The effects of dietary energy restriction on overloaded skeletal muscle in rats‡

Khalid Almurshed† and Katharine Grunewald*

Department of Human Nutrition, Kansas State University, Manhattan, KS 66506-1407, USA

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We evaluated the effects of three levels of energy intake, 73 % (CON73), 81 % (CON81) and 100 % (CON100) of the *ad libitum* intake of the control diet, on skeletal muscle growth induced by functional overload in male rats. Unlike most previous studies which have employed chronic or acute food restriction where all nutrients are reduced in the diet, the present study tested the effects of energy deprivation as a single factor without inducing other nutritional deficiencies. Muscular growth of plantaris and soleus muscles was induced by removal of synergist gastrocnemius muscles in one hindlimb; muscles in the other leg were used as sham-operated intra-animal controls. After 30 d, rats on the energy-restricted CON73 and CON81 diets gained less weight and had smaller livers, kidneys, hearts and fat pads (epididymal, retroperitoneal and omental) than CON100 rats ($P < 0{\cdot}05$). They also had smaller sham-operated plantaris muscles (CON73 −13 %, CON81 −9 %) containing less total protein (CON73 −14 %; CON81 −10 %) than CON100 rats ($P < 0{\cdot}05$). However, the same measurements in overloaded plantaris muscles were similar among groups. Soleus muscle mass and protein contents were not significantly affected by energy restriction in our study. Percentage distributions of myosin heavy-chain isoforms (types I, IIa, IIx and IIb) were similar among rats in CON100, CON81 and CON73 groups for both plantaris and soleus muscles. We conclude that the growth reduction of plantaris muscle induced by energy restriction at 73 % and 81 % for 30 d was prevented by functional overload in male rats.

Dietary energy: Skeletal muscle: Muscular overload

Growth of skeletal muscle is a normal biological process that occurs in man and other animal species (Young, 1970; Goldspink, 1980; Layman *et al.* 1980). However, muscular growth can be reduced or compromised by a nutritionally-inadequate diet. For example, a reduction in energy intake decreases several indices of muscular growth, including overall size (Hegarty & Kim, 1981; Bedi *et al.* 1982; Lewis & Sieck, 1992), cross-sectional area (Glore & Layman, 1983; Lanz *et al.* 1992; Lewis & Sieck, 1992) and functional characteristics such as contractile and relaxation properties (Lewis *et al.* 1986; Nishio & Jeejeebhoy, 1991), aerobic capacity (Layman *et al.* 1981) and resistance to fatigue (Barclay & Loiselle, 1992). Restricted food intake of rats resulted in reduced muscle contents of total protein (Howarth & Baldwin, 1971; Glore & Layman, 1983; El Haj *et al.* 1986; Ocken & Grunewald, 1988) and myofibrillar protein (Mosoni *et al.* 1996). Effects are generally more severe for fast-twitch than slow-twitch muscles and muscle fibres (Li & Goldberg, 1976; Hansen-Smith *et al.* 1978; Goodman *et al.* 1981; Layman *et al.* 1981; Bedi *et al.* 1982).

Muscular growth can also be induced to varying degrees by the addition of load through exercise programmes or other experimental interventions. For example, plantaris or soleus muscles are forced to grow by functional overload after tenotomy or removal of synergist muscles (Noble *et al.* 1984; Tsika *et al.* 1987; Ianuzzo *et al.* 1991; Sugiura *et al.* 1993). This approach results in increased muscle mass, total protein and myofibrillar protein in the overloaded muscles (Noble *et al.* 1984; Tsika *et al.* 1987).

The overall objective of the present study was to investigate the effects of energy restriction on growth of

Abbreviations: CON100, CON81 and CON73, energy intake at 100, 81 and 73 % of the *ad libitum* intake of the control diet respectively; MHC, myosin heavy chain.

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†**Present address:** Department of Community Health Sciences, King Saud University, Riyadh 11433, Saudi Arabia.

