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ARTICLE

Plasma Creatine Kinetics After Ingestion of Microencapsulated Creatine Monohydrate with Enhanced Stability in Aqueous Solutions

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ABSTRACT

Creatine monohydrate represents one of the largest sports supplement markets. Enhancing creatine (CRE) stability in aqueous solutions, such as with microencapsulation, represents innovation potential. Ten physically active male volunteers were randomly assigned in a double-blind design to either placebo (PLA) (3-g maltodextrin; $n = 5$) or microencapsulated CRE (3-g creatine monohydrate; $n = 5$) conditions. Experimental conditions involved ingestion of the samples in a 70-mL ready-to-drink format. CRE was delivered in a novel microencapsulation matrix material consisting entirely of hydrolyzed milk protein. Three hours after ingestion, plasma creatine concentrations were unchanged during PLA, and averaged ~45 μM. During CRE, plasma creatine concentration peaked after 30 min at 101.6 ± 14.9 μM ($p < 0.05$), representing a 2.3-fold increase over PLA. Thereafter, plasma creatine concentration gradually trended downwards but remained significantly elevated (~50% above resting levels) 3 hr after ingestion. These results demonstrate that the microencapsulated form of creatine monohydrate reported herein remains bioavailable when delivered in aqueous conditions, and has potential utility in ready-to-drink formulations for creatine supplementation.

KEYWORDS
bioavailability; creatine; microencapsulation; milk protein; plasma kinetics; supplementation

Introduction

Creatine monohydrate supplementation has long-established benefits for improving high intensity exercise training and sports performance (Buford et al., 2007; Cooper et al., 2012), and therefore represents one of the largest sports supplement markets (Jager et al., 2011). In addition, growing evidence suggests that creatine supplementation in elderly, cachexia, or trauma patients may offset the loss of lean tissue mass (primarily skeletal muscle) that occurs in such states (Gualano et al., 2012).

The breakdown of creatine to creatinine when solubilized is a major concern for the sports supplement and nutrition aid industries, since this is a clear indication of creatine instability. This, therefore, reduces the effectiveness of oral supplementation when delivered in

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ready-to-drink products (Jager et al., 2011). Moreover, solubility of creatine powders in water is low but can be increased by lowering the pH of the solution, a principle that forms the basis for the improved solubility of creatine salt, but this does not solve the problem with creatine stability in solution (Jager et al., 2011). New forms of creatine supplements aim to improve on the standard set by creatine monohydrate with regard to chemical, physical, and physiological properties. These improvements include stability in a liquid form, improved solubility, and increased bioavailability. Although numerous claims regarding new forms of creatine have been made, there is little scientific evidence indicating that any form offers an improvement over creatine monohydrate (Buford et al., 2007; Cooper et al., 2012; Jager et al., 2011).

Similarly, the development of dietary supplements presents a number of challenges to manufacturers as many of these nutrients can be adversely affected by extrinsic conditions experienced during processing, storage, and ingestion, and thus rendered ineffective (Champagne & Fustier, 2007; Cook et al., 2012; Dias, Ferreira, & Barreiro, 2015). Parameters such as sensitivity to oxidation, heat, pH, and low in vivo bioavailability may curtail the development of an efficacious product. Microencapsulation is a technique that has shown great potential for the protection and appropriate delivery of drugs and sensitive nutrients (Champagne & Fustier, 2007; Dias, Ferreira, & Barreiro, 2015; Singh et al., 2010). The technology is based on the immobilization of an ingredient (core material) in a miniature-sealed capsule that offers protection from environmental conditions and maintains structural integrity until degrading and releasing the ingredient at an appropriate time or site in the body (Dias, Ferreira, & Barreiro, 2015). This degradation should occur in a controlled manner under the influence of an extrinsic factor (pH, enzyme, etc.; Champagne & Fustier, 2007; Dias, Ferreira, & Barreiro, 2015). An array of materials can be used as encapsulation matrices and have been extensively reviewed elsewhere (Champagne & Fustier, 2007; Dias, Ferreira, & Barreiro, 2015; Singh et al., 2010). Milk protein is currently receiving significant interest as a matrix material, primarily because milk proteins are inexpensive, generally recognized as safe (GRAS) food ingredients, which are widely recognized and accepted by consumers. Some of these proteins possess excellent gelation and capsule forming properties and have excellent solubility in water (Heidebach, Forst, & Kuzolik, 2009; Livney, 2010).

Microencapsulation techniques may offer an alternative method to improve the performance of various forms of creatine in products by increasing shelf-life stability and bioavailability. Therefore, the aims of the present study were to establish a microencapsulated form of creatine monohydrate using a milk protein-based encapsulation matrix, and subsequently examine the plasma creatine kinetics of this novel form delivered in a convenient ready-to-drink preparation in physically active young males.

