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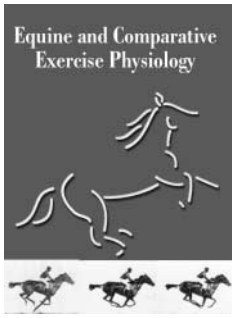

Effects of chromium supplementation on selected metabolic responses in resting and exercising horses

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Abstract

Chromium (Cr) is required for insulin function in the control of cellular glucose uptake. Other functions of Cr relate to its effects on growth, lipid metabolism, immune responses and interactions with nucleic acids. This study was conducted to obtain information on the effect of Cr supplementation on the metabolic responses of five exercising Standardbred horses. During the experiment, horses were fed every day for a 21-day period in a randomized order either a yeast product without Cr (control) or with 4.15 or 8.3 mg Cr day^{-1}. Horses were exercised on a treadmill, alternating a work day of low-speed exercise at 5 m s^{-1} on a 3% incline for 45 min with a rest day. Each horse was adapted over a 21-day period to his or her respective supplementation before undergoing a standardized exercise test (SET). The SET comprised five incremental steps, each of 4 min duration, on a treadmill with a 3% incline; the first step was at 5 m s^{-1} and was followed with increments of 1 m s^{-1}. Blood samples were taken for lactate, plasma glucose, serum insulin and cortisol estimation before, during and after each SET (30, 120 min and 24 h post-exercise). Blood Cr was estimated 2 h after feeding the control or Cr-enriched yeast (intake 8.3 mg Cr) in two horses. Heart rate was monitored throughout each SET. Blood lactate and plasma glucose peaks were highest at 8 and 9 m s^{-1} during the SET when 8.3 mg Cr was supplied. Serum insulin levels declined during the SET and there were no treatment-related changes. Twenty-four hours after exercise, plasma glucose and serum cortisol concentrations returned to basal levels or lower. Serum insulin rebounded 30 min after exercise but 24 h later, serum insulin concentrations were below resting levels. During the recovery period, Cr supplementation did not clearly affect metabolic responses. These results suggest that Cr supplementation had no beneficial effect in healthy, exercising horses.

Keywords: Chromium; glucose metabolism; exercise; horse

Introduction

Chromium (Cr) is an essential trace element that is involved in the metabolism of carbohydrates, lipids and proteins by amplifying the activity of insulin[1,2]. In diabetic humans and rats, Cr supplementation has been shown to increase the cellular uptake of glucose and stimulate insulin metabolism[1–3]. In that context it is hypothesized that Cr forms a complex between insulin and insulin receptors that facilitates the insulin–tissue interaction[1]. Other functions of Cr relate to its effect on growth, lipid metabolism, immune response and interactions with nucleic acids[4].

Cr is present in several oxidation states, but is most stable in the trivalent state, the predominant form in biological systems[4]. However, the Cr content of foods has not been clearly defined and inorganic Cr^{3+} is poorly absorbed (Table 1). In contrast, Cr^{6+} that is derived mainly from industrial exposure is absorbed more readily[4]. The daily dietary Cr intake by humans is generally low, whereas in animals it is considered to be adequate to meet physiological need. There is little retention of Cr and that which is absorbed is excreted primarily via the kidneys[4]. In general, marginal Cr intakes can be

Table 1 The biology of Cr^{4-7}

Feedstuffs ($mg\,kg^{-1}$):	Cereals: 0.1–20.0 Kernels: oat 0.49, barley 0.35, wheat 0.64, rye 0.6 Meat: 0.15 Milk: 0.015 Egg: 0.005–0.02.0 (egg yolk > 20 times) Hay: 0.1–10.0 Water: 0.001–100 Minerals: 60–500
Specification:	Cr^{3+}: most stable oxidation state, predominant form in biological systems Cr^{6+}: bound to oxygen, strongly oxidizing, toxic properties Cr^{0}, Cr^{2+}: no relevance
Absorption:	Passive absorption (small intestine), presumably active transport mechanism, transport of Cr in blood via transferrin Inorganic Cr^{3+}: < 0.5–2.0% (e.g. $CrCl_3$) Organic Cr^{3+}: 25–30% (e.g. high Cr-yeast, CrNic, CrPic)
Excretion:	After absorption: mainly via urinary tract with small losses in faeces, sweat and hair
Storage ($mg\,kg^{-1}$):	Liver: 0.04–1.00 Kidney: 0.05–6.20 Spleen: 0.05–0.30 Hair: 0.20–3.30 Muscle: 0.10–0.20
Requirements:	Humans: 50.0–200 $\mu g\,day^{-1}$ Horses: no available information
Deficiency:	Decreased sensitivity of peripheral tissues to insulin Impaired protein metabolism
Toxicity:	Cr^{3+}: very low toxicity Cr^{6+}: allergic dermatitis, skin ulcers, bronchogenic carcinoma by industrial exposure to Cr

