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Protein synthesis of muscle fractions from the small intestine in alcohol fed rats

V R Preedy, T J Peters

Abstract

The effects of chronic ethanol feeding on the amounts and synthesis rates of cytoplasmic, contractile, and stromal protein fractions were investigated in the small intestine of eight pairs of immature and seven pairs of mature rats. Treated rats were fed ethanol as 36% of total energy in a nutritionally adequate liquid diet. Paired controls were fed isovolumetric amounts of the same diet in which ethanol was substituted by isocaloric glucose. After six weeks the total cytoplasmic and contractile protein content in immature rats was reduced by 18% and 31%, respectively ($p \leq 0.007$). The decline in the stromal protein content (26%) was not statistically significant ($p = 0.130$). In mature rats the protein contents were also reduced in the cytoplasmic (25%, $p = 0.035$) and contractile (27%, $p = 0.005$) protein fractions, though the stromal protein fraction was unaltered ($p = 0.913$). In immature rats fractional rates of protein synthesis in cytoplasmic and contractile protein fractions of the small intestine were unaltered by chronic ethanol feeding ($p \leq 0.853$). In mature rats, the synthesis rates of corresponding fractions declined, by 18% and 31%, respectively, but were also not statistically significant ($p \leq 0.369$). Absolute rates of protein synthesis in immature rats fell by 6% ($p = 0.549$) in the cytoplasmic and 31% in the contractile protein fraction ($p = 0.045$). In mature rats, the corresponding reductions were 38% ($p = 0.106$) and 48% ($p = 0.033$), respectively. Virtually no radioactivity could be detected in the stromal fraction, signifying very low synthesis rates. Chronic ethanol feeding reduces the amount of protein in the small intestine of the immature and mature rat with the contractile protein fraction showing the greatest decrease. In the absence of statistically significant reductions in fractional synthesis rates a partial adaptation in turnover rates may have occurred.

One of the major symptomatic effects of chronic alcohol abuse is disturbances in gastrointestinal function, with up to a third of patients being affected.[1] The suggestion that the small intestine is a primary target organ of ethanol toxicity is supported by a variety of clinical and experimental studies which have shown that exocrine, endocrine, absorptive and metabolic functions of the small intestine are impaired.[2–6] Alterations in the amount or functional integrity of the contractile apparatus may also be important in the development of the alcohol induced gastrointestinal dysfunction. For example, alcohol causes alterations in the normal characteristic pattern of intestinal motility[7 8] and this may be caused by a reduction in the amount of contractile apparatus. Evidence to support this was obtained from our recent study which showed that acute ethanol preferentially reduces the synthesis of contractile proteins in the small intestine of the young (100 g body weight) rat.[9] It is, however, difficult to translate the experimental results of acute studies to events occurring as a result of long term treatments. Furthermore, observations in rapidly growing, immature, alcohol fed rats may not be relevant to the human situation characterised by the sexually mature, slowly growing that is, adolescent – or non-growing – that is, adult – chronic alcohol abuser. We therefore investigated the chronic effects of ethanol on the cytoplasmic, contractile and stromal protein fraction in the small intestine of both immature and mature rats.

Methods

SOURCES OF ANIMALS AND MATERIAL

Two groups of male Wistar rats, initially weighing approximately 50–70 g or 230–270 g body weight were obtained from the National Institute of Medical Research (Mill Hill, London, UK); L[4^{3}H]-phenylalanine was obtained from Amersham International (Amersham, Bucks, UK) and other biochemicals were from Sigma Company Ltd (Poole, Dorset, UK).

