Creatine supplementation does not affect kidney function in an animal model with pre-existing renal failure

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Abstract

Background. Creatine is widely used as an ergogenic substance among athletes. Safety of prolonged creatine intake has been questioned, based upon case reports and animal data. We investigated the effect of prolonged creatine ingestion on renal function in animals with normal kidney function or pre-existing kidney failure, respectively.

Methods. Male Wistar rats were randomly allocated to four experimental groups: (i) sham-operated, control diet; (ii) sham-operated, creatine-supplemented diet (2% w/w (0.9±0.2 g creatine/kg body weight/day)); (iii) two-thirds nephrectomized, control diet; and (iv) two-thirds nephrectomized, creatine supplemented diet. Glomerular filtration rate was determined using inulin and creatinine clearance, together with albumin excretion, urea clearance, muscle and serum creatine and serum cystatin C concentrations.

Results. In contrast to previous reports, no detrimental effects of creatine supplementation on the renal function indices were observed in two-thirds nephrectomized or sham-operated animals. No differences were observed in inulin (0.28±0.08 vs 0.25±0.08 ml/min/100 g; P=NS) or creatinine clearance rates. Serum cystatin C concentration, urinary protein excretion, and albumin and urea clearance were comparable between creatine-supplemented and control-diet fed animals in both sham-operated and two-thirds nephrectomized animals. Serum creatine and intramuscular total creatine concentrations were higher in creatine-supplemented groups (P<0.05).

Conclusions. Creatine supplementation at a dosage of 2% w/w for 4 weeks does not impair kidney function in animals with pre-existing renal failure or in control animals.

Keywords: creatine supplementation; kidney function

Introduction

In recent years creatine has received considerable attention in the medical and lay press and is widely used as an ergogenic substance. Numerous publications have reported the beneficial effect of creatine supplementation on exercise performance, in healthy subjects or animals as well as in patients or animal models of neurodegenerative diseases [1–3].

As early as 1926, Chanutin [4] reported increased creatinine excretion after creatine ingestion in humans. Several studies have described the non-enzymatic degradation of creatine to creatinine, without evidence of altering kidney function. Phosphocreatine is converted to creatinine at a rate of 2.6%/day and creatine at 1.1%/day in vivo [2,4].

Based upon two case reports, safety of prolonged high-dose creatine intake has recently been questioned [5–7], but no causal relationship between creatine ingestion and renal failure can be attributed on the basis of these case reports. Controlled studies [5,8–11] could not demonstrate major adverse effects or health risks of creatine supplementation in humans. Muscle cramps, stomach upset or diarrhoea were reported as sporadic complaints. No biochemical evidence is present on eventual impairment of kidney or liver function in humans. In contrast, in Han:SPRD-cy rats, an animal model of human polycystic kidney disease, creatinine clearance diminished and renal impairment was found to be accelerated upon creatine supplementation [12].

Exact determination of glomerular filtration rate (GFR) in small laboratory animals can be a cumbersome task [13]. Isotope-based methods and inulin clearance are still regarded as gold-standard methods. Creatinine clearance is easier to perform; however, creatinine is partly secreted by the tubules and analytical
interferences exist in serum creatinine determination. The Jaffe reaction is influenced by protein, bilirubin, glucose and other non-specific chromogens [14]. The magnitude of the interference varies from method to method. Moreover, creatinine production is dependent on body composition and increases upon creatinine supplementation. Several low-molecular-weight proteins have been described as good alternatives to serum creatinine in determining kidney function, independent of body composition. Cystatin C is a 13.3 kDa cysteine-protease inhibitor present in plasma. It has been regarded as the product of a ‘housekeeping’ gene and therefore production is considered to be stable. Cystatin C is freely filtered and neither reabsorbed intact nor secreted. Using anti-human cystatin C antibodies, cystatin C concentrations have been measured in rat sera with good linearity [15]. This study investigates the effects of creatine supplementation on kidney function in rats with normal kidney function and in rats with pre-existing renal impairment. Glomerular filtration was investigated using inulin and creatinine clearances, together with serum cystatin C concentration, 24 h protein excretion, urea and albumin clearance.

Subjects and methods

High-pressure liquid chromatography of the creatine formulation

Creatine monohydrate (99% pure) was obtained from Sigma (St Louis, MO, USA). Possible creatinine contamination of this formulation was assessed by high-pressure liquid chromatography (HPLC) analysis as described previously [16].

