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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATION

The Three Hundred and Forty-fourth Meeting of the Nutrition Society was held at the Royal Society of Medicine, London on Thursday 22nd May 1980, *when the following papers were read:*

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The effect of protein content and meal frequency of isoenergetic diets on nitrogen loss and weight loss of obese inpatients. By MERRIL L. DURRANT, SANDRA E. BLAZA, SHIRLEY SUNKIN, DEBORAH Y. WILKINS and J. S. GARROW, *Clinical Research Centre, Watford Road, Harrow, Middlesex*

Thirty-eight obese subjects were admitted to a hospital metabolic unit for a 3 week course of weight reduction. Mean age (±SD) was 39±13 years; mean initial weight was 99·8±19·7 kg and obesity index 37·3±6·8 kg/m^2.

Subjects were given isoenergetic diets of mean energy content 3·3±0·2 MJ (780±39 kcal). All subjects had a diet containing 13 protein energy per cent (13PE) with three meals (3M) in week 1. Ten subjects ate 15 protein energy per cent (15PE) or 10 protein energy per cent (10PE) in weeks 2 and 3. Meal frequency was held constant (3M). Fourteen subjects ate five meals/d (5M) or one meal/d (1M) in weeks 2 and 3. Protein content was held constant (13PE). Fourteen subjects ate 15PE and 5M or 10PE and 1M in weeks 2 and 3. On all trials weeks 2 and 3 were cross over designs alternated for every patient.

Patients were weighed fasting each weekday morning on a beam balance to an accuracy of ±50 g. Nitrogen output was measured by Kjeldahl analysis of urine and stool. 1% samples of the 24 h collections were pooled and analysed at the end of each 7 d test period. N balance was derived by subtracting N output from N input (samples of food checked against food tables by Kjeldahl analysis and stored for trials). The protocol was approved by the Northwick Park Hospital Ethical Committee.

Test week					Nitrogen balance (g/d)			Weight loss (g/d)		
1	2	3	*n*		1	2	3	1	2	3
13PE	15PE	10PE	10	Mean	−4·34	−1·52**	−2·51	326	176	226
3M	3M	3M		SD	0·82	0·75	0·64	96	107	106
13PE	13PE	13PE	14	Mean	−4·11	−1·30***	−2·07	353	224	255
3M	5M	1M		SD	1·69	1·38	2·34	154	74	107
13PE	15PE	10PE	14	Mean	−3·76	−0·62***	−2·66	332	193*	271
3M	5M	1M		SD	1·57	1·15	1·68	158	63	110

*P<0·05, **P<0·01, ***P<0·001.

N loss was significantly less in the high-protein than the low-protein week (P<0·01) and in the five meals/d than the one meal/d week (P<0·001). Combining high-protein diet with frequent meals reduced N loss even further. Weight loss was lower in the high-protein and frequent-meal experiments although this difference reached significance only in the combined experiment (P<0·05). Despite the slightly lower weight loss, the high-protein, frequent-meal diet is more beneficial owing to the more favourable composition of the weight lost.

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The voluntary food intake of young growing pigs given diets containing a high proportion of rapeseed meal. By R. HILL and PAULINE LEE, *The Royal Veterinary College, Boltons Park, Potters Bar, Herts*

The low acceptability by young animals of diets containing rapeseed meal has been recognized for a number of years (Bowland, 1965). In this study with growing pigs, the initial object was to compare voluntary food intake for diets containing rapeseed meals from four well-defined sources of seed; (1) British *B. napus*, (2) Span, *B. campestris* variety grown in Canada, (3) Tower, new Canadian variety, (4) Erglu, new European variety. The diets contained 250 g rapeseed meal/kg and met the recommended nutrient allowances of the Agricultural Research Council (1967). The control diet contained soya-bean meal in place of rapeseed meal.

The diets were given singly, one day at a time to each group of pigs and the daily pattern of feeding was such that the mean intake of any particular diet was not biased by the diet given on the previous day.

The quantity of diet given at each feed, twice daily, was the weight of the control soya bean diet eaten readily in 30 min. The acceptability of the diet was measured as the proportion of the weight offered, eaten in 30 min. The adjusted means from eight experiments and *t* values versus soya bean are given in the Table.