TREATMENT OF ANIMALS AND LIQUID FEEDING REGIME

Rats were housed in a temperature controlled and humidified animal house on a 12 h light: 12 h dark schedule, commencing at 07.00 h. A standard laboratory chow was fed *ad libitum*, and rats were allowed free access to water for approximately three to five days. At 85 g ('immature') and 285 g body weight ('mature') animals were 'pair matched' on the basis of weight before starting the experimental feeding. One animal from each pair was then fed a nutritionally adequate – that is, able to sustain normal growth patterns when administered *ad libitum*[10] – liquid diet containing 36% of total energy as ethanol, as originally described by Leiber.[11 12] The other animal from each pair – that is, controls – was fed identical volumes of the same diet, in which the ethanol was replaced by isoenergetic glucose. The protein, carbohydrate and fat composition of the two diets, (15, 19 and 66% of total calories, respectively) and micronutrients (vitamins, etc.) were otherwise identical.[10] The liquid diets were freshly prepared each day and the volumes consumed by alcohol fed rats were recorded daily so that control animals could receive the corres-

Department of Clinical Biochemistry, King's College School of Medicine and Dentistry, London SE5 9PJ
V R Preedy
T J Peters

Correspondence to:
Dr Victor R Preedy, Department of Clinical Biochemistry, King's College School of Medicine, and Dentistry, Bessemer Road, London SE5 9PJ.

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ponding volumes of control diet the next day.[10] Feeding continued for six weeks, at the end of which rates of protein synthesis and the composition of the small intestine were measured as described below.

MEASUREMENT OF PROTEIN SYNTHESIS

The 'flooding dose' technique using L[4^{3}H]-phenylalanine was used to measure rates of protein synthesis. Rats were killed 15 minutes after intraperitoneal injection of isotope (150 mmol/l, 0·1 Ci/mol, 20 ml/kg body weight) and blood was collected for separation of plasma by centrifugation. The entire small intestine (from stomach to caecum) was rapidly dissected out, and immersed in an ice/water mixture. Connective tissue and fat were removed and the intestine was flushed with 30–40 ml of 0·15 mol/l NaCl (0–4°C), blotted dry and weighed. Tissues were stored at −70°C until subsequent analysis.

SEPARATION OF PROTEIN COMPARTMENTS

The entire small intestine was homogenised in 30 ml water, using three to four, 10 second bursts of an ultrasonic tissue polytron homogeniser (The Northern Media Supply Co Ltd, Hull, UK). All steps and apparatus, unless indicated otherwise, were kept at between 0 and 4°C. An aliquot of homogenate containing 200–400 mg tissue was removed for precipitation with 10 ml 0·2 mol/l perchloric acid for subsequent measurement of specific radioactivity of phenylalanine in acid supernatants of intestinal homogenate (S_i), defined as the specific radioactivity of free phenylalanine in acid supernatants of small intestinal homogenates. A sample of plasma was similarly treated with 0·2 mol/l perchloric acid to obtain specific radioactivity of phenylalanine in acid supernatants of plasma (Sp). Another aliquot of homogenate containing 200–400 mg tissue was also transferred to a test tube containing 20 ml of buffer containing 10 mmol/l imidazole; 60 mmol/l KCl; 0·5 mmol/l ethyleneglycolbis(aminoethylether)tetra-acetate; 4 mmol/l $MgCl_2$; 1 mmol/l sodium azide; 1 mmol/l dithiothreitol and 0·5% (v/v) Triton X-100, pH 7·0.

After centrifugation (33 000 g, 10 min) the supernatant was removed ('cytoplasmic' fraction). The pellet was homogenised in a ground glass homogeniser, with 10 ml buffer containing: 100 mmol/l potassium dihydrogen orthophosphate; 50 mmol/l dipotassium hydrogen orthophosphate; 300 mmol/l KCl; 1 mmol/l ethylenediamine-tetra-acetate and 5 mmol/l adenosine 5′-triphosphate, pH 6·3. After centrifugation (33 000 g, 10 min) the supernatant was decanted. The extraction procedure with 10 ml of high ionic strength buffer was repeated and the supernatants combined ('contractile' protein fraction). The pellet was the 'stromal' protein fraction.[9]

The terminology 'cytoplasmic' 'contractile' and 'stromal' was defined in detail in our previous paper.[9] Briefly, the cytoplasmic fraction contains principally soluble enzyme proteins; the 'contractile' fraction contains proteins such as actin and myosin and other myofibrillar proteins; the 'stromal' fraction comprises of connective tissue proteins such as collagen, and possibly traces of unextractable contractile protein.

