Creatine Supplementation Alters Insulin Secretion and Glucose Homeostasis In Vivo

Kieron Rooney, Janet Bryson, Jenny Phuyal, Gareth Denyer, Ian Caterson, and Campbell Thompson

Dietary creatine supplementation has been used to improve skeletal muscle performance. However, dietary creatine manipulation also affects glucose homeostasis. The aim of this study was to investigate the effect of dietary creatine supplementation on insulin secretion, glucose tolerance, and quadriceps glycogen metabolism in chow-fed rats. Forty-eight rats in total were divided into 2 groups of 24 and were then subdivided into 6 groups of 8. Rats were fed a diet supplemented with 0% (CON) or 2% (CREAT) creatine for 2, 4, or 8 weeks. At these 3 time points an oral glucose tolerance test was performed. Two days later, rats were euthanized and the pancreas and quadriceps muscles were collected. The peak insulin response to a glucose challenge was significantly elevated after both 4 (CON 327 ± 72 *v* CREAT 735 ± 140 pmol/L, *P* = .01) and 8 (CON 248 ± 48 *v* CREAT 588 ± 136 pmol/L, *P* = .02) weeks. Fasting insulin levels were also increased by creatine supplementation for 8 weeks (CON 78 ± 14 *v* CREAT 139 ± 14 pmol/L, *P* = .01). Glucose tolerance was not affected until 8 weeks at which point the peak plasma glucose was elevated in the creatine supplemented group (CON 10.1 ± 0.6 *v* CREAT 13.5 ± 1.5 mmol/L, *P* = .05). A significant increase in pancreatic total creatine content was seen in supplemented animals at 2 (CON 1.2 ± 0.1 *v* CREAT 2.7 ± 0.1 μmol/g wet wt, *P* = .005), 4 (CON 1.5 ± 0.2 *v* CREAT 2.7 ± 0.3 μmol/g wet wt, *P* = .02) and 8 (CON 1.5 ± 0.1 *v* CREAT 2.6 ± 0.1 μmol/g wet wt, *P* = .005) weeks, whereas no change in quadriceps total creatine or glycogen content was observed at any individual time point. This study shows that prolonged creatine supplementation induces abnormalities in pancreatic insulin secretion and changes in glucose homeostasis.

CREATINE SUPPLEMENTATION is used by athletes as it has been shown to improve performance in a number of sports. The overall benefits of dietary creatine supplementation on muscle performance are generally related to an increase in muscle phosphocreatine content.[1,2] Creatine in the muscle acts as a buffer for adenosine triphosphate (ATP) levels within the muscle cytosol at the beginning and end of exercise.[3,4] Although skeletal muscle is the major reservoir for creatine in the body, creatine supplementation has resulted in increased total creatine stores in a number of other tissues such as brain,[5] heart, and kidney,[6] as well as skeletal muscle.

In addition to having a role in ATP buffering in exercise, creatine supplementation affects muscle carbohydrate metabolism. The administration of creatine intraperitoneally causes hypoglycemia,[7] and dietary creatine supplementation has also been shown to improve impaired glucose tolerance[8] and increase resting glycogen stores.[9] Furthermore, creatine supplementation was shown to increase glycogen resynthesis[10] and increase glucose transporter protein (GLUT4)[11] after exercise. It has been speculated that creatine supplementation may enhance insulin-mediated skeletal muscle glucose uptake and glycogen synthesis.[11] However, the basis of these effects of creatine supplementation on glucose metabolism may be a relationship between creatine and pancreatic insulin secretion.

Glucose is accepted as the primary stimulant for insulin secretion, however insulin secretion can also be induced by protein[12] and specific amino acids.[13,14] In vitro studies have shown that creatine directly stimulates insulin secretion from the pancreas.[15,16] The potential for short-term creatine administration to stimulate insulin secretion in vivo has been studied in humans. Insulin secretion was not enhanced by either one 5-g dose of creatine[17] or 3 days of creatine supplementation.[18] However, creatine is usually taken as a dietary supplement for longer than 3 days.