Materials and methods

Materials

BiPro, a commercial whey protein isolate (WPI) obtained from Davisco Foods International Inc. (MN, U.S.A.) containing 98% (w/w) protein, was utilized for production of cell immobilization matrices. β-Lactoglobulin (β-Lg) and α-Lactalbumin (α-La) content were analyzed by reversed-phase high performance liquid chromatography (HPLC) and estimated at 82% and 16%, respectively. Flavourzyme, a protease-peptidase complex produced by submerged fermentation of a selected strain of *Aspergillusoryzae*, was purchased from Novo Nordisk A/S, Bagsvaerd, Denmark for enzymatic hydrolysis. Highly purified water (MilliQ, BioSciences,

Cork, Ireland) was used in all cases for dispersion of samples, culture media, and buffer solutions. The chemical products used in HPLC were acetonitrile (ACN) and trifluoroacetic acid (TFA), both of HPLC grade. Milli-Q water (Millipore, Cork, Ireland) was sterilized and utilized in all cases for dispersion of samples, media, and buffer solutions.

Preparation of protein hydrolysate and characterization

An untreated WPI solution (8.5% w/v) was prepared using the protease–peptidase complex, Flavourzyme, which was standardized in Leucine Amino Peptidase Units (LAPU) by the manufacturer. Hydrolysis was performed batch-wise in a thermostated reaction vessel (B. Braun Biotech., Melsungen, Germany). The protein solution was pre-heated (50°C) and a measured amount of Flavourzyme (1000 LAPU) was pre-suspended in WPI solution and subsequently adjusted to the hydrolysis conditions (50°C). The reaction was terminated after 3 hr by heat treatment at 80°C for 20 min to facilitate permanent inactivation of the enzymatic reaction. This was followed by cooling and storage of the hydrolysate at 4°C. Size exclusion chromatography was performed using an automated 2695 Waters™ HPLC system (Millipore, Middlesex, UK) equipped with a TSK G2000 SW column (600 × 7.5 mm; Tosoh Corporation, Tokyo, Japan). Samples were filtered (0.2 μm) and eluted at a flow rate of 1 mL/min using 30% acetonitrile containing 0.1% (v/v) TFA. A molecular weight calibration curve was prepared from the average retention time of standard proteins, peptides, and amino acids. Following this, hydrolysate samples were subsequently utilized as an encapsulation milieu.

The degree of hydrolysis was assayed directly by quantification of cleaved peptide bonds as assessed by the o-phthaldialdehyde (OPA) spectrophotometric assay, which involved using N-acetyl-L-cysteine (NAC) as a thiol reagent (Garcia et al., 1989). The OPA–NAC reagent was prepared as described previously (Spellman et al., 2003), and the reagent was covered and stirred overnight at room temperature. To assay proteolysis, 100 μL of each hydrolysate sample was added to an equal volume of 24% (w/v) trichloroacetic acid (TCA) and allowed to stand at ambient temperature for 10 min. A small aliquot (20 μL) of the supernatant, obtained after centrifugation (14,000× *g* for 3 min), was added directly to 3 mL of OPA reagent. The solution was vortexed, incubated at ambient temperature for 30 min, and the reaction product was subsequently detected by absorbance at 340 nm.

Preparation of microencapsulated creatine monohydrate

BiPro was dissolved in sterile water (9% w/v) for 16 hr at 4°C under slight agitation (180 rpm); the solution was adjusted to pH 7 with 100 mM HCl and filtered through Durapore® 0.45-μm HVLP (Millipore Ireland BV, Cork, Ireland). The appropriate formulation for a curing medium was investigated using calcium chloride, acetate, and citrate buffers systems. Uniform size whey protein micro-particulates were prepared for the encapsulation and extrusion of creatine monohydrate using an extrusion ratio of 95:5 of creatine monohydrate:WPI. Optimum parameters for a given protein–creatine monohydrate suspension were logged and utilized without adjustment during further batch production. The extrusion process utilized no organic, acids, solids, or excipients. Process temperatures were maintained at 35°C to optimize encapsulation efficiency and to prevent the conversion of creatine to creatinine. For the generation of placebo (PLA) condition, microencapsulation of maltodextrin was performed under similar circumstances. Micro-particulate analysis was performed during five independent trials with mean value and standard deviation (SD) being reported in triplicate for respective conditions.

Microscopic and morphological analysis

Atomic Force Microscopy (AFM) images were obtained using Asylum Research MFP-3D-AFM (Asylum Research UK Ltd. Oxford, UK) in AC-mode. Prior to imaging, all encapsulated samples were diluted ($\times 100$) in MilliQ H_2O and 10-µL aliquots were deposited onto freshly cleaved mica surfaces and subsequently dried in a desiccator. An aluminium reflex-coated cantilever with a tetrahedral tip (AC 240), spring constant of 1.8 N/m (Olympus Optical Co. Ltd, Tokyo, Japan), working frequency of 50–90 kHz, and scan rate at 1 Hz was used for air-dried samples. The radius of curvature of the tetrahedral tip was 10 ($\pm$3) nm. Bright-field light microscopy measurements were also carried out using a BX51 light microscope (Olympus, Essex, UK). Samples were deposited on glass slides and analyzed.