PROCESSING OF TISSUE SAMPLES FOR MEASUREMENT OF PHENYLALANINE SPECIFIC RADIOACTIVITIES

Acid supernatants of whole intestine homogenates were neutralised with tripotassium citrate and, after centrifugation (2000 g, 10 min), the solution was incubated with phenylalanine decarboxylase in sodium citrate (pH 6·3, 1·5 mol/l, 52°C) as described previously.[13] The β-phenylethylamine formed was solvent, then acid, extracted and assayed by scintillation spectrophotometry (for radioactivity) and fluorimetry (for β-phenylethylamine).

Buffers containing the extracted protein fractions were initially precipitated with 0·33 mol/l trichloracetic acid. After centrifugation (2000 g, 10 min), the protein pellets were washed with 12–14 ml 0·2 mol/l perchloric acid. The protein was then digested in 10 ml 0·3 N NaOH (1 h, 37°C) to solubilise all the protein and an aliquot (1 ml) removed for subsequent assay of total protein content as described by Gornall.[14] The alkaline digest was reprecipitated in 2 ml 20% perchloric acid. After centrifugation (2000 g, 10 min), the protein pellet was washed a further six to eight times with 0·2 mmol/l perchloric acid.

TABLE I *Effect of chronic ethanol feeding on protein composition in the small intestine of the rat*

		Immature rats				*Mature rats*			
Parameter	*Protein fraction*	*Control*	*Ethanol*	*Difference between means %*	*Statistical significance (p)*	*Control*	*Ethanol*	*Difference between means %*	*Statistical significance (p)*
Protein concentration (mg/g wet weight)	Cytoplasmic	23·9 (1·0)	24·5 (1·0)	+2	0·710	31·1 (2·1)	25·5 (1·7)	−18	0·092
	Contractile	7·5 (0·9)	6·3 (0·6)	−16	0·028	9·3 (0·7)	7·4 (0·4)	−20	0·019
	Stromal (S_B)	4·3 (0·5)	3·9 (0·9)	−9	0·595	5·1 (0·3)	5·6 (0·5)	+10	0·441
	Total	35·7 (1·9)	34·6 (1·5)	−3	0·440	44·1 (3·5)	37·5 (2·5)	−15	0·125
	Recovery (%)	65 (2)	66 (2)	+1	0·659	75 (14)	67 (3)	−11	0·118
Total protein (mg)	Cytoplasmic	234 (7)	193 (11)	−18	0·007	320 (22)	240 (14)	−25	0·035
	Contractile	72 (7)	49 (5)	−31	0·003	95 (7)	69 (3)	−27	0·005
	Stromal	41 (4)	30 (7)	−26	0·130	52 (3)	53 (5)	+1	0·913
Total protein/ body weight (mg/kg)	Cytoplasmic	1040 (37)	991 (51)	−5	0·301	820 (57)	669 (46)	−18	0·080
	Contractile	319 (27)	252 (22)	−21	0·010	243 (18)	192 (10)	−21	0·012
	Stromal	185 (18)	155 (33)	−16	0·341	132 (7)	147 (14)	+10	0·471

Male Wistar rats were fed a control or ethanol containing liquid diet for six weeks as described in Methods. Cytoplasmic, contractile and stromal proteins were separated on the basis of differential solubility. All data are presented as mean (SEM) of six to eight observation. Differences between control and ethanol fed rats were assessed by paired statistics (Student's *t* test as all differences were normally distributed).