It was hypothesised that creatine supplementation may benefit whole body glucose metabolism and could be relevant to the prevention and/or treatment of type 2 diabetes.[11] This might be true if long-term creatine supplementation could enhance pancreatic insulin secretion in vivo and improve glucose tolerance. However, long-term insulin hypersecretion may not be beneficial as obesity and insulin resistance could develop.[19]

Since creatine supplementation is often taken by athletes for periods exceeding 3 days, the earlier short-term studies on insulin secretion may be inadequate. We suggest that long term creatine supplementation can alter insulin secretion in vivo and that this could provide the mechanism for the observed effects on glucose transport and storage in skeletal muscle. Therefore, this study investigated the effect of 2, 4, and 8 weeks of dietary creatine supplementation on insulin secretion, glucose tolerance, and glycogen metabolism in quadriceps skeletal muscle of rats.

MATERIALS AND METHODS

Animals and Experimental Procedure

This study was approved and performed in accordance with guidelines set out by the Animal Ethics Committee of the University of Sydney, NSW, Australia. Male Wistar rats (obtained from The Animal Resource Centre Perth, Western Australia) were randomly divided into 2 study groups. All rats were housed in cages (4 per cage) on a 12-hour day/night cycle. The control group (CON; n = 8) was fed 20 g of standard chow diet per rat per day. The test group (CREAT; n = 8) was fed 20 g of chow of which 2% by weight was creatine hydrate (Sigma

From the Human Nutrition Unit, Department of Biochemistry, University of Sydney, Sydney, Australia.

Submitted July 25, 2001; accepted October 10, 2001.

Supported by the Diabetes Australia Research Trust, the Institute for Magnetic Resonance Research, Sydney, Australia, and the National Health and Medical Research Council of Australia.

Address reprint requests to Kieron Rooney, Human Nutrition Unit, Department of Biochemistry, Building G08, The University of Sydney, NSW, Australia 2006.

0026-0495/02/5104-0014$35.00/0

doi:10.1053/meta.2002.31330

Chemical, St Louis, MO). Both groups were allowed free access to water.

Two, 4, or 8 weeks after initiation of dietary intervention, an oral glucose tolerance test was performed. After an overnight fast, a blood sample was collected from the tail vein of each rat. The rats were then given a glucose load of 3 g/kg body weight by oral gavage. Blood samples (100 μL) were collected and pooled with 50 μL heparinized saline (10 mg/mL) 15, 30, 45, and 60 minutes postgavage. Blood samples were centrifuged (12,000 rpm, 30 seconds) and plasma was frozen for subsequent glucose and insulin assay.

Two days later, the rats were anesthetized in a nonfasted state, using an intraperitoneal injection of pentobarbitone sodium (60 mg/kg). Mixed quadriceps muscles from the hind limbs were removed, freeze-clamped and stored at -80°C for subsequent assay of creatine and glycogen content, glycogen synthase, and phosphorylase-a activity. The pancreas was also removed, freeze-clamped, and stored at -80°C for subsequent assay of creatine content. The rats were then killed by heart excision.

Analysis

Glucose was analyzed by a glucose oxidase method. Insulin was assayed using a radioimmunoassay (Linco Research, St Louis MO). The total creatine content of the muscle and pancreas was determined in neutralized perchloric acid extracts by a fluorimetric assay.[20] Glycogen extraction and measurement were carried out using a method adapted from Chan and Exton.[21] Glycogen synthase (EC 2.4.1.11) activity was measured using a previously described filter paper method.[22,23] The activity of glycogen phosphorylase-a (EC 2.4.1.1) was determined by a method which involved the precipitation of synthesized glycogen onto the fibres in filter paper.[24]

Statistics

All values in the text are expressed as the mean ± SEM. Significance was assumed if the P value was less than .05 after applying an unpaired Student's t test to fasting blood or to muscle samples or 2-way repeated-measures analysis of variance (ANOVA) testing for the effects of treatment over time with a post-hoc Student's t test as appropriate.

