High-performance capillary electrophoresis-pure creatine monohydrate reduces blood lipids in men and women

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1. A randomized, double-blind, placebo-controlled trial utilizing creatine as a potential lipid-lowering agent was conducted to determine plasma lipid, lipoprotein, glucose, urea nitrogen and creatinine profiles in men and women ranging in age from 32 to 70 years.

2. Thirty-four subjects (18 men and 16 women) with total cholesterol concentrations exceeding 200 mg/dl received either a creatine supplement (5 g of creatine plus 1 g of glucose) or a glucose placebo (6 g of glucose) for 56 days. Creatine and placebo were taken orally four times a day for 5 days and then twice a day for 51 days. Plasma analyses were measured at baseline, and at 4 weeks after cessation of treatment (week 12).

3. Significant reductions in plasma total cholesterol, triacylglycerols and very-low-density lipoprotein-C occurred within the creatine monohydrate group. Minor reductions in plasma total cholesterol from baseline (233 ± 9 mg/dl) of 6% and 5% occurred at weeks 4 and 8, respectively, before returning to baseline at week 12. Baseline triacylglycerols (194 ± 21 mg/dl) and very-low-density lipoprotein-C (39 ± 4 mg/dl) were reduced by 23% and 22% at weeks 4 and 8, respectively, and remained attenuated by 26% at week 12. These results remained consistent when data were separated and analysed by gender. Finally, a small, but statistically significant increase in urea nitrogen was observed in women between baseline (11.8 ± 0.7 mg/dl) and week 8 (13.8 ± 0.7 mg/dl, P < 0.05). No significant differences were noted for low-density lipoprotein-C, high-density lipoprotein-C, total cholesterol/high-density lipoprotein ratio, glucose, creatinine, body mass, body mass index or physical activity within or between the experimental and placebo groups. However, a trend towards reduced blood glucose levels was present in males given creatine monohydrate (P = 0.051).

4. These preliminary data suggest that creatine monohydrate may modulate lipid metabolism in certain individuals. These observations may demonstrate practical efficacy to the hyperlipidaemic patient as well as providing possible new mechanistic insights into the cellular regulation of blood lipid concentrations.

INTRODUCTION
Recently, the use of creatine monohydrate (CR) has received much attention as an ergogenic aid. Harris et al. [1] were the first to demonstrate that orally ingested CR can increase both intramuscular total creatine and phosphocreatine. Further investigations have revealed that supplementation potentiates short duration, high intensity (anaerobic) work efforts [2–4]. In an earlier study, we demonstrated a positive effect of CR on anaerobic power indices including muscular power, muscular strength and muscular endurance in young, strength trained men [4]. To date, no side or 'secondary' effects have been associated with the use of CR, with the exception of increased total body mass [3–5]. In this regard, we analysed the blood chemistries of the subjects as a screening method to determine potential side effects utilizing a non-fasting SMAC (Sequential Multiple Assays and Chemistries) blood profile. While no acute side effects were noted, we did observe a tendency for the CR group to show a reduction in blood plasma total cholesterol concentrations after 4 weeks of supplementation. These data were not reported because the blood samples were not obtained in a fasting state. Still, this phenomenon was sufficiently intriguing to undertake the current investigation to ascertain whether CR does indeed have an effect on blood lipids. To date, we are unaware of any investigation showing a similar finding. Moreover, because elevated blood cholesterol has been implicated in coronary heart disease [6], the use of CR as a dietary adjunct in blood lipid management has important implications both practically for hyperlipidaemic patients and mechanistically via an undetermined pathway; the latter suggesting a role of cellular bioenergetics in lipid metabolism.

Key words: cholesterol, lipid metabolism, triacylglycerols.
Abbreviations: BMI, body mass index; CR, creatine monohydrate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAG, triacylglycerol; TC, total cholesterol; VLDL-C, very-low-density lipoprotein cholesterol.
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PATIENTS AND METHODS

Subjects

In order to determine whether CR has a significant effect on blood lipid parameters, sixty-five male and female subjects with plasma total cholesterol (TC) exceeding 200 mg/dl were recruited from the Dallas, Texas area for this study. Each participant was screened before entering the investigation. Entry criteria were established so that each participant was not currently taking any cholesterol-lowering medications or over-the-counter agents such as niacin. Furthermore, participants who were pregnant or presented with a history of kidney, cardiovascular, metabolic, or thyroid disease were also excluded from the study. Of the initial 65 subjects that began the investigation, 34 participants completed the entire investigative protocol. Completion was designated as full compliance with all delineated testing protocols (see below).