compensated by Cr-containing supplements that contain inorganic $CrCl_3$ or the more available organic forms such as Cr yeast, Cr methionine, Cr nicotinate (CrNic), Cr tripicolinate (CrTri) and Cr picolinate (CrPic).

Studies with humans undergoing strenuous exercise have shown that the urinary excretion of Cr was increased and, as a result, exercise may deplete Cr stores[8–10]. Aside from the compensation of Cr losses, Cr supplementation in athletes would be expected to increase muscle mass by increasing amino acid uptake into cells for incorporation into muscle protein via the potentiating effects of insulin[10]. The only reported study with exercising horses suggested that the daily intake of Cr-enriched yeast (5 mg Cr per horse over a 14-day period) had some beneficial effect on glucose metabolism[11].

Therefore, the aim of this study was to try to confirm the effects of Cr previously reported in horses and to examine the effect of feeding different levels of Cr-enriched yeast on the metabolism of trained performance horses. We hypothesized that, in healthy exercising horses, Cr supplementation would not improve glucose metabolism.

Material and methods

Animals and diets

Horses

Five trained Standardbred horses–three geldings and two mares–were used in this experiment, with a mean body weight of 412 ± 46 kg, a body condition score of 4^{12} and a mean age of 3 ± 2.2 years. The horses were individually housed in box stalls and had free access to a sand paddock 3 h daily. During the experiment the horses were fed a constant basal diet formulated to meet or exceed energy and nutrient requirements according to GEH[13] (1989) recommendations for the performance horse (5 kg grass hay, 2 kg oats, 0.4 l soybean oil, 0.1 kg soybean meal and 0.04 kg NaCl daily). This project was approved by the ethics committee on animal welfare of the Hannover District Government in accordance with German legislation on animal rights and welfare.

Supplementation

In a cross-over design, horses were fed in randomized order each supplement as follows: a yeast product without Cr (= control), a yeast with 4.15 mg Cr (= 4.15 mg Cr, 10.1 ± 1.3 $\mu g\,kg^{-1}$ BW, organic Cr^{3+}) or a yeast with 8.3 mg Cr (= 8.3 mg Cr, 20.2 ± 2.4 $\mu g\,kg^{-1}$ BW, organic Cr^{3+}). The supplement was given at 700 h and the horses were adapted to each supplement over a 21-day period before undertaking the SET (Fig. 1).

Experimental design

All exercise workouts were performed on a high-speed treadmill (Mustang 2200, Kagra AG, Switzerland). Each horse completed an identical 3-month training programme on the treadmill before the experiment started.

All standardized exercise tests (SET 1–3) started with a warm-up phase in which each horse was exercised at 1.8 $m\,s^{-1}$ for 10 min and then at 4.0 $m\,s^{-1}$ for 5 min. Each SET continued with five incremental steps, each of 4 min duration. The initial speed was 5 $m\,s^{-1}$ and it was increased by 1 $m\,s^{-1}$ at each step so that the speed on the last step was 9 $m\,s^{-1}$. All exercise workouts were performed on a treadmill with a 3% incline. Exercise tests were started 3 h after the morning feed. All horses completed three SETs during the whole experimental period.

A 3-week training programme (Fig. 1) was initiated before SET 1 and was continued between the SETs.