RESULTS

When compared to age-matched chow-fed CON rats, 2, 4, or 8 weeks of dietary creatine supplementation did not result in a difference in body weight or fasting plasma glucose levels (unpaired Student's t test, Table 1). Fifteen minutes following an oral glucose load, plasma glucose levels were elevated in the CREAT group after both 4 weeks (unpaired Student's t test, $P = .07$) and 8 weeks of supplementation (unpaired Student's t test, $P = .05$) when compared to the appropriate CON group (Table 1). The plasma glucose response to an oral glucose load was not different between the CON and CREAT groups when assessed throughout 60 minutes (repeated-measures ANOVA).

Fasting plasma insulin levels were not different between groups at either the 2-week (CON 59.1 ± 2.4 *v* CREAT 59.9 ± 3.2 μmol/L) or 4-week (CON 85.3 ± 6.6 *v* CREAT 101.9 ± 14.6 μmol/L) time point. Fasting plasma insulin levels were increased after 8 weeks of creatine supplementation (CON 78.3 ± 13.5 *v* CREAT 139.0 ± 14.2 μmol/L, unpaired Student's t test, $P = .01$, Fig 1). Fifteen minutes following administration of an oral glucose load, the plasma insulin was significantly elevated in the CREAT compared to CON rats (Fig 1) at 4 weeks (unpaired Student's t test, $P = .04$) and 8 weeks (unpaired Student's t test, $P = .02$) of dietary intervention. When the plasma insulin levels were compared by repeated-measures ANOVA over the entire 60 minutes after an oral glucose load, there was no significant difference between CON and CREAT groups following 2 ($P = .75$), 4 ($P = .10$), or 8 ($P = .13$) weeks of dietary intervention (Fig 1).

Pancreatic creatine content was significantly elevated after 2, 4, and 8 weeks of dietary creatine supplementation (repeated-measures ANOVA, $P < .0001$ followed by post hoc unpaired Student's t test, Fig 2). Quadriceps muscle creatine content in the creatine-supplemented groups was elevated above that in the relevant CON groups by 17%, 5%, and 10 % at 2, 4, and 8 weeks, respectively (repeated-measures ANOVA, $P = .046$); however, the difference was not statistically significant at any of the time points studied (post hoc unpaired Student's t test, Table 2). There was no effect of dietary creatine content on quadriceps glycogen content after 2, 4, or 8 weeks' intervention (repeated-measures ANOVA, $P = .94$, Table 2). There was no change in the glycogen synthase activity or glycogen phosphorylase-a activity in the quadriceps muscles (Table 2).

DISCUSSION

The novel findings of this study are that dietary creatine supplementation increased the total creatine content of the pancreas and that prolonged creatine supplementation resulted in elevated insulin secretion in response to an oral glucose challenge. Furthermore, fasting hyperinsulinemia and altered glucose homeostasis developed after 8 weeks of creatine supplementation.

Creatine administration affects glucose homeostasis and insulin levels. Hill (1928) induced a hypoglycemic response in dogs in vivo following creatine injection suggesting increased glucose disposal. Creatine stimulated insulin secretion directly

Table 1. Body Weight and Plasma Biochemistry

	2 Weeks		4 Weeks		8 Weeks	
	Control	Creatine	Control	Creatine	Control	Creatine
Body weight (g)	142 ± 4	139 ± 3	216 ± 11	195 ± 10	287 ± 6	276 ± 8
Fasting plasma glucose (mmol/L)	4.5 ± 0.2	5.5 ± 0.5	5.1 ± 0.2	5.8 ± 0.3	7.2 ± 0.7	7.5 ± 0.9
15-minute plasma glucose (mmol/L)	11.9 ± 0.5	11.6 ± 0.7	11.9 ± 1.0	15.5 ± 1.3	10.1 ± 0.6	13.5 ± 1.5*

NOTE. Values are mean ± SEM.
*$P < .05$ (Student's t test).

