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Creatine supplementation decreased homocysteine plasma levels in rats but not humans: A critical review with meta-analysis

Rafael Deminice*, Flávia Troncon Rosa

Department of Physical Education, State University of Londrina, Londrina PR, Brazil

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ABSTRACT

Background: Increasing evidence has shown that an elevated a level of homocysteine (Hcy) in the blood is related to several diseases. Over the last few years, studies have demonstrated creatine (Cr) synthesis and Hcy formation are metabolically connected; and Cr supplementation can decrease Hcy blood levels in different situations. This data however is inconsistent and still controversial.
Objective: The aim of this critical review with meta-analysis was to discuss and ascertain the effects of Cr supplementation on blood Hcy levels.
Method: A review was conducted according to the PRISMA guidelines using PubMed Discuss and Scielo online databases to identify relevant studies through November 2015. RevMan was used to calculate the effect size of the change in Hcy plasma/serum concentration from baseline to post-supplementation with Cr vs. placebo groups. Weighted mean differences were calculated using random effect models.
Results: Cr supplemented trials were divided into two subgroups according to whether the experimental design included animals or humans participants. Overall, 14 studies were included in the meta-analysis. The six rodent included studies reported decreased plasma Hcy concentration after Cr supplementation with a mean effect size equal to −2.43 μmol/l (95% CI: 3.60, −1.26, $P < 0.01$). The humans studies involved 483 participants (242 Cr and 241 placebo supplemented subjects) and indicated no changes in plasma Hcy concentration after Cr supplementation compared to placebo (0.09 μmol/l, 95% CI: −0.47, 0.66, $P = 0.18$).
Conclusions: Our data demonstrated Cr supplementation is effective in decrease Hcy blood concentration in rats; the same effect however, is not demonstrated humans studies. Human and rats particularities in Hcy metabolism and poorly controlled humans studies may contribute to the divergence of results.

Contents

* Corresponding author. Department of Physical Education, State University of Londrina, Rodovia Celso Garcia Cid, Pr 445 Km 380, Campus Universitário, Londrina, Paraná, Brazil.
E-mail address: rdeminice@uel.br (R. Deminice).

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1. Introduction

Homocysteine (Hcy) is a sulfur amino acid synthesized in the liver as a byproduct of methionine metabolism [1]. Hcy has recently gained attention in the literature due to its association with several diseases that may increase the risk of mortality [2,3]. Humphrey et al. [4] demonstrated that each increase of 5 μmol/l in plasma Hcy concentration increased the risk of cardiovascular events by approximately 20%. Bostom et al. [5] demonstrated elevated blood Hcy concentration is independently associated with an elevated risk of mortality (54% for all-cause mortality and 52% for cardiovascular mortality).

Creatine (Cr) occurs naturally in food, especially in meat and fish. In human omnivores, one-half of Cr required is provided in the diet and the remainder is endogenously synthesized [6]. Recent studies have shown Cr synthesis and Hcy formation are metabolically connected (Fig. 1) [7,8]. Cr synthesis involves the reversible transfer of the amidino group of arginine to glycine to form guanidinoacetic acid (GAA) and ornithine in a reaction catalyzed by the enzyme arginine: glycine amidinotransferase (AGAT), which is very active in kidneys. Next, the irreversible transfer of a methyl group from S-adenosylmethionine (SAM) to GAA is catalyzed by the enzyme guanidinoacetate N-methyltransferase (GAMT) in the liver [6] and Cr is formed. SAM acts primarily as a universal methyl donor in the synthesis of several others methylated compounds such as neurotransmitters (adrenaline, noradrenaline), DNA, RNA, phosphatidylcholine and others. A byproduct of these methylation reactions is S-adenosylhomocysteine, which is hydrolyzed to adenosine and Hcy. Thus, Cr synthesis and Hcy formation are metabolically linked by methyl metabolism. Previous studies have shown that Cr supplementation downregulates AGAT activity [7,9] and the endogenous synthesis of Cr; because Cr synthesis is responsible for a considerable consumption of SAM (at least 40%) and Hcy formation [7,10], Cr supplementation leads to reduced Hcys formation in rats [7,10–12]. There have only been a small number of studies regarding the effect of Cr supplementation in humans, and the results from these studies were quite inconsistent.

