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Dietary whey protein increases liver and skeletal muscle glycogen levels in exercise-trained rats

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We investigated the effect of different types of dietary protein on glycogen content in liver and skeletal muscle of exercise-trained rats. Twenty-four male Sprague-Dawley rats (approximately 100 g; *n* 6 per group) were divided into sedentary or exercise-trained groups with each group being fed either casein or whey protein as the source of dietary protein. Rats in the exercised groups were trained during 2 weeks using swimming exercise for 120 min/d, 6 d/week. Exercise training resulted in an increase in the skeletal muscle glycogen content. Furthermore, the whey protein group significantly increased the skeletal muscle glycogen content compared with the casein group. The increase in glycogen content in liver was significantly greater in rats fed the whey protein diet compared with those fed the casein diet. We also found that the whey protein diet increased the activity of liver glucokinase, whereas it decreased the activities of 6-phosphofructokinase and pyruvate kinase compared with the casein diet. However, hepatic total glycogen synthase activity and mRNA expression were similar with the two diets. In the skeletal muscle, whey protein decreased only 6-phosphofructokinase activity compared with casein. Total glycogen synthase activity in the skeletal muscle in the whey protein group was significantly higher than that in the casein group. The present study is the first to demonstrate that a diet based on whey protein may increase glycogen content in liver and skeletal muscle of exercise-trained rats. We also observed that whey protein regulated glycogen metabolism in these two tissues by different mechanisms.

Whey protein: Glycogen: Glycoregulatory enzymes: Exercise-trained rats

During both sprint and prolonged endurance exercise, the body must carefully balance the availability and fuel utilization in liver and skeletal muscle. In the liver, several important pathways play a role in the control of glucose homeostasis by maintaining a balance between glucose uptake and storage that is determined by glucogenesis and glycolysis, and glucose release that is determined by glycogenolysis and gluconeogenesis (Levine & Haft, 1970). Glycolysis also has a role in determining the activity of key enzymes of the opposing glycolytic and gluconeogenic pathways, which must be controlled and regulated in order to achieve a net flux in the appropriate direction. Glycolysis shares almost all of the enzymes of the gluconeogenic pathway, except for pyruvate kinase, 6-phosphofructokinase and hexokinase/glucokinase. These three reactions of glycolysis proceed with a large negative free energy change and are bypassed during gluconeogenesis using different enzymes.

In skeletal muscle, glucose transport and glycogen synthase activity are considered the key regulatory factors for glycogen synthesis (Bogardus *et al.* 1984; Cartee *et al.* 1989) with defects in GLUT4 and hexokinase, two major components of skeletal muscle carbohydrate metabolism, being linked to insulin resistance (Shepherd & Kahn, 1999). Exercise training in rats has been shown to increase GLUT4 (Neufer & Dohm, 1993; Ren *et al.* 1994; Kuo *et al.* 1999) and hexokinase II gene expression and protein levels (O'Doherty *et al.* 1994, 1996). The major fate of skeletal muscle glucose uptake during the post-exercise period is storage as muscle glycogen, the magnitude of which correlates with the levels of skeletal muscle GLUT4 and glycogen synthase activity (McCoy *et al.* 1996). Glycogen depletion in the post-exercise period has been associated with enhanced insulin action, while reversal of glucose transport following exercise is correlated with muscle glycogen levels (Richter *et al.* 1982). Depletion of glycogen stores has been associated with fatigue during both sprint and endurance exercise (Hermansen *et al.* 1967; Karlsson & Saltin, 1971; Terjung *et al.* 1974) and therefore it is important to maintain adequate stores of glycogen in tissues.

It is recognized that dietary carbohydrate is an effective source for tissue glycogen. Furthermore, recent studies showed that combination of carbohydrate and protein was more effective than carbohydrate alone in the replenishment of muscle glycogen during the 4 h immediately after exercise (Zawadzki *et al.* 1992; Ivy *et al.* 2002). However, it is not clear if different types of dietary protein affect glycogen content or the activity of glycoregulatory enzymes in tissues. Casein and whey protein are used mainly as the source of protein in dietary supplements. Accordingly, the aim of the present study was to compare the effect of casein and whey protein as the source of dietary protein on the content

Abbreviation: C_T, cycle threshold.

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of glycogen, activity of glycoregulatory enzymes and mRNA expression in the liver and skeletal muscle of exercise-trained rats.