	Soya bean	British	Tower	Span	Erglu
Adjusted mean	0·92	0·65	0·85	0·81	0·95
t		7·3	2·8	3·0	0·9

The results show that a diet containing rapeseed meal from British seed as the protein supplement was much less readily accepted by growing pigs than a similar diet based on soya-bean meal. Meals from the new varieties, Tower and Erglu, were superior to that from British seed.

In similar experiments, flavouring substances (molassine meal, sucrose and four proprietary preparations) were added to the British rapeseed meal. One of these improved intake but in general, the results showed that the effects of the substances responsible for low intake were not readily masked by commonly used flavouring supplements.

Substances present in rapeseed meal that may be associated with low palatability are glucosinolates, tannins and sinapine. From the results of analyses of the four rapeseed meals used in this study, of these three substances, glucosinolates seemed most likely to be associated with reduced food intake.

Agricultural Research Council (1967). *Nutrient requirements of farm livestock, no. 3 Pigs.* London: Agricultural Research Council.

Bowland, J. P. (1965). *Canada Department of Agriculture,* Publ. 1257, p. 69. Ottawa, Canada.

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The effects of treatments of rapeseed meal and of extracts of the meal on the voluntary food intake of young growing pigs. By PAULINE LEE, SHARON PITTAM and R. HILL, *The Royal Veterinary College, Boltons Park, Potters Bar, Herts*

The low palatability to young pigs of rapeseed meals from seed of *B. napus* varieties grown in Britain may be associated with their high glucosinolate content and possibly the presence of sinapine and tannin (Hill & Lee, 1980). Treated meals from British *B. napus* seed and extracts of these meals were prepared and used in feeding experiments to provide more specific information on the relation between food intake and the presence of 'toxic' substances. The treated meals were dried and included at 250 g/kg diet and compared with similar diets containing untreated meal or soya-bean meal. The extracts were added to the soya-bean meal diet and compared with a similar mix containing water.

Batches of rapeseed meal were extracted from hot water either once or twice to reduce the concentrations of glucosinolates and sinapine, and others were treated with calcium hydroxide and water to reduce the concentration of sinapine. Intakes of these diets were determined in the manner described earlier (Hill & Lee, 1980).

Soya bean	British untreated	British extracted x1	British extracted x2
0·93	0·54	0·67	0·82

Soya bean	British untreated	British treated with calcium hydroxide
0·96	0·64	0·71

Water extraction increased intake markedly and significantly while the increase after calcium hydroxide treatment was small and non-significant. The proportions of glucosinolate and sinapine removed by water extraction were about 80 and 95% respectively and the corresponding values for calcium hydroxide treatment were about 25 and 80% respectively.

Water extracts of British meal were prepared and divided into three, one remained untreated, one was passed through anion exchange resin to remove glucosinolates and the other through cation exchange resin to remove sinapine. Intakes of the soya-bean meal diet with water, untreated extract, anion treated and cation treated extracts were 0·96, 0·45, 0·98 and 0·47 respectively.

The untreated extract caused a pronounced and significant reduction in food intake, and the anion exchange resin removed this effect while the cation resin did not. Analyses of the extracts for glucosinolate showed that the high concentration in the untreated extract was greatly reduced by the anion resin and unaltered by the cation resin. These results provide further evidence for the proposal that the glucosinolates of rapeseed meal are a major factor reducing its palatability.

Hill, R. & Lee, P. (1980). *Proc. Nutr. Soc.* **39**, 75A.

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An epidemiological study of the relation between milk consumption and ischaemic heart disease. By J. J. SEGALL, (Introduced by PATRICIA P. SCOTT), 308 *Cricklewood Lane, London NW2 2PX*

The dietary habits of patients suffering from ischaemic heart disease (IHD) were studied in a North London practice, and showed a relation between IHD and milk intake (Segall, 1978). The author therefore examined the relation between available information (United Nations, 1972, 1973; OECD, 1975; World Health Organization, 1976; Simoons, 1978) on milk consumption and some other related nutritional factors with epidemiological information on IHD (see Table). Knox (1977) has suggested that correlations between consumption of particular foodstuffs and disease, having a correlation coefficient greater than 0·7, might be causal.

