CHAPTER

40

Oral Bioavailability of Creatine Supplements

Insights into Mechanism and Implications for Improved Absorption

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INTRODUCTION

Creatine is found primarily in skeletal muscle as both free creatine and creatine phosphate. Creatine phosphate comprises 70% of the total creatine found in skeletal muscle. The cellular importance of creatine phosphate is as a readily available source of phosphate for regeneration of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). In the absence of creatine phosphate, ATP cannot be regenerated, resulting in impaired muscle function due to lack of an available energy source in the muscle cells. Studies have demonstrated that dietary supplementation with creatine can increase total skeletal muscle creatine levels by approximately 20% [1–3]. Of the increased deposition of creatine in the muscle following dietary supplementation, approximately one third is in the form of creatine phosphate and available for immediate use [1,4,5]. The correlation between increased muscle stores of creatine and improved muscle performance is well established [6], and dietary supplementation with creatine is widely used and accepted by most governing sports bodies [7].

The performance benefits that result from creatine supplementation include (a) more cellular energy for short bursts of high intensity exercise, (b) improved energy transfer in muscle cells, and (c) greater buffering capacity resulting in less fatigue and shorter recovery time following intense exercise. [8–12]. While there is certainly individual variation regarding creatine response, the above-described effects are obtainable with daily dosing of creatine ranging from 3 to 10 grams. In addition to the well-known effects of creatine supplementation on muscle performance, more recent studies have reported potential use of creatine supplements in muscle repair following injury [13,14], as well as anti-inflammatory effects [15–17], that apply to both endurance and power athlete. The muscle repair and anti-inflammatory effects appear to require higher doses of creatine supplements, often in the 20–30 gram daily dose range [13–17].

There is little available scientific evidence to refute the effects of creatine supplementation on muscle performance. However, the relatively large amounts of creatine supplementation required to produce these desired effects suggests inefficiencies in either the bioavailability and/or tissue distribution of current creatine products. While there has been much research devoted to enhancing creatine uptake into muscle cells through co-administration of glucose [18], various fatty acids [19], and insulin-stimulating products [20], until recently, little effort was devoted to enhancing the oral bioavailability of creatine supplements. This is due in large part to the assumption that creatine monohydrate, the most widely used form of creatine, is completely absorbed from the gastrointestinal tract. However, there is sufficient evidence to suggest that the oral bioavailability of creatine monohydrate is far

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from complete. This chapter reviews the mechanisms governing creatine absorption in the epithelial cells of the gastrointestinal tract and the evidence supporting passive diffusion of creatine as the route for oral absorption. Based on this information, a critical re-examination of the bioavailability of creatine monohydrate, especially in comparison with newer salt forms of creatine, will be undertaken to provide potential insight into ways to improve the efficiency of the available dosage forms of creatine.

CELLULAR MECHANISM OF INTESTINAL ABSORPTION

The gastrointestinal tract (GIT) is well suited for the absorption of nutrients. Ingested material first enters the stomach where excreted enzymes and the low pH environment begin breaking down the material into more absorbable nutritional units such as glucose, amino acids and peptides. For any compound to be absorbed in the GIT, whether it is a nutrient, drug, or potential toxin, it must be in solution. The primary function of the digestive processes in the stomach is to solubilize the ingested material so that it can be absorbed in the small intestines. The basic anatomical features of the small intestine result in a large surface area for the absorption of nutrients. The small intestine is divided into three segments: the duodenum is the first part, the jejunum is the middle segment, and the ileum is the third and final segment adjacent to the large intestine.

There are three basic absorption pathways by which a nutrient can be taken up from the GIT and enter into the systemic circulation (Figure 40.1). These include transcellular diffusion, paracellular diffusion and transcellular transport. The transcellular diffusion route is a passive process governed by the concentration gradient that exists for the solute of interest (in this case creatine) in the lumen of the intestine and the epithelial cell interface and the permeability of the solute across a lipid bilayer. Permeability is determined by the physico-chemical properties of a solute, with parameters such as size/surface area, lipophilicity, charge and hydrogen bonding potential of the solute influencing passive diffusion across the cellular interface [21]. Indeed, based on solutes with high oral bioavailability through the transcellular diffusion route, ideal properties include a molecular weight less than 300 Daltons, a log P value (measure of lipophilicity) between 1 and 3, nonionized and with fewer than five hydrogen-bond acceptors [22].