* **Corresponding author:** Dr Katharine Grunewald, fax +1 785 532 3132, email grunew@humec.ksu.edu

two muscles subjected to weight-bearing exercise through functional overload. Although endurance training has been shown to reduce the muscle-wasting effects of food restriction (Sakamoto & Grunewald, 1987; Ballor *et al.* 1990), relatively few studies have employed weight-bearing or resistance exercise designed to maximize muscular growth. Furthermore, most previous investigations employed chronic or acute feed restriction, i.e. the whole diet was reduced so that intake of all nutrients was reduced. Consequently, effects on muscular growth could not be attributed to energy restriction as a single effective factor. This information should strengthen and support our current understanding of muscular growth and how it is affected by the nutritional environment. In addition, it might benefit individuals who follow dietary practices designed to enhance muscular growth (Bazzarre *et al.* 1990; Kleiner *et al.* 1990; Grunewald & Bailey, 1993).

In the present study we examined the effects of three levels of energy intake (100, 81, and 73 % of the *ad libitum* intake of the control diet) on growth of fast-twitch plantaris and slow-twitch soleus muscles subjected to functional overload. The energy-restricted diets were formulated to be nutritionally adequate except for energy. Growth indices studied were muscle mass, total and myofibrillar protein contents and myosin heavy-chain (MHC) isoform expression in male rats.

Materials and methods

Experimental protocol

The protocols for animal care and use were approved by the Institutional Animal Care and Use Committee of Kansas State University, Manhattan, KS, USA. The animals were cared for according to the guidelines of the National Institutes of Health on the experimental use of laboratory animals (National Research Council, 1985).

Male Sprague Dawley weanling rats (Harlan Sprague Dawley, Indianapolis, IN, USA) were housed in individual cages under a controlled temperature of 24°C and a 12 h light–dark cycle. At body weights of 200–220 g, the rats were anaesthetized with halothane and the gastrocnemius muscle was removed from the left hindlimb to induce functional overload of the underlying plantaris and soleus muscles. The right hindlimb was sham-operated to serve as an intra-animal control.

Immediately following the surgical procedure, the rats were randomly allocated to one of the three experimental diets (Harlan Teklad, Madison, WI, USA; Table 1). Effects of three different levels of energy were tested. Control rats had free access to the AIN-76A diet (American Institute of Nutrition, 1977, 1980) and thus received a baseline 100 % level of dietary energy (CON100). Rats in the two energy-restricted groups were fed either 75 % or 90 % of the daily amount of feed consumed by CON100 rats. The energy-restricted diets were formulated to be nutritionally similar to the control diet except for energy, when fed at the designated amounts. Animals in the three groups had free access to water.

After 30 d on their respective diets, the rats were fasted for 12–16 h and killed by exsanguination. Blood samples were collected into small vials and stored at −70°C. Plantaris and soleus muscles were removed from both hindlimbs, weighed, submerged in ice-cold glycerol and stored at −70°C until required for analysis. Organ (liver, heart and kidneys) weights were recorded and the organs were wrapped in Al foil and stored at −70°C. Fat pad (epididymal, retroperitoneal and omental) weights were also recorded.

Table 1. Composition of the experimental diets

Diet*…	CON100	CON81	CON73
Ingredients (g/kg)			
Casein, high-protein	200	222·23	266·67
DL-Methionine	3·00	3·34	4·00
Sucrose	499·99	473·4	420·1
Maize starch	150	142	126
Maize oil	50	55·56	66·67
Fibre (cellulose)	50	51·22	53·87
Mineral mix, AIN-76†	35·00	38·89	46·67
Vitamin mix, AIN-76A‡	10·00	11·12	13·34
Choline bitartrate	2·00	2·23	2·67
Ethoxyquin (antioxidant)	0·0100	0·0112	0·0134
Energy: kJ/g	15·7	15·7	15·7
kJ/g protein	90·0	81·3	67·8
Composition by energy (%)§			
Protein	18·54	20·57	24·68
Fat	12·50	13·86	16·62
Carbohydrate	68·95	65·57	58·70

CON100, CON81, CON73, energy intake at 100, 81 and 73 % of the *ad libitum* intake of the control diet respectively.

