Amino Acids (2016) 48:1807–1817
DOI 10.1007/s00726-016-2199-y

REVIEW ARTICLE

Creatine for women: a review of the relationship between creatine and the reproductive cycle and female-specific benefits of creatine therapy

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Received: 19 November 2015 / Accepted: 8 February 2016 / Published online: 22 February 2016

Abstract The creatine/phosphocreatine/creatine kinase circuit is instrumental in regulating high-energy phosphate metabolism, and the maintenance of cellular energy turnover. The mechanisms by which creatine is able to buffer and regulate cellular energy balance, maintain acid–base balance, and reduce the effects of oxidative stress have led to a large number of studies into the use of creatine supplementation in exercise performance and to treat diseases associated with cellular energy depletion. Some of these studies have identified sex-specific responses to creatine supplementation, as such; there is the perception, that females might be less receptive to the benefits of creatine supplementation and therapy, compared to males. This review will describe the differences in male and female physique and physiology that may account for such differences, and discuss the apparent endocrine modulation of creatine metabolism in females. Hormone-driven changes to endogenous creatine synthesis, creatine transport and creatine kinase expression suggest that significant changes in this cellular energy circuit occur during specific stages of a female's reproductive life, including pregnancy and menopause. Recent studies suggest that creatine supplementation may be highly beneficial for women under certain conditions, such as depression. A greater understanding of these pathways, and the consequences of alterations to creatine bioavailability in females are needed to ensure that creatine is used to full advantage as a dietary supplement to optimize and enhance health outcomes for women.

Keywords Women's health · Nutrition · Reproduction

Handling Editor: T. Wallimann and R. Harris.

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Introduction

The creatine/phosphocreatine/creatine kinase circuit is integral to the maintenance of cellular energy (ATP) turnover, and thus cellular function (Wallimann et al. 2007), and it has particular importance in tissues with high and fluctuating energy demands, such as skeletal muscle, cardiac muscle and the brain (Wallimann et al. 1992). Creatine (Cr) is readily obtained from a diet containing meat and fish, and is also synthesized endogenously by the body, via a two-step enzymatic reaction that consumes arginine, glycine and methionine (Brosnan and Brosnan 2007). Once absorbed or synthesized, creatine is released into the circulation and actively transported into tissues by the Creatine Transporter 1 (CrT1), encoded by the *SLC6A8* gene (Guimbal and Kilimann 1993). Within the cell ~75 % of creatine is phosphorylated via creatine kinase to produce phosphocreatine (PCr), which then acts as a phosphate donor for the regeneration of ATP from ADP.

The phosphagen system provides support to mitochondrial oxidative phosphorylation and cellular ATP turnover by mitigating temporal and spatial imbalances in ATP supply and demand (Ellington 1989). The creatine/phosphocreatine/creatine kinase circuit is also tightly coupled with mitochondrial structure and bioenergetics (Guidi et al. 2008), and the activity of this biochemical reaction has a mild antioxidant effect because the rephosphorylation of ADP via PCr consumes a proton (H^+). These properties give the creatine/phosphocreatine reaction the ability

Table 1 Summary of reported differences in creatine metabolism between men and women

	Adult male	Adult female	Source
Circulating creatine			
Circulating Guanidinoacetate (GAA)	3.12 ± 0.66 μmol/l	2.02 ± 0.54 μmol/l	Kalhan et al. (2015)
Creatine synthesis	3.7–7.7 mmol day^{-1}	2.6–6.2 mmol day^{-1}	Brosnan and Brosnan (2007)
Serum creatine	40.8 ± 19.0 μmol/l	50.2 ± 20.6 μmol/l	Delanghe et al. (1989)
Creatinine clearance	1.0	0.75	Cockcroft and Gault (1976)
Dietary intake			
Daily meat consumption	146 g	107 g	Delanghe et al. (1989)
Daily creatine intake	7.9 mmol/day	5.0 mmol/day	Brosnan and Brosnan (2007)
Physique			
Skeletal muscle mass	33 kg	21 kg	Janssen et al. (2000)
Muscle Creatine content (vastus lateralis)	132 ± 10 mmol/kg	145 ± 10 mmol/kg	Forsberg et al. (1991)

to buffer the pH of the cytosol, thus protecting cells from damage associated with internal acidification and ATP depletion (Sestili et al. 2011).