While paracellular diffusion is also dependent on a concentration gradient, instead of moving through the epithelial cell, the solute travels in a bulk flow manner in the spaces that exist between the epithelial cells. For the absorptive epithelial cells that line the intestine, the junctions between the cells have a collection of membrane proteins that interact with each other to form what is referred to as a tight junction [23]. While the bulk flow movement of solutes through the tight junctions is restricted, the complexity and restrictiveness of the tight junctions vary depending on the location within the small intestine. Thus, tight junctions between epithelial cells in the duodenum and jejunum have a larger pore opening (approximately 8–13 Ångstroms in diameter) than in the ileum, where pore sizes of approximately 4 Ångstroms in diameter are observed [24]. Those solutes most likely to be absorbed via paracellular diffusion processes in the GIT are

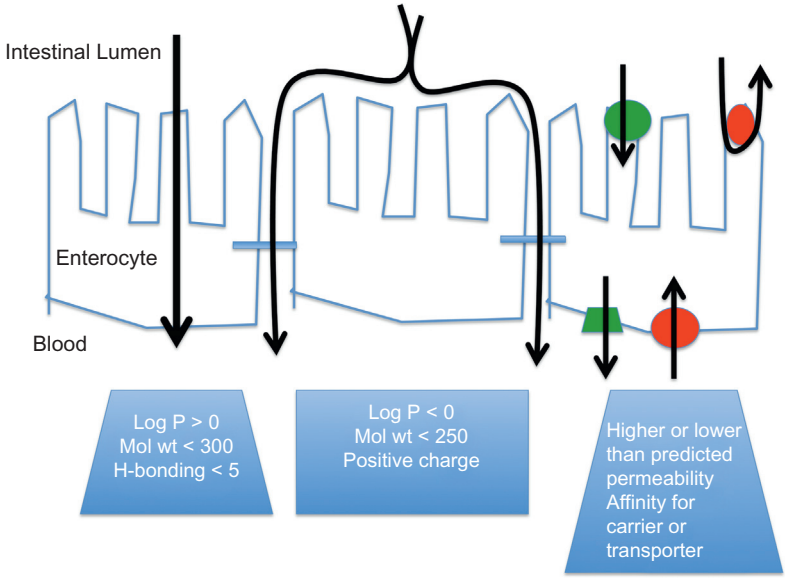

FIGURE 40.1 **Schematic of different solute absorption pathways in the gastrointestinal tract and the general properties governing absorption through each specific pathway.**

relatively small in size (<250 Daltons in molecular weight) with positive charged solutes having greater potential for movement through the paracellular pore than negative charged or zwitterionic solutes [21,23,25]. Another important feature of solutes that are absorbed through paracellular diffusion is that they typically display regionally specific and incomplete absorption characteristics due to the limited sites for paracellular diffusion within the GIT [23,25].

Transcellular transport of solutes requires specific membrane proteins that act as carriers to move the solute across the biological membrane. These carriers or transporters facilitate the movement of the solute into or out of the cell at a rate that is far greater than that possible with simple passive diffusion of the solute. Transcellular transport processes for solutes are often coupled with the movement of ions in either a co-transport or counter-transport fashion [26]. Examples in the small intestine include vitamin and peptide transporters that are driven by the co-transport of sodium and hydrogen ions, respectively [27,28]. There are also solute transporters that utilize adenosine triphosphate (ATP) as the cellular energy source to move solutes across the cell membrane [29]. Regardless of whether the solute transporter is driven by electrochemical gradients or hydrolysis of ATP, a feature of all solute transporters is selectivity and saturability of transport.