* The CON100 diet is the AIN-76A diet (American Institute of Nutrition, 1977, 1980). The CON81 and CON73 diets were formulated to be nutritionally similar to the control diet except for energy. They were fed at 90 and 75 % by weight of the CON100 diet respectively. All diets were purchased from Harlan Teklad, Madison, WI, USA.

† The AIN-76 mineral mix supplied the following (per kg mixture): calcium phosphate dibasic 500 g, sodium chloride 74 g, potassium citrate monohydrate 220 g, potassium sulfate 52 g, magnesium oxide 24 g, manganous carbonate 3·5 g, ferric citrate 6 g, zinc carbonate 1·6 g, cupric carbonate 300 mg, potassium iodate 10 mg, $Na_2SeO_{3zrad;}5H_2O$ 10 mg, $KCr(SO_4)_{2zrad;}12H_2O$ 550 mg, sucrose 118·03 g.

‡ The AIN-76A vitamin mix supplied the following (per kg mixture): thiamin hydrochloride 600 mg, riboflavin 600 mg, pyridoxine hydrochloride 700 mg, niacin 3 g, calcium pantothenate 1·6 g, folic acid 200 mg, biotin 20 mg, vitamin B_{12} (0·1 % trituration in mannitol) 1 g, dry retinyl palmitate 800 mg, dry α-tocopheryl acetate 10 g, cholecalciferol trituration 250 mg, menadione–sodium bisulfite complex 150 mg, sucrose 981·08 g.

§ Estimations based on calculated energy values.

Serum and tissue measurements

Serum free fatty acids were assayed by the colorimetric method of Duncombe (1964) with modifications by Noma *et al.* (1973) and Laurell & Tibbling (1966). Serum albumin was assayed using a colorimetric procedure (Sigma Kit no. 631; Sigma Chemical Co., St Louis, MO, USA). Serum glucose was determined by the glucose oxidase method (Sigma Kit no. 510). Blood urea-N was measured by a colorimetric procedure (Sigma Kit no. 535). Total protein contents of livers, kidneys, and hearts were determined using a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

Washed myofibrils preparation

We used the method of Talmadge & Roy (1993) to wash

and prepare myofibrils. Frozen muscles were minced with scissors in 10 vol. (per unit muscle weight) ice-cold homogenization buffer (250 mM-sucrose, 100 mM-KCl, 5 mM-EDTA 20 mM-Tris (base), pH 6·8). The muscle minces were then homogenized using a high-speed Brinkmann Homogenizer (Sybron Corporation, Westbury, NY, USA). Total protein assays were performed on 0·1 ml of the homogenate using the Bio-Rad protein assay employed for other tissues. The remainder of the homogenate was centrifuged in 4°C at 1000 ***g*** for 10 min. The supernatant fraction was discarded and the myofibril pellet was resuspended in wash solution (175 mM-KCl, 20 mM-Tris (base), 2 mM-EDTA, 5 g Triton X-100/l, pH 6·8, at 6°C) using the same volume as for the homogenization buffer. It was then centrifuged as described earlier and the supernatant fraction was discarded. The washing step was repeated and the resultant myofibril pellet was resuspended in 0·5 vol. (relative to the volume of the previous wash) resuspension buffer (150 mM-KCl, 20 mM-Tris (base), pH 7·0, at 6°C). This myofibril solution was used to determine myofibrillar protein content and MHC ratios in electrophoretic assays.

Electrophoresis

Electrophoresis was run in a Bio-Rad Mini-Protean II Dual Slab Cell electrophoretic system utilizing a Bio-Rad 1000/500 power supply (Bio-Rad Laboratories). Preparation of separating gels, stacking gels and running buffers was performed as described previously by Talmadge & Roy (1993). Polymerization was initiated with 0·5 g N,N,N′,N′-tetramethylethylenediamine/l and 1 g ammonium persulfate/l. The washed myofibrils were boiled in sample buffer (10 ml mercaptoethanol/l, 138·7 mM-SDS, 160 mM-Tris (base), 250 ml glycerol/l, 2 g bromophenol blue/l) for 1 min at a final concentration of 0·125 μg/l immediately before loading, cooled down to room temperature, and loaded at 5 μl per well. The entire electrophoresis apparatus was placed in the refrigerator for the period of running (24 h) at 80 V (constant voltage). The gels were then Ag stained using the Bio-Rad silver stain plus kit (Bio-Rad Laboratories). Bands were quantified using a Hewlett Packard 6100C ScanJet scanner (Hewlett Packard, Palo Alto, CA, USA) and Sigma Gel version 1·1 software (John Dell Scientific, Chicago, IL, USA).