Vertebrates express four different creatine kinase (CK) isoforms, and it is generally the expression pattern of these isoforms that govern the way in which creatine is used by a cell (Eppenberger et al. 1964, 1967; Dawson et al. 1967; Patra et al. 2012). Muscle creatine kinase (MCK) is a cytosolic isoform of CK expressed solely in sarcomeric skeletal and cardiac muscle cells (Turner et al. 1973; Wallimann et al. 1977). All other non-muscle cells, including the kidney, bone and neuronal tissue express the ubiquitous brain (BCK) isoform of creatine kinase (Wallimann et al. 2011). In addition to these cytosolic isoforms two mitochondrial isoforms of creatine kinase, located between the inner and outer mitochondrial membranes have been characterized (Jacobs et al. 1964). Sarcomeric mitochondrial CK (sMITCK) is expressed alongside MCK in striated skeletal and cardiac muscle cells, whilst mitochondria of other tissue types express a ubiquitous isoform (uMITCK) along with BCK (Wyss and Kaddurah-Daouk 2000). Despite variations in location and expression patterns, all CK isoforms catalyse the reversible transfer of the γ-phosphate group of ATP to the guanidine group of creatine, to yield PCr and ADP, and vice versa (Wyss and Kaddurah-Daouk 2000).

Increasing dietary consumption of creatine increases the intracellular pool of creatine/phosphocreatine available for ATP re-synthesis and can prolong cellular energy homeostasis. The use of dietary creatine supplementation as an ergogenic aid for exercise performance, and as a targeted therapeutic for a wide range of conditions where mitochondrial demise and depleted ATP underlie the pathology has been widely studied (see reviews, Feldman 1999; Gualano et al. 2010; Wallimann et al. 2011). Despite the fundamental role this phosphagen circuit plays at a cellular level, from time-to-time studies into creatine homeostasis and the benefits of dietary creatine supplementation for exercise performance and disorders of metabolism have identified differences in the sex-specific responses to creatine loading, with the benefits for women, particularly in regard to exercise physiology, being less than those reported for men (Mihic et al. 2000).

Here we review and discuss the physiological differences between males and females that may lead to differences in creatine metabolism between the sexes, with a focus on how the female utilises creatine under different physiological conditions, including age, sexual maturity and pregnancy. We discuss the areas where significant knowledge gaps exist, and highlight the need to overcome these to ensure that creatine is an effective ergogenic aid for female athletes; that creatine supply during pregnancy is maintained for appropriate fetal growth and development; and its use as a therapeutic intervention to treat a variety of diseases or conditions, such as clinical depression and sarcopenia, that are underpinned by cellular energy failure are realised.

Comparison of creatine metabolism between men and women

A number of differences in the storage and utilization of creatine have been identified between healthy males and females (Brosnan and Brosnan 2007). These are summarized in Table 1. When assessing creatine synthesis rates in the population, based on age, females produced amounts of endogenous creatine that were consistently 70–80 % lower than males (Brosnan and Brosnan 2007). Dietary intake of creatine of adult females aged 20–39 is also lower than their male counterparts (Brosnan and Brosnan 2007). This lowered rate of synthesis and consumption of creatine is the likely driver behind the reduced mean excretion rate of creatinine in females, which is ~80 % of the rate of excretion in males (Cockcroft and Gault 1976).