CREATINE ABSORPTION IN THE GIT

Creatine is a zwitterion with a positively charged guanidine functional group and a negatively charged carboxylic acid functional group. Within the acidic environment of the stomach and jejunum of the small intestine, the carboxylic acid functional group is likely to be protonated and the predominant form of ingested creatine is the positively charged species. As the ingested creatine progresses down the intestinal lumen the pH becomes neutral and the zwitterion and negative charged species will become more prevalent. Because creatine exists primarily as a charged molecule, its ability to partition into a lipophilic environment such as the plasma membrane of the intestinal epithelial cell is limited. Indeed, the logP value for creatine monohydrate, the most common form of creatine supplement, is approximately −1.0. By comparison, only solutes displaying LogP values between 1 and 3 are likely to be absorbed by transcellular diffusion in the gastrointestinal tract [22]. Thus, in considering the absorption of creatine from the gastrointestinal tract, passive diffusion via the transcellular route is likely to be minimal.

There are potentially multiple transporters for creatine within the GIT. The creatine transporter (CRT) is a solute transporter that selectively transports creatine and creatine analogs in a sodium dependent manner [30]. The CRT is expressed in high amounts in the brain, intestine and skeletal muscle, where it plays a crucial role in the distribution of creatine to target tissue [31,32]. Indeed, CRT genetic abnormalities are linked to reduced creatine distribution to the brain and the development of mental retardation [33]. Expression of CRT at both the messenger RNA and protein level has been reported in epithelial cells of the intestine [34]. Recent studies examining the expression and function of CRT during development in the rat, showed multiple forms of CRT within the colon during development [34]. However, the activity and expression level of CRT in the colon diminished during maturation, with little CRT activity observed in the adult rat [34]. If similar developmental patterns exist in humans, this would suggest that creatine absorption through CRT1 in the GIT is lowest in adulthood.

An additional consideration is the localization of CRT within the GIT. To aid in the oral absorption of creatine, CRT would need to be localized in the brush border membrane of the intestinal epithelial cells. Previous studies have reported brush border expression of CRT in the jejunal and ileal segments of the small intestine [35,36]. However, while brush border CRT is the first step in absorbing dietary sources of creatine, there would need to be transporters positioned on the basolateral membrane of the intestinal epithelial cells to move the absorbed creatine into the bloodstream. While an extensive search for CRT on the basolateral membrane did reveal a sodium-dependent transporter for creatine the directionality of transport was inward [37]. The directionality of this transporter means that it would only be able to transport creatine from the blood into the intestinal epithelial cell, and thus would not aid in the oral absorption of creatine.

A final consideration for transporter-mediated absorption of creatine in the GIT is the kinetics of the CRT. As absorption through CRT is saturable at high concentrations, the extent to which CRT can efficiently absorb creatine will be dependent on the amount of creatine in the gastrointestinal fluid. Given that creatine supplements are consumed at doses of 5−10 grams or more, the concentration of creatine in the gastrointestinal fluid is likely to be above those required for optimal transport function of CRT. Indeed, this may explain some of the dose-dependent observations where low doses of creatine administered more frequently appear to provide better results [38]. For this reason, and those discussed above,

transcellular transport pathways for the absorption of creatine supplements are likely to be minimal.

Based on creatine permeability studies conducted in various intestinal models, the most likely route for creatine absorption in the GIT is through paracellular diffusion. Studies by Orsenigo and colleagues [37] examined creatine permeability across inverted jejunal segments of the rat intestine. While transporter-mediated uptake of creatine was observed in both brush border and basolateral intestinal membrane preparations, permeability across intact intestinal tissue was not transporter dependent, as demonstrated by the absence of concentration dependency and the inability to influence creatine permeability with various CRT inhibitors [37]. The paracellular diffusion pathway also fits from the perspective of what is known about the characteristics of solutes most likely to undergo paracellular diffusion. As creatine is below the 250 molecular weight cut-off and is primarily positively charged in the early portion of the GIT, where pH of the intestinal fluid is slightly acidic, solute diffusion through the paracellular route is ideal. Together, these studies provide compelling evidence for paracellular diffusion of creatine as the primary mechanism for oral absorption.