Mechanical strength of micro-particles

The mechanical strength of micro-particles was examined using a texture analyzer (TA-XT2i, Stable Micro Systems, Godalming, UK). Briefly, a specific force was applied to a mono-layer and the quantity of deformation/rupture of micro-particles was assigned as a measure of mechanical stability. Strength assays were performed using a 20-mm diameter cylindrical aluminium probe at a mobile speed of 0.3 mm/s in compression mode. A rupture distance of 95% was applied and the peak force (grams) exerted by the probe on the micro-bead monolayer was recorded as a function of compression distance leading to a force versus curing time relation. Analysis was conducted on 15 monolayer samples per batch and a total of 10 replicate batches were analyzed at each time point (5 hr and five days post-production) to obtain statistically relevant data.

Micro-particle size distribution and sample viscosity

The mean size distribution and D (v, 0.9) (size at which the cumulative volume reaches 90% of the total volume) of micro-particles were determined using a laser diffractometer (Mastersizer 2000, Stable Micro Systems, Surrey, UK) with a range of 70 to 120 μm. This represents the most practical size range applicable in a beverage "shot" (70 mL) or long-drink (237 mL), an emerging market segment that requires very specific sensory, physical, and chemical characterization for quality control and product development. For particle size analysis, batches were re-suspended in Milli-Q water, and size distribution was calculated based on the light intensity distribution data of scattered light. Measurement of bead size was performed at 25°C and three runs were performed for each replicate batch. Viscosity of PLA and creatine (CRE) "shots" was measured by dynamic viscometer (RVDI, Brookfield, USA) to confirm beverage viscosity within the range 7.5 to 9.0 cp. These rheology values are required for optimum dispersion of micro-particles in the beverage for suitable flowability, sensorial properties, and micro-particle dispersion.

Storage and ex vivo digestion of microencapsulated creatine samples

Following storage for 28 days at 25°C, 70-mL shots containing encapsulated creatine monohydrate were tested for gastric tolerance and stability. Gastric contents from three porcine stomachs were collected and pooled within 2 hr of slaughter. The fasted animals (12 hr prior to slaughter) were not prescribed any medicated feed prior to/at the time of collection, and gastric juice was subjected to centrifugation and filtration. Samples of creatine encapsulated

in native, hydrolyzed, and denatured milk protein were tested for gastric stability for comparative purposes in pre-warmed gastric juice (1:10 dilution; pH 1.8 at 37°C for 3 hr). Orbital agitation (100 rpm) in a controlled environment incubator was maintained during the gastric testing. At appropriate time intervals, samples were withdrawn and creatine monohydrate values (mg/mL) were tested. Data generated dictated the encapsulation system to be utilized in the in vivo trial.

Participants for in vivo human study

Ten healthy young males volunteered to participate in the study. Each was self-reported as physically active during recruitment, as defined by an inclusion criterion of habitually performing at least 30 min of exercise on five or more days each week. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human volunteers were approved by the University College Dublin (UCD) Research Ethics Committee (permit: LS-13-29-Egan-DeVito). Written informed consent was obtained from all volunteers prior to participation.

Experimental procedures

All procedures took place at the Human Physiology Laboratory at the Institute for Sport & Health, UCD (Figure 1). Participants visited the laboratory on a single occasion, were randomly assigned to either PLA or CRE conditions in a double-blind design. The CRE condition provided a 3-g dose of creatine monohydrate, while the PLA condition provided a 3-g dose of maltodextrin, both in microencapsulated form. Randomization was performed by drawing envelopes from an opaque container containing an equal distribution of PLA and CRE. When an envelope was selected, it was not returned to the container prior to the randomization of subsequent participants.

Participants were asked to abstain from caffeine and alcohol and refrain from strenuous exercise for 24 hr prior to their visit, and kept a two-day portion size estimate food diary on the two days prior to their visit (analyzed using the Nutritics Professional Diet Analysis Software package). In addition, the European Prospective Investigation into Cancer and Nutrition Norfolk Food Frequency Questionnaire (EPIC Norfolk FFQ) was administered to quantify the participants' usual food intake during the previous year. Food Frequency Questionnaire European Prospective Investigation into Cancer and Nutrition Tool for Analysis (FETA) software was used to analyze the questionnaires to determine daily averages of nutrition intake.

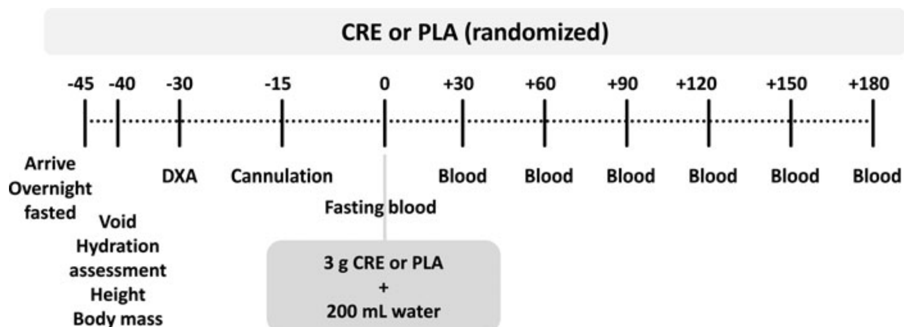

Figure 1. Experimental design schematic.