Experimental model of moderate chronic renal failure

Male Wistar rats weighing 200–230 g were obtained from Iffa Credo (Brussels, Belgium). The animals had free access to drinking water and were fed ad libitum. After acclimatization, animals were randomly allocated to four experimental groups: (i) sham-operated, normal diet (n=10); (ii) sham-operated, creatine fed (n=10); (iii) renal failure, control diet (n=12); and (iv) renal failure, creatine fed (n=11). All animal care was in accordance with local prescriptions and the NIH Guide for the Care and Use of Laboratory Animals.

Renal failure was induced using a standard procedure of tissue removal (two-thirds nephrectomy), as described previously [17]. In brief, rats were anaesthetized with halothane (Fluothane; AstraZeneca, Södertälje, Sweden) and a flank incision was made exposing the kidney. The upper and lower pole of the left kidney were cryoablated, followed 1 week later by a right nephrectomy. Sham procedure consisted of flank incision and manipulation of the kidney without destruction of tissue. The cumulative mortality, including nephrectomy/sham procedure, was 14%, mostly due to post-operative bleeding or anaesthesia-associated deaths [17]. Renal failure was confirmed by a serum creatinine determination 1 week after the last incision. Dietary manipulation was started 1 week after last surgery/incision.

Control animals received a soy-based grounded maintenance chow (RM1; Special Diet Services, Witham, UK) containing 14% protein. Creatine monohydrate (2% w/w) was added to this diet in the creatine-supplemented groups.

Rats were housed in metabolic cages for 24 h during the study at two occasions: at the start and after 4 weeks of creatine supplementation. Urine and blood samples were collected and food intake noted on these occasions. Body weight was measured once a week. Creatine intake in the supplemented groups was calculated from food intake. Creatine intake in the control-diet groups is negligible due to the absence of meat products in the diet.

Measurement of glomerular filtration rate and proteinuria

Creatinine clearance. Serum and urinary creatinine concentrations were determined using an enzymatic method (Roche Diagnostics, Mannheim, Germany) on a Hitachi 911 Autoanalyzer according to the manufacturer’s procedures. Creatinine clearances were calculated based upon 24 h urine collections.

Inulin clearance. After 4 weeks of creatine supplementation, animals (n=27) were anaesthetized using pentobarbital (5 mg/100 g) (Nembutal; Sanofi, Libourne, France) and placed on a temperature-controlled heating pad. The trachea was intubated, the left carotid artery was canulated using a PE50 catheter for repeated blood sampling and the left jugular vein was canulated for continuous saline administration at a rate matching diuresis. A single bolus (80 mg/kg) of fluorescein isothiocyanate (FITC)-inulin (Sigma) was administered intravenously and plasma samples were obtained at t=0, 3, 30, 120, 140, 160 and 180 min. The FITC-inulin concentration was determined using a 96-well fluorometer (Fluoroscan; Tiertek, Huntsville, AL, 35805). Inulin clearance was calculated as described previously [13].

Cystatin C concentrations were measured using anti-human cystatin C antibodies (Dade Behring, Marburg, Germany) on a BN nephelometer (Dade Behring). Relative values are reported, expressed as μg/l of human cystatin C, as described previously [15]. Using this cystatin C assay, correlations between renal function indices were in accordance with published data (serum creatinine vs cystatin C: r=0.92, P<0.001; creatinine clearance vs 1/cystatin C: r=0.94, P<0.001; inulin clearance vs 1/cystatin C: r=0.82; P<0.001). Using these animal samples, the coefficient of variance (CV) value for this assay was 5.0%.

Serum and urine urea were determined enzymatically using Roche diagnostics kits on a modular autoanalyser. Urea clearances were calculated using 24 h urine collections. Serum albumin was determined using the bromocresol green method. Total urinary protein was measured by pyrogal-lol red. Urine albumin concentration was determined as described before using a Protur Hisi kit [18].

Tissue collection and processing

After the inulin clearance determinations, the animals were killed by cervical dislocation under pentobarbital anaesthesia.
and hindlimb muscles (soleus and gastrocnemius) were quickly excised, trimmed of fat and connective tissue, washed in phosphate-buffered saline (pH 7.4, 0.075 M), blotted dry, weighed and stored at −70 °C for further analysis.

Serum creatine was determined enzymatically [19] and total muscle creatine concentrations were determined colorimetrically [20].