The routine training programme started with a warm-up period (1.8 $m\,s^{-1}$ for 10 min and then 4.0 $m\,s^{-1}$ for the next 5 min) and was followed by a low-speed (5 $m\,s^{-1}$, fast trot) exercise session for

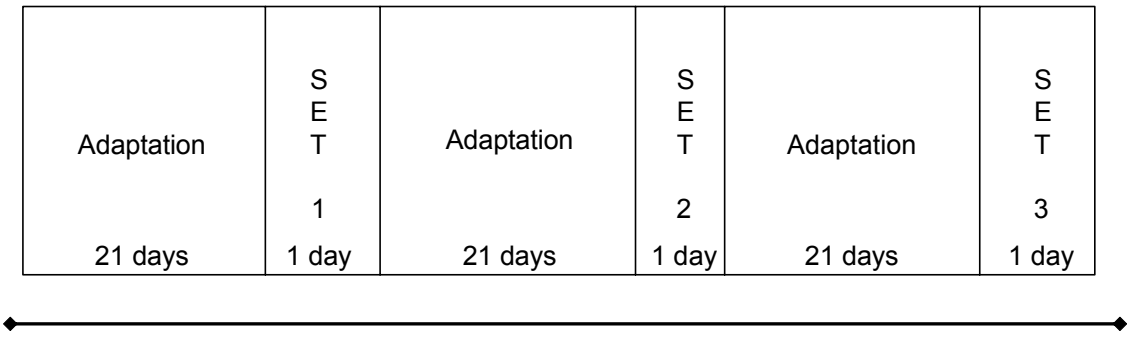


Fig. 1 Experimental protocol

45 min on a 3% incline with a day of rest after each workday. The whole experiment, including SETs and routine training programme, lasted for 10 weeks.

Sample collection

Two hours before the SET began, an indwelling catheter (1.8 × 2.35 mm/14 G, Braun, Melsungen, Germany) was inserted into the *vena jugularis externa*, connected to a 50 cm-long extension set (Fa. Vygon GmbH, Aachen, Germany) and then sutured into place. The extension set and catheter were flushed with physiological saline after every blood sampling.

For the determination of glucose, blood samples were collected immediately before a SET (at rest, 2 h after feeding), immediately before the first step (warm-up) and after each velocity step during a SET and, finally, after the SET (30 and 120 min and 24 h later).

Blood lactate was measured in samples obtained at rest, immediately before the first step (warm-up) and after each velocity step during a SET. Blood samples for insulin and cortisol estimation were taken before a SET (rest), immediately after the last velocity step of a SET as well as at 30 and 120 min and 24 h after a SET. Blood Cr was only measured in samples from two horses, 2 h after intake of the control or the Cr-enriched yeast (8.3 mg Cr) to test if there was a clear difference in plasma content between different levels of supplementation.

Blood samples were centrifuged (12 000 × *g* for 10 min) within 30 min of collection and serum and plasma were harvested and stored at − 20°C for a maximum of 2 months prior to analysis.

Analysis

Lactate analysis of whole blood was performed using a dry chemistry device (Accusport®, Boehringer, Ingelheim, Germany) and plasma glucose was determined using a glucose hexokinase assay (NobiFlow Glucose-HK®, Hitado Diagnostic Systems, Möhnesee, Germany, coefficient of variation = 1.8 ± 0.7%).

Serum insulin and cortisol concentrations were measured using RIA (Insulin-RIA, Fa. DPC Biermann GmbH, Bad Nauheim, Germany, coefficient of variation = 7.9 ± 6.8%; Cortisol RIA according to Klein *et al.*[14], coefficient of variation = 5.8 ± 4.3%). Serum Cr was measured by atomic absorption spectrophotometry (limit of detection: 0.5 $\mu g\,l^{-1}$, Unicam Solar 969, Unicam, Offenbach, Germany).

Heart rate was monitored continuously during SETs by telemetry (monitoring interval of 15 s, Polar®, Fa. Polar Elektro GmbH, Groß Gerau, Germany).

Statistics

Serum insulin and serum cortisol are presented as means ± SD. Data for blood lactate and plasma glucose are described as means and pooled SD. The mean heart rate data were pooled for each velocity step and data are reported as means ± SD. The coefficient of variation (%) for plasma glucose, serum insulin and serum cortisol was calculated as: 100 × (SD)/(mean value of set). The effects of time and differences between the control and Cr supplementation were tested by analysis of variance for repeated measurements (ANOVA). When *F*-values were significant, further analysis was made using the least significance difference test. Multiple regression was performed to analyse the relationship between serum cortisol and insulin as independent variables and plasma glucose as a dependent variable. Statistical significance was considered at $P < 0.05$.