Participants arrived at the laboratory between 0730 and 0900 hr after an overnight fast, and provided a urine sample for hydration assessment (PEN Refractometer, Atago Instruments, Japan). Height, using a wall-mounted stadiometer to the nearest 1 cm, and body mass, using a digital scale to the nearest 0.1 kg, were measured. Participants then underwent a dual-energy X-ray absorptiometry (DXA) scan (Lunar iDXA, GE Healthcare, UK) to assess body composition, i.e., fat-free mass, fat mass, and percentage of body fat. Next, a fasting blood sample was taken via a cannula inserted into a superficial forearm vein for serial blood sampling. Blood samples (4 mL) were collected in plastic tubes containing lithium heparin (Vacuette, Greiner Bio-One, Germany) at each sampling point followed by centrifugation for 10 min at 4,000 rpm at 4°C. Plasma was stored at −80°C until subsequent analysis.

Assessment of plasma creatine concentrations in response to an oral creatine load

After providing a fasting blood sample, participants ingested a 70-mL "shot" of either PLA or CRE as designated, together with a 200-mL bolus of plain water. In a double-blind design, PLA and CRE were identical in taste, appearance, and flavor, and were contained in identical bottles with branded commercial labels but were identifiable to only one of the research team by differences in batch number. This researcher was not party to the subsequent blood analysis. Every 30 min for 3 hr after ingestion, a blood sample was taken. Participants remained in the lab at quiet rest throughout this period. Between samples, the cannula was kept patent with isotonic saline (0.9% w/v sodium chloride; Baxter Healthcare, Ireland).

Analysis of plasma creatine concentrations

The creatine concentrations of the blood samples were analyzed using a commercially available creatine assay kit (ab65339, Abcam, UK). This colorimetric assay involves creatine being enzymatically converted to sarcosine, which is then specifically oxidized to generate a product that converts a colorless probe into an intensely red color at 570 nm. Since high protein concentrations affect the performance of the assay, 500 μL of each plasma sample was processed through a 10-kDa molecular weight cut-off filter (Amicon Ultra, Millipore, Cork, Ireland). The filtrate was collected and the remainder of the assay was performed in duplicate according to the manufacturer's instructions.

Statistical analysis

Data were analyzed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) and reported as mean $\pm$ SEM. Two-way (treatment $\times$ time) repeated measures ANOVA, with Student–Neuman–Keuls post hoc pair-wise comparisons were performed to identify differences within and between the supplementation regimens for variables with serial measurement, i.e., blood samples. An independent samples t-test was performed to identify differences between variables with single measurements, i.e., anthropometry. The significance level was set at $\alpha = 0.05$ for all statistical tests.

Results

Physicochemical characterization of protein hydrolysate

The milk protein hydrolysate was characterized using the OPA method (Spellman et al., 2003), which established a degree of hydrolysis (DH) of 9.33 $\pm$ 0.29%. Size exclusion

chromatography illustrated a predominant exo-peptidase activity because of the presence of a peptide fraction <4 kDa coupled with the enhanced liberation of free amino acids (12.2 μmol/mL) after 3-hr hydrolysis. A significant proportion of the intact monomeric form continued to prevail at the end of hydrolysis, which is a requirement for efficient encapsulation.

The technique selected for successful encapsulation of creatine monohydrate commands a high rate of encapsulation efficiency (Figure 2A). In the event that low encapsulation efficiency occurs (Figure 2B), an incorrect and unstable encapsulation coating is generated, which subsequently compromises the stability of creatine monohydrate. This ultimately results in the production of an ineffective encapsulation system. When the encapsulation physico-chemical parameters are balanced and compatible (i.e. time, temperature, flow rate, protein source, structure, etc.), a high rate of encapsulation efficiency can be achieved for creatine monohydrate (Figure 2A).

The mechanical strength profile was measured on creatine micro-particles 5 hr and five days post-production. No break or rupture was observed for micro-particles following 95% compression stress tested on either time-point. The stable strength profile endorses the shear resistance and mechanical strength of creatine micro-particles as a function of time and compression distance.

The stability of this system in aqueous environments was further tested (Figure 3), with the importance and significance of protein source and secondary structure on encapsulation efficiency demonstrated. Once this creatine encapsulation system is optimized, the controlled release of creatine can be controlled. Thus, the pulsative liberation of creatine during ex vivo porcine intestinal contents (pH 7.1; 37°C) for rapid absorption and assimilation can be illustrated (Figure 2C). Hence, this research is novel due to the unique mechanism employed for the delivery, protection, and release of creatine monohydrate in aqueous environments.