Materials and methods

Animals

Male Sprague-Dawley rats (CLEA Japan Inc., Tokyo, Japan) were used in this study. All the rats were housed individually in temperature-controlled rooms (22°C), with light from 08.00 to 20.00 hours and dark from 20.00 to 08.00 hours. The study was approved by the Animal Committee of Meiji Seika Kaisha Ltd, Health & Bioscience Laboratories, with the animals receiving care under the guidelines laid down by this committee.

Diets

The design of the experimental diets followed the AIN-93 protocol (Reeves *et al.* 1993) with the composition of the diets shown in Table 1. Casein and whey protein were used as the source of dietary protein. The protein content calculated as N concentration × 6·38 was measured using the Kjeldahl method. Casein (87·7 g crude protein/100 g) and whey protein (79·3 g crude protein/100 g) were added as 200 g protein per 1 kg to the diets. The difference in the protein content between the two diets was compensated for by the addition of corn starch.

Experimental protocol

Twenty-four male Sprague-Dawley rats (*n* 6 per group), each with a body weight of about 100 g were allowed free access to food and water for 2 weeks. The rats were then divided into sedentary or exercise-trained groups and were fed either the casein or whey protein diet. Rats in the exercise-trained groups swam simultaneously without a load 6 d/week for 120 min/d in a barrel filled with water maintained at 35°C to a depth of 50 cm so that the average surface area available to each animal was 170 cm^2. At the end of the 2 weeks of training, 15 h after the end of the last training session, all the rats were fasted for 3 h. Holness & Sugden (1989) showed that fasting for 3 h does not affect the glycogen content in tissues. The rats were killed between 09.00 and 10.00 hours. Arteriovenous blood samples were collected from all the animals under ether anaesthesia, centrifuged at 3000 ***g*** for 15 min and the serum then stored at − 80°C.

Table 1. Composition of the two protein diets (g/kg diet)

	Casein	Whey protein
Casein*	228	–
Whey protein†	–	252
Vitamin mixture‡	10	10
Choline bitartrate§	2·5	2·5
Mineral mixture‡	35	35
Corn oil‖	70	70
Corn starch¶	504·5	480·5
Sucrose**	100	100
Cellulose††	50	50

* Oriental Yeast Co. Ltd, Tokyo, Japan.
† Nihon NZMP Co. Ltd, Tokyo, Japan.
‡ AIN-93 diet, Nosan Corporation, Kanagawa, Japan.
§ Wako Pure Chemical Industries Ltd, Osaka, Japan.
‖ Ajinomoto Co. Inc., Tokyo, Japan.
¶ Taiyo Kagaku Co. Ltd, Mie, Japan.
** Nippon Beet Sugar Manufacturing Co. Ltd, Tokyo, Japan.
†† Asahi Kasei Corporation, Tokyo, Japan.

After blood collection, the abdominal cavity was opened and the liver and gastrocnemius muscle were quickly excised, washed, weighed and frozen at − 80°C until assay.

Serum analyses

Serum glucose concentration was measured using a glucose oxidase assay (Miwa *et al.* 1972), serum insulin concentration by an ELISA kit obtained from Mercodia AB (Uppsala, Sweden) and serum glucagon level by an ELISA kit purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan).

Glycogen analysis

Tissue glycogen was isolated and purified by precipitation with ethanol from a digest formed by the addition of 5·3 M-KOH, and then quantified by the phenol–sulphuric acid method (Lo *et al.* 1970).

Enzyme activities

Aliquots of liver or skeletal muscle were homogenized in 0·1 M-Tris-HCl buffer (pH 7·4) using a Potter-Elvhjem-type homogenizer fitted with a Teflon pestle. The homogenate was centrifuged for 30 min at 12 000 ***g*** at 4°C and the supernant was used immediately to determine the enzyme activities.

