea caused by cathartic agents such as magnesium citrate and phenolphthalein²⁸ or lactose.¹⁴ In our model, concomitant changes included reductions in muscle weights and the amount of muscle protein and RNA. Diarrhoea induced significant reductions in muscle tissue protein synthesis. These changes occurred in the absence of infection, and histologically, the muscles from control and treated rats seemed normal. Also, the type II fibre predominant plantaris was more sensitive compared with the type I fibre predominant soleus and this was also confirmed by inspecting soleus/plantaris ratios.

Although our study was based on the experimental technique described by Nunez *et al.*,¹⁴ we included another set of rats (group (3)), which were subjected to controlled feeding (analogous to pair feeding). Thus, our data showed that reductions in protein synthesis occurred even when diarrhoea induced rats (group (2)) were compared with either pair fed controls or rats fed the liquid diet ad libitum. Group (3) rats were given glucose instead of lactose, but the amount of diet they received was identical to that of the anorexic animals in group (2). Therefore group (3) rats were deficient in calories when compared with group (1) rats fed ad libitum, hence it is difficult to postulate a mechanism how muscle protein synthesis (k_s) in group (3) was comparable with group (1). Consideration should be given to the fact that degradation may also have been involved in mediating the changes in protein content. Alternatively, the endocrine status may have had an influence on k_s . Though a more important influence is the prevailing nutritional state of the rat.

Effectively, rats in group (3) were in a re-fed state while rats in groups (1) and (2) were postabsorptive at the time of synthesis measurement. Problems inherent in interpreting data in muscle from pair feeding regimens have been reviewed previously.²⁹ Though one could argue that acute transitions in nutritional status influenced k_s values, comparison of data clearly shows that the RNA composition, effectively an indicator of the protein synthetic potential, was reduced in plantaris muscles of groups (2) and (3) in comparison with group (1). Indeed the RNA/DNA ratio in plantaris muscle was 0.40 (0.02) and 0.46 (0.03) ($p < 0.01$) in groups (2) and (3), respectively, clearly showing that protein synthetic potential was reduced.

Nunez *et al.*¹⁴ reported that the lower bowel showed signs of inflammation and Bueno *et al.*⁷ have shown that when diarrhoeal episodes have abated, there was still loss of enterocyte microvillar surface, lymphocyte infiltration, and abnormal mitochondria. The more

insidious changes in the model must be considered: endotoxaemia may have occurred, or the diarrhoea may have induced perturbations in permeability, facilitating the ingress of toxins.³⁰ It would not be inconceivable for the malabsorption of one or more micro-nutrients or electrolyte abnormalities to be responsible for the reduced protein synthesis in group (2).

In conclusion, the results showed loss of skeletal muscle protein caused by osmotic diarrhoea induced by lactose. As we were able to study the response in pair fed animals the contribution of anorexia was controlled. The model provides a suitable means of further examining the superimposition of other metabolic processes, such as endotoxaemia.

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