Statistical analysis

Multiple-sample comparison was performed using Kruskal–Wallis testing. One-sample Kolmogorov–Smirnov test was used to pre-test normal distribution. Student’s $t$-test (means±SD) or Mann–Whitney $U$-test [median (interquartile range)] were used to compare separate groups when appropriate. Differences were considered significant at $P<0.05$. Correlation between parameters was examined using regression analysis.

Results

High-pressure liquid chromatography of the creatine formulation

The creatine monohydrate formulation (Sigma) used in this study for supplementation was found to contain 0.37% creatinine. No other side products were detected in the formulation.

Effect of creatine supplementation on glomerular filtration rate

No detrimental effect of creatine supplementation on kidney function could be observed during the study period.

Table 1 summarizes serum and urine renal function indices. Serum creatinine concentrations were higher in two-thirds nephrectomized creatine-supplemented animals, compared with two-thirds nephrectomized control-diet fed animals $(0.72 ± 0.19 \, \text{mg/dl} \, P<0.001)$. Sham-operated animals did not differ in serum creatinine concentrations upon creatine supplementation. No significant difference was observed in serum urea or cystatin C concentrations or in 24 h protein excretion between supplemented and control-diet fed animals. Two-thirds nephrectomized animals had significantly different serum creatinine, cystatin C (Figure 1) and serum urea concentrations ($P<0.01$) and exhibited higher 24 h proteinuria [$19.3 ± 7.5 \, \text{mg/dl}; \, P<0.05$ (pooled data)] and 24 h albuminuria [$5.5 ± 4.5 \, \text{mg/dl}; \, P<0.01$ (pooled data)].

Creatine-supplemented groups exhibited higher serum creatinine concentrations [$11.66 ± 5.48 \, \text{mg/dl}; \, P<0.0001$ (pooled data)]. No significant differences in creatine concentrations were observed between two-thirds nephrectomized and sham-operated animals for either creatine-supplemented or control-diet groups.

Inulin and creatinine clearance rates, corrected for body weight and serum cystatin C concentrations, are illustrated in Figure 1. No differences were observed in either inulin, creatinine clearance [absolute values (data not shown) or relative to body weight] or serum cystatin C values (P = NS) between creatine-supplemented and control-diet fed animals. Glomerular filtration rate in two-thirds nephrectomized animals was significantly lower compared with sham-operated animals [inulin clearance: $0.21 ± 0.04 \, \text{ml/min/100 g, P}<0.001$; creatinine clearance: $0.30 ± 0.07 \, \text{ml/min/100 g, P}<0.001$; cystatin C: $97 ± 21 \, \mu g/l, P<0.001$ (pooled data)].

Creatine clearance rates at the start and after 4 weeks of creatine supplementation are illustrated in Table 2. Creatinine clearance was comparable at the start and after 4 weeks of creatine supplementation between supplemented and control-diet fed rats in either sham-operated or two-thirds nephrectomized animals.

Recuperation of kidney function after renal ablation/nephrectomy was comparable in the creatine-supplemented group vs control-diet fed rats ($0.69 ± 0.34 \, \text{ml/min/100 g, P} = \text{NS}$). 24 h proteinuria was significantly higher in the renal failure group.

<table>
<thead>
<tr>
<th>Table 1. Serum and urine renal function indices after 4 weeks of creatine supplementation</th>
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<tbody>
<tr>
<td>Serum creatinine (mg/dl)</td>
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<tr>
<td>Serum creatinine (mg/dl)</td>
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<tr>
<td>Serum creatinine (mg/dl)$^b$</td>
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<tr>
<td>Serum urea (g/l)$^b$</td>
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<tr>
<td>24 h urinary protein excretion (mg/24 h)$^b$</td>
</tr>
<tr>
<td>24 h urinary albumin excretion (mg/24 h)$^b$</td>
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</tbody>
</table>

Kruskal–Wallis multiple group comparison was significant for all parameters ($P<0.001$).

$^aP<0.05$, creatine-loaded animals compared with their respective control-diet fed animals.

$^bP<0.05$, two-thirds nephrectomized animals had significantly different renal function indices compared with their respective controls. Serum creatine did not differ significantly between two-thirds nephrectomized and sham-operated animals.
Effect of creatine supplementation on albumin and urea excretion

Urea and albumin clearance rates are illustrated in Figure 2. No differences were observed in either urea or albumin clearance between creatine-supplemented and control-diet fed animals [urea clearance: $0.21 \pm 0.06$ vs $0.18 \pm 0.07$ ml/min/100 g, $P=NS$; albumin...
clearance: $0.19 (0.013–0.036)$ vs $0.18 (0.011–0.042)$ \(\mu \text{mol/min/100 g} \), \(P = \text{NS} \) (Mann–Whitney \(U\)-test) (pooled data)).