Results

As expected, heart rate rose significantly during each SET (Table 2). A lower heart rate was recorded at 5 $m\,s^{-1}$ for the 4.15 mg Cr intake compared to the control ($P < 0.05$), but at 8 and 9 $m\,s^{-1}$, heart rate was lower for the control compared to horses fed Cr.

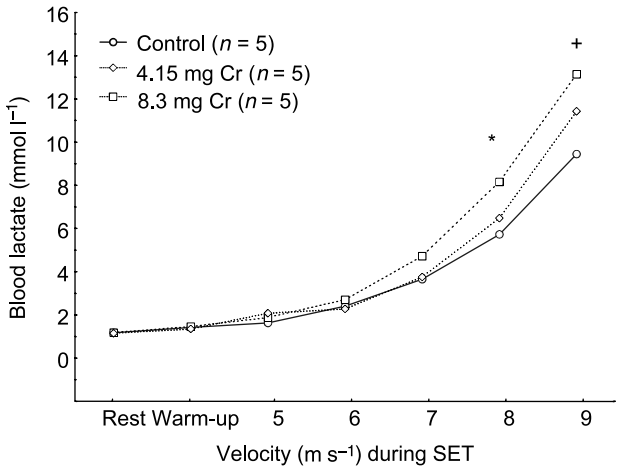
Exercise-related changes in blood lactate during SETs showed higher blood lactate peaks for Cr supplementation at 8 and 9 $m\,s^{-1}$ (Fig. 2).

Table 2 Mean heart rate (beats per min) during SETs for the different treatments ($N = 5$, means ± SD) and at different treadmill speeds

Treatment	*n*	5 ($m\,s^{-1}$)	6 ($m\,s^{-1}$)	7 ($m\,s^{-1}$)	8 ($m\,s^{-1}$)	9 ($m\,s^{-1}$)
Control	5	$150 \pm 21^{a*}$	$161 \pm 14^{b*}$	$169 \pm 14^{c\#*}$	$182 \pm 15^{d*}$	$196 \pm 14^{e*}$
4.15 mg Cr	5	$142 \pm 17^{a+}$	$152 \pm 11^{b+}$	$165 \pm 14^{c\#}$	$181 \pm 11^{d*}$	$199 \pm 14^{e*+}$
8.3 mg Cr	5	$143 \pm 6^{a*+}$	$159 \pm 7^{b*}$	$176 \pm 11^{c*}$	$190 \pm 17^{d+}$	$204 \pm 9^{e+}$

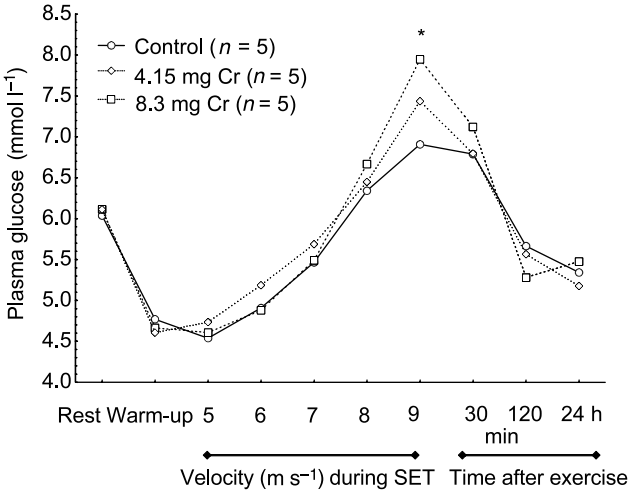
Mean values in the same row with unlike lower-case superscripts are significantly different ($P < 0.05$); mean values in the same column with unlike symbols are significantly different ($P < 0.05$).