A paracellular diffusion pathway for creatine absorption in the GIT is also supported by Caco-2 cell permeability studies [39–41]. The Caco-2 cell line is a human transformed cell that is widely used to examine oral absorption within the pharmaceutical industry [42]. It expresses many of the transporters involved in absorption of nutrients in the GIT [42]. From the standpoint of creatine absorption, Caco-2 express CRT at the mRNA level [43], although potential changes in expression were observed during differentiation, consistent with the developmental expression analysis reported for CRT in rats [34]. Studies using radiolabeled creatine showed a very low permeability across Caco-2 monolayers, consistent with a solute with poor oral absorption profile [39]. Permeability of various creatine salt forms across Caco-2 monolayers was also consistent with minimal permeability [40]. As the Caco-2 have highly developed tight junctions, paracellular diffusion would be limited in this model and solutes undergoing paracellular diffusion would have low permeability [21]. Thus, the results with creatine monohydrate and various creatine salt forms in the Caco-2 reflect poorly permeable solutes. For solutes undergoing paracellular diffusion as their mechanism of permeability in the GIT, the Caco-2 system may underestimate actual intestinal permeability. Interestingly, recent studies with creatine ethyl ester, an esterified form of creatine with improved solubility and lipophilicity properties, demonstrated significantly greater permeability than did either creatine monohydrate or creatinine in Caco-2 monolayers [41].

HUMAN ORAL BIOAVAILABILITY OF CREATINE SUPPLEMENTS

Bioavailability is defined as the amount of an administered agent that is absorbed and present in the systemic bloodstream for distribution and use by the various tissues. It is typically expressed as a percentage or fraction of the amount administered. As creatine supplements are ingested, the oral bioavailability represents the fraction of the administered dose that is absorbed in the GIT and available in the system circulation for distribution to various tissue sites. The oral bioavailability of any compound is determined definitively by calculating the area under the curve (AUC) of the plasma concentration versus time profile following oral administration and comparing this to the resulting AUC plasma concentration versus time profile for the compound following intravenous injection. The direct intravenous administration of the compound results in a 100% bioavailability and the resulting plasma concentration profile provides the necessary data for comparison of absorption from other routes.

While there is abundant evidence in the literature for creatine supplementation and improved muscle performance using creatine monohydrate (CM), considerably less is known about the oral bioavailability of CM. Indeed, there are no published reports of the definitive oral bioavailability of any creatine supplement. Given the physico-chemical properties of CM, the relatively high doses required, and the previously discussed studies in the various intestinal absorption models that report low permeability of CM [37,39–41], the oral absorption of creatine supplements are likely to be incomplete. Despite this, CM based supplements are generally considered to have nearly complete absorption in the GIT. Such claims of complete oral absorption of CM are based on studies in which CM was administered orally and the increases in tissue levels of creatine combined with the increases in creatinine elimination in the urine were used to provide an index of the body burden of CM [44,45]. These methods used to obtain the estimates of oral bioavailability for CM have at least two major limitations. First, the accuracy of using urinary creatinine levels as an index of the amount of CM absorbed depends on the extent to which the creatinine excreted in the urine originated from the conversion of systemically absorbed creatine. The assumption is that increased levels of urinary creatinine following CM supplementation are the result of the conversion of creatine in the various tissues to

creatinine which is readily excreted into the urine. However, both the intestinal epithelial cells and bacteria have the ability to take up and process creatine [31,34,46,47]. This ability to acquire and metabolically process creatine within the intestine provides a potential source of creatinine in the GIT. Thus the conversion of creatine to creatinine within the lumen of the GIT, and its subsequent absorption into the bloodstream would result in a potential overestimate of the amount of creatine that has been systemically absorbed.

A second issue with previous studies reporting complete oral absorption of CM is equating an absence of creatine or creatinine in fecal matter with the complete absorption of CM from the intestine. Such interpretations ignore the ability of bacteria to utilize creatine and its metabolic products [31,46,47]. The extent of creatine utilization in the GIT provides a potential pathway for the pre-systemic removal of ingested creatine and results in an overestimate of CM oral bioavailability.