All reported values are mean ± SEM. The subjects ranged in age from 32 to 70 years (mean 50 ± 2 years). For the CR group (n = 20), mean baseline data were: age 50.9 ± 3.2 years, weight 74.2 ± 3.6 kg, and body mass index (BMI, weight in kg divided by height in meters, squared) 25.4 ± 1.2 kg/m². For the placebo group (n = 14), mean baseline data were: age 49.2 ± 2.7 years, weight 78.6 ± 5.6 kg, and BMI 26.3 ± 1.9 kg/m². Activity levels were determined for each individual via a health history questionnaire and were determined to be 4.0 ± 0.1 and 4.2 ± 0.3 days per week for the CR and placebo group, respectively. All subjects were encouraged to maintain their baseline exercise and dietary patterns throughout the study duration; however, dietary records were not collected. Before participation all subjects signed a written informed consent statement.

Outline of the study

All subjects who participated in this investigation were examined over a 12 week period. Upon entry into the study, subjects reported to the Cooper Clinic (Dallas, TX, U.S.A.) for all blood testing. The Cooper Clinic laboratory is certified by the College of American Pathologists. Subjects reported to the laboratory after an overnight fast (12 h) that included abstinence from alcohol and caffeine consumption, as well as exercise participation, during this fasting time period. Initial baseline screening consisted of two fasting blood tests within a 5 day period in order to determine a stable baseline measure of TC, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TC/HDL-C ratio, triacylglycerols (TAG) and calculated very-low-density lipoproteins (VLDL-C). In this regard, VLDL-C was calculated as TAGs divided by 5. Additional values were also obtained for blood glucose, urea nitrogen and creatinine. No subject demonstrated a baseline plasma TC value differing 10% or greater from the first test, and thus baseline measures are reported as the mean value of these two initial baseline tests. Measures for body mass and BMI were also performed at this time. Baseline data for plasma lipid, lipoprotein, glucose and creatinine profiles are presented in Table 1.

After obtaining baseline measurements, subjects were randomly assigned, in a double-blind fashion, to receive either high-performance capillary electrophoresis pure CR (Phosphagen; Experimental and Applied Sciences, Inc., Golden, CO, U.S.A.) or a glucose placebo. CR was ingested orally in two phases. Phase one entailed a 5 day loading sequence consisting of four servings of 5 g of CR daily (20 g/day), with 1 g of glucose added to each serving in order to mask the identity of the supplement. Previous research has shown this dose regimen to be effective in elevating intramuscular free creatine and phosphocreatine stores [1]. During phase two, subjects were instructed to take half of the loading dose or two servings per day (10 g of CR/day) for the remainder of the study (51 days). This lower dose was chosen to reinforce usage compliance over the remaining duration of the study. The placebo group followed the same sequence of supplementation, but received doses of 6 g of glucose. All supplements were provided in blinded and coded bottles by the manufacturer and their identity was not revealed until all data collection and analysis were completed. Each participant was given enough supplement to last for 8 weeks. Treatment was
administered for 8 weeks with additional blood samples being taken at weeks 4 and 8 of the investigation. After week 8, subjects were taken off their respective supplements and asked to return at week 12 for further blood analysis to determine if any observed treatment effects would be altered after supplement withdrawal. All reported blood parameters were analysed via a Boehringer Mannheim/Hitachi 911 Analyzer. Body mass and BMI were also assessed at weeks 8 and 12. To aid in compliance, each subject was given a treatment diary that was set up in a 12 week calendar format. This diary served the purpose of (i) establishing a scheduled time and date for follow-up testing and (ii) instituting a method for recording daily doses and compliance to the treatment format. Subjects were asked to record their daily doses as well as any changes in dietary and exercise patterns. Study protocols were approved by the human subjects and internal review boards of Texas Woman’s University (Denton, TX, U.S.A.) and The Cooper Clinic (Dallas, TX, U.S.A.), respectively, in accordance with the Declaration of Helsinki (1989) of the World Medical Association.