At rest, plasma glucose concentrations were similar for the different treatments (control: $6.0 \pm 0.3\,mmol\,l^{-1}$; 4.15 mg Cr: $6.1 \pm 0.06\,mmol\,l^{-1}$; 8.3 mg Cr: $6.1 \pm 0.1\,mmol\,l^{-1}$). A fall in plasma glucose occurred after the warm-up period and was followed by a rise during the SETs (time $P < 0.05$; Fig. 3). The highest plasma glucose value was measured at $9\,m\,s^{-1}$ ($7.9 \pm 0.9\,mmol\,l^{-1}$) in horses fed 8.3 mg Cr, whereas the control-fed animals concurrently peaked at up to $6.9 \pm 0.7\,mmol\,l^{-1}$ (treatment: $P < 0.05$). During the



Time: $P<0.05$
*Treatment 8 m/s: control *vs* 8.3 mg Cr: $P<0.05$, and 4.15 mg Cr *vs* 8.3 mg Cr: $P<0.05$
+Treatment 9 m/s: control *vs* 4.15 mg Cr and 8.3 mg Cr: $P<0.05$, and 1.15 mg Cr *vs* 8.3 mg Cr: $P<0.05$

Fig. 2 Blood lactate concentrations ($mmol\,l^{-1}$) before, during and after SETs, for the different treatments ($n = 5$, means, pooled SD: $3.8\,mmol\,l^{-1}$)



Time: $P<0.05$
*Treatment 9 m s^{-1}: control *vs* 8.3 mg Cr: $P<0.05$

Fig. 3 Plasma glucose concentrations ($mmol\,l^{-1}$) before, during and after SETs, and during recovery period for the different treatments ($n = 5$, means, pooled SD: $0.95\,mmol\,l^{-1}$)

recovery period, plasma glucose concentrations fell in all horses irrespective of treatment and 24 h after the SETs, plasma glucose levels were below resting values (time: $P < 0.05$).

Resting serum insulin levels did not vary across the treatments (Table 3). A SET caused a sharp drop in serum insulin, which rebounded 30 min after finishing the SET. At this time, the lowest peak value was measured in horses fed 4.15 mg Cr. During the course of the recovery period, serum insulin concentrations fell again (120 min post-exercise); the fall was most marked in horses fed the control or 8.3 mg Cr. Twenty-four hours after a SET, serum insulin levels were lower than those measured at rest before a SET; there were no treatment-related differences (time: $P < 0.05$). There was no close relationship between serum insulin and plasma glucose values $y = 6.0480 + 0.02\,179x$, where y, plasma glucose; x, serum insulin ($N = 75$, $r = 0.2$, ß $= 0.048$, $P > 0.05$).

Slightly lower serum cortisol concentrations (non-significant, Table 3) were measured in horses fed 8.3 mg Cr at rest than for those fed the control or 4.15 mg Cr. Exercise caused an expected increase in serum cortisol with a further rise 30 min after finishing a SET (time $P < 0.05$). At this point, the highest value was measured in control-fed animals; the rise was least in those fed 4.15 mg (treatment $P < 0.05$). During the course of recovery, serum cortisol levels fell and similar basal levels were observed 24 h after exercise for all treatments.

There was a strong relationship between serum cortisol and plasma glucose that was best described by the linear regression equation: $y = 4.907 + 0.0207x$, where y, plasma glucose; x, serum cortisol ($N = 75$, $r = 0.61$, ß $= 0.614$, $P < 0.001$).

Mean values in the same row with unlike lower-case superscripts are significantly different ($P < 0.05$); mean values in the same column with unlike symbols are significantly different ($P < 0.05$).

The serum Cr content in two horses, 2 h after the consumption of the control yeast or the Cr-enriched yeast, was the same (data not shown).

Discussion

The resting state

The trace element Cr has attracted attention because Cr supplements have been shown to benefit biological functionality and health in both humans and animals.

It has been suggested that Cr decreases insulin levels and improves glucose disposal in Type 2 diabetic and obese humans (Table 4), whereas in healthy humans, Cr supplements did not benefit glucose metabolism.

Furthermore, Cr supplements can benefit production efficiency and immune responses in cattle, poultry and pigs. Cr picolinate supplements increased muscle area and percentage of muscle in the longissimus muscle of growing pigs[32,33] and litter size in sows[33]. Immune function, stimulated by Cr supplementation, was manifested by higher immunoglobulin production, lowered cortisol levels and reduced morbidity in feeder calves[34–36] and dairy cows[37].