While definitive oral bioavailability data are lacking for CM, there are studies examining the relative bioavailability of various CM formulations [45,48] that suggest absorption is less than complete. Studies by Harris and colleagues [48] examined the oral absorption and pharmacokinetics of single-dose CM supplements when given either in solution, as a suspension or as a solid dosage form lozenge. In these studies, CM delivered in solution in liquid formulation resulted in faster absorption of CM as well as more extensive absorption represented by the larger AUC of the plasma creatine concentration versus time profile compared with either lozenge or suspension formulations. Based on these data, the authors suggested that there was a decrease in oral bioavailability of CM when in suspension or solid dosage form. The importance of this is underappreciated. Given that the aqueous solubility of CM is approximately 12–15 mg/mL, athletes taking a standard dose of CM (ranging from 5 to 10 grams) would require 400–800 mL of fluid to ensure the dose is completely solubilized. As a result of this, most CM products are taken as suspensions and would be incompletely absorbed in the GIT. The authors also compared absorption of CM in solution to that of equivalent amounts of creatine contained within red meat. While the absorption of creatine was delayed, compared with CM in aqueous solution, the amount of creatine absorbed from the red meat was similar [48]. Although more studies are required, there may be advantages to a delayed but sustained absorption of creatine supplements in terms of more efficient loading into skeletal muscle [48,49].

This is in contrast to the recent studies of Deldicque et al. [45] examining the oral absorption and resulting plasma pharmacokinetics of CM when administered in aqueous solution compared with either protein or beta-glucan nutritional bars. The investigators reported a significant difference in the rate of absorption of CM based on the dosage formulation used, with the aqueous solution providing the most rapid intestinal absorption. However, when looking at the extent of intestinal absorption, despite the appearance of moderate alterations in bioavailability, there was no significant difference in the resulting AUC for plasma creatine with any of the formulations examined.

ABSORPTION CHARACTERISTICS OF ALTERNATIVE FORMS OF CREATINE

Perhaps the most convincing evidence that the oral absorption of CM is less than complete comes from the comparison of oral bioavailability of various creatine salt forms. As different salt forms have potentially different solubility parameters [49,50], identification of creatine salts with increased aqueous solubility is likely to result in improvements in oral absorption and more efficient dosage formulations. Unfortunately there is considerably less information on the performance of these newer forms of creatine supplements in terms of bioavailability and biological response. Of the many different creatine salt forms, there are three—creatine pyruvate (CPyr), creatine citrate (CCit) and creatine hydrochloride (CHCl)—for which human oral bioavailability studies have been reported [49,50]. The aqueous solubility and relative bioavailability of these three creatine salt forms in comparison with CM is summarized in Figure 40.2. Studies by Jagar and

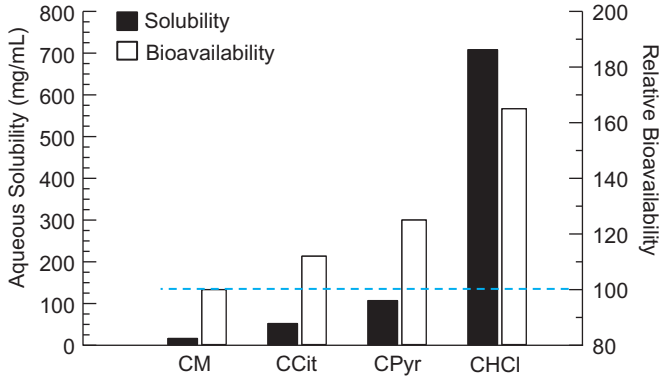

FIGURE 40.2 **Comparison of aqueous solubility and human bioavailability of various creatine salt forms.** The mean aqueous solubility values were obtained from [40]. Relative bioavailability was determined from the reported areas under the curve (AUC) for plasma creatine obtained with various creatine salt forms compared with that of creatine monohydrate (CM). The AUC values were taken from data in [49] for creatine citrate (CCit) and creatine pyruvate (CPyr) and [50] for creatine hydrochloride (CHCl).