Statistical analysis

We used a randomized complete block design, blocking for body weight and day of surgery. There were three diet groups, two legs (overloaded *v.* sham) and ten rats for each diet group. The response tested was the difference between the overloaded and sham-operated legs. ANOVA followed by the least squares means option in Statistical Analysis Systems statistical software package version 6·12 (SAS Institute, Cary, NC, USA) were used. Both linear and quadratic contrast were used to detect the main effect of the diet. Results were considered statistically significant at $P < 0·05$.

Table 2. Weight gain and feed intake of rats fed for 30 d of 100 % (CON100), 81 % (CON81) and 73 % (CON73) of the *ad libitum* energy intake*

(Mean values with their pooled standard errors for ten rats)

Diet group…	CON100	CON81	CON73	Pooled SEM
Initial body wt (g)	215	213	214	2
Final body wt (g)†	346[a]	303[b]	289[c]	4
Body-wt gain (g/30 d)†	132[a]	91[b]	75[c]	4
Total feed intake (g)†	589[a]	481[b]	427[c]	8
Daily feed intake (g/d)†	19·6[a]	16·0[b]	14·2[c]	0·3
Feed efficiency (g/g)†‡	0·22[a]	0·19[b]	0·18[b]	0·01
Energy intake (kJ/d)†	310[a]	251[b]	226[c]	4
Macronutrient intake (g/d)				
Protein†	3·42[a]	3·10[b]	3·31[a]	0·05
Fat†	1·04[a]	0·93[b]	0·99[c]	0·01
Carbohydrate†	12·7[a]	9·9[b]	7·9[c]	0·2

[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and p. 698.
† Main treatment effect of diet was significant ($P < 0·05$).
‡ Body weight gain/total feed intake.

Results

Body-weight gain and feed intake

Weight gain and feed intake of rats fed the three different energy levels are shown in Table 2. Over the 30 d period rats fed 75 % and 90 % the amount of feed consumed by CON100 rats consumed 73 % and 81 % of the energy respectively of CON100 rats ($P < 0·05$). Thus, the energy-restricted groups are referred to as CON73 or CON81 to reflect the actual energy intake of the animals. Rats in the CON81 and CON73 groups respectively had lower feed efficiencies (−14 % and −18 %), weighed less (−12 % and −16 % and gained less weight (−31 % and −43 %) than CON100 rats ($P < 0·05$).

Tissue measurements

Table 3 shows dietary energy effects on tissue and organ growth and serum measurements. Rats fed the energy-restricted CON81 and CON73 diets respectively had smaller livers (−13 % and −19 %), kidneys (−14 % and −17 %) and hearts (−9 % and −14 %), and lower epididymal (−27 % and −27 %), retroperitoneal (−45 % and −35 %) and omental (−21 % and −23 %) fat pad weights than CON100 rats ($P < 0·05$). However, when normalized for body weight (g/100 g body weight), liver, kidney and heart weights were similar among the three dietary groups. Serum albumin, blood urea-N, free fatty acids, and glucose levels were also similar when comparing both groups of energy-restricted rats to CON100 rats.