The proposed mode of action of Cr is that increases in blood insulin levels stimulate its uptake by insulin-dependent cells, resulting in the binding of Cr to a low-molecular-weight, chromium-binding substance, chromodulin. The binding of four Cr ions to chromodulin enables the link to the insulin-stimulated, insulin receptor, and thereby amplifies insulin signalling[38–41]. In the present study, Cr supplementation did not affect glucose or insulin concentrations in healthy horses 2 h after a meal. These results were not unexpected as Cr supplementation appears to have its greatest influence in cases of impaired glucose metabolism, such as in diabetes Type 2; normal glucose tolerance is not enhanced[1–3,42,43]. In humans, the daily dietary Cr intake is often below the recommendations of 50–200 $\mu g\,day^{-1}$ in adults (0.7–2.8 $\mu g\,kg^{-1}$ BW)[4,8]. Cr deficiency is evidenced by impaired glucose tolerance, a fasting hyperglycaemia, elevated circulating insulin levels and high blood cholesterol and triglyceride concentrations[43]. As resting glucose metabolism was normal in the current study, Cr intake seemed to be adequate under resting, control conditions. The serum Cr

Table 3 Serum insulin ($\mu U\,ml^{-1}$) and cortisol ($ng\,ml^{-1}$) concentrations before and after SETs, and during the recovery period for the different treatments ($n = 5$, means ± SD)

			SET		Post-exercise recovery period		
Parameter	Treatment	*n*	Before[z]	After	30 min	120 min	24 h
Insulin	Control	5	14.8 ± 2.9^{a}	1.2 ± 0.2^{b}	20.6 ± 7.7^{c*+}	4.9 ± 3.3^{d*+}	2.3 ± 0.7^{d}
	4.15 mg Cr	5	15.2 ± 7.1^{a}	1.2 ± 0.2^{b}	14.7 ± 12.8^{c+}	11.7 ± 10.0^{d+}	3.4 ± 2.4^{e}
	8.3 mg Cr	5	17.3 ± 10.0^{a}	1.1 ± 0.3^{b}	21.7 ± 6.8^{c*}	4.8 ± 5.2^{d*}	4.2 ± 1.5^{d}
Cortisol	Control	5	45 ± 22^{a}	89 ± 22^{b}	110 ± 31^{c*}	62 ± 13^{d}	35 ± 19^{a}
	4.15 mg Cr	5	47 ± 21^{a}	81 ± 21^{b}	92 ± 15^{c*+}	62 ± 19^{d}	35 ± 14^{a}
	8.3 mg Cr	5	37 ± 12^{a}	83 ± 12^{b}	99 ± 22^{c+}	61 ± 11^{d}	30 ± 6^{a}

Mean values in the same row with unlike lower-case superscripts are significantly different ($P < 0.05$), mean values in the same column with unlike symbols are significantly different ($P < 0.05$).

[z] Blood sampling at rest.

Table 4 Effects of Cr supplementation on carbohydrate metabolism in humans[2,3]

Reference	Study type	Study duration	Dose ($\mu g\,day^{-1}$)	Subjects	*n*	Method	Results: Glucose	Results: Insulin
Supplementation of $CrCl_3$								
15	OL	2 months	100	D II	12	OGTT	↔	↔
16	DB	4 months	150	D I, II, NonD	14	OGTT	↔	NA
17	DB	4 months	150	D I, II	43	Meal challenge	↔	↔
18	OL	18–133 days	180–3000	D I, II	6	IVGTT, OGTT	↓	NA
19	DB	6 weeks	200	D II	10	OGTT	↔	↓
20	OL	3 months	200	IGT	5	Hyperglycaemic clamp	↔	↔
21	DB	3 months	220	NonD	26	FBG/insulin	↔	↔
22	OL	2 months	500	D II	12	OGTT	↓	↓
23	DB	3 months	600	D I, II	26	FBG	↓	NA
Supplementation CrPic								
24	OL	1–7 days	600	D	3	FBG	↓	NA
25	DB	6 weeks	200	D II	11	FBG	↓	NA
26	DB	2 months	200	D II	30	FBG	↔	NA
27	DB	4 months	200, 1000	D II	180	OGTT	↓	↓
28	DB	2 months	320, 640	GestD	20	OGTT	↓	↓
29	OL	3 months	400	D II	5	Insulin tolerance	↔	↓
30	OL	1–10 months	500	D II	833	Fasting, post-meal	↓	NA
Supplementation Cr yeast								
31	DB	6 months	160	IGT	26	OGTT	↔	↔

OL, open labelled; DB, double-blind; D, diabetic conditions Type 1 or 2; nonD, non-diabetic; GestD, gestation diabetic; OGTT, oral glucose tolerance test; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; FBG, fasting blood glucose; NA, not assessed; ↓, decrease; ↔, no effect.