colleagues compared the oral bioavailability of CCit and CPyr with that of CM. In these studies subjects were given 5-gram doses of CCit, CPyr or CM as an aqueous solution and the resulting plasma creatine levels were measured over time. For CCit, which has a slightly greater solubility than CM, there was a small increase (approximately 10%) in oral bioavailability compared with CM (Figure 40.2). For CPyr, which has an approximately 8-fold higher aqueous solubility, there was a modest, approximately 25%, increase in oral bioavailability compared with CM (Figure 40.2). Given the improved aqueous solubility of CPyr, an increase in oral bioavailability would be expected. Interestingly, the improved oral absorption observed with CPyr and CCit was statistically significant, though the differences were not considered important, primarily due to the perceived complete oral absorption of CM [49]. In this respect, regulatory standards indicate that different salt forms of a compound are considered bioequivalent when the relative bioavailability of one salt form is 75–120% of that observed with another salt form [51]. Under this criterion, CPyr, with an approximately 25% increase in oral bioavailability, would not be considered to have the same oral absorption properties as CM and would not be considered bioequivalent.

While the increases in oral bioavailability observed with CCit and CPyr could be classified as relatively modest, oral absorption studies with CHCl provide even more compelling evidence to suggest that improvements in bioavailability of creatine supplements are possible [50]. In these studies volunteers were given a 5-gram dose of either CM or CHCl in 8 ounces of water. A cross-over design was employed to allow direct comparisons of differences in oral absorption of the two creatine salts within the same subject. The results of these studies reported an approximately 60% increase in oral absorption of CHCl compared with CM [50]. With such increases in bioavailability observed with CHCl, it is difficult to claim complete oral absorption of CM. Together, these studies provide two important and fundamental findings. First, with increased oral absorption of the various creatine salts ranging from 10% to 60% over that of CM, it is clear that the bioavailability of CM is not nearly complete. Second, as CM bioavailability is not complete, there is potential for development of creatine supplements with improved oral absorption, which in turn could provide significant advancements in performance benefits, and allow for reduced dosages and more flexible dosing formulations (sports drinks and bars, fortified foods, etc.).

In addition to the multiple salt forms of creatine, there are modified forms of creatine such as creatine ethyl ester (CEE). In addition to having improved aqueous solubility compared with CM, the ester form of creatine also has improved octanol–water partitioning, an index of cell permeability (Table 40.1). As there are esterases throughout the blood and tissue, CEE was designed to be a pronutrient with enhanced oral absorption, which could then be hydrolyzed to creatine once absorbed into the body. There are few studies examining either the biological effects or oral absorption properties of CEE. The only direct comparison study of CEE and CM reported that CEE was "...not as effective at increasing serum and muscle creatine levels or in improving body composition, muscle mass, strength, and power" [52]. The study followed healthy volunteers over a 48-day period in which subjects were supplemented daily with 300 mg/kg of CM, CEE or placebo and underwent an exercise weight training regimen. Furthermore, the study reported high levels of creatinine in serum samples from the CEE-supplemented treatment group [52]. However, re-examination of the data looking at changes in muscle creatine, and peak and mean power measurements that occurred in the CEE, CM and placebo groups over the course of the 48-day study show CEE performance was as good or better than CM (Figure 40.3). This is due in part to the lower starting values for subjects in the CEE treatment group in terms of muscle creatine levels and power assessments [52]. As for the high serum creatinine levels in the CEE group, recent reports demonstrate that CEE is rapidly converted to creatinine in aqueous solutions at neutral pH [53]. Interestingly, CEE is very stable in aqueous solutions at low pH and appears to be more stable in lipophilic environments at neutral pH ranges [53], suggesting that CEE is intact during absorption in the GIT and stable within the membrane environment of cells. Thus, while initial findings suggested CEE is less effective, more studies are required to definitively address the issue.