Plantaris measurements

Plantaris muscle measurements are shown in Table 4. Data are presented separately for sham-operated (right hindlimb) and overloaded (left hindlimb) muscles, as well as the difference between those muscles (expressed as a percentage of the sham-operated value). In general, overloaded muscles weighed more (range 31–47 %) and had more total

Table 3. Organ, tissue and serum measurements for rats fed for 30 d at 100 % (CON100), 81 % (CON81) and 73 % (CON73) of the *ad libitum* energy intake*

(Mean values with their pooled standard errors for ten rats)

Diet group…	CON100	CON81	CON73	Pooled SEM
Liver				
Wt: g†	11·3[a]	9·8[b]	9·1[b]	0·3
g/100 g body wt	3·3	3·2	3·1	0·1
Total protein (g)†	1·98[a]	1·95[a]	1·58[b]	0·05
Kidneys				
Wt: g†	2·58[a]	2·23[b]	2·13[b]	0·05
g/100 g body wt	0·75	0·73	0·74	0·01
Total protein, (g)	0·26	0·24	0·23	0·01
Heart				
Wt: g†	1·05[a]	0·96[b]	0·90[b]	0·02
g/100 g body wt	0·30	0·32	0·31	0·01
Total protein, (g)†	0·14[a]	0·12[b]	0·12[b]	0·01
Fat pads (g)				
Epididymal†	4·52[a]	3·29[b]	3·28[b]	0·14
Retroperitoneal†	2·85[a]	1·56[b]	1·85[b]	0·19
Omental†	4·13[a]	3·27[b]	3·18[b]	0·12
Serum measurements				
Albumin (g/l)	35	35	35	1
Blood urea-N (mg/l)	138	148	157	8
Free fatty acids (mg/l)	86	83	93	11
Glucose (mg/l)	990	880	890	40

[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and p. 698.
† Main treatment effect of diet was significant ($P < 0·05$).

protein (range 31–52 %) and myofibrillar protein (range 27–51 %) than sham-operated muscles in all three treatment groups. Rats on the energy-restricted diets had smaller sham-operated plantaris muscles (CON73 −13 %, CON81 −9 %) containing less total protein (CON73 −14 %, CON81 −10 %) and myofibrillar protein (CON73 −12 %) than CON100 rats ($P < 0·05$). In contrast, energy restriction did not affect growth of the plantaris muscles subjected to functional overload because muscle mass (mg), total protein, and myofibrillar protein measurements were similar among the three dietary groups. Furthermore, normalized weights (mg/100 g body weight) of the overloaded plantaris muscles were greater for CON73 and CON81 rats than for CON100 rats ($P < 0·05$).

Table 4. Weights and protein contents of sham-operated (sham) and functionally-overloaded (overloaded) plantaris muscle from rats fed for 30 d at 100 % (CON100), 81 % (CON81) and 73 % (CON73) of *ad libitum* energy intake*

(Mean values with their pooled standard errors for ten rats)

Diet group…	CON100	CON81	CON73	Pooled SEM
Wt (mg)				
Sham†	389[a]	354[b]	339[b]	8
Overloaded	510	498	497	16
Difference (%)‡	+31	+42	+47	4
Wt (mg/100 g body wt)				
Sham	112	116	117	2
Overloaded†	147[a]	165[b]	172[b]	5
Difference (%)‡	+31	+42	+47	4
Total protein (mg)				
Sham†	70[a]	63[b]	60[b]	2
Overloaded	92	87	91	3
Difference (%)‡	+31	+39	+52	5
Myofibrillar protein (mg)				
Sham†	32·4[a]	29·2[ab]	28·5[b]	1·2
Overloaded	40·8	41·4	43·1	2·0
Difference (%)‡	+27	+43	+51	8

[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and p. 698.
† Main treatment effect of diet significant ($P < 0·05$).
‡ Difference between overloaded value and sham value expressed as a percentage of the sham value.