levels were similar in supplemented ($\sim 20.2\,\mu g\ Cr\,kg^{-1}$ BW) and non-supplemented horses. In obese women, Cr picolinate supplementation ($\sim 4.5\,\mu g\ Cr\,kg^{-1}$ BW) resulted in higher serum Cr levels in the fasted state (Cr: $2.62 \pm 2.7\,\mu g\,l^{-1}$, placebo: $0.4 \pm 0.41\,\mu g\,l^{-1}$), at one (Cr: $1.24 \pm 1.3\,\mu g\,l^{-1}$, placebo: $0.38 \pm 0.45\,\mu g\,l^{-1}$) and at 2 h (Cr $1.73 \pm 1.28\,\mu g\,l^{-1}$, placebo: $0.5 \pm 0.8\,\mu g\,l^{-1}$) after a meal. However, there was no difference between the placebo and a Cr supplement on blood insulin or glucose levels[10]. Despite changes in serum Cr levels after Cr picolinate supplementation, it is generally accepted that circulating Cr does not reflect tissue Cr concentrations[43]. There is no adequate tool to assess Cr status and, currently, the best method to diagnose Cr deficiency is based on the improvement in glucose tolerance following Cr supplementation[1]; the effects of different Cr supplements on post-prandial serum Cr levels have not been established in horses.

The resting glucose and insulin values were similar to those measured by Gentry *et al.*[44], who found no significant effects of Cr on glucose metabolizm in healthy adult mares following intravenous glucose challenge. In contrast, Ott and Kivipelto[45] showed that Cr supplementation increased the rate at which glucose was metabolized during intravenous glucose tolerance and insulin sensitivity tests in growing horses, with no apparent effects on growth parameters.

The exercise and recovery states

Studies with human athletes have shown that urinary Cr concentrations were increased nearly fivefold 2 h after exercise and, moreover, 24 h urinary Cr losses were about twofold higher on a run day compared to a rest day[8–10,46]. These data suggest that Cr needs may be increased during strenuous exercise. Training has an effect in that basal urinary Cr losses seemed to be about 50% lower in trained than in untrained subjects[8]. Urinary Cr losses reflect Cr that has been mobilized and then lost, since Cr is not reabsorbed in the kidneys[2].

It has been speculated that lower basal urinary Cr losses might be due to a partial depletion of Cr body stores, or that they might reflect an adaptive mechanism to compensate increased Cr losses in exercising human subjects. Aside from the compensation of Cr losses, Cr supplementation in athletes would be expected to increase muscle mass by increasing amino acid uptake into cells for incorporation into muscle protein via the potentiating effects of insulin[47,48]. However, the effect of Cr supplementation on the body composition of human athletes is equivocal (Table 5); some studies have shown benefits whilst more recent work has failed to demonstrate changes in body composition and glucose metabolism during exercise and training.

In the current study, during exercise, similar insulin concentrations were measured in control and Cr-supplemented horses, but higher glucose peaks occurred at the end of the exercise period in Cr-supplemented horses compared to those fed the control diet. Since serum insulin dropped significantly during exercise, it would seem that Cr did not amplify the effect of insulin and, thus, there was insufficient insulin to stimulate the insulin-dependent receptor and Cr transport into the cells[41]. During exercise, and in the early period of recovery, there was an increase in cortisol that would be antagonistic to insulin. This would

Table 5 Effects of supplemental Cr on body composition, minerals and glucose tolerance in exercising humans[2]

Reference	Subjects	Cr suppl. ($\mu g\,day^{-1}$)	Exercise	Duration (weeks)	Method	Results
25	10 males	CrPic: 200	Resistive	5–6	Anthropometry	FFM: ↑
25	31 football players	CrPic: 200	Resistive	6	Anthropometry	FFM: ↑
49	64 adults	CrPic: 200	Resistive	6	Anthropometry	Circumferences: ↑
50	36 football players	CrPic: 200	Resistive	9	Densitometry	No effects
51	95 males and females	CrPic: 200	Aerobic	16	Anthropometry	No effects
52	36 males	CrPic, $CrCl_3$: 170–180	Resistive	8	DXA minerals	DXA: no effects; Iron, copper: ↓
47	16 males	CrPic: 200	Resistive	12	Densitometry	No effects
53	43 obese females	CrPic, CrNic: 200	Aerobic	9	Densitometry GT	CrPic without execise: BW ↑ CrNic with exercise: BW ↓ GT: no effects
47	122 adults	CrPic: 400	Variable	9	Densitometry	Weight: ↓, fat: ↓
54	18 elderly males	CrPic: 1000	Resistive	12	Densitometry	No effects