Countries	No. of countries	Nutritional factor (per capita)	IHD	Correlation coefficient (r)
Global	43	Total milk	MR	0·75**
	43	Energy value	MR	0·72**
OECD	21	Total milk	MR	0·72**
	21	Butter	MR	0·50*
European, Australia & Israel	21	Total milk	Attacks	0·79**
Global	23	LA (%)	MR	0·73**

Total milk, total dairy products excluding butter.
MR, national mortality rates for IHD.
OECD countries; West European, Australia, Canada, Japan, New Zealand, USA.
Attacks, age-standardized myocardial infarction attack rates in defined areas (WHO).
LA, ethnic prevalence of lactose absorbers in adults, matched to countries.
*$P<0.05$; **$P<0.001$.

There is an indication from the results that the correlation of total milk consumption with IHD, if causal, may not be due solely to dairy fats; lactose and the contribution of milk to dietary energy value are other possible factors.

Knox, E. G. (1977). *Br. J. prev. soc. Med.* **31**, 71.
Segall, J. J. (1978). *Br. J. Clin. Pract.* **32**, 15.
Simoons, F. J. (1978). *Am. J. dig. Dis.* **23**, 963.
OECD (1975). *Food Consumption Statistics.*
United Nations (1972). *Statistical Yearbook*, p. 524.
United Nations (1973). *Demographic Yearbook*, p. 322.
World Health Organization (1976). *Myocardial infarction community registers.*

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Quantification of protein, fat and lactose concentrations in milk samples using a liquid scintillation counter. By R. C. NOBLE and J. H. SHAND, *Hannah Research Institute, Ayr KA6 5HL*

The use of a liquid scintillation counter in the quantification of coloured solutions has recently been highlighted (Noble *et al.* 1979, 1980). This has been achieved by the insertion of a sealed radioactive standard of known count rate into the centre of a standard liquid scintillation vial; using the space between the outer vial and the sealed miniature standard to contain the coloured solution, attenuation of count rate provides an accurate means of quantification.

Major amongst the several advantages that are inherent in quantification using a liquid scintillation counter is the ability to measure without dilution a range of substrate concentrations considerably in excess of that possible by means of a spectrophotometer. The analysis of milk for its content of protein, fat and lactose ranges from the use of dedicated and expensive instrumentation to a variety of separate and often time-consuming techniques usually based on entirely different analytical parameters. Using the procedure involving the liquid scintillation counter as outlined above, it was felt that a series of simplified methods could be evolved that would be able to provide a combination of technical simplicity, accuracy and automation to the analysis of milk protein, fat and lactose concentrations.

Quantification in each case was based on a colorimetric procedure, that of protein through the formation of a blue biuret complex, fat by the formation of an opalescent emulsion containing a water soluble yellow dye and lactose by the formation of a yellow lactosazone. Adaptation of the procedures to meet the requirements of the quantifying process were minimal whilst, through the inherent ability of the process to accommodate extremes of substrate concentration, simplification of procedure was able to be adopted and exploited. In the case of fat and lactose determinations, highly accurate standard curves for substrate concentration were produced from the measurement of the decreased count rate of a ^{14}C scintillation source and that for protein through the use of a ^{3}H source. Accuracy of quantification for the methods was assessed by comparing the results obtained for a series of milks of varying protein, fat and lactose contents with results obtained using recommended standard analytical techniques for milk analysis. Highly comparable results were obtained throughout. In all cases the concentration range covered by the standard curves far exceeded that liable to be encountered in the average milk sample and therefore enabled extremes in composition to be readily quantified, e.g. concentration of fat in excess of 8 g per 100 ml were easily accommodated. Through the use of the method full advantage could be taken of the present state of sophistication of modern liquid scintillation spectrometry.