Table 5. Weights and protein contents of sham-operated (sham) and functionally-overloaded (overloaded) soleus muscle from rats fed for 30 d at 100 % (CON100), 81 % (CON81) and 73 % (CON73) of the *ad libitum* energy intake*

(Mean values with their pooled standard errors for ten rats)

Diet group…	CON100	CON81	CON73	Pooled SEM
Wt (mg)				
Sham	112	114	105	3
Overloaded	133	145	130	6
Difference (%)†	+19	+26	+25	5
Wt (mg/100 g body wt)				
Sham‡	32[a]	38[b]	36[b]	1
Overloaded‡	38[a]	48[b]	45[b]	2
Difference, (%)†	+19	+26	+25	5
Total protein (mg)				
Sham	19	20	19	1
Overloaded	24	26	23	1
Difference (%)†	+22	+29	+26	4
Myofibrillar protein (mg)				
Sham‡	9·1[ab]	9·6[a]	8·4[b]	0·3
Overloaded	10·7	11·9	10·8	0·4
Difference (%)†	+18	+23	+29	5

[a,b,c]Mean values within a row not sharing a common superscript letter were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and p. 698.
† Difference between overloaded value and sham value expressed as a percentage of the sham value.
‡ Main treatment effect of diet was significant ($P < 0·05$).

Soleus measurements

Measurements for soleus muscles are shown in Table 5. Overloaded soleus muscles weighed more than sham-operated muscles (range 19–25 %), and contained more total protein (range 22–29 %) and myofibrillar protein (range 18–29 %) in all three treatment groups. However energy-restricted diets had little effect on soleus muscle growth. Soleus weights (mg) and total protein contents were similar among CON100, CON81 and CON73 groups for both sham-operated and overloaded muscles. Sham-operated soleus of CON73 rats had less myofibrillar protein (−8 %) than those in CON100 rats ($P < 0·05$). Normalized soleus weights (mg/100 g body weight) were greater for rats in the CON73 and CON81 groups than for those in the CON100 group for both sham-operated and overloaded muscles ($P < 0·05$).

Myosin heavy chain isoforms

Fig. 1 shows sample MHC isoform separations of plantaris and soleus muscles using the minigel system and Ag staining. We obtained good resolution of the four MHC bands of the plantaris (types I, IIa, IIx, IIb) and the two

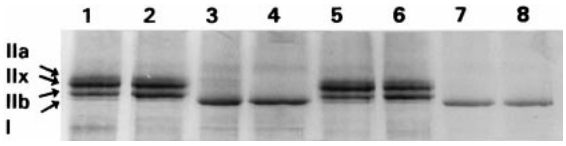

Fig. 1. SDS–PAGE of myosin heavy-chain (MHC) isoforms (types I, IIa, IIx, IIb) in functionally-overloaded and sham-operated plantaris and soleus muscles of rats fed for 30 d at 100 % (CON100), 81 % (CON81) and 73 % (CON73) of the *ad libitum* energy intake. Lane 1, CON100 overloaded plantaris; lane 2, CON100 sham plantaris; lane 3, CON100 overloaded soleus; lane 4, CON100 sham soleus; lane 5, CON81 overloaded plantaris; lane 6, CON81 sham plantaris; lane 7, CON81 overloaded soleus; lane 8, CON81 sham soleus. Good resolution was obtained for all four MHC bands in the plantaris and for two MHC bonds (types I, IIa) in the soleus. For details of diets and procedures, see Table 1 and p. 698.

MHC bands of the soleus (types I, IIa). Fig. 2 shows the distribution of MHC isoforms in plantaris and soleus muscles. Rats fed the CON100, CON81, and CON73 diets for 30 d had similar MHC distribution in plantaris muscles (types I, IIa, IIx, IIb) and in soleus muscles (types I, IIa). The MHC distribution of those muscles was also similar in sham-operated and overloaded muscles.