TABLE II *Effect of chronic ethanol feeding on specific radioactivities and fractional synthesis rates in small intestine protein fractions*

Parameter	Protein fraction	Immature rats: Control	Immature rats: Ethanol	Immature rats: Difference between means %	Immature rats: Statistical significance (p)	Mature rats: Control	Mature rats: Ethanol	Mature rats: Difference between means %	Mature rats: Statistical significance (p)
Specific radioactivities (dpm/nmol)	Cytoplasmic (S_B)	1.515 (0·069)	1·440 (0·030)	−5	0·394	1·247 (0·081)	1·338 (0·040)	+7	0·413
	Contractile (S_B)	0·738 (0·023)	0·674 (0·054)	−9*	0·673	0·679 (0·035)	0·592 (0·043)	−13	0·068
	Stromal (S_B)	0·103 (0·029)				ND	ND		
	Tissue supernatant (S_i)	68 (2)	65 (5)	−4	0·586	64 (9)	69 (4)	+7	0·538
	Plasma (S_p)	189 (2)	190 (4)	0	0·816	181 (11)	191 (2)	+5	0·418
k_s, calculated from S_i (%/day)	Cytoplasmic	178 (12)	183 (17)	+3	0·754	193 (46)	157 (12)	−18	0·369
	Contractile	86 (4)	84 (8)	−2	0·853	103 (19)	71 (7)	−31	0·162
	Stromal	12 (4)				ND	ND		
k_s, calculated from S_p (%/day)	Cytoplasmic	63 (3)	60 (2)	−4	0·582	56 (5)	56 (1)	0	0·891
	Contractile	31 (1)	28 (2)	−7*	0·933	31 (1)	24 (2)	−22	0·021
	Stromal	4 (1)				ND	ND		

Rates of protein synthesis were measured by injection of a large concentration of L[4-^{3}H] phenylalanine, at the end of six weeks liquid feeding, as described in Methods. All data are presented as mean (SEM) of four to seven observation. Differences between means were assessed by paired statistics.
*Indicates statistical significances were evaluated by Wilcoxon's signed-rank test, otherwise all other significances were obtained by Student's *t* test.
ND=no counts were detectable in protein hydrolysates.

The protein pellets were then hydrolysed in 6 mol/l HCl (36 h, 105°C) and the hydrolysates dried over solid P_2O_5 and NaOH *in vacuo*. The residues were suspended in 2 ml sodium citrate buffer (1·5 mol/l, pH 6·3), incubated with phenylalanine decarboxylase and solvent then acid extracted.[15] The acid phase was assayed by scintillation spectrophotometry and fluorimetry to obtain the specific radioactivity of phenylalanine in intestinal protein (S_B, which was defined as the specific radioactivity of phenylalanine in protein hydrolysates).

CALCULATION OF SYNTHESIS RATES

Fractional rates of protein synthesis (k_s), were defined as the percentage of tissue protein renewed each day by synthesis, and calculated from the formula

$$k_s = \frac{S_B \times 100}{(S_p \text{ or } S_i) \times t}, \text{ (\%/day)}$$

where the incorporation period of radioisotope (t) was the period between injection of isotope and immersion of the entire small intestine in the mixture of ice and water.[15]

Absolute rates of protein synthesis – that is, V_s, – were defined as the total amount of protein synthesised per day (mg/d) and was calculated from k_s and total protein content.

STATISTICAL ANALYSIS

As a pair feeding regime was used on rats from a single batch of animals and rats were paired before starting the experimental treatments, differences between means were assessed with Student's *t* test for paired samples as described by Snedecor and Cochran.[15] The conditions for using paired statistics in animal studies are discussed by these authors. This was only applied to data where differences between pairs of observations were normally distributed. When differences were non-normally distributed the Wilcoxon's signed-rank-test was used.[15] All data are presented as mean (SEM) of four to eight pairs of observations.