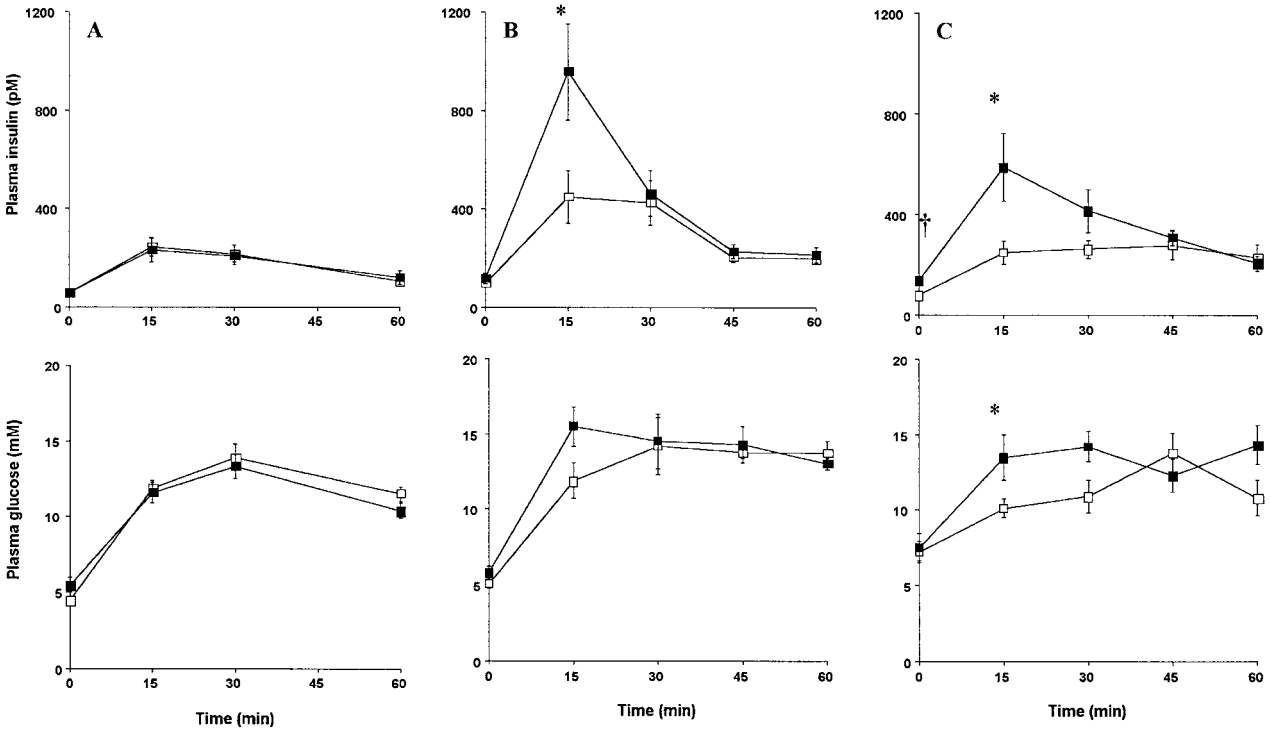

Fig 1. Plasma insulin response (top panels) and plasma glucose response (bottom panels) of rats given an oral glucose load following (A) 2 weeks, (B) 4 weeks and (C) 8 weeks of a diet consisting of standard rat chow (□) or standard rat chow supplemented with creatine (■). Data are expressed as the mean ± SEM. †$P < .01$, *$P < .05$ (post hoc, unpaired Student's *t* test).