Storage stability and subsequent gastric resistance of creatine monohydrate encapsulated in native, hydrolyzed, and denatured milk protein were elucidated (Figure 3). Following 28 days storage at 25°C, creatine concentrations are highest in hydrolyzed protein encapsulation systems, relative to native and denatured protein preparations. Creatine micro-particles have the most stable and robust structure when hydrolyzed milk protein is utilized as an encapsulation matrix. Storage and ex vivo gastric exposure confirmed the inherent stability of creatine monohydrate micro-particles encapsulated using hydrolyzed milk protein, and that is why it was the encapsulation material of choice for in vivo trial.

In vivo human trial – plasma creatine concentrations in response to an oral creatine load

On average, participants reported to the lab in a dehydrated state defined by urine osmolality >700 mOsm/kg and urine specific gravity >1.020 (Sawka et al., 2007), but hydration status did not differ between groups (urine osmolality: PLA, 760 ± 50 vs. CRE 864 ± 33 mOsm/kg; USG: PLA, 1.021 ± 0.001 vs. CRE, 1.023 ± 0.001). For all components of body composition analyzed (height, body mass, body mass index (BMI), percentage of body fat, body fat mass, and fat-free mass), there were no significant differences between PLA compared with CRE (Table 1).

The two-day average intake as a percentage of total energy intake for the PLA group was 33 ± 5%, 31 ± 5%, and 36 ± 3% for carbohydrate, protein, and fat, respectively. For the CRE group, contributions from carbohydrate, protein, and fat were 29 ± 5%, 29 ± 5%, and 42 ± 5%,

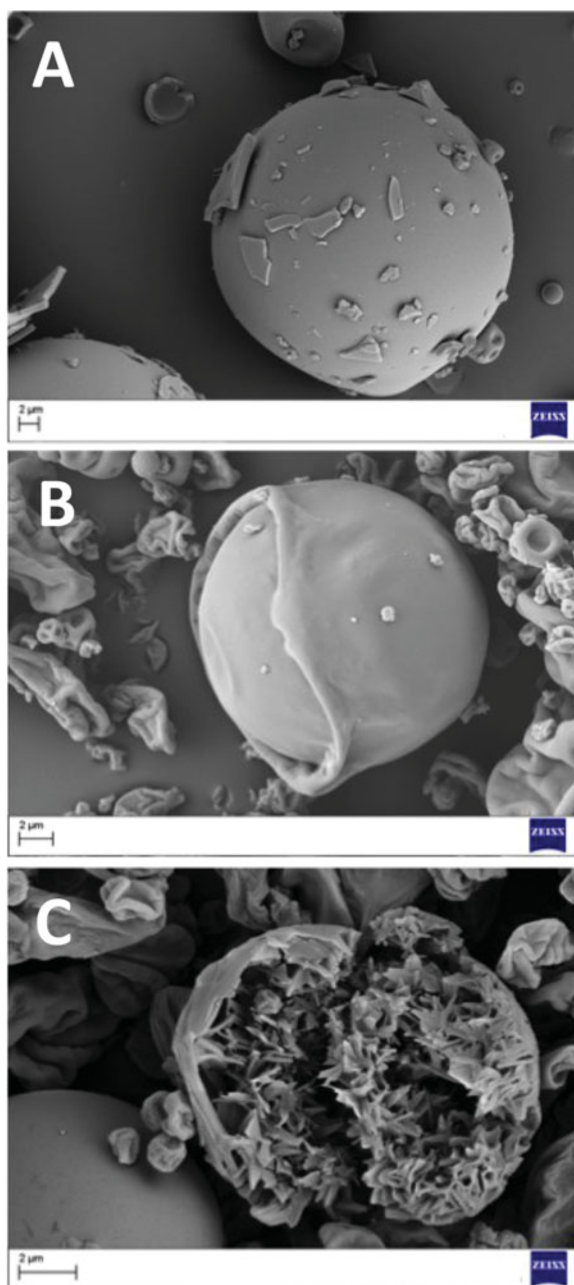

Figure 2. Scanning electron microscope images of creatine micro-capsules demonstrating (A) a creatine micro-capsule with optimum capsule protection and high encapsulation efficiency; (B) a creatine micro-capsule with low encapsulation efficiency and incomplete capsule coating; and (C) initial intestinal digestion of a creatine micro-capsule after ex vivo intestinal digestion. The creatine micro-capsules are described by International Patent Application Number PCT/EP2014/062154.

respectively. There were no significant differences between groups for any of these parameters, nor when intake was expressed on a gram per kilogram body mass basis (data not shown). Intake of fish and meat products did not differ between the groups (PLA, 259 $\pm$ 44 g vs. CRE, 322 $\pm$ 56 g).