Results

PROTEIN COMPOSITION IN CONTROL AND ETHANOL FED RATS

The protein concentrations of cytoplasmic, contractile and stromal fractions are displayed in Table I. Inspection of data for control animals showed that the amount of protein in the cytoplasmic fraction was considerably greater – that is, three to four times – than the amount of protein in the contractile protein fraction of both immature ($p<0·001$) and mature ($p<0·001$) rats. The amount of contractile protein was also greater than the stromal protein in immature ($p<0·0001$) and mature ($p<0·001$) rats. This is

TABLE III *Effect of chronic ethanol feeding on fractional and absolute rates of protein synthesis (V_s) in fractions of the small intestine.*

Parameter	Protein fraction	Immature rats: Control	Immature rats: Ethanol	Immature rats: Difference between means %	Immature rats: Statistical significance (p)	Mature rats: Control	Mature rats: Ethanol	Mature rats: Difference between means %	Mature rats: Statistical significance (p)
V_s, from S_i, (mg/day)	Cytoplasmic	419 (37	392 (55)	−6	0·549	601 (131)	374 (24)	−38	0·106
	Contractile	60 (6)	42 (6)	−31	0·045	94 (14)	49 (4)	−48	0·033
	Stromal	6 (3)				ND	ND		
V_s, from S_p, (mg/day)	Cytoplasmic	146 (8)	121 (10)	−17	0·021	180 (19)	133 (5)	−26	0·061
	Contractile	21 (2)	13 (2)	−37	0·020	30 (3)	17 (1)	−43	0·006
	Stromal	2 (1)				ND	ND		
V_s per unit body weight, from S_i (mg/day/kg)	Cytoplasmic	1885 (170)	1994 (286)	+6	0·679	1524 (319)	1042 (80)	−32	0·143
	Contractile	269 (28)	210 (28)	−22	0·134	237 (29)	136 (15)	−43	0·034
	Stromal	28 (16)				ND	ND		
V_s per unit body weight, from S_p (mg/day/kg)	Cytoplasmic	656 (38)	617 (53)	−6	0·471	461 (48)	370 (19)	−20	0·115
	Contractile	95 (10)	67 (10)	−29	0·052	76 (6)	47 (4)	−38	0·008
	Stromal	9 (5)				ND	ND		

Details are described in the caption to Tables I and II. All data are presented as mean (SEM) of between four to seven observations. Differences between means were assessed by paired statistics using Student's *t* test as all differences between treatments were normally distributed.
ND=no counts detected in hydrolysates.

compatible with the various histological studies which showed that the muscle layers constitute a small proportion of the small intestine, with the connective tissue contributing a relatively minor portion of the tissue bulk. The extraction procedure however, will also solubilise the actin and myosin in microfilaments of the intestinal villi. The recoveries of the extraction procedures were between 65 and 75% of total intestinal protein. Although this figure was lower than expected, the losses were spread over the three fractions and presumably occurred during the numerous stages of acid precipitation and washing of the protein pellet. There was no difference in recoveries between control and treated groups.

Chronic ethanol feeding caused significant reductions in the concentrations (mg/g wet weight) of contractile proteins in both immature and mature rats but not in any of the other fractions. The concentration of protein in the stromal fraction of the immature rats and in both the cytoplasmic and stromal fractions of the mature rats were not significantly altered by ethanol feeding. The total protein contents of both cytoplasmic and contractile protein fractions were significantly smaller in ethanol-fed rats of both groups, by between 18–31%, but changes in the stromal protein fractions of mature rats were negligible. Although there was an apparent decrease in the total protein content of the stromal fraction of immature rats of 26% this did not reach statistical significance as one of the seven pairs of alcohol fed rats showed an increase. When total protein contents were expressed relative to body weights, statistically significant reductions occurred for the contractile protein fraction only, by 21%.