↑, increase; ↓, decrease; FFM, fat-free mass; DXA, dual X-ray absorptiometry; GT, glucose tolerance; BW, body weight.

prevent the entry of glucose into muscle cells and adipose tissue, thereby making it available for tissues of high demand. Exercise-induced changes in cortisol have been frequently reported in horses[55] and are characterized by a cortisol peak 30 min after finishing strenuous exercise. The close relationship between cortisol and glucose ($r = 0.61$, $P < 0.05$) supports the conclusion that effects of insulin on glucose metabolism ($r = 0.2$, $P > 0.05$) were overridden during exercise and recovery. In stressed calves, reduced cortisol concentrations were measured following Cr supplementation[34–36], but the precise mechanism of this effect is unclear. A similar result was obtained in the present investigation; the control-fed group had higher cortisol levels 30 min post-exercise in comparison to horses fed 4.15 mg Cr. However, the higher Cr intake ($8.15\,mg\,day^{-1}$) did not cause a significant reduction in cortisol and, thus, the effect of Cr appears to be dose independent. The results of the current study contrast with those of Pagan *et al.*[11], who measured lowered blood levels of insulin, cortisol and glucose during exercise in trained Thoroughbreds after feeding a Cr-enriched yeast (5 mg $Cr\,day^{-1}$). Plasma glucose fell below $4\,mmol\,l^{-1}$ during high-speed exercise in Pagan's experiment and hypoglycaemia of this nature during exercise may have a negative impact on performance[56]. Furthermore, the decrease in plasma glucose measured by Pagan (even in the control group) was rather surprising in view of the fact that the plasma glucose level in horses performing high-speed exercise normally increases[57–59].

The most striking results in the present study were the high lactate and glucose peaks, together with high heart rates measured at the end of exercise in the Cr-supplemented animals. Our results confirm those of Gentry *et al.*[44], who measured higher lactate levels in Cr-supplemented horses post-exercise. High heart rates and lactate levels are known to reflect an impaired capacity for exercise in horses. The results obtained in the current study may reflect an interaction between Cr and iron. *In vivo* it has been shown that transferrin is the major transport protein for Cr in blood and, as iron competes with Cr for binding sites, Cr supplementation might inhibit iron binding[60]. In human athletes, a reduction in iron binding to transferrin was measured 8 weeks after the daily supplementation of $\sim 2.8\,\mu g\ Cr\,kg^{-1}$ BW, either as picolinate or chloride[52]. In addition, Cr supplementation significantly affected iron excretion. Compared with a placebo, Cr supplementation reduced urinary iron; a greater reduction was obtained with the Cr picolinate than with the Cr chloride[52]. There were significant decreases in serum iron, ferritin and haemoglobin in rats supplemented with Cr over a 45-day period when compared with control animals[61]. In contrast, iron metabolism was unchanged during a 12-week Cr picolinate supplementation ($13.2\,\mu g\,kg^{-1}$ BW) in human adults engaged in a resistive training programme[62]. In the present study, it was assumed that a decreased haemoglobin concentration would negatively impact oxygen transport, resulting in higher heart rates and the upregulation of anaerobic metabolic pathways during exercise. It should be emphasized that the level of Cr supplementation in the current study was somewhat higher than that given to humans and the effects on lactate and heart rate seemed to be dose dependent. Unfortunately, there was no estimation of transferrin saturation or haemoglobin during the current work; interactions between Cr and iron warrant further study in exercising performance horses.

In conclusion, Cr supplementation in healthy trained horses elicited no beneficial effects on glucose and insulin metabolism during both rest and exercise. However, Cr supplementation may have beneficial

effects in fat and diabetic horses, and this requires further investigation.

The negative impact of Cr supplementation on heart rate and lactate accumulation during exercise needs further elucidation as there might be an adverse effect of Cr on iron metabolism.

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