Noble, R. C., Shand, J. H. & West, I. G. (1979). *Lab. Pract.* **28**, 393.
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An assessment of the nutritional status of preschool vegan children. By REBECCA PURVES and T. A. B. SANDERS, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Vegans eschew the use of food of animal origin for ethical reasons and select diets comprising cereals, vegetables, pulses, nuts and fruit. While a vegan diet seems to be adequate for adults providing it is supplemented with vitamin B_{12} (Sanders, 1978), far less is known about its suitability for young children. Four cases of severe malnutrition in infants fed extremely restricted diets led Roberts *et al.* (1979) to conclude that vegan diets were obviously inadequate for growing children. As we had access to a number of vegans with preschool children, we decided to make an assessment of their nutritional status.

Parents were contacted through the Vegan Society and the response was excellent: nineteen families participated in the study. The mothers had all followed a vegan diet during pregnancy and lactation; twelve had been subjects in an earlier study on the effect of a vegan diet on pregnancy and lactation (T. A. B. Sanders, unpublished results). A total of twenty-five children who had been reared on a vegan diet were studied (nine boys and sixteen girls): their average ages were 26 months (11–42) and 30 months (9–55). Families were visited at home and anthropometric measurements were made using standardized techniques and parents were given comprehensive instructions on how to record their child's food intake using a dietary balance. Nutrient intakes were calculated from 7-d-weighed intake measurements and estimates of breast milk supplied where applicable (seven children were still receiving breast milk).

All the children appeared to be healthy and the anthropometric measurements suggested that their growth was normal. However, they did tend to be shorter in stature and lighter in weight when compared with the standards of Tanner *et al.* (1966). All the children had been or were being breast-fed usually well into the second year of life. In most cases, food other than breast milk, generally vegetable and fruit purees and baby cereals, was introduced between 4 and 6 months. Their diets comprised a wide variety of plant foods and were generally supplemented with vitamin B_{12} either as foods fortified with with vitamin, such as Plamil, Barmene and Tastex, or as Cytacon syrup (Glaxo). Although energy and calcium intakes were below those recommended, their diets were generally satisfactory. However, a few children had low intakes of riboflavin and vitamin B_{12}. There is obviously a need for vegan mothers to ensure that their children receive an adequate supply of these vitamins. It is concluded that a properly constructed vegan diet can meet the requirements of preschool children.

We are grateful to the Vegan Society for a grant.

Roberts, I. F., West, R. J., Ogilvie, D. & Dillon, M. J. (1979). *Br. med. J.* **1**, 296.
Sanders, T. A. B. (1978). *Plant Foods for Man.* **2**, 181.
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The effect of altering the linoleic:α-linolenic acid ratio in the maternal diet on foetal brain lipids. By T. A. B. SANDERS and D. J. NAISMITH, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

The consequences of changing the composition of the dietary fat to conform with recommendations for the prevention of coronary heart disease (Royal College of Physicians/British Cardiac Society, 1976) have been studied in the pregnant rat. The brains of foetal rats whose mothers had been fed on a soft margarine, rich in linoleic acid (18:2,n-6), contained only half as much docosahexaenoic acid (22:6,n-3) as did those from animals fed a similar amount of a mixture of lard and butter (Sanders & Naismith, 1979). Docosahexaenoic acid is the major C_{22} polyunsaturated fatty acid in human brain phosphoglycerides (Svennerholm, 1968). It was suggested that a high linoleic:α-linolenic value (18:3,n-3) acid in the diet suppressed the conversion of 18:3,n-3 to 22:6,n-3 by competitive inhibition. In this report, we describe the effects of varying this ratio in the maternal diet on foetal brain lipids.

Virgin rats of the Sprague-Dawley strain were divided into five groups on a littermate basis, mated and transferred from stock to experimental diet and killed on day 22 of pregnancy. Five experimental diets were used and were similar in all respects except for the linoleic:α-linolenic acid ratio. The diets contained (g/kg) casein 200, fat 200, sucrose 500, vitamin and mineral mix 100. Maternal food intakes were similar as were pup body and brain weights. The results of the analysis of foetal brain phosphoglycerides for six litters/group are shown in the Table.