Given the inconsistence of the published data, it is important to investigate whether Cr supplementation modulate Hcy metabolism in different situations. The increasing number of published studies allows us to apply strict methodological analysis and summarize the main results. Thus, we propose a meta-analysis approach to provide a statistical summary of comparable studies in order to consolidate a quantitative review of the effects of Cr supplementation on blood Hcy concentration.

2. Methods

2.1. Search approach and study selection

This review was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) statement guidelines [13,14]. The PubMed database was searched for English-language articles, using the combination of

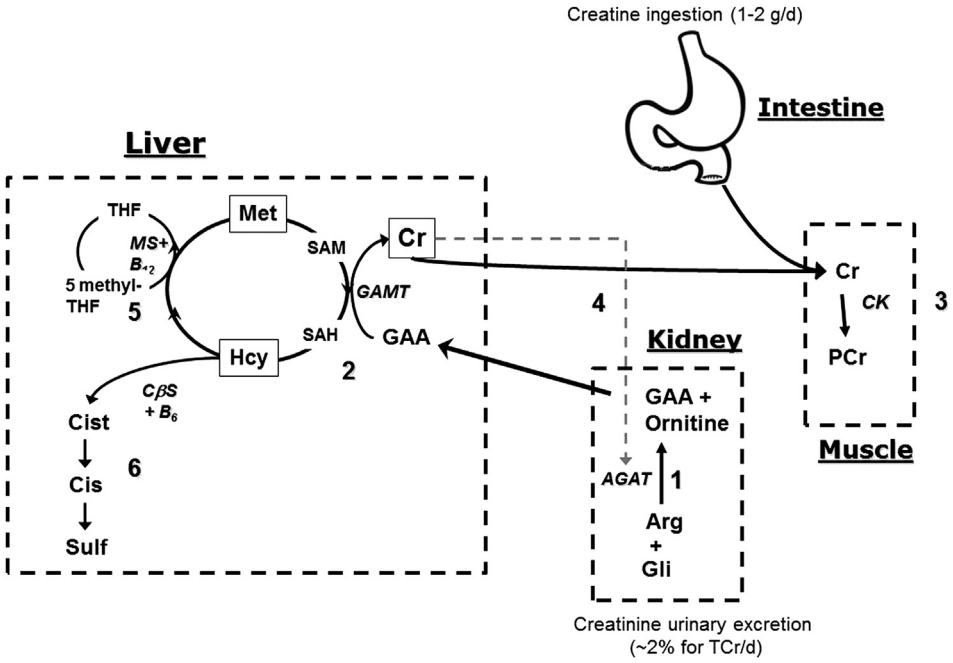

Fig. 1. Schematic presentation of the interaction of Hcy and Cr metabolism in rats. 1) action of kidney L-arginine: glycine amidinotransferase AGAT for guanidineacetic acid (GAA) formation as the first step of Cr synthesis; 2) the irreversible transfer of a methyl group from S-adenosylmethionine (SAM) to GAA is catalyzed by the enzyme guanidinoacetate N-methyltransferase (GAMT) in the liver; 3) Cr/PCr muscle system; 4) an increase in Cr intake down-regulates kidney AGAT in reaction 1 and decreases methyl flux for Hcy formation (reaction 2); 5) remethylation pathway; 6) transsulfuration pathway. THF, tetrahydrofolate; MS, methionine synthase; GNMT, glycine N-methyltransferase; GAMT, guanidinoacetate methyl-transferase; CbS, cystathionine-b-synthase; GSH, reduced glutathione.