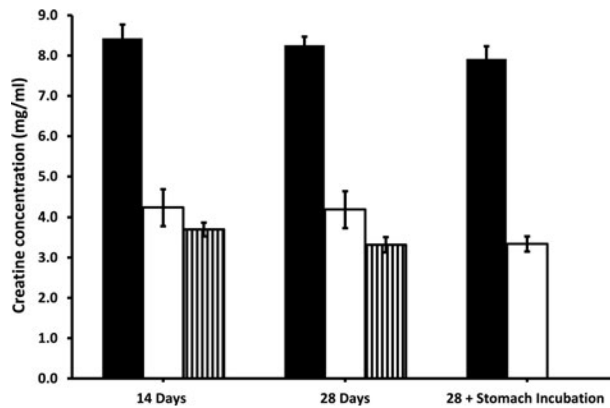

Figure 3. Concentration of creatine monohydrate during 28-day storage in aqueous solution at pH 4.0 at room temperature. Treatments include hydrolyzed milk protein capsules (■); native milk protein capsules (□), and creatine in denatured milk protein micro-capsules (▥) at 25°C for up to 28 days followed by exposure to ex vivo stomach contents (pH 1.6; 3 hr).

Plasma creatine concentrations were unchanged after ingestion of 3 g of maltodextrin during PLA, and averaged ∼45 μM (Figure 4). During CRE, plasma creatine concentration peaked after 30 min at 101.6 ± 14.9 μM ($p < 0.05$), representing a 2.3-fold increase over resting concentrations, thereafter gradually trending downwards toward resting concentrations, but remained significantly elevated (∼50% above resting levels) at 3 hr after ingestion (Figure 4).

Discussion

Micro- and nano-encapsulation are technologies capable of enhancing nutrient stability in aqueous solutions with potentially enhanced bioavailability in blood and to target tissues after ingestion (Champagne & Fustier, 2007; Dias, Ferreira, & Barreiro, 2015). These encapsulation systems have acceptable organoleptic properties with improved bioavailability for food and beverage applications. Previous in vitro and ex vivo studies of these systems demonstrated protection of phytochemicals and bioactives against digestion, proteolysis, and lipolysis in the stomach and the intestine (Champagne & Fustier, 2007; Dias, Ferreira, & Barreiro, 2015). Specific to creatine, microencapsulation technology may reduce the risk associated with creatine degradation to creatinine with concomitant potential for incorporation into food matrices or ready-to-drink formulations appropriate for long-term supplementation to enhance sports performance and/or offset muscle wasting as described elsewhere (Buford et al., 2007;

Table 1. Participant anthropometrics.

	PLA	CRE
Age (years)	23 ± 2	22 ± 1
Height (m)	1.74 ± 0.02	1.75 ± 0.03
Body mass (kg)	79.0 ± 5.3	81.6 ± 5.0
BMI (kg m^{-2})	26.0 ± 1.4	26.8 ± 1.3
Body fat (%)	16.8 ± 1.9	20.9 ± 3.5
Fat mass (kg)	12.99 ± 2.02	16.92 ± 3.94
Fat-free mass (kg)	62.67 ± 3.44	61.12 ± 2.14

BMI: body mass index; PLA: placebo; CRE: creatine. Data are mean ± SEM, $n = 5$ in each group. PLA vs. CRE, nonsignificant for all variables (independent sample t-test).

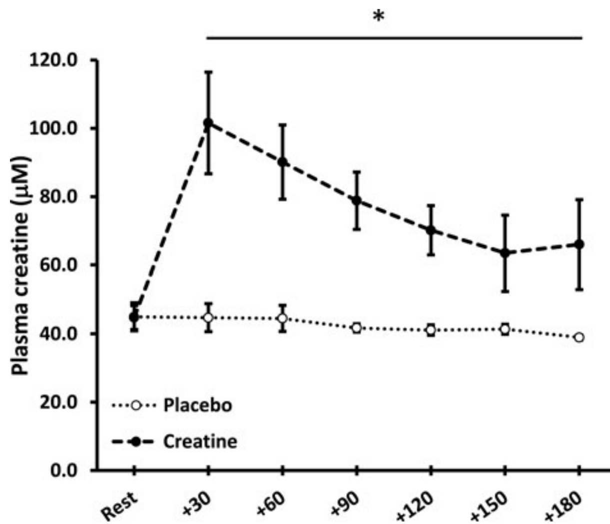

Figure 4. Plasma creatine concentrations in response to the ingestion of CRE or PLA. $^{*}p < 0.05$ PLA vs. CRE.

Cooper et al., 2012; Gualano et al., 2012). The aim of the present study was to investigate the stability and subsequent bioavailability of microencapsulated creatine within a polymerized hydrolyzed milk protein matrix delivered in an aqueous solution (i.e., ready-to-drink delivery mechanism) in physically active young males.