Energy in maternal diet (%)		Foetal brain phosphoglyceride fatty acids (% wt)			
		docosapentaenoic		docosahexaenoic	
linoleic	α-linolenic	Mean	SE	Mean	SE
30·3	0·2	10·0	0·48	5·0	0·21
29·7	0·5	8·7	0·13	6·0	0·16
29·0	0·9	7·1	0·15	8·3	0·21
28·4	1·5	5·1	0·13	10·3	0·15
27·1	2·7	3·9	0·13	11·0	0·15

As the value of linoleic:linolenic was decreased, the proportion of docosahexaenoic acid increased and that of docosapentaenoic acid (22:5,n-6) was correspondingly reduced. Whether this change alters brain function is not known.

We are grateful to the Rank Prize Funds for a grant and to Miss S. K. Rana and Mrs N. Buckland for their technical assistance.

Royal College of Physicians/British Cardiac Society. (1976). *Jl R. Coll. Phycns* **10**, 213.
Sanders, T. A. B. & Naismith, D. J. (1979). *Proc. Nutr. Soc.* **38**, 100A.
Svennerholm, L. (1968). *J. Lipid Res.* **9**, 570.

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Dietary modification of heat acclimatization in the fowl. By A. H. SYKES and A. A. FATAFITAH, *Wye College, University of London, Ashford, Kent TN25 5AH*

Exposure of laying hens to a dry bulb temperature of 38° for 4 h/d brings about a state of acclimatization within 7 to 10 d as shown by a lower mean rectal temperature (T_r) and a lower rate of rise of T_r (RRT_r), (Hutchinson & Sykes, 1953). When groups of six hens resided at a constant temperature (5°, 20° or 30°), except for the periods of heat exposure, they became well acclimatized (RRT_r day 1, 4·6±0·3°/h; day 14, 0·4±0·1°/h); when similar groups of hens were transferred to a colder residential climate (30° to 20° or 30° to 5°) at or shortly before the heat exposure periods, they failed to become acclimatized (RRT_r day 12, 3·6±0·2°/h). The explanation of this was considered to be the higher metabolic rate which followed from the increased dietary energy intake in the cooler climate. If, on transferring hens from 30° to 20°, food intake was restricted to that found at 30°, then normal heat acclimatization was achieved (RRT_r day 7, 0·63±0·1°/h). Fully acclimatized hens, residing at 20°, lost their acclimatization when energy intake was increased following the addition of 75 g corn oil/kg diet (RRT_r before corn oil, 0·6±0·04/h; 4 d after corn oil, 3·5±0·6°/h). As the voluntary food intake decreased, to adjust for the dietary energy concentration, heat tolerance increased until almost complete acclimatization was reached 15 d after the addition of corn oil (RRT_r 0·81±0·03°/h).

AAF is a British Council Scholar on leave from the University of Jordan.

Hutchinson, J. C. D. & Sykes, A. H. (1953). *J. agric. Sci., Camb.* **43**, 294.

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Glucocorticoid administration and muscle protein turnover. By B. ODEDRA,[1] P. C. BATES,[1] M. NATHAN,[2] M. RENNIE[2] and D. J. MILLWARD,[1] [1]*Clinical Nutrition and Metabolism Unit, London School of Hygiene and Tropical Medicine, London NW1 2PE,* [2]*Department of Human Metabolism, University College Hospital, University Street, London WC1*

It is generally agreed that glucocorticoids induce net muscle protein degradation through a suppression of protein synthesis (e.g. Millward *et al.* 1976) but any effects on degradation are more equivocal. N^{τ}-methyl histidine (MH) excretion rates are increased in rats following corticosterone administration (Tomas *et al.* 1979) but this may reflect release from non-muscle sources (Bates *et al.* 1979). We report here on further measurements of muscle protein turnover in rats treated with corticosterone. We have measured protein synthesis and degradation in vivo as previously reported (Millward *et al.* 1976) and the muscle:plasma concentration gradient of free MH as an indirect index of the muscle degradation rate.