the following keywords: “homocysteine” and “creatine supplementation” through November 2015. Any case–control, cross-sectional study, clinical trial or cohort study that assayed the blood concentrations of Hcy in rodents and/or humans using Cr supplementation condition were first analyzed. The references of all review articles and original papers were examined and cross-checked. Relevant articles cited in the publications were carefully reviewed and included in the meta-analysis if they matched the inclusion criteria. After exclusion of duplicate publications, the identified articles were included in the review if they matched the following criteria: 1) available in English, 2) performed in rodents and/or humans, 3) performed a Cr supplementation design, and 4) contained quantitative information regarding Hcy plasma or serum concentrations. The records were excluded if they presented the following characteristics: 1) provided a review and/or meta-analysis, 2) studies without a placebo/control group. The selection criteria were not limited by supplementation duration, Cr supplementation dosage, included physical exercise group or intervention, B-vitamins intake control. Two authors (RD and FTR) independently conducted this review search using the same search strategy. A total of 44 articles were identified during the initial search process. The search strategy considered two main outcomes: 1) Effects of Cr supplementation on Hcy plasma/serum concentrations in rodents, and 2) in humans. The study selection process is described in Fig. 2.

2.2. Data extraction

The following data were extracted from the articles included in the rodent meta-analysis: authors and year of publication; country where the study was conducted; animal model; supplementation regime; principal results (Table 2). When humans were considered, the following data were extracted: authors and year of publication; country where the study was conducted; number of subjects included in the study; participants' characteristics; supplementation regimen; and principal results (Table 3). All meta-analysis procedures were conducted as described by Stroup et al. [15].

2.3. Data analyses

Meta-analysis was conducted using Review Manager Software (RevMan software package version 5.0). RevMan was used to calculate the effect size of the change in Hcy plasma/serum concentration from baseline to post-supplementation with Cr vs. placebo groups in human studies. When rodent studies were analyzed, RevMan was used to calculate the effect size of the change in Hcy plasma/serum concentration from Cr supplement group compared to comtrol. In circumstances when the change from baseline data or corresponding standard deviations were not available, these values were calculated using standard statistical methods assuming a correlation of 0.50 between the baseline and post-intervention scores within each subject [16]. In situations where studies reported standard error, the values were converted to standard deviation (SD). For studies with non-parametric data reporting median and range, the equations of Hozo et al. [17] were used to estimate mean and SD.

Data from all included studies were used to calculate the weighted mean difference and 95% confidence interval (CI) using a continuous random effects model. Weighted percentages were based on the sample sizes of respective studies. Statistical significance was assumed as $p < 0.05$ in a Z test analysis, to examine whether effect size was significantly different from zero. Study heterogeneity was evaluated using the I^2 statistic and Cochrane's Q. Values of I^2 higher than 50 and 75% were considered moderate and high heterogeneity. For Cochrane's Q, significant heterogeneity was considered to exist when the Q value exceeds the degrees of freedom (df) of the estimate. When meta-analysis was considered to be moderate to high heterogeneity, and the random-effects model was used [18], publication bias was tested visually using a funnel plot.

3. Results

3.1. Study search

The initial search resulted in 44 abstracts; Fig. 2 describes how they are arranged. First, repeated studies and reviews were excluded (n = 10). Thirty-four studies were then assessed for eligibility by applying the inclusion and exclusion criteria, as a result of which 15 studies were excluded because they did not present adequate information to calculate effect size or aimed to correlate Hcy and Cr levels, only. Then, nineteen articles received more detailed evaluation. After extensive discussions between the authors, 5 studies were excluded; Table 1 summarizes the excluded articles. Finally, a total of 14 articles were identified for inclusion in the meta-analysis: six rodent and eight human studies. Table 2 describes the characteristics of the 6 animals studies included and Table 3 summarizes the 8 humans studies included in the meta-analysis. PRISMA flow diagram of the study selection process is presented in Fig. 2.