Statistical analysis was performed using a two-way analysis of variance for repeated measures; (treatment x time) in addition to (gender x time) for each blood parameter, as well as measures of body mass and BMI. Normal statistical assumptions were considered. If the assumption of sphericity was not met the degrees of freedom were corrected using a Greenhouse-Geisser adjustment. An $\alpha$ level of 0.05 was chosen to determine statistical significance. If significance was found, a Tukey post-hoc analysis was employed to determine where the treatment differences were observed both within and between groups. Furthermore, an independent $t$-test was utilized to determine any differences between treatment groups with regard to baseline participation in physical activity (days/week).

RESULTS

No significant differences were noted for between-within-group values with respect to age and baseline participation in physical activity. Furthermore, no differences were noted for baseline, 8 week and 12 week values of body mass and BMI. With respect to plasma lipid concentrations, no significant differences from baseline were noted for LDL-C, HDL-C or the TC/HDL-C ratio, either between or within the CR and placebo groups, during the investigative period (Table 1). However, significant reductions were observed for plasma TC, TAG and VLDL-C both within the CR group and between the CR and placebo groups ($P<0.01$).

Significant reductions were also noted for the CR group with respect to TAG and VLDL-C ($P<0.01$). These differences remained consistent for men and women when analysed separately.

At baseline the CR group and female TAG/VLDL-C levels were significantly higher than the placebo group ($P<0.05$, Fig. 2 and Fig. 3). Within the CR group, an overall group reduction in TAGs from baseline values of $194\pm21$ mg/dl at 4 weeks (23%, $P<0.01$), 8 weeks (22%, $P<0.01$) and 12 weeks (26%, $P<0.01$) was noted (Fig. 2). Men in the CR group showed a significant reduction in TAGs from baseline values of $212\pm33$ mg/dl at 4 weeks.
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Baseline Week 4 Week 8 Week 12

Fig. 3. TAG levels for women in the CR and placebo groups. Values are mean ± SEM. Statistical significance: *P < 0.01 lower value than baseline within the CR group; †P < 0.05 lower value than corresponding treatment group for the same time period.

Fig. 4. TAG levels for men in the CR and placebo groups. Values are mean ± SEM. Statistical significance: *P < 0.01 lower value than baseline within the CR group.

(28%, P < 0.01), 8 weeks (27%, P < 0.01) and 12 weeks (31%, P < 0.01; Fig. 4). Women in the CR group also demonstrated a significant reduction from baseline values of 179 ± 6 mg/dl at week 4 (19%, P < 0.01), week 8 (16%, P < 0.01) and week 12 (22%, P < 0.01; Fig. 3). This pattern remained consistent for calculated VLDL-C.

No significant differences were noted for values between or within groups, or gender values, for blood glucose or creatinine (Table 1). Furthermore, no significant differences were observed for between or within group values for urea nitrogen. However, a significant increase in urea nitrogen was observed in CR women by week 8 (P < 0.05). Urea nitrogen values for CR women are baseline (11.8 ± 0.7 mg/dl), week 4 (12.9 ± 0.9 mg/dl), week 8 (13.8 ± 0.7 mg/dl) and week 12 (11.2 ± 1.1 mg/dl), respectively. Finally, men in the CR group showed a declining trend in blood glucose levels with week 8 values approaching a level significantly lower than baseline values (P = 0.051). Finally, individual subject reports revealed supplement compliance to be 96% with no reported changes in dietary or exercise patterns.

DISCUSSION

Tolerance to chronic consumption was excellent with three men in the CR group reporting mild asthmatic symptoms and gastrointestinal distress, while none were reported in the placebo group. Of the three men who reported asthmatic symptoms, one was a known asthma sufferer. In two of the cases, symptoms were relieved when the supplement was taken with a meal. Serum creatinine was not elevated at any time during the study. After CR loading (20–30 g of CR/day for 5–7 days) only transient elevations in serum creatinine have been described. However, urinary creatinine excretion increases in parallel with intramuscular creatine concentrations and is probably due to the increased release and cyclization of intramuscular creatine as a normal consequence of myofibrillar protein turnover (P. Greenhaff, personal communication).