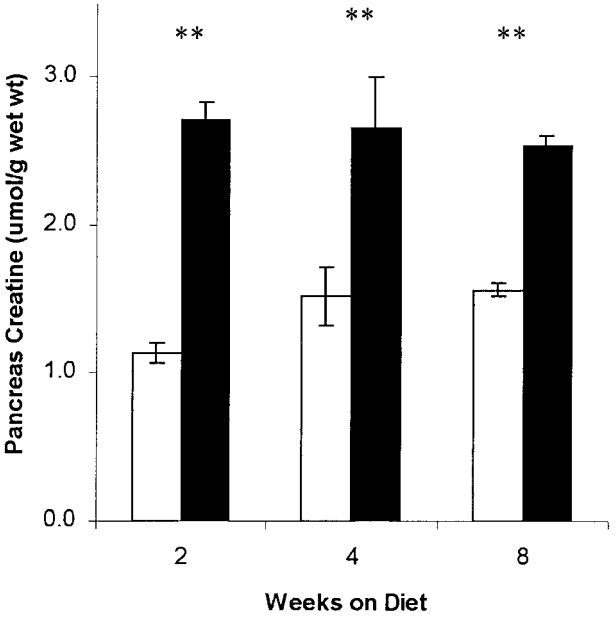

Fig 2. Pancreas total creatine (μmol creatine/g wet wt). Rats were fed a diet consisting of standard rat chow (□) or standard rat chow supplemented with creatine (■) for 2, 4, or 8 weeks. Data are expressed as the mean ± SEM. **$P < .005$ (unpaired Student's *t* test).

in vitro in isolated perfused rat pancreas[15] and isolated mouse islet[16] studies. In humans, the effect of creatine on glucose and insulin is less well examined. Increased glucose disposal may be inferred but cannot be assumed from the increase in GLUT4 protein and glycogen resynthesis observed in previous studies.[10,11] Because of the design of these studies, their results are more suggestive of a synergistic effect of creatine and exercise on GLUT4 protein, glucose disposal, and glycogen storage as opposed to a creatine supplementation-induced increase in insulin sensitivity. Abnormal hyperinsulinemia after creatine ingestion or creatine and glucose ingestion combined was not seen in vivo in human studies in which subjects ingested one 5-g dose of creatine[17] or after 3 days of creatine supplementation.[18] However, these studies do not answer the question of whether longer periods of creatine supplementation affect glucose-stimulated insulin secretion in vivo. The present study, which incorporates a longer term of supplementation with an oral glucose tolerance test, shows abnormal insulin secretion without a significantly lower plasma glucose response after 4 and 8 weeks of 2% dietary creatine supplementation.

In the present study, pancreatic creatine content was measured and was elevated as early as 2 weeks following creatine supplementation. The guanidinium group in creatine produces a structure similar to a known, potent stimulator of insulin secretion, arginine.[15] Specific amino acids, including arginine, enhance insulin release from mouse pancreatic islets incubated with glucose.[13] A glucose drink combined with arginine produced a higher plasma insulin response from humans than

Table 2. Muscle Creatine and Glycogen Metabolism

	2 Weeks		4 Weeks		8 Weeks	
	Control	Creatine	Control	Creatine	Control	Creatine
Creatine (μmol/g wet wt)	23.1 ± 1.7	27.2 ± 1.4	25.7 ± 0.8	26.3 ± 0.6	27.7 ± 0.6	30.5 ± 1.2
Glycogen (nmol glucosyl residues/g wet wt)	40.6 ± 2.2	39.0 ± 2.6	30.7 ± 2.3	34.1 ± 2.8	23.2 ± 1.1	22.3 ± 0.7
Glycogen synthase fractional velocity	0.33 ± 0.04	0.30 ± 0.04	0.37 ± 0.02	0.31 ± 0.05	0.42 ± 0.06	0.36 ± 0.03
Glycogen phosphorylase-a (U/mg wet wt)	58.6 ± 4.2	55.2 ± 5.0	76.6 ± 5.4	72.3 ± 4.4	84.5 ± 6.2	79.1 ± 5.4

NOTE. Values are mean ± SEM.