3.2. Overview of included studies

All rodent studies included in the meta-analysis used rats. Cr supplementation regimens were diverse in supplementation duration (from 2 to 4 weeks) and dosage (from 0.4 to 2% added to the diet). Cr supplementation was able to decrease Hcy in all rat studies identified under different situations including healthy conditions [10,7]; elevated Hcy induced by pathological models including chronic renal failure [11] and fatty-liver disease [12,24]; and elevated Hcy induced by high-intensity exercise [25] (Table 2).

Sample size of human studies ranged from 5 to 101 subjects per treatment. Five of the human studies included healthy participants; others included Hemodialysis [26], coronary artery disease [27] and Rett syndrome [28] patients. Four studies used long-term Cr supplementation [26,28–30] ranging from 1 to 6 months. Only two studies used short-tern supplementation regimens from 3 to 7 days of supplementation [27,31], all of them with high Cr dosages (>20 g/d). Other two used the classical supplementation protocol proposed by Hultman et al. [32] including load and maintenance supplementation phases [33,34] (Table 3).

3.3. Meta-analysis

The six rodent studies included reported decreased plasma Hcy concentration after Cr supplementation with an average effects size of −2.43 μmol/l (95% CI: −3.60, −1.26, $P < 0.01$; Fig. 3) and a moderate degree of heterogeneity ($I^2 = 67\%$; Cochrane's $Q = 15.1$, $df = 5$, $P < 0.01$). Funnel plot inspection demonstrated symmetric distribution and an absence of publication bias. Conversely, only 2 human studies agree that Cr supplementation decreases Hcy plasma concentration, 5 demonstrated no changes and 1 found increased plasma Hcy levels after Cr supplementation. The eight human studies included a total of 483 participants (242 Cr and 241 placebo supplemented subjects). The meta-analysis demonstrated no changes in plasma Hcy concentration of humans after Cr supplementation with a mean effect size equal to 0.09 μmol/l (95% CI: −0.47, 0.66, $P = 0.75$; Fig. 3) and a low degree of heterogeneity ($I^2 = 4\%$; Cochrane's $Q = 7.31$, df = 7, P = 0.40). Funnel plot