**Effect of creatine supplementation on body mass, food intake and muscular creatine concentrations**

Table 3 summarizes food intake and body mass in the four experimental groups.

No difference in body weight or food intake was observed after 4 weeks of creatine supplementation in either sham-operated or in the two-thirds nephrectomized group.

Two-thirds nephrectomized animals exhibited lower body mass than sham-operated controls \([387 \pm 22 \text{ vs } 421 \pm 21 \text{ g}; P < 0.001 \) (pooled data)]. Daily food was not different between sham-operated and two-thirds nephrectomized animals \([17.5 \pm 2.6 \text{ vs } 17.4 \pm 3.7 \text{ g}; P = \text{NS} \) (pooled data)]. Creatine intake, expressed as absolute intake \((353 \pm 69 \text{ vs } 333 \pm 85 \text{ mg}; P = \text{NS} \) (pooled data)] or relative to body weight \((853 \pm 173 \text{ vs } 870 \pm 242 \text{ mg/kg}; P = \text{NS} \) (pooled data)] in supplemented animals did not differ between two-thirds nephrectomized and sham-operated animals.

Total creatine intramuscular concentrations are increased upon supplementation \([23.9 \pm 4.0 \text{ vs } 21.5 \pm 2.3 \mu \text{mol/g}; P = 0.02] \), gastrocnemius: \(35.8 \pm 2.9 \text{ vs } 32.8 \pm 1.6 \mu \text{mol/g}, P < 0.001 \) (pooled data)]. No difference in muscular creatine concentration was observed comparing two-thirds nephrectomized animals with sham-operated animals \([24.0 \pm 4.2 \text{ vs } 21.9 \pm 2.6 \mu \text{mol/g}, P = \text{NS} \) (pooled data)]

**Discussion**

The present study could not demonstrate any deleterious effect of creatine supplementation on kidney function. No changes in GFR or renal protein handling were observed. Creatine supplementation was shown to increase intramuscular total creatine concentrations in sham-operated and nephrectomized animals.

Creatinine determinations are susceptible to analytical interferences. These in vitro interferences can make up a substantial fraction of the apparent creatinine concentrations. The Jaffé reaction is particularly susceptible to non-specific chromogens, such as bilirubin and cephalosporins [14]. Creatine supplementation can interfere with creatinine determinations by Jaffé’s method, with apparent increases in creatinine concentrations up to 8% (Y. Taes, unpublished data). No analytical interferences were found using the enzymatic assay with increased creatine concentrations, as found in animals or subjects who ingest creatine (Y. Taes, unpublished data).

As creatine feeding could interfere with creatinine determination, we investigated GFR by means of the gold-standard procedure (inulin clearance), together with creatinine clearance and serum cystatin C. No significant alterations in any of the markers were observed upon creatine supplementation, indicating no effect on GFR. Urea clearance was comparable in creatine-supplemented and control-diet animals. No changes in 24 h proteinuria or albumin excretion were noted in both sham-operated and two-thirds nephrectomized animals upon creatine supplementation, indicating absence of structural glomerular changes.

Serum creatinine concentrations were found to be higher in nephrectomized supplemented animals but not in creatine-supplemented sham-operated animals. With declining kidney function, increased serum creatinine concentrations are attained in order to excrete the increased creatinine load, due to creatine–creatinine interconversion. However, care is warranted as creatinine has been described as a weak uraemic toxin. Inhibitory effects on metabolic processes have been described, however, at supraphysiological concentrations and not observed in vivo [21].

Upon creatine supplementation, Edmunds et al. [12] demonstrated renal disease progression in Han:SPRD-cy rats. These authors described increases in serum creatinine concentrations in male (but not in female) rats. Unfortunately, no independent renal function measurements were performed. Creatinine clearances were diminished in both sexes. Creatinine

**Table 3. Effect of 4 weeks of creatine supplementation on body mass, food intake and total creatine intramuscular concentration**