Glucokinase (EC 2.7.1.1), hexokinase (EC 2.7.1.1), 6-phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40) were assayed spectrophotometrically. Glucokinase was assayed according to Grossman *et al.* (1974) with the assay mixture containing 0·1 M-Tris-HCl (pH 7·5), 5 mM-$MgCl_2$, 5 mM-ATP, 100 mM-glucose, 0·4 mM-NADP and 0·3 U glucose 6-phosphate dehydrogenase. Hexokinase was assayed according to Burcelin *et al.* (1993) using an assay mixture containing 0·1 M-Tris-HCl (pH 7·5), 5 mM-$MgCl_2$, 5 mM-ATP, 5 mM-glucose, 0·4 mM-NADP and 0·3 U glucose 6-phosphate dehydrogenase. The method of Karadsheh *et al.* (1977) was used to analyse 6-phosphofructokinase activity with the assay mixture containing 50 mM-Tris-HCl (pH 8·2), 1 mM-fructose 6-phosphate, 1 mM-ATP, 0·16 mM-NADH, 1 mM-EDTA, 2·5 mM-dithiothreitol, 2 mM-$MgCl_2$, 5 mM-ammonium sulphate, 0·4 U aldolase, 2·4 U triosephosphate isomerase and 0·4 U glycerophosphate dehydrogenase. Pyruvate kinase was determined according to the method described by Harada *et al.* (1978) using an assay mixture containing 50 mM-Tris-HCl (pH 7·5), 100 mM-KCl, 5 mM-$MgSO_4$, 2 mM-phosphoenolpyruvate, 2 mM-ADP, 0·5 mM-fructose 1,6-bisphosphate, 0·18 mM-NADH and 8 U lactate dehydrogenase. Total glycogen synthase activity (EC 2.4.1.11) was measured by the method of Danforth (1965) with enzyme activity assayed at pH 7·4 and 30°C in a reaction mixture containing 60 mM-Tris-HCl (pH 7·4), 1·2 mM-EDTA, 3 mM-mercaptoethanol, 1·2 mM-NaF, 7·5 mM-UDP-glucose and 1·2 % (w/v) glycogen. The assay was carried out in the presence of 12 mM-glucose 6-phosphate in order to measure total glycogen synthase activity with the reaction being terminated by heating for 2 min in a boiling water-bath. The denatured protein was removed by centrifugation and UDP concentration in the supernatant solution assayed enzymatically by allowing UDP to react with phosphoenolpyruvate in the presence of pyruvate kinase.

The total protein concentration of the tissue homogenate supernatant was measured using bicinchoninic acid with bovine serum albumin as the standard (Smith *et al.* 1985).

Total RNA isolation and cDNA

Total RNA was isolated from the liver and skeletal muscle by the guanidine thiocyanate method of Chomczynski & Sacchi (1987) using Isogen solution (Nippon Gene Co. Ltd, Tokyo, Japan). The extracted RNA was dissolved in diethylpyrocarbonate-treated water and quantified spectrophotometrically at a wavelength of 260 nm. Reverse transcription was used to produce cDNA from RNA using a first standard cDNA synthesis kit (Fermentas Inc.). The cDNA was stored at −80°C for subsequent analysis.

Quantitative real-time RT-PCR analysis

Real-time PCR was performed using the ABI PRISM 7000 sequence detection system (Applied Biosystems). Primers and probes (TaqMan® Assays-on-Demand™ Gene Expression Products) were designed at Applied Biosystems (Foster City, CA, USA) from gene sequences obtained from GenBank (GLUT2, NM-012879; glycogen synthase II, NM-013089; glucokinase, NM-012565; GLUT4, NM-012751; hexokinase, NM-012735; glycogen synthase I, XM-341858). DNA amplification was carried out in 12·5 μl TaqMan Universal PCR Master Mix, 1·25 μl primer and probes, 2·5 μl cDNA and 8·75 μl RNase and DNase free water in a final volume of 25 μl/well. The samples were loaded in a MicroAmp 96-well reaction plate and then run using the ABI sequence detection system. After 2 min at 50°C and 10 min at 95°C, the plates were co-amplified by 50 repeated cycles, with each cycle consisting of a 30 s denaturing step at 95°C and a 1 min annealing/extending step at 59°C. Data were analysed by ABI software using the cycle threshold (C_T), a value that is calculated as the time, measured as cycle number, at which the reporter fluorescent emission increased beyond a threshold level, defined as the background number at which cDNA amplification was first detected. Fluorescent emission data were captured and mRNA levels quantified for each gene using the C_T value. The ΔC_T was calculated by subtracting the C_T for β-actin from the C_T for the gene of interest. The relative expression of the gene of interest was then calculated using the expression $2^{-\Delta CT}$ with the results being expressed as arbitrary units.

Statistics

Data were subjected to two-way ANOVA with *post hoc* analyses being carried out by Tukey's honestly significant difference test. Associations between the variables were examined using Pearson's correlation coefficient. Differences between groups were considered to be significant at $P<0·05$.