glucose alone.[14] The basis of this effect of arginine on insulin secretion may be related to a stimulatory effect of arginine on nitric oxide production. It is possible that, since arginine and creatine share structural similarities, elevated levels of creatine might result in altered pancreatic insulin response to glucose ingestion in vivo. Alternatively, an increase in total creatine could result in an increase in the amount of phosphocreatine present within the pancreas in a similar way to the effect of creatine on muscle. The mechanism of insulin secretion is based on the closing of ATP-sensitive channels and an influx of calcium into islet cells which then secrete insulin. Insulin secretion is sensitive to changes in ATP concentration. Any increase in phosphocreatine content resulting from creatine supplementation could alter the energy status within the pancreatic islet and affect ATP flux in times of glucose stimulation. This could result in a greater insulin secretory response to glucose. Interestingly, no change in insulin secretion following glucose ingestion was observed until 4 weeks of dietary supplementation, suggesting that chronic exposure to creatine in vivo is required for a change in insulin secretion, not just a rise in pancreatic creatine content alone. Presumably, some chronic event occurs, such as upregulation of synthesis of proteins important in the regulation of, for example, ATP concentrations or glucose flux into the islet cell.

The experimental diet in the present study was 2% by weight as creatine hydrate. This resembles the dose of creatine used during the initial loading phase of an athlete (20 to 40 g daily). Creatine supplementation of 2% for 2 weeks resulted in a statistically significant increase in total creatine of 20% in rat gastrocnemius muscle.[25] A study in which rats were supplemented with creatine at 3% for 40 days showed no change in heart, brain, or skeletal muscle creatine, yet observed an increase in liver and kidney creatine content.[6] In the present study we saw a 17% increase in quadriceps total creatine content following 2 weeks of supplementation. The difference between control and creatine groups was smaller after 4 and 8 weeks of supplementation. This supports the previous work[6] proving that prolonged administration of dietary creatine has little effect on rat muscle creatine. It is possible that the rat muscles in the present study are close to their maximum total creatine capacity. The muscle creatine transporter is downregulated by creatine supplementation[26] and other tissues such as the pancreas, liver, and kidneys[6] may preferentially take up the available creatine. In previous work, dietary intervention that elevates muscle creatine content can also produce elevated glycogen content,[9,27] but the increase in glycogen appears to be muscle-specific and may reflect the fiber type proportion of the muscle.[11,28,29] In the current study, there was no change in glycogen content and no change in the activity of the enzymes responsible for glycogen synthesis or glycogen degradation was observed.

In conclusion, the present study has shown that sustained dietary creatine supplementation increased pancreas total creatine content. Increased pancreas creatine content was later accompanied by elevated pancreatic insulin secretion in response to a glucose load. Fasting hyperinsulinemia developed subsequent to this, accompanied by an elevated peak plasma glucose following an oral glucose load. Glycogen content of the quadriceps muscle was not altered by dietary creatine supplementation or the resulting changes in insulin secretion. The altered insulin homeostasis induced by creatine supplementation suggests that metabolic abnormalities of the pancreas may accompany prolonged ingestion of creatine.

REFERENCES

1. Feldman EB: Creatine: A dietary supplement and ergogenic aid (review). Nutr Rev 57:45-50, 1999

2. Greenhaff PL: The nutritional biochemistry of creatine (review). J Nutr Biochem 8:610-618, 1997

3. Andres LPA, Sacheck J, Tapia S: A review of creatine supplementation: Side effects and improvements in athletic performance. Nutr Clin Care 2:73-81, 1999

4. Casey A, Constantin-Teodosiu D, Howell S, et al: Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. Am J Physiol 271:E31-37, 1996

5. Stockler S, Holzbach U, Hanefeld F, et al: Creatine deficiency in the brain: A new, treatable inborn error of metabolism. Pediatr Res 36:409-413, 1994

6. Horn M, Frantz S, Remkes H, et al: Effects of chronic dietary creatine feeding on cardiac energy metabolism and on creatine content in heart, skeletal muscle, brain, liver and kidney. J Mol Cell Cardiol 30:277-284, 1998