<table>
<thead>
<tr>
<th></th>
<th>Sham-operated</th>
<th>Two-thirds nephrectomized</th>
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<tbody>
<tr>
<td></td>
<td>Control diet ((n = 10))</td>
<td>Creatine loaded ((n = 10))</td>
</tr>
<tr>
<td>Body mass (g) ((t = 0 \text{ days}))</td>
<td>259 ± 15</td>
<td>262 ± 16</td>
</tr>
<tr>
<td>Body mass (g) ((t = 28 \text{ days}))*</td>
<td>416 ± 23</td>
<td>426 ± 18</td>
</tr>
<tr>
<td>Food intake (g) ((t = 0 \text{ days}))</td>
<td>17.3 ± 2.5</td>
<td>18.5 ± 2.0</td>
</tr>
<tr>
<td>Food intake (g) ((t = 28 \text{ days}))</td>
<td>17.2 ± 1.3</td>
<td>17.7 ± 3.4</td>
</tr>
<tr>
<td>Total creatine, soleus (µmol/g)</td>
<td>22.1 ± 1.9</td>
<td>25.4 ± 5.0</td>
</tr>
<tr>
<td>Total creatine, white gastrocnemius (µmol/g)*</td>
<td>32.2 ± 0.9</td>
<td>35.9 ± 3.5*</td>
</tr>
</tbody>
</table>

Creatine concentrations are expressed as µmol/g wet weight.

*\(P < 0.05\), Kruskal–Wallis multiple group comparison.

b\(P < 0.01\), two-thirds nephrectomized animals compared with their respective sham-operated control animals.

c\(P < 0.01\), creatine loaded animals compared with their respective control-diet fed animals.
was determined using an end point Jaffé-based method (alkaline picrate; Sigma Diagnostics). The Jaffé method is susceptible to interference from non-specific chromogens [14], varying from method to method. The creatinine values reported by Edmunds et al. [12] might have been influenced by creatine concentrations. Unfortunately, no creatine determinations were communicated in the former study. Creatine was supplemented as a mixture of creatine and glutamine in an over-the-counter formulation. This formulation could contain traces of contaminants or toxic products. Purity of this formulation has not been established.

The Han:SPRD-cy rat is a well-established animal model of human polycystic kidney disease. Several interventional studies reported effects on kidney function in this animal. Dietary interventions have been shown to delay the progression of renal disease [22]. While this model mimics human polycystic disease well, it cannot be used as an animal model for general renal functional impairment.

The remnant kidney model is a well-established animal model for kidney function impairment, by means of kidney tissue ablation. Intrinsic renal disease is, however, absent in these animals and the kidney function can partly recover over time. These differences in animal model can account for the differences found between the present study and the study by Edmunds et al. [12].

In humans, no evidence from controlled studies is available describing adverse effects of creatine supplementation. Poortmans [5, 8, 9, 11] investigated kidney function in young healthy subjects. No alterations in creatinine, urea or albumin clearance were reported in short-term (5 days, 20 g creatine daily [8]) or long-term studies (1 month to 5 years, with 1–80 g creatine daily [9]; 63 days with 21 g creatine daily [11]). Robinson et al. [10] reported only transient increases in serum creatinine during creatine ingestion. Six weeks after cessation of the creatine supplementation, serum creatinine had returned to baseline. No gold-standard procedures for determining GFR, such as inulin or isotope-based methods, have been performed in humans.

Creatine is regarded as a nutritional supplement and available over-the-counter. The different industrially prepared formulations are not subjected to adequate quality control. Toxic side products or contaminants could be present in these formulations. No toxic side products were detected in the creatine monohydrate formulation by our HPLC method [16]. Creatinine contamination was found to be 0.37%. Intake of creatinine is therefore very low in comparison to creatine intake.

This study demonstrates that creatine supplementation can increase intracellular creatine concentration in skeletal muscle of renal failure animals, as described in animals with normal kidney function [1]. Serum and muscle creatine concentrations were found to increase upon creatine supplementation in animals with kidney function impairment. Creatine supplementation in animals has been shown to increase intracellular creatine concentration (total, free and phosphocreatine) in both fast-twitch and slow-twitch skeletal muscles, together with an improved running performance [1].

Differences in creatine concentrations between the soleus and gastrocnemius are in accordance with reported values [1], reflecting differences in energy metabolism. No intramuscular phosphocreatine or high energy nucleotides are reported in the present study as the prolonged anaesthesia during inulin clearance determination could have influenced these high energy metabolites.

In conclusion, we could not demonstrate any harmful effects of prolonged high dose creatine supplementation on glomerular filtration or protein excretion in an animal model with pre-existing moderate renal dysfunction. Muscle intracellular creatine concentration was increased upon creatine supplementation in these animals.

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