EFFECT OF ETHANOL FEEDING ON RATES OF SYNTHESIS

Specific radioactivities of phenylalanine in protein hydrolysates are shown in Table II. An important feature of these data was the observations that only very low levels of radioactivity could be assayed in the stromal protein fraction in immature rats whilst in mature rats, no radioactivity could be found. In immature rats sufficient radioactivity could only be obtained for four control and two treated rats, so data relating only to control rats are presented. In control rats fractional rates of protein synthesis were considerably higher in the cytoplasmic fraction than in the contractile protein fraction which was in agreement with our previous observations.[9] In response to ethanol feeding the fractional synthesis rates of cytoplasmic and contractile protein fractions were unaltered in immature rats. In mature rats, synthesis rates were reduced slightly, but these differences only reached statistical significance for the contractile fraction when k_s was calculated from S_p.

Table III shows that as a result of reduced protein contents, absolute rates of protein synthesis absolute rate of protein synthesis (V_s) were reduced by ethanol feeding, particularly in the contractile protein fraction of both immature and mature rats. Absolute synthesis rates relative to body weight were similarly reduced by chronic ethanol feeding, but the magnitude of the effects was much smaller. The same directional changes in protein synthesis as a result of ethanol feeding, were obtained when data were calculated on the basis that the precursor amino-acids were derived from extracellular compartments – that is, by substituting S_i by S_p (defined as the specific radioactivity of free phenylalanine in plasma) in the formula used to calculate synthesis.

Discussion

The rationale for using immature and mature rats requires a brief discussion. Basically, the standard experimental technique within our research group is to use either 80–110 g or 250–300 g sets of animals for a variety of alcohol feeding studies. These include the development of skeletal muscle myopathy[16] and of fatty liver.[17] A frequent criticism of using small, rapidly growing rats, however, is that they bear no relation to the clinical situation and therefore data derived from such studies may be difficult to interpret. The rationale for their continued use in nutritional studies is that pathophysiological effects in response to experimental treatments are often readily manifested in young, rapidly growing, rats and alcohol consumption in growing adolescents is increasingly becoming a major problem.

The data showed that chronic ethanol feeding reduced the protein content of the various fractions of the small intestine, particularly the contractile protein fraction. This was observed in both immature and mature rats. The rates of protein synthesis were therefore examined using an accurate and reliable technique, the large 'flooding-dose' technique of McNurlan *et al*[18] as adapted by Garlick *et al*[13] for phenylalanine. The theory behind the measurement of protein synthesis *in vivo* has been adequately reviewed by Waterlow *et al*[19]

One of the main advantages of the flooding dose technique is the fact that differences between S_i and S_p are minimised, thus reducing uncertainty as to the true synthesis rate of the tissue. In this study, however, k_s calculated from S_p was a third of the value of k_s calculated from S_i. This contrasts with the relative values of S_i and S_p in tissues with low turnover rates, such as skeletal muscle, in which the ratio S_i/S_p approaches unity.[13] It is possible that in this study S_i and S_p differed due to the route of administration of the isotope. Reeds *et al*[20] however, also injected a large concentration of [^{3}H] phenylalanine intraperitoneally, and S_i was only 60% of the value of S_p at the end of synthesis. Furthermore, we have shown that in 48 h starved rats the value of S_i at the end of synthesis was approximately 50% of S_p, even though the isotope was administered intravenously.[21] It is very likely that there may be a range of values for the S_i/S_p ratio, which are dependent on the concentration of endogenous phenylalanine or the rate of protein breakdown or even phenylalanine derived from the digestion of the diet. For this reason synthesis data – that is, k_s and V_s, have been presented as calculated from both S_i and S_p.