TABLE 40.1 Comparison of Aqueous Solubility and Octanol–Water Partitioning of Creatine Ethyl Ester (CEE) and Creatine Monohydrate (CM)

Property	CEE HCl	CRT Monohydrate
Molecular weight g/mol	195.6	149.7
Percent by weight creatine	67	88
Aqueous solubility 25°C mg/mL	396	14.5
Ratio of solubility (relative to monohydrate)	27.4	1.00
Octanol–water partition coefficient	0.205	0.102
Ratio of Partition coefficient (relative to monohydrate)	2.01	1.00

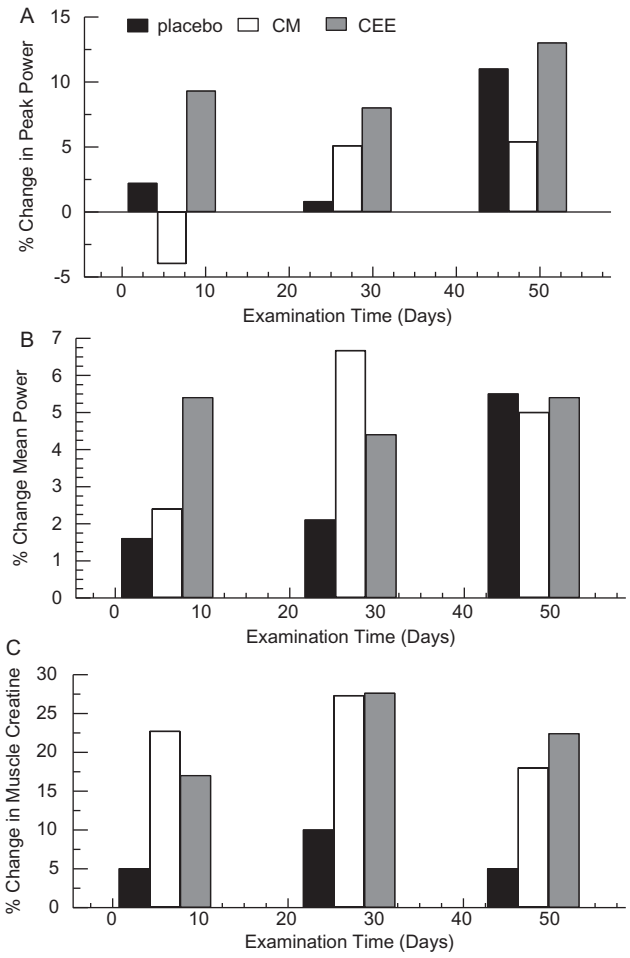

FIGURE 40.3 **Comparison of changes in (A) peak power, (B) mean power and (C) muscle creatine observed during various days of supplementation with either placebo (black bars), creatine monohydrate (white bars) or creatine ethyl ester (grey bars).** Values reported represent the changes from baseline measurements prior to initiation of the supplementation. The values reported were taken from data first reported in [52].

CONCLUSIONS

Creatine supplementation is an accepted and effective way to increase power output and speed muscle recovery. Because of these effects on skeletal muscle, creatine supplements are commonly used to improve athletic performance. While much effort has been given to improving the cellular processes governing accrual of creatine into the tissue, significantly less effort has been placed on increasing the oral absorption of creatine dietary supplements. Much of the reason for this is the perpetuated assumption that CM, the most widely used and studied form of creatine supplement, is completely absorbed from the GIT and is thus 100% bioavailable. Based on the mechanism of absorption of creatine in the GIT, there is ample evidence to suggest that creatine is not likely to have 100% bioavailability. Furthermore, based on the available human pharmacokinetic data, there is mounting evidence pointing to the less than complete absorption of creatine supplements.

Given the relatively large daily doses administered, improving the efficiency of creatine absorption could result in reductions in dosage as well as a more diverse range of formulations including sports drinks, bars and potentially fortified foods. Efforts to identify ways to increase creatine oral absorption, whether based on altering the absorption profile of CM through reduced doses given more frequently [38], or formulations with delayed release matrixes [45,48], or through identification of different creatine salt forms and compositions [49,50,53], have the potential to improve creatine supplementation options. Such efforts, in combination with appropriate safety and efficacy studies, will ultimately provide consumers and athletes with better options for creatine supplementation.

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