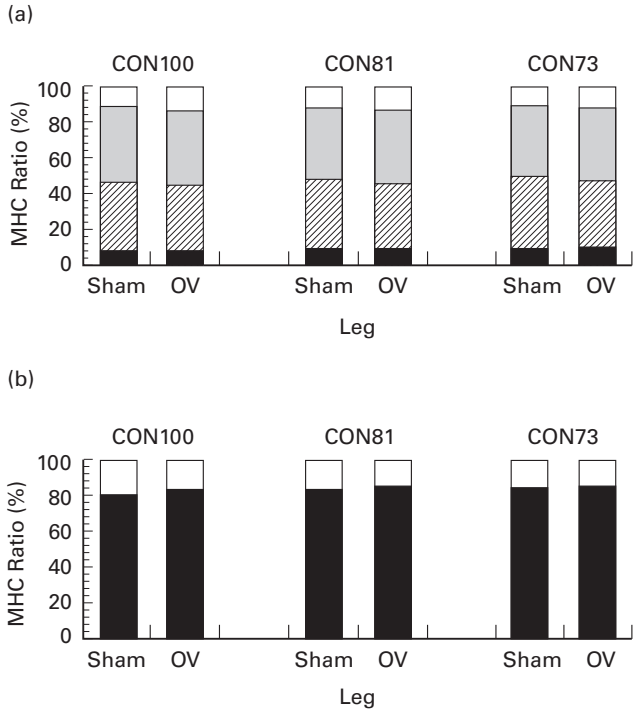

Fig. 2. Myosin heavy-chain (MHC) isoform ratios in sham-operated (sham) and functionally-overloaded (OV) (a) plantaris and (b) soleus leg muscles of rats fed for 30 d at 100 % (CON100), 81 % (CON81) and 73 % (CON73) of the *ab libitum* energy intake. MHC isoforms are □ Type IIa, ▨ Type IIx, ▨ Type IIb and ■ Type I. Values are means for ten rats. No significant differences were observed between dietary treatment groups. For details of diets and procedures, see Table 1 and p. 698.

Discussion

Our study showed that two levels of energy restriction, moderate and severe, did not compromise or affect growth of plantaris or soleus muscles subjected to functional overload in rats; as values for muscle mass, total protein and myofibrillar protein were similar in overloaded muscles among rats fed the different energy levels for a 30 d period. These effects were noted in both plantaris and soleus muscles. In contrast, sham-operated muscles were more sensitive to the growth-limiting effects of energy deprivation. Sham-operated plantaris muscles of rats fed the energy-restricted diets weighed less and contained less protein than those of control rats. The differences were not observed in soleus muscles. Other indices of reduced growth exhibited by the energy-restricted animals included lower body weight, smaller visceral organs and smaller fat depots. These effects have been shown in previous studies (Hill *et al.* 1970; Hegarty & Kim, 1980, 1981; Ocken & Grunewald, 1988).

The growth maintenance by overloaded, but not sham-operated, muscles in our study suggests that the additional muscular activity made them more resistant to the effects of energy deprivation. This finding is supported by data from other studies. For example, enforced stretch made *extensor digitorum longus* muscles more resistant to the wasting effects of starvation (Goldspink, 1978), feed-restricted mice trained to pull a cord had larger *biceps brachii* muscles than untrained feed-restricted mice (Goldspink, 1964), and treadmill exercise helped to conserve lean body mass in rats fed restricted diets (Sakamoto & Grunewald, 1987; Ballor *et al.* 1990). Human studies have also shown that resistance training during energy restriction enhances the maintenance of lean body weight (Ballor *et al.* 1988; Bryner *et al.* 1999).

In the absence of loading or weight-bearing activity, effects of feed deprivation appear to be more severe on fast-twitch plantaris muscles than slow-twitch soleus muscles. In the present study, the sham-operated plantaris muscles were smaller in energy-restricted rats than in control rats; but these differences were not observed in soleus muscles. Thus, soleus muscles were relatively resistant to the effects of energy deprivation. There are several explanations for this finding. First, the soleus is primarily an anti-gravitational muscle, and the constant loading probably attenuated the growth-reducing effects of energy restriction. In contrast, the plantaris is primarily a locomotion muscle that is used less frequently. Second, there is an abundance of information which shows that nutritional deprivation effects are generally more severe for fast-twitch than slow-twitch muscles or muscle fibres (Goodman *et al.* 1981; Layman *et al.* 1981; Bedi *et al.* 1982). The reduction of protein synthesis was more severe in pale *extensor digitorum longus* muscles than in dark soleus muscles (Li & Goldberg, 1976). It has been suggested that the preservation of slow-twitch muscle over fast-twitch muscle is more economical from an energy standpoint, because slow-twitch fibres have a lower energy cost for contraction or isometric tension (Crow & Kushmerick, 1982; Henriksson, 1990).