Our findings indicate that daily supplementation with CR can reduce plasma TC, TAGs and VLDL-C in mildly hypertriglyceridaemic and hypercholesterolaemic individuals. We believe this to be the first report describing hypolipidaemic effects of CR. The decline in TAGs and VLDL-C became apparent in the CR group after 4 weeks of supplementation, and a downward trend continued through the eighth week. Interestingly, the greatest depressions in both TAG and VLDL-C manifested 4 weeks post-supplementation in both men and women. These observations were made in the light of no change in body mass or BMI within the CR group. Moreover, after expressing the data for the women as a function of oestrogen status (i.e. premenopausal menstruating, premenopausal oligomenorrhoeic, postmenopausal with or without oestrogen replacement therapy) no relationships to blood lipid response were seen (data not shown), despite the well known hypertriglyceridaemic effects of exogenous oestrogens [7]. Women receiving oestrogen replacement therapy did so in an oral fashion and were not otherwise receiving conjunctive progestin therapy. Progestin therapy has been shown to reduce TAG levels [8] or attenuate the rise in TAG [9], when taken alone or in combination with oestrogen regimens, respectively. Women in the CR group showed significant declines in both TAG and VLDL-C, beginning at 4 weeks and persisting through week 12. These results were observed in the light of no reported changes in physical activity or dietary patterns for either group.

In an earlier study, menstruating collegiate women were shown to display increases in muscular power after CR administration [10], similar to that described in men [2]. Although sex steroids have been described as being capable of modulating de novo creatine biosynthesis in animal systems [11], we are unaware of differences in human creatine...
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metabolism between men and women, nor of the influence of androgenic and oestrogenic steroids upon creatine transporter protein expression and/or activity [12]. However, cross-sectional studies analysing quadriceps muscle total creatine content in men and women (age range 19–85 years) noted significantly greater concentrations in women, when expressed per unit fat-free dry weight or as a function of cell protein content [13].

In relation to lipid metabolism, recent studies in 40–65-year-old males with mild testosterone insufficiency suggest that testosterone can modulate increased export of TAG from adipose tissue, coupled with reduced skeletal muscle lipoprotein lipase and/or reduced peripheral insulin sensitivity, could ultimately manifest as increased TAG and VLDL-C (see below).

Recently, we have described the body mass gain attending CR supplementation to be due exclusively to increases in fat-free mass as determined by hydrostatic weighing [4]. While none of the subjects (men or women) in our current study showed alterations in total body mass, it is probable that creatine disposal may be regulated by sex steroids and may compartmentalize in different regions that could ultimately alter lipid metabolism, muscle bioenergetics and body composition independently of one another. Alternatively, body composition may have been altered (i.e. increased fat-free mass and decreased fat mass) without a change in total body mass.

The mechanism of CR’s hypolipidaemic effects remains enigmatic. Neither group displayed any changes in HDL-C or LDL-C throughout the duration of the study. The absence of any changes in these lipid subfractions is reminiscent of the hypolipidaemic effects of fibrate derivatives (clofibrate, gemfibrozil), which elicit falls in both TAGs and VLDL-C, with mild influences upon LDL-C and HDL-C [15–18]. Animal studies have described the effects of creatine administration (infusion and feeding) upon hepatic creatine distribution and retention. Recent studies in mice receiving a 10% creatine-enriched diet for 5 days revealed a marked increase in hepatic total creatine (creatine and phosphocreatine) concentrations, although no functional assessment was performed [18]. We are unaware of any studies describing alterations of hepatic function in animals [19] or humans. In this study we observed a transient elevation in urea nitrogen for CR women by week 8 of the supplementation period. It should be noted, however, that while statistically significant, these values do not exceed the normal reference ranges for urea nitrogen in adults (10–20mg/dl), reside at the low end of the normal range, and may be subject to normal physiological variance. Moreover, during this investigation, we did not measure dietary protein intake and the timing of menstrual activity in relation to the timing of blood sampling. Therefore, increased dietary protein intake cannot be ruled out as a mechanism for the minor, but statistically significant increase in urea nitrogen. Oestrogen status and timing of menstrual activity may also account for the change in urea nitrogen observed in the female group. Clinically, these types of changes occur via intravascular compartmental hydration fluctuations (hypovolaemia) encountered during menstrual cycle activity.