In control animals, the small intestine showed

considerable heterogeneity in synthesis rates. The fractional synthesis rate in the cytoplasmic fraction was approximately twice that in the contractile protein fraction. These differences between fractions are in agreement with our previous studies which showed that in normal chow fed rats, synthesis rates were also higher in the cytoplasmic fraction, when compared with the contractile fraction.[9] An interesting and additional observation, however, was that protein synthesis in the stromal fraction was considerably less than either contractile or cytoplasmic fraction. This is quite different to skeletal muscle, where synthesis rates in the stromal fraction is of the same order of magnitude as synthesis rates in the contractile fraction.[16] A possible explanation for this difference between tissues is that the same extraction procedures applied to different muscle types solubilise different protein components. This difference between smooth and skeletal muscle obviously requires further investigation. It may be related to the types of proteins contained in the stromal fraction.

It is possible that the failure of chronic ethanol feeding to alter fractional synthesis rates of intestinal protein in small rats (which showed the greatest reductions in protein content) may have been caused by methodological errors or limitations. The technique we used, however, to measure synthesis is the most accurate currently available. Thus, we are left with two other possibilities; the first is that large changes in fractional rates of protein synthesis occurred during the initial stages of the ethanol feeding. It is important to note that in acute studies the fractional rate of protein synthesis of contractile proteins decline by approximately 40% in response to 2½ h ethanol exposure in immature rats.[9] This implies that subsequent reductions in protein synthesis in response to ethanol feeding may have abated, because of a biochemical adaptation. It is noteworthy that in this study the absolute rates of protein synthesis, either *per se* or relative to body weight, were considerably reduced by chronic ethanol feeding, in both immature and mature animals. The second possibility, of course, is that ethanol feeding increased the rate of intestinal protein breakdown. This is entirely consistent with the concept of protein turnover, which implies that changes in protein content must be due to alterations in synthesis and/or degradation.[19] The difficulties lie in measuring the rate of protein degradation. There are no reliable methods for directly measuring this process *in vivo*. Estimation of protease activity may provide speculative information in this regard, but will only indicate the tissues capacity for proteolytic activity *in vitro* and not its dynamic rate *in vivo*. If the rate of protein breakdown is increased by ethanol feeding then the data also indicate that ethanol may cause preferential degradation of contractile proteins compared with the cytoplasmic function – that is, independent control mechanisms, to explain the selective decrease in contractile protein content. Studies in both heart[22] and skeletal muscle[23] have supported the contention that protein degradation of the myofibrillar protein fractions may be regulated separately from other protein fractions, but there is no evidence for a similar phenomena in smooth muscle.

The reason for the differences in the magnitude of the alcohol induced effects between immature and mature rats is difficult to ascertain. A plausible explanation is that blood alcohol concentrations attain higher levels in immature rats than in mature rats.[24] Differences in alcohol concentration, or its metabolites such as acetaldehyde, within different regions of the small intestine may be another contributing factor. If the changes in protein turnover were mediated by humoral factors then different types and numbers of receptors in different regions may also explain differential sensitivity.

As far as we are aware, this is the first report showing a fall in the total amount of relatively purified contractile protein in the entire small intestine of an experimental animal in response to a chronic pathophysiological stimuli, albeit an adverse one. The results indicate a preferential reduction in the amount of contractile protein, when compared with other protein fractions. The experimental observations also implies early adaptive responses or the involvement of enhanced degradative pathways in response to ethanol. If similar changes in contractile protein content were to occur in the chronic alcohol abuser then further studies must be directed to the hypothesis that preferential effects on the contractile apparatus may be a causal factor for gastrointestinal disturbances.