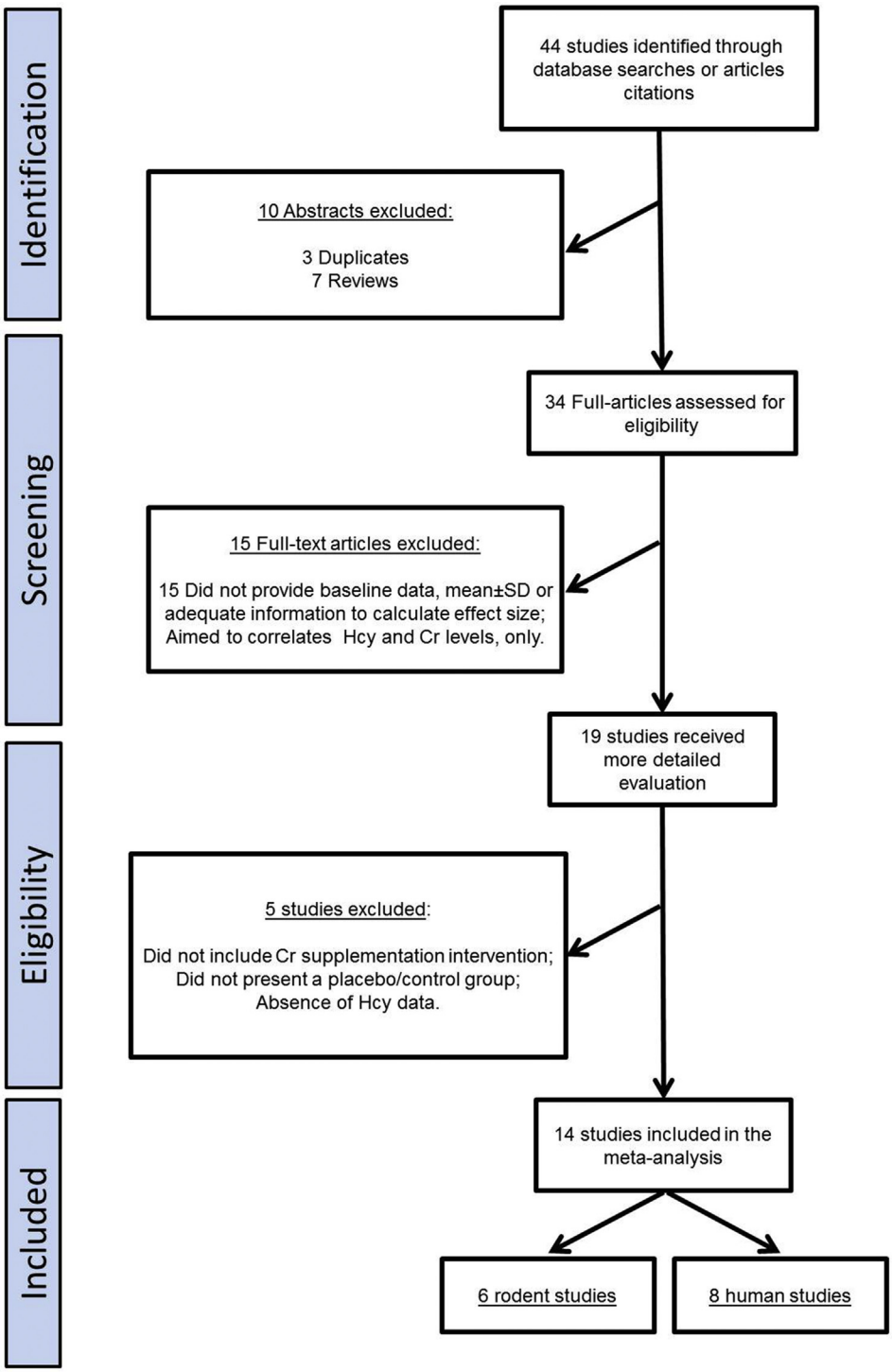

Fig. 2. PRISMA flow diagram of the study selection process. After careful discussion between the 2 reviewers, two outcomes were identified and included in the meta-analysis.

inspection demonstrated symmetric distribution and lack of publication bias.

4. Discussion

The present study sought to investigate whether Cr supplementation influences blood levels of Hcy through a meta-analysis. We conducted the analysis using two pre-specified outcomes: studies including rodents and humans. The rodent studies meta-analysis revealed Cr supplementation is able to decrease Hcy plasma concentration; the magnitude of the Hcy plasma decrease was −2.43 μmol/l (~30%, Fig. 3). This observation is particularly relevant considering the moderate heterogeneity among the studies with regard to the supplementation period and dosage (Table 2). In addition, Cr could decrease or prevent elevated Hcy under different animal models such as increased Hcy induced by acute exercise [25], and some pathological conditions such as renal chronic failure [11] and fatty-liver disease [24]. In contrast, meta-

Table 1
Articles (n = 12) excluded from the study.

Study	Aim	Reason for exclusion
Norlund et al., 1998 [19]	To explore the relation between plasma Hcy concentration and serum creatinine in health subjects	Did not included Cr supplementation intervention
Navrátil et al., 2010 [20]	To investigate the levels of various compounds in blood or urine, and body parameters connected with CR metabolism.	Absence of a placebo/control group
Petr et al., 2013 [21]	To evaluate homocysteinemia following Cr supplementation in relation to *MTHFR* 677C/T genotype.	Absence of a placebo/control group
Moraes et al., 2014 [22]	To investigated the effects of Cr supplementation on the systemic microcirculation and on Hcy levels in healthy young individuals.	Absence of a placebo/control group
Peters et al., 2015 [23]	To determine whether folic acid and/or creatine supplementation lowers blood arsenic in an arsenic-exposed Bangladeshi population.	Lack of Hcy concentration post Cr supplementation intervention

Table 2
Characteristics of rodents studies included in the meta-analysis.