The observed resting plasma creatine concentrations of ∼45 μM on both PLA and CRE are comparable with previous reports (Deldicque et al., 2008; Harris et al., 2002, 2004; Jager et al., 2007; Rawson et al., 2004; Schedel et al., 1999). Dietary factors are typically considered in supplementation studies rather than acute dosing designs such as the present study. This is because in creatine supplementation studies, large inter-subject variability in change in muscle creatine content is observed (e.g., no change up to 30% increase), with initial muscle creatine content likely to be the most important determinant of muscle creatine uptake after supplementation (Greenhaff et al., 1994; Harris, Soderlund, & Hultman, 1992; Rawson et al., 2002). For instance, subjects with lower muscle creatine concentrations have the largest increase in muscle creatine after supplementation, whereas subjects with higher muscle creatine concentrations have little or no increase in muscle creatine after supplementation (Greenhaff et al., 1994; Harris, Soderlund, & Hultman, 1992; Lukaszuk et al., 2002).

The temporal pattern of plasma creatine elevation (peaking in 30 min, and a half-life of ∼1.5 hr) and magnitude of increase (approximately two-fold) compare favorably with previous reports examining plasma kinetics in response to 2- to 3-g doses of creatine (Deldicque et al., 2008; Harris et al., 2002, 2004). Reports of 10- to 50-fold increase in plasma creatine concentrations has involved 5- to 20-g doses of creatine (Jager et al., 2007; Persky, Brazeau, & Hochhaus, 2003; Rawson et al., 2004; Schedel et al., 1999), i.e., a clear dose-response relationship exists between the amount ingested and the change in plasma concentrations (Persky, Brazeau, & Hochhaus, 2003). These data indicate that microencapsulated creatine monohydrate is stable in an aqueous solution, and ingestion of 3 g of creatine in a 70-mL bolus increases plasma creatine concentration for up to 3 hr after ingestion. Therefore, contrary to the prevailing understanding that creatine monohydrate is unstable in solutions that are neither pH-adjusted nor refrigerated (Jager et al., 2007), microencapsulation of creatine monohydrate using the method herein produces a form of creatine that is stable in solution at ambient temperatures and can provide a physiologically relevant dose of creatine.

Creatine is formed from methionine, glycine, and arginine, amino acids that are found primarily in meat and fish. Moreover, dietary-free creatine is predominantly obtained from meat and fish, so dietary analysis or control focuses on fish and meat intake of participants (Lukaszuk et al., 2002, 2005). In the absence of differences between groups for intake of fish and meat products, it is unlikely that the plasma creatine response is influenced by the dietary intake of participants, consistent with previous findings (Lukaszuk et al., 2005). Moreover, similar plasma creatine kinetics are observed after ingestion a 5-g oral creatine bolus on two occasions separated by 30 days, in spite of muscle phosphocreatine being 23% higher at the ingestion of the second bolus (Rawson et al., 2004), suggesting that plasma levels of creatine after acute ingestion are neither influenced by muscle creatine content nor indicative of muscle creatine uptake.

Many studies have demonstrated that oral creatine supplementation can maximize muscle creatine levels by either a “loading” phase for five days followed by a “maintenance dose,” or by a “maintenance dose” for approximately 30 days (Buford et al., 2007; Cooper et al., 2012). For example, traditional supplementation protocols include a brief (∼5 to 7 days), high dose (∼20 g/day or 0.3 g/kg body mass/day, “loading”) supplementation protocol followed by a “maintenance dose” of 2 g/day, or a longer duration (∼3 to 6 weeks) low dose (∼3 g/d or 0.03 g/kg body mass/day) supplementation protocol (Harris, Soderlund, & Hultman, 1992; Hultman et al., 1996). In either case, similar increase in muscle creatine content is observed (Buford et al., 2007; Cooper et al., 2012). In the present study, a 3-g bolus was chosen because the ingestion of 3-g creatine per day is likely to be as effective at raising muscle content with longer term supplementation as the loading protocol but represents a more practical quantity to incorporate into food matrices or ready-to-drink formulations via a microencapsulation approach.

Conclusive proof of an increase in relevant creatine bioavailability can only be gained by assessing the amount of creatine reaching the target tissue, the muscle, measured by muscle biopsy and/or whole body creatine retention assessed by measuring the difference between creatine intake and urinary excretion (Jager et al., 2011). Therefore, the effects on muscle creatine concentration and subsequent exercise performance benefits remain to be confirmed. The future studies should investigate the efficacy of this novel form of creatine using a >4-week daily supplementation protocol (3 g per day), combined with analysis of muscle creatine concentrations before and after supplementation and effects on repeated sprint and high intensity exercise performance similar to previous works (Buford et al., 2007; Cooper et al., 2012). However, the present study demonstrates clear preliminary evidence that microencapsulation of creatine monohydrate within a polymerized hydrolyzed milk protein matrix provides a stable source of creatine delivered in an aqueous, ready-to-drink product.

Declaration of interest

Sinead B. Bleiel is a founder and shareholder in Anabio Technologies, creators of the microencapsulated creatine described in the article. This formulation is covered by International Patent Application Number PCT/EP2014/062154 published by WIPO on December 18, 2014. Robert M. Kent is an employee of Anabio Technologies. All other authors declare no conflicts of interests.