Among the men receiving CR, the decline in fasting plasma glucose approached a priori designations for statistical significance after 8 weeks (P = 0.051). No downward trends were noted in the women. However, one study conducted in insulin-dependent diabetic patients found an acute oral administration of creatine (3g in 200ml of an undisclosed fluid) significantly reduced circulating glucose concentrations at 60 and 120min after ingestion, without changes in serum insulin or C-peptide concentrations [20]. No changes in glycemia were seen in age-matched non-diabetic control subjects. These data suggest that oral creatine can acutely enhance glucose disposal and/or reduce gluconeogenesis in hyperglycaemic Type I diabetics. The latter study did not evaluate the effects of creatine upon glucose disposal after a glucose load, nor did the authors attempt to elucidate a possible mechanism of the hypoglycaemia.

If creatine promotes acute increases in peripheral and/or hepatic insulin sensitivity and/or post-receptor signalling in postprandially hyperglycaemic non-diabetic subjects, this may foster decreases in de novo TAG production, especially if accompanied by reductions in fasting insulin concentrations [21]. A positive relationship between elevated fasting insulin or glucose, or decreased insulin sensitivity, and TAG and VLDL-C concentrations has been described by several groups [22–24]. Moreover, guanidine-containing compounds, including creatine, have been reported to have hypoglycaemic effects [25, 26].

Finally, the decline in the TAGs and VLDL-C persisted through the 4 week post-supplementation period, unlike that seen with the use of fibrates/gemfibrozil. However, in this population the subjects would be considered as being mildly hypertriglyceridaemic, which in response to CR (or fibrate derivatives/nicotinate-based agents) may elicit different lipid turnover kinetics relative to that observed in severe hypertriglyceridaemia.

The National Cholesterol Education Program (U.S.A.) has defined individuals with total cholesterol concentrations greater than or equal to 240mg/dl, or LDL-C levels greater than or equal to 160mg/dl in adults without evidence of coronary heart disease to be at high risk for the development of coronary heart disease [27].

Additional data suggest elevated TAG to be a complementary risk factor [28]. Coupling these
lipid parameters with lifestyle risk factors (e.g., smoking, physical inactivity and obesity) and hyperinsulinaemia could produce an additive or multiplicative increase in the risk of coronary heart disease. The elucidation of strategies capable of producing a normalization of one or more of these lipid parameters has focused both on pharmacological and lifestyle intervention. In this study among mildly hypercholesterolaemic/dyslipidaemic males and females without a history of coronary vascular disease, dietary supplementation with CR produced marked blood-lipid modifying effects in a majority of individuals, without any appreciable side effects. In the light of more conventional methods of cholesterol reduction, the observation that CR appears to alter lipid metabolism is indeed intriguing. Its efficacy in reducing coronary heart disease remains to be seen pending further investigation.

Until now, CR has largely been used as an ergogenic aid for anaerobic type workouts. Current research has shown that supplementation with CR can produce increases in both peak and total power output [2–4], body mass [3–5] and lean body mass [4]. In this regard, no deleterious side effects have been noted, in contrast to those typically observed with illegal ergogenic aids claiming and demonstrating similar performance enhancing benefits. The most controversial and severely criticized among these have been anabolic steroids [29]. Considering creatine’s traditional biochemical role as a means of ‘buffering’ ATP production in contracting skeletal muscle, and its still newer role as an ergogenic aid for those participating in short duration, high intensity (anaerobic) exercise, these new findings, demonstrating positive alterations in lipid metabolism, may offer additional insights into the mechanisms that regulate lipid metabolism and provide a new strategy in the primary prevention of cardiovascular disease manifestations.

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REFERENCES