Results

Initial body weight, food intake and body weight gain

Table 2 shows the changes in initial body weight, food intake, body weight gain and liver and gastrocnemius muscle weights. Food intake and body weight gain were not different between the casein and the whey protein diets. Exercise training for 2 weeks significantly decreased the gain in body weight. The liver weight was significantly higher in the whey protein group compared with the casein group.

Serum glucose, insulin and glucagon

Serum glucose and serum insulin levels were significantly lower in the exercise training groups compared with the sedentary groups. However, serum glucagon level was not affected by exercise training. Fig. 1 shows that the type of dietary protein had no effect on the serum levels of glucose, insulin or glucagon.

Liver and skeletal muscle glycogen

Liver glycogen content in the whey protein groups was increased significantly compared with the casein groups. Rats receiving exercise training had significant increases in the glycogen content of skeletal muscle compared with sedentary animals. The level of skeletal muscle glycogen in the whey protein group was also higher than levels in the casein group (Fig. 2).

Liver glycoregulatory enzyme activities

Glucokinase activity was increased significantly in the whey protein groups compared with the casein groups. In contrast, the groups fed whey protein significantly lowered pyruvate kinase and 6-phosphofructokinase activities compared with the groups fed casein. Exercise training decreased pyruvate kinase activity, but had no effect on glucokinase or 6-phosphofructokinase activity. Hepatic total glycogen synthase activity was similar in all the groups (Table 3).

Table 2. Initial body weight, food intake and body weight gain
(Mean values with their standard errors)

	Initial body wt (g)		Food intake (g/14 d)		Body wt gain (g/14 d)		Liver wt (g/100 g body wt)		Gastrocnemius muscle wt (g/100 g body wt)	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Sedentary – casein	101	2	257	5	114	3	4·98	0·18	1·06	0·02
Sedentary – whey protein	101	1	240	7	111	2	5·41	0·15	1·10	0·02
Exercise-trained – casein	99	2	249	5	104	2	4·66	0·09	1·09	0·02
Exercise-trained – whey protein	99	2	240	4	101	3	5·19	0·12	1·04	0·02
Two-way ANOVA										
Diet	0·990		0·081		0·896		0·003		0·777	
Exercise	0·166		0·785		<0·001		0·070		0·689	
Diet × exercise	0·876		0·479		0·252		0·690		0·052	

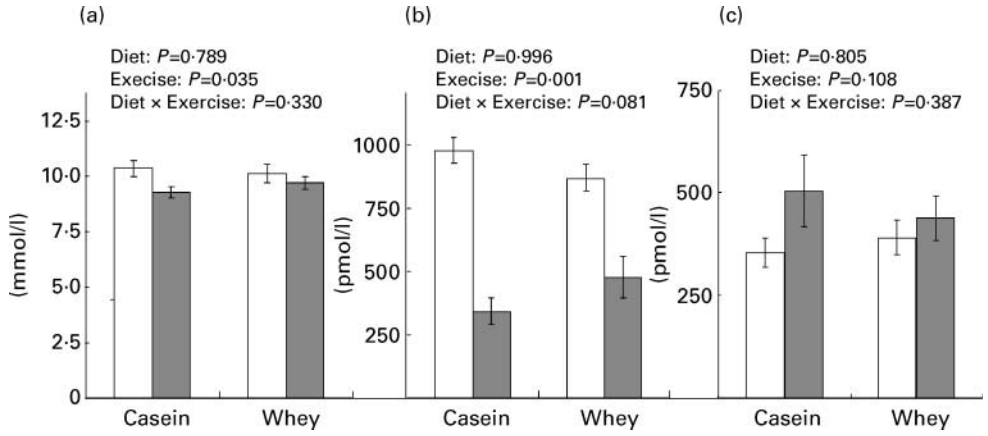

Fig. 1. Effect of dietary protein on (a) serum glucose, (b) serum insulin and (c) serum glucagon concentrations in sedentary (□) or exercise-trained (■) rats. Values are means with their standard errors depicted by vertical bars.

Liver mRNA levels

A significant decrease in hepatic GLUT2 mRNA levels was observed in exercise training rats compared with sedentary rats, whereas glucokinase and glycogen synthase I mRNA levels did not vary among the groups (Table 4).