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References

Buford TW, Kreider RB, Stout JR, Greenwood M, Campbell B, Spano M, et al. International Society of Sports Nutrition position stand: creatine supplementation and exercise. J Int Soc Sports Nutr 2007;4:6.

Champagne CP, Fustier P. Microencapsulation for the improved delivery of bioactive compounds into foods. Curr Opin Biotechnol 2007;18:184–190.

Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV. Microencapsulation of probiotics for gastrointestinal delivery. J Control Release 2012;162:56–67.

Cooper R, Naclerio F, Allgrove J, Jimenez A. Creatine supplementation with specific view to exercise/sports performance: an update. J Int Soc Sports Nutr 2012;9:33.

Deldicque L, Decombaz J, Zbinden FH, Vuichoud J, Poortmans JR, Francaux M. Kinetics of creatine ingested as a food ingredient. Eur J Appl Physiol. 2008;102:133–143.

Dias MI, Ferreira IC, Barreiro MF. Microencapsulation of bioactives for food applications. Food Funct. 2015;6:1035–1052.

Garcia Alvarez-Coque MC, Medina Hernandez MJ, Villanueva Camanas RM, Mongay Fernandez C. Spectrophotometric determination of N-acetylcysteine in drug formulations with o-phthalaldehyde and isoleucine. Analyst. 1989;114:975–977.

Greenhaff PL, Bodin K, Soderlund K, Hultman E. Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. Am J Physiol 1994;266:E725–E730.

Gualano B, Roschel H, Lancha Jr AH, Brightbill CE, Rawson ES. In sickness and in health: the widespread application of creatine supplementation. Amino Acids. 2012;43:519–529.

Harris RC, Almada AL, Harris DB, Dunnett M, Hespel P. The creatine content of creatine serum and the change in the plasma concentration with ingestion of a single dose. J Sports Sci. 2004;22:851–857.

Harris RC, Nevill M, Harris DB, Fallowfield JL, Bogdanis GC, Wise JA. Absorption of creatine supplied as a drink, in meat or in solid form. J Sports Sci. 2002;20:147–151.

Harris RC, Soderlund K, Hultman E. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. Clin Sci (Lond). 1992;83:367–374.

Heidebach T, Forst P, Kuzolik U. Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. Food Hydrocolloids. 2009;23:1670–1677.
Hultman E, Soderlund K, Timmons JA, Cederblad G, Greenhaff PL. Muscle creatine loading in men. J Appl Physiol. 1996;81:232–237.
Jager R, Harris RC, Purpura M, Francaux M. Comparison of new forms of creatine in raising plasma creatine levels. J Int Soc Sports Nutr 2007;4:17.
Jager R, Purpura M, Shao A, Inoue T, Kreider RB. Analysis of the efficacy, safety, and regulatory status of novel forms of creatine. Amino Acids. 2011;40:1369–1383.
Livney YD. Milk proteins as vehicles for bioactives. Curr Opin Colloid Interface Sci. 2010;15:73–83.
Lukaszuk JM, Robertson RJ, Arch JE, Moore GE, Yaw KM, Kelley DE, et al. Effect of creatine supplementation and a lacto-ovo-vegetarian diet on muscle creatine concentration. Int J Sport Nutr Exerc Metab. 2002;12:336–348.
Lukaszuk JM, Robertson RJ, Arch JE, Moyna NM. Effect of a defined lacto-ovo-vegetarian diet and oral creatine monohydrate supplementation on plasma creatine concentration. J Strength Cond Res. 2005;19:735–740.
Persky AM, Brazeau GA, Hochhaus G. Pharmacokinetics of the dietary supplement creatine. Clin Pharmacokinet 2003;42:557–574.
Rawson ES, Clarkson PM, Price TB, Miles MP. Differential response of muscle phosphocreatine to creatine supplementation in young and old subjects. Acta Physiol Scand. 2002;174:57–65.
Rawson ES, Persky AM, Price TB, Clarkson PM. Effects of repeated creatine supplementation on muscle, plasma, and urine creatine levels. J Strength Cond Res. 2004;18:162–167.
Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc. 2007;39:377–390.
Schedel JM, Tanaka H, Kiyonaga A, Shindo M, Schutz Y. Acute creatine ingestion in human: consequences on serum creatine and creatinine concentrations. Life Sci. 1999;65:2463–2470.
Singh MN, Hemant KS, Ram M, Shivakumar HG. Microencapsulation: a promising technique for controlled drug delivery. Res Pharm Sci. 2010;5:65–77.
Spellman D, McEvoy E, O'Cuinn G, FitzGerald RJ. Proteinase and exopeptidase hydrolysis of whey protein: comparison of the TNBS, OPA and pH stat methods for quantification of degree of hydrolysis. Int Dairy J. 2003;13:447–453.