7. Hill R: The effect of the administration of creatine on the blood sugar. J Biol Chem 78:iv, 1928 (abstr)

8. Ferrante RJ, Andreassen OA, Jenkins BG, et al: Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. J Neurosci 20:4389-4397, 2000

9. Zehnder M, Rico-Sanz G, Kuhne G, et al: Muscle phosphocre-

atine and glycogen concentrations in humans after creatine and glucose polymer supplementation measured non-invasively by ^{31}P and ^{13}C-MRS. Med Sci Sports Exerc 30:S264, 1998 (suppl, abstr)

10. Robinson TM, Sewell DA, Hultman E, et al: Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. J Appl Physiol 87:598-604, 1999

11. Op't Eijnde B, Urso B, Richter EA, et al: Effect of oral creatine supplementation on human muscle GLUT4 protein content after immobilization. Diabetes 50:18-23, 2001

12. Steenge GR, Simpson EJ, Greenhaff PL: Protein- and carbohydrate-induced augmentation of whole body creatine retention in humans. J Appl Physiol 89:1165-1171, 2000

13. Bolea S, Pertusa JA, Martin F, et al: Regulation of pancreatic beta-cell electrical activity and insulin release by physiological amino acid concentrations. Pflugers Arch Eur J Physiol 433:699-704, 1997

14. Van Loon LJ, Saris WH, Verhagen H, et al: Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. Am J Clin Nutr 72:96-105, 2000

15. Alsever RN, Georg RH, Sussman KE: Stimulation of insulin secretion by guanidinoacetic acid and other guanidine derivatives. Endocrinology 86:332-336, 1970

16. Marco J, Calle C, Hedo JA, et al: Glucagon-releasing activity of guanidine compounds in mouse pancreatic islets. FEBS Lett 64:52-54, 1976

17. Green AL, Hultman E, Macdonald IA, et al: Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. Am J Physiol 271:E821-826, 1996

18. Green AL, Simpson EJ, Littlewood JJ, et al: Carbohydrate ingestion augments creatine retention during creatine feeding in humans. Acta Physiol Scand 158:195-202, 1996

19. Ferranini E, Natali A, Bell P, et al: Insulin resistance and hypersecretion in obesity European Group for the Study of Insulin Resistance (EGIR). J Clin Invest 100:1166-1173, 1997

20. Conn RB: Fluorimetric determination of creatine. Clin Chem 6:537-548, 1960

21. Chan TM, Exton JH: A rapid method for the determination of glycogen content and radioactivity in small quantities of tissue or isolated hepatocytes. Anal Biochem 71:96-105, 1976

22. Oakes ND, Cooney GJ, Camilleri S, et al: Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. Diabetes 46:1768-1774, 1997

23. Thomas JA, Schlender KK, Larner J: A rapid filter paper assay for UDPglucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-14C-glucose. Anal Biochem 25:486-499, 1968

24. Gilboe DP, Larson KL, Nuttall FQ: Radioactive method for the assay of glycogen phosphorylases. Anal Biochem 47:20-27, 1972

25. McMillen J, Donovan CM, Messer JI, et al: Energetic driving forces are maintained in resting rat skeletal muscle after dietary creatine supplementation. J Appl Physiol 90:62-66, 2001

26. Guerrero-Ontiveros ML, Wallimann T: Creatine supplementation in health and disease—Effects of chronic creatine ingestion in vivo—Down-regulation of the expression of creatine transporter isoforms in skeletal muscle. Mol Cell Biochem 184:427-437, 1998

27. Op't Eijnde B, Richter EA, Kiens B, et al: Effect of creatine on glucose disposal in rat skeletal muscle. J Sports Sci 17:561-562, 1999

28. Snow RJ, McKenna MJ, Selig SE, et al: Effect of creatine supplementation on sprint exercise performance and muscle metabolism. J Appl Physiol 84:1667-1673, 1998

29. Tanaka T, Ohira Y, Danda M, et al: Improved fatigue resistance not associated with maximum oxygen consumption in creatine-depleted rats. J Appl Physiol 82:1911-1917, 1997