Skeletal muscle glycoregulatory enzyme activities

Hexokinase and total glycogen synthase activities were higher in exercise training rats than in sedentary rats. In contrast, 6-phosphofructokinase and pyruvate kinase activities were not altered by exercise training. However, groups fed whey protein had increased total glycogen synthase activity and decreased 6-phosphofructokinase activity compared with the groups fed casein (Table 5).

Skeletal muscle mRNA levels

While exercise training resulted in a significant increase in mRNA levels of GLUT4, hexokinase II and glycogen synthase I, the type of dietary protein had little effect on mRNA expression of these enzymes (Table 6).

Discussion

Depletion of glycogen stores has been associated with fatigue during both sprint and endurance exercise (Hermansen *et al.* 1967; Karlsson & Saltin, 1971; Terjung *et al.* 1974). Storage of glycogen in tissues is therefore of great importance, with dietary carbohydrate being the main source of tissue glycogen. However, it is not clear if different types of dietary protein affect glycogen content or the activity of glycoregulatory enzymes in tissues. The present study showed for the first time that the type of dietary protein affects both liver and muscle glycogen content, as exercise-trained rats fed a whey protein diet were shown to accumulate more glycogen than those fed a casein diet.

Control of hepatic glycogen storage is governed by two related pathways, namely glycogen metabolism that incorporates both synthesis and degradation, gluconeogenesis and glycolysis, the latter pathway essentially being a reversal of gluconeogenesis. The same enzymes catalyse glycolysis and gluconeogenesis except at three

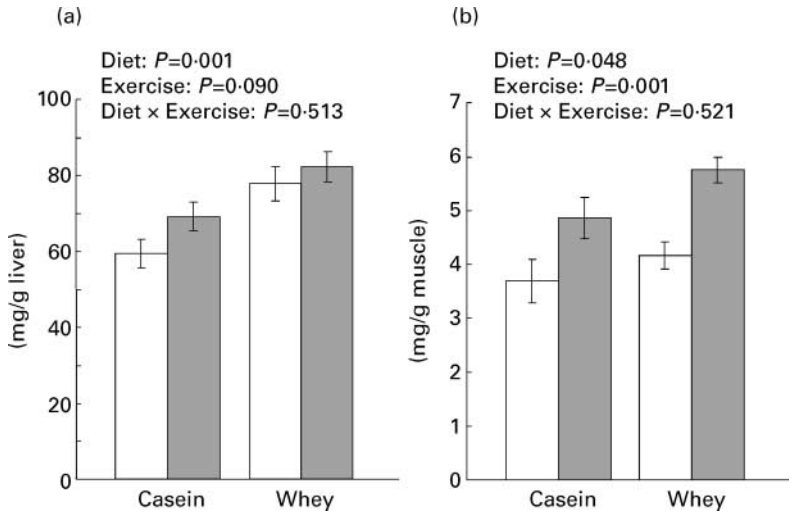

Fig. 2. Effect of dietary protein on (a) liver and (b) gastrocnemius muscle glycogen content in sedentary (□) or exercise-trained (■) rats. Values are means with their standard errors depicted by vertical bars.

Table 3. Liver glycoregulatory enzyme activities

(Mean values with their standard errors)

	Glucokinase*		6-Phosphofructokinase*		Pyruvate kinase*	
Group	Mean	SE	Mean	SE	Mean	SE
Sedentary – casein	65·0	7·5	11·6	0·7	687	37
Sedentary – whey protein	98·7	4·3	9·8	0·7	527	18
Exercise-trained – casein	66·4	5·2	12·6	0·3	584	44
Exercise-trained – whey protein	84·5	6·8	9·6	0·4	383	23
Two-way ANOVA						
Diet	<0·001		0·001		<0·001	
Exercise	0·305		0·226		0·002	
Diet × exercise	0·214		0·125		0·564	

* Data are expressed in nmol/min per mg protein.

regulated sites that involve either glucokinase or glucose 6-phosphatase, phosphofructokinase or fructose 1,6-bisphosphatase and pyruvate kinase or phosphoenolpyruvate carboxykinase. These rate-limiting enzymes are therefore of great importance as they occupy key positions in carbohydrate metabolism. We demonstrated in groups fed whey protein that hepatic glucokinase activity was increased and 6-phosphofructokinase and pyruvate kinase activities decreased compared with casein-fed groups. However, total glycogen synthase was not affected by either dietary protein. Recently, de la Iglesia *et al.* (2000) showed that glucokinase has a number of unique regulatory properties and exerts considerable control over hepatic glucose uptake and glycogen synthesis. In the present study, we observed a significant correlation (r 0·53, P=0·001) between hepatic glycogen level and glucokinase activity, a finding that suggests activation of glucokinase activity resulting from the whey protein diet may lead to an increase in hepatic glycogen. Furthermore, inhibition of 6-phosphofructokinase and pyruvate kinase in the whey protein groups may have conserved utilization of glucose by glycogenolysis.