Study	Country	Specie	Animal model	Supplementation regimen	Principal results
Stead et al., 2001 [7]	Canada	Sprague–Dawley rats	Healthy rats	0.4% wt/wt Cr monohydrate added to AIN-93 diet for 2 wk	Cr-supplemented rats exhibited a significantly lower (~25%) plasma Hcy level compared to control.
Taes et al., 2003 [11]	Belgium	Wistar rats	Nephrectomized rats	2% wt/wt Cr monohydrate added to the diet for 2 wk	Plasma Hcy was higher in nephrectomized rats. Cr supplementation reduced (18%) Hcy in nephrectomized compared to nephrectomized control diet–fed rats
Deminice et al., 2009 [10]	Brazil	Wistar rats	Healthy rats	0.4% wt/wt Cr monohydrate added to AIN-93 diet for 4 wk	Plasma Hcy and TBARS were significantly lower in CR-supplemented group compared to control
Deminice et al., 2011 [25]	Brazil	Wistar rats	Acute exercised rats	2% wt/wt Cr monohydrate added to AIN-93 diet for 4 wk	Cr supplementation inhibited increased Hcy plasma concentration induced by acute exercise.
Deminice et al., 2011 [12]	Canada	Sprague–Dawley rats	Fatty liver induced by high-fat diet	1% wt/vol Cr monohydrate added to high-fat liquid diet for 3 wk	Cr-supplemented rats had lower (~25%) plasma Hcy level compared to control; high-fat diet did not changed Hcy plasma levels
Deminice et al., 2015 [24]	Brazil	Wistar rats	Fatty liver induced by choline deficient diet	2% wt/wt Cr monohydrate added to AIN-93 choline-deficient diet for 4 wk	Cr supplementation prevented increased Hcy plasma levels and fatty liver induced by a choline-deficient diet

wt, weight; vol, volume; wk, week.

Table 3
Characteristics of human studies included in the meta-analysis.

Study	Country	Total n (M/F)	Participants characteristics	Supplementation regimen	Principal results
Steenge et al., 2001 [33]	Netherlands	12 (0/12)	Moderated active woman aged 19–38 years	20 g/d for 5 days, followed by another 8 wk with 3 g/d	Cr supplementation resulted in a small non-significant decrease in HCy plasma levels
Korzun 2004 [29]	USA	16 (7/9)	Apparently healthy university volunteers	Cr supplementation was twice the daily creatinine excretion for 4 wk	Cr supplementation lowered Hcy after 4 wk period
Taes et al., 2004 [26]	Belgium	49 (24/21)	Hemodialysis patients, performed dialysis 4–5 h, 3 times weekly	2 g/d for 4 wk in a placebo controlled, double-blind and crossover design (4 wk washout)	All hemodialysis patients had elevated Hcy; Cr did not affect Hcy plasma concentration.
Jahangir et al., 2009 [27]	USA	55 (–)	Subjects with proven coronary artery disease	21 g/d for 3 days in a placebo controlled double-blind manner	Cr supplementation (alone or in combination with L-arginine) was associated with an 11–20% increase in Hcy.
Freilinger et al., 2011 [28]	Austria	21 (0/21)	Patients with Rett syndrome	200 mg/kg/d for 6 months in a placebo controlled, double-blind and crossover design (1 month washout)	Plasma Hcy had no significant changes after Cr supplementation
Deminice et al., 2013 [31]	Brazil	23 (23/0)	Healthy and well-trained and young soccer players	0.3 g/kg/d for 7 days in a double-blind, placebo controlled manner	Hcy was significant increased (18%) after acute exercise; Cr supplementation did not change Hcy plasma level
Bereket-Yucel et al., 2015 [34]	Turkey	16 (16/0)	Non-vegetarian university students	25 g/d for 5 days followed by another 51 days with 5 g/d	Hcy was significant lower in Cr supplemented resistance exercised subjects compared to placebo
Peters et al., 2015 [30]	Bangladesh[a]	203 (103/101)	Adults between 20 and 65 years of age	3 g/d for 12 wk in a randomized, double-blind, placebo-controlled trial	Cr supplementation decreased plasma GAA but not Hcy

G, grams; d, day; wk, week; GAA, guanidineacetic acid.
[a] Authors from USA.