Muscle fibre type has been correlated with the content of

specific MHC isoforms (Staron, 1991; Oishi, 1993) and so MHC isoform measurements were performed in our study. Myosins comprise a major portion of skeletal muscle proteins; and four MHC isoforms are expressed in rodent muscle (Pette & Staron, 1990). Fast-twitch muscle such as the plantaris expresses types IIa, IIb and IIx MHC, whereas slow-twitch muscle such as the soleus expresses primarily type I and smaller amounts of type IIa MHC (Talmadge & Roy, 1993; Sullivan *et al.* 1995; Demirel *et al.* 1999). These observations were shown and supported by our data. We also demonstrated that the MHC ratios expressed in soleus and plantaris muscles were not altered by energy deprivation over a 30 d period. Thus, this component of skeletal muscle appears to be resistant to the levels of energy deprivation used in our study.

The energy-restricted diets in our study were formulated to be nutritionally adequate except for energy. Thus, the two energy-restricted diets contained higher concentrations of protein, vitamins and minerals so that they could be fed in smaller amounts (90 % and 75 % by weight of that fed to the control animals). We could therefore test the effects of energy deprivation as a single factor without inducing other nutritional deficiencies. This approach was taken because most previous studies had employed chronic or acute feed restriction where all nutrients were reduced in the diet, and so energy could not be tested as a single factor. However, the animals fed the energy-restricted diets ate less feed than that offered; the CON90 rats ate 81 %, and the CON75 rats ate 73 % of that consumed by control rats. This lower intake can be attributed mostly to feed spillage of the CON81 and CON73 rats since all animals in the restricted groups were fed controlled amounts of feed each day. Protein intake of the CON81 rats was slightly less than that of the CON100 rats, but still nutritionally adequate. Nonetheless, we were able to test the effects of two different energy-restricted diets that were nutritionally adequate in other respects. The growth-reduction effects observed in the present study are similar to those published for acute or chronic feed restriction (Hill *et al.* 1970; Hegarty & Kim, 1980, 1981; Ocken & Grunewald, 1988).

Several methods have been used to experimentally induce skeletal muscle growth with varying degrees of success. Animals have been trained to work using food as a reward (Goldspink, 1964; Watt *et al.* 1982; Yarasheski *et al.* 1990). Other models include electrical stimulation (Garner *et al.* 1991; Caiozzo *et al.* 1992; Tamaki *et al.* 1992) and weighted stretch (Gollnick *et al.* 1983; Antonio & Gonyea, 1993). The approach used in our investigation, functional overload by removal of synergist muscles, has well-documented effects on muscle growth and composition (Noble *et al.* 1984; Tsika *et al.* 1987; Ianuzzo *et al.* 1991; Sugiura *et al*. 1993). This method was particularly appropriate for our study because it produced rapid growth in a short period of time, an important feature for nutritional deprivation studies. Furthermore, food was not given as a reward for work, as this approach may be problematic if rats become physically compromised by the diet and are therefore less able to work (e.g. pull a lever, jump for food).

Another strength of our experimental approach was the two-leg (sham, overloaded) intra-animal design. This approach allowed us to observe the effects of energy restriction on two types of skeletal muscle growth: (1) normal growth occurring in sham-operated muscles, and (2) experimentally-induced rapid growth induced in overloaded muscles. Studying muscles from both hindlimbs in the same animal also allowed for comparison of genetically-identical muscles exposed to the same nutritional environment.

In conclusion, the present study shows the effects of two levels of energy restriction on several indices of growth of slow-twitch soleus and fast-twitch plantaris skeletal muscles in rats. We examined effects on normal growth of sham-operated muscles and on experimentally-induced growth of overloaded muscles. In the absence of loading, growth of fast-twitch plantaris muscle was reduced by energy restriction. Plantaris muscles subjected to functional overload, however, were not affected by energy restriction. Soleus muscles were more resistant to the effects of energy deprivation.

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