The liver expresses unique proteins for the three pathways involved in glycogen storage. Glucose transport is carried out by GLUT2, a member of the facilitative glucose transporter family (Burcelin *et al.* 2000). The GLUT2 system moves glucose down a concentration gradient with net flux dependent on the relative intra- and extracellular glucose concentration. However, it remains unclear whether exercise training affects the hepatic levels of GLUT2. We found that exercise training caused a significant decrease in GLUT2 mRNA levels that may have been a consequence of the reduction in serum glucose concentration. In addition, we observed a significant positive correlation (r 0·78, P<0·001) between GLUT2 and glycogen synthase II mRNA expression. Although there was only minimal correlation between hepatic glycogen content and glycogen synthase II mRNA expression (r −0·249, P=0·121), we observed a strong negative correlation between liver glycogen content and GLUT2 mRNA level (r −0·52, P=0·005). These results indicate that regulation of hepatic glycogen resulting from a whey protein diet is dependent on modulation of glycolytic and gluconeogenic enzymes, but not on an increase in the activity of GLUT2 or glycogen synthase.

We also observed that swimming training increased the mRNA level of GLUT4, hexokinase and glycogen synthase, and the activities of hexokinase and total glycogen synthase in skeletal muscle. A number of previous studies have provided evidence that there is a relationship between skeletal muscle glycogen and glucose transport (Bogardus *et al.* 1984; Cartee *et al.* 1989; Neufer & Dohm, 1993; O'Doherty *et al.* 1994, 1996; Ren *et al.* 1994; Kuo *et al.* 1999; Shepherd & Kahn, 1999). Exercise training is known to increase GLUT4 content and improve insulin-stimulated glucose uptake, with a single exercise session having been shown to increase the rate of insulin-stimulated glucose uptake and metabolism in skeletal muscle for 24–48 h. This increase was attributed to an effect of exercise on translocation of the GLUT4 glucose transporter (Ren *et al.* 1994; Phillips *et al.* 1996), hexokinase (O'Doherty *et al.* 1994, 1996) or glycogen synthase activity (Devlin *et al.* 1987). Moreover, skeletal muscle glycogen accumulation has been shown to correlate with the relative proportion of glycogen synthase. In the present study, dietary whey protein induced the accumulation of skeletal muscle glycogen in exercise-trained rats,

Table 4. Liver mRNA levels

(Mean values with their standard errors)

	GLUT2*		Glucokinase*		Glycogen synthase II*	
Group	Mean	SE	Mean	SE	Mean	SE
Sedentary – casein	100	11	100	20	100	5
Sedentary – whey protein	84·0	7·0	115	34	103	7
Exercise-trained – casein	81·3	6·7	90·1	15·9	96·1	6·7
Exercise-trained – whey protein	67·9	5·1	134	24	84·4	6·0
Two-way ANOVA						
Diet	0·069		0·247		0·475	
Exercise	0·034		0·860		0·088	
Diet × exercise	0·862		0·566		0·256	

* Data are expressed in arbitrary units.

Table 5. Skeletal muscle glycoregulatory enzyme activities
(Mean values with their standard errors)

	Hexokinase*		6-Phosphofructokinase*		Pyruvate kinase†		Glycogen synthase*	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Sedentary – casein	18·5	0·8	28·7	2·1	5·67	0·13	1·28	0·36
Sedentary – whey protein	18·2	1·5	20·9	1·0	6·02	0·23	3·33	0·40
Exercise-trained – casein	24·6	0·9	31·7	3·5	6·07	0·26	5·11	0·54
Exercise-trained – whey protein	25·4	1·0	15·2	2·4	5·63	0·20	6·37	0·52
Two-way ANOVA								
Diet	0·861		<0·001		0·815		0·002	
Exercise	<0·001		0·592		0·972		<0·001	
Diet × exercise	0·622		0·090		0·075		0·406	

* Data are expressed in nmol/min per mg protein.
† Data are expressed in μmol/min per mg protein.