Treatment (daily injection)	Body wt		Gastrocnemius wt (g/100 g)	Protein turnover		N^{τ}-methyl histidine: muscle:plasma
	Initial (g)	Final (g)		Synthesis (%/d)	Degradation (%/d)	
Saline	137	200	0·452	11·1	6·6	0·8
Corticosterone (5 mg/100 g)	150	164	0·439	5·6	4·1	2·3
Corticosterone (10 mg/100 g)	140	140	0·393	5·6	8·1	5·5

After six days of treatment of adrenalectomized rats muscle growth was either suppressed (at 5 mg/100 g per d) or actual wasting was induced (at 10 mg/100 g per d). Protein synthesis was equally depressed at each dose, but changes in degradation were dose dependent, being suppressed at the low and elevated at the high dose. However, muscle:plasma concentration gradients of MH were increased at each dose level (as a result of increased intracellular concentrations) indicating a progressive increase in myofibrillar protein degradation. Thus, these results do indicate increased degradation (and depressed protein synthesis) at the highest dose although at the lower dose changes in degradation are still equivocal. This may reflect the fact that changes in the myofibrillar degradation rate are disproportionate to changes in the over-all rate of degradation as we have previously suggested (Bates & Millward, 1978).

Supported by the Muscular Dystrophy Group of Great Britain.

Bates, P. C., Grimble, G. K. & Millward, D. J. (1979). *Proc. Nutr. Soc.* **38,** 136A.
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Sensitivities of rat gastrocnemius and soleus muscles to starvation, insulin and glucagon. By V. R. PREEDY, V. M. PAIN and P. J. GARLICK, *Department of Human Nutrition, The London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

In vivo measurements showed that a 25 h period of starvation caused a 30% fall in the rate of protein synthesis in gastrocnemius muscle of young male rats (body-weight 150–190 g). However, the synthesis rate in the soleus did not alter after 25 h of food deprivation. This difference in response may result from the variable functions of the muscles. The gastrocnemius has equal tonic and phasic capabilities whilst the soleus is predominantly tonic. Continual activity of the soleus in maintaining posture may act protectively towards nutritional stress, (Goldberg, 1979). An additional factor could be that muscles respond differently to the hormonal changes that occur during starvation. To test this hypothesis the relative sensitivities of the gastrocnemius and soleus muscles to insulin and glucagon in the rat hemicorpus (Preedy *et al.* 1979) were examined.

Fractional synthesis rates of mixed muscle proteins (%/d)

Labelling period (min) ...	25 h starved preparations, 15–50						Fed preparations, 15–50				Fed preparations, 50–90			
	Control		+ Insulin 25 mU/ml		+ Glucagon 1 μg/ml		Control		+ Glucagon 1 μg/ml		Control		+ Insulin 25 mU/ml	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Soleus	24·3	1·2	27·8	3·9	24·6	1·5	24·2	1·4	23·8	1·3	17·7	2·1	22·7	2·9
Gastrocnemius	7·4	0·8	11·0	1·1*	6·9	0·4	10·8	0·8	9·1	0·4*	6·4	0·8	12·4	0·5**

*$P<0.05$, **$P<0.001$ (Student's t test; treatment *v.* control).

The results demonstrate that in preparations from starved rats the gastrocnemius muscle was more responsive to insulin than the soleus, when synthesis was measured between 15 and 50 min of perfusion. When glucagon was added to these preparations the gastrocnemius also appeared to be more sensitive than the soleus, although the fall in synthesis in the gastrocnemius was not statistically significant. However, when glucagon was added to preparations from fed rats there was a significant decrease in the synthesis of gastrocnemius muscle proteins while the soleus remained unaffected. The effect of insulin was also examined in fed preparations but because previous publications indicated that muscle from fed animals was insensitive to insulin during the first stages of perfusion (Jefferson *et al.* 1976) a 50–90 min period of study was selected. The results showed the gastrocnemius to be more sensitive to the effects of insulin than soleus.

In conclusion, protein synthesis in soleus is less sensitive to starvation than in gastrocnemius muscle. This could result from different sensitivities to the changing hormonal levels that occur during starvation.

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Jefferson, L. S., Li, J. B. & Rannels, S. R. (1977). *J. biol. Chem.* **252**, 1476.
Preedy, V. R., Pain, V. M. & Garlick, P. J. (1979). *Biochem. Soc. Trans.* **7**, 1040.

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