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1 Levi AJ, Chalmers DM. Recognition of alcoholic liver disease in distinct general hospital. *Gut* 1978; **19**: 521–5.
2 Lindenbaum J, Lieber CS. Effect of chronic ethanol administration on intestinal absorption in man in the absence of nutritional deficiency. *Ann NY Acad Sci* 1975; **252**: 238–44.
3 Roggin GM, Iber FL, Kater RMH, *et al.* Malabsorption in the chronic alcoholic. *Johns Hopkins Med J* 1969; **125**: 321–30.
4 Baraona E, Pirola RC, Lieber CS. Small intestinal damage and changes in cell population produced by ethanol ingestion in the rat. *Gastroenterology* 1974; **66**: 226–34.
5 Bjarnason I, Smethurst P, Levi AJ, *et al.* Intestinal permeability to ^{51}Cr-EDTA in rats with experimental induced enteropathy. *Gut* 1985; **26**: 579–85.
6 Mazzanti R, Jenkins WJ. Effect of chronic ethanol ingestion on enterocyte turnover in rat small intestine. *Gut* 1987; **28**: 52–5.
7 Robles EA, Mezey E, Halsted CH, *et al.* Effect of ethanol on motility of the small intestine. *Johns Hopkins Med J* 1974; **135**: 17–24.
8 Angel F, Sava P, Crenner F, *et al.* Modifications de la mortricité intestinale apres injection intravelneuse d'alcool. *C R Soc Biol* (Paris) 1980; **174**: 192–8.
9 Preedy VR, Duane P, Peters TJ. Acute ethanol dosage reduces the synthesis of smooth muscle contractile proteins in the small intestine of the rat. *Gut* 1988; **29**: 1244–8.
10 Preedy VR, Duane P, Peters TJ. Biological effects of chronic ethanol consumption. A reappraisal of the Lieber-De Carli liquid diet model with reference to skeletal muscle. *Alcohol Alcohol* 1988; **23**: 151–4.
11 De Carli LM, Lieber CS. Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate new liquid diet. *J Nutr* 1967; **91**: 331–6.
12 Lieber CS, Jones DP, De Carli LM. Fatty liver, hyperlipemia and hyperuricaemia produced by prolonged alcohol consumption despite adequate dietary intake. *Trans Assoc Am Physicians* 1963; **76**: 289–300.
13 Garlick PJ, McNurlan MA, Preedy VR. A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [^{3}H]phenylalanine. *Biochem J* 1980; **192**: 719–23.
14 Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. *J Biol Chem* 1949; **177**: 751–66.
15 Snedecor GW, Cochran WG. *Statistical methods.* 6th edition. USA: Iowa State University Press 1976.
16 Preedy VR, Peters TJ. Acute effects of ethanol on protein synthesis in different muscles and muscle protein fractions of the rat. *Clin Sci* 1988; **74**: 461–6.
17 Venkatesan S, Ward RJ, Peters TJ. Fatty acid synthesis and triacylglycerol accumulation in rat liver after chronic ethanol consumption. *Clin Sci* 1987; **73**: 159–63.

18 McNurlan MA, Tomkins AM, Garlick PJ. The effect of starvation on the rate of protein synthesis in rat liver and small intestine. *Biochem J* 1979; **178**: 373–9.
19 Waterlow JC, Garlick PJ, Millward DJ. Protein turnover in mammalian tissues and in the whole-body. Amsterdam: North-Holland 1978.
20 Reeds PJ, Haggarty JP, Wahle KWJ, Fletcher JM. Tissue and whole-body protein synthesis in immature Zucker rats and their relationship to protein deposition. *Biochem J* 1982; **204**: 393–8.
21 Preedy VR, Paska L, Sugden PH, Schofield PS, Sugden MC. The effects of surgical stress and short-term fasting on protein synthesis *in vivo* in diverse tissues of the mature rat. *Biochem J* 1988; **250**: 179–88.
22 Smith DM, Sugden PH. Contrasting response of protein degradation to starvation and insulin as measured by release of N^1 methylhistidine or phenylalanine from the perfused rat heart. *Biochem J* 1986; **237**: 391–5.
23 Lowell BB, Ruderman NB, Goodman MN. Evidence that lysosomes are not involved in the degradation of myofibrillar proteins in rat skeletal muscle. *Biochem J* 1986; **234**: 237–40.
24 Preedy VR, Peters TJ. The effect of chronic ethanol feeding on body and plasma composition and rates of skeletal muscle protein turnover in the rat. *Alcohol Alcohol* 1988; **23**: 217–24.