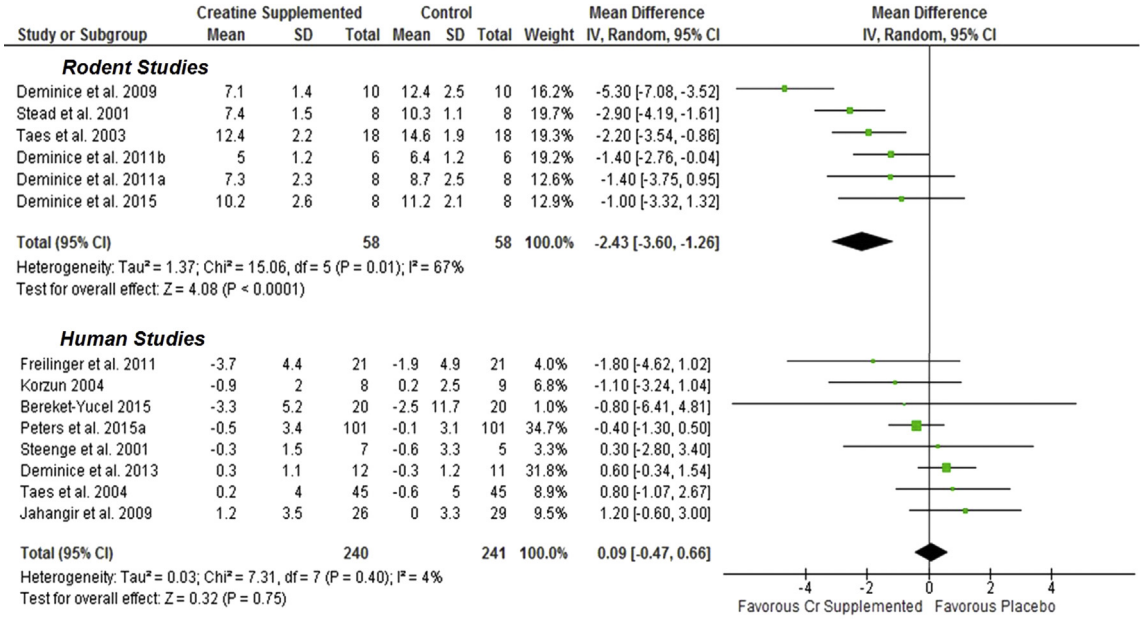

Fig. 3. Meta-analysis performed on the effects of Cr supplementation on blood Hcy concentration in rodents and humans (mean ± SD). For rodent studies, effect size was calculated using the change in Hcy plasma/serum concentration from Cr supplement group compared to control. For humans, effect size of the change in Hcy plasma/serum concentration from baseline to post-supplementation with Cr vs placebo groups is presented. Results are expressed as weighted mean difference (WMD) of Hcy (μmol/l) and 95% confidence intervals (95% CI).

analysis of human studies demonstrated no changes in Hcy plasma concentration after Cr supplementation (Fig. 3). Indeed, the effect of Cr supplementation on Hcy blood levels in humans is demonstrated to be inconsistent. Our review demonstrated two human studies found decreased Hcy after Cr supplementation [29,34], five demonstrated no changes [26,28,30,31,33], and one [27] demonstrated increased Hcy after Cr supplementation.