Table 6. Skeletal muscle mRNA levels
(Mean values with their standard errors)

	GLUT4*		Hexokinase II*		Glycogen synthase I*	
Group	Mean	SE	Mean	SE	Mean	SE
Sedentary – casein	100	7	100	10	100	43
Sedentary – whey protein	104	8	81	9	422	139
Exercise-trained – casein	150	3	133	6	1019	220
Exercise-trained – whey protein	139	7	134	9	1270	433
Two-way ANOVA						
Diet	0·578		0·273		0·31	
Exercise	<0·001		<0·001		<0·001	
Diet × exercise	0·246		0·262		0·889	

* Data are expressed in arbitrary units.

and increased glycogen synthase activity with a significant positive correlation (r 0·86; $P<0·001$) between skeletal muscle glycogen synthase and glycogen content being observed. However, we were unable to demonstrate any effect of the type of dietary protein on GLUT4 mRNA level, and hexokinase activity and mRNA expression. In contrast to liver carbohydrate metabolism, skeletal muscle pyruvate kinase activity was similar with the casein and whey protein diets, while skeletal muscle 6-phosphofructokinase activity was lower in the whey groups than in the casein groups. We also observed a significant negative correlation between skeletal muscle 6-phosphofructokinase activity and glycogen content (r −0·37; $P=0·036$). Taken together these findings indicate that whey protein increases skeletal muscle glycogen content as a result of an increase in total glycogen synthase activity or a decrease in 6-phosphofructokinase activity.

The regulation of glycogenesis is also mediated by hormonal changes, especially insulin and glucagon. Zawadzki *et al.* (1992) reported that addition of an intact protein to a carbohydrate-containing solution resulted in higher glycogen synthesis rates in subjects after exercise than did ingestion of carbohydrate only. This was explained by the observed additional increase in plasma insulin concentrations after ingestion of the carbohydrate–protein mixture. Elevated insulin concentrations may lead to increased glucose uptake (Ivy & Kuo, 1998) and to an increase in glycogen synthase activity (Bak *et al.* 1991). However, in the present study, the serum levels of glucose, insulin and glucagon were similar with the two protein diets. Despite these similarities, we found dietary whey protein increased the activity of hepatic glucokinase and skeletal muscle glycogen synthase, and decreased the activities of liver and skeletal muscle 6-phosphofructokinase compared with dietary casein. This finding implies that the effect of the whey protein diet on the activity of tissue glycolytic enzymes is not related to changes in the concentration of serum hormones.

There is evidence that certain amino acids may also stimulate glycolytic and gluconeogenic enzyme activity in liver. In isolated hepatocytes, several amino acids such as glutamine, proline, alanine and histidine have been shown to stimulate glycogen synthesis from glucose and gluconeogenic precursors (Baquet *et al.* 1990). Contrary to the liver, it is established that certain amino acids have an important role in glucose uptake and glycogen synthesis in skeletal muscle, as previous studies in myoblast L6 cells (Peyrollier *et al.* 2000) and cultured human muscle have shown that leucine and total amino acid concentration activates glycogen synthase via mammalian target of rapamycin (mTOR) activation (Armstrong *et al.* 2001). Furthermore, our previous report demonstrated in exercise-trained rats that a whey protein diet, compared with casein and soy protein diets, had beneficial effects on liver glycogen storage as a result of regulation of the activity of glycolytic and gluconeogenic enzymes and aminotransferase. Compared with casein, whey protein caused significant increases in the level of various amino acids, especially alanine and also enhanced hepatic alanine aminotransferase activity. This increase in alanine aminotransferase indicated enhancement of glycogenesis from alanine (Morifuji *et al.* 2005). In addition, the significant changes we observed in this metabolism

in rats fed whey protein may be related to other dietary ingredients of whey protein because whey protein is not purified protein and consists of various proteins, such as β-lactoglobulin (50 %), α-lactalbumin (25 %), serum albumin (7 %) and immunoglobulins (5 %) (Heine *et al.* 1991). However, the manner in which these ingredients may exert this effect is not fully understood.

In conclusion, this is the first study to show that a diet based on whey protein increases glycogen content in liver and skeletal muscle in exercise-trained rats. We consider that a daily intake of whey protein may play an important role in increasing the glycogen content of tissues in exercised-trained rats.

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