Studies have demonstrated that Cr synthesis is responsible for considerable SAM consumption in the liver and Hcy formation [6]. Stead et al. [8] based on studies using stable isotopes to determine the methylation flux, demonstrated that the synthesis of phosphatidylcholine and Cr are both the major consumers of SAM in the liver; and Cr is responsible for at least 40% of the methylation demand and consequently for Hcy formation. Cr supplementation increases plasma, muscle and liver Cr concentration. As a consequence, a remarkable (~80%) down-regulation of renal AGAT activity and decreased GAA concentration are found [12], showing a reduction in the endogenous formation of Cr [7,10,12]. This Cr synthesis suppression by Cr supplementation leads to decreasing GAA availability and SAM consumption in rats, decreasing Hcy formation as consequence [7,10,12] (Fig. 1). Stead et al. [7] studied how methylation demand and Hcy formation were modulated by Cr and GAA intake, using rats receiving either 0.4% Cr, 0.36% GAA or a control diet. Hcy levels were reduced 25% by Cr, and increased 50% with GAA, while plasma methionine levels were unchanged.

The divergence between rodent and human studies found in our meta-analysis seems paradoxical since the Cr synthesis suppression by Cr supplementation appears be the same in rats and humans. Although no human studies have determined renal AGAT activity after Cr supplementation, Derave et al. [35] showed decreased GAA (50%) after 1 week of Cr supplementation, beside an inverse relationship between plasma Cr and GAA levels measured in 10-week Cr-supplemented humans. More recently, Deminice et al. [31] and Peters et al. [30] demonstrated ~33% and ~11% significant decreased GAA plasma concentration after Cr supplementation, respectively. Controversially, none of the cited human studies demonstrated that Cr supplementation lowers plasma Hcy. Some studies have pointed differences in creatine and Hcy metabolism between humans and rats and try to explain the controversy in the results [36–39]. As an example, in rats, around 30% of plasma Hcy is protein bound. In normal humans, however, 80% of total Hcy bound to form mixed disulfide and the remaining is found in free form [36]. In addition, human but not rat kidney contains BHMT activity. Conversely, kidney of humans presents very small activity of cystathionine-b-synthase compared with rats [38]. However, whether these differences are responsible for the different responses of Cr supplementation on Hcy metabolism in rats and humans is not known.

Despite the metabolic differences presented above, we consider the inconsistence regarding supplementation protocol, presence of disease, age and gender of the studied population, presence of exercise protocols, control of B-vitamins intake or vitamins supplementation contribute significantly to the controversy in the results found between rats and human studies. The low number of controlled studies for inclusion in human outcome analysis may contribute to the discrepancy in the results and appears to be the major limitation of the present study. In addition, differences in Cr supplementation dosage may be also considered. Studies from our laboratory demonstrated a Hcy-lowering effect with 3.1 g/kg/d of Cr [12], which is appreciably higher than the ~0.3 g/kg/d performed in humans studies and classically proposed for humans [32,40]. It should be recognized, however, the allometric scaling factors between rats and humans. In general, mass-specific metabolic rate scales as the 0.75 exponent of mass. Therefore, a 300 g rat has ~4 times the mass-specific metabolic rate of a 70-kg human. When expressed on this physiological basis, the rats received 2–3 fold the Cr supplementation recommended for humans in load phase (~0.3 g/kg/d). Peters et al. [30] considers the hypothesis that Cr dosage used in human studies did not sufficiently inhibit AGAT. Indeed, rats and human studies must be compared with caution.

5. Conclusion

Over the last few years, interest in Cr supplementation has increased markedly in the medical field because of the beneficial effects found in a number of muscular, neurological and metabolic diseases [41]. Our meta-analysis revealed that studies conducted on animals have demonstrated that inhibition of the endogenous methylation demand by Cr supplementation can reduce Hcy levels, but these results have not been reliably reproduced in humans. Human and rats particularities in Hcy metabolism have been speculated as the reason for differences in the results. However, inconsistence regarding supplementation protocol, presence of disease, age and gender of the studied population, presence of exercise protocols, and intake control or supplementation with B-vitamins also may contribute significantly to the controversy in the results found between rats and human studies.

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Disclosure

Disclosure none.

Author's contributions

RD and FTR designed the project; RD and FTR conducted search; RD and FTR analyzed data; RD and FTR wrote the paper; RD had primary responsibility for final content. All authors read and approved the final manuscript.

Conflict of interest

None.

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