As skeletal muscle is the major storage compartment of creatine in the human body variation in the physical make up of men and women may be a major determinant of the difference of creatine homeostasis between the sexes. Janssen et al. (2000) measured skeletal muscle mass and distribution between 468 healthy adult males and females, and reported that males had significantly more skeletal muscle, both in terms of total mass (33 kg for men and 21 kg for women) and percentage body composition (38.4 % mens and 30.6 % for womens) (Janssen et al. 2000). Interestingly, in a study where biopsies of the vastus lateralis muscle were assessed for creatine content, females appeared to store about 10 % more creatine compared to males, relative to alkali-soluble protein (ASP) (Forsberg et al. 1991). This finding held irrespective of subject age and could not be attributed to differences in the proportion of fast and slow twitch fibre types or fibre cross sectional area. The higher percentage of stored creatine in female skeletal muscle, but the larger mass of skeletal muscle in men may account for the fact that on a standard western diet, which includes animal products, and where females consume around 25 % less meat than males, serum creatine levels are relatively similar between the sexes (Delanghe et al. 1989). A recent comparison between males and females showed that baseline guanidinoacetate (GAA) levels were lower in women than men and directly correlated with muscle mass; absolute synthesis rate of creatine was less in females than males; and after a 5-day creatine loading regime, women gained weight and men did not (Kalhan et al. 2015). The authors concluded that the increased body weight of female subjects was likely due to water retention (Powers et al. 2003) and did not report any other physiological variables associated with fluid retention, such as changes in blood pressure or renal function.

Creatine metabolism and exercise performance in males and females

Concerns about the ergogenic potential of supplementary creatine in women have been raised, as a higher resting total creatine content in skeletal muscle could diminish the capacity for creatine loading prior to exercise, and a lower total muscle mass has been correlated to lower CK activity (Norton et al. 1985; Forsberg et al. 1991). A study conducted by Mihic et al. (2000) directly assessed potential sex differences of acute dietary creatine loading on fat-free mass, blood pressure, plasma creatinine and CK activity, and concluded that increased creatine consumption increased total body mass and fat free mass for males and females, but that the effect was significantly greater in men (Mihic et al. 2000). In addition, only creatine supplementation in men has been shown to reduce amino acid oxidation and protein breakdown following strenuous exercise (Parise et al. 2001). This study concluded that the reasons for the differences between male and female participants were unclear, but not likely associated with muscle total creatine or phosphocreatine concentrations (Parise et al. 2001). While the consensus of these studies is that the performance-enhancing effect of creatine in females is less than it is in males, they have generally failed to consider the role of sex hormones and the stage of the menstrual cycle of the female subjects at the time of the study.

Sex hormone regulation of creatine homeostasis

There is substantial evidence supporting the contention that estrogens and progesterone influence female skeletal muscle metabolism (Volek et al. 2006). Under standard exercise conditions there are significant differences (albeit, small) in substrate utilization between males and females, with females favoring lipid metabolism over carbohydrate metabolism (Braun and Horton 2001). These differences have been attributed to the expression of female sex hormones, with progesterone down-regulating glucose production and estrogens mobilizing lipids, with the overall effect of shifting metabolism to conserve carbohydrates (Godsland 1996). The degree of these hormone driven shifts in female muscle metabolism have been linked to stages of the menstrual cycle, with affects most prominent in the luteal phase of the menstrual cycle when the level of estrogens are at their peak (Volek et al. 2006). Stages of the menstrual cycle and thus circulating levels of estrogens have also been linked to reduced muscle damage after eccentric exercise through preventing CK release (Williams et al. 2015). Considering these fundamental shifts associated with sex hormones for skeletal muscle metabolism, the roles that estrogens and progesterone have for the overall storage and metabolism of creatine for women certainly deserves further study.

Creatine kinase activities, along with expression of key enzymes for the endogenous synthesis of creatine, are affected by sex hormones (Wyss and Kaddurah-Daouk 2000). Studies conducted in the rat kidney, testis and decidua have shown that estrogens, diethylstilbesterol and testosterone all influence the expression of arginine-glycine aminotransferase (AGAT) (Walker 1979; Hasegawa et al. 1992). As AGAT is the rate-limiting step of creatine synthesis, up-regulation of AGAT expression is indicative of increased de novo creatine synthesis. In the tissues that express receptors for estrogens or androgen, estradiol and testosterone have also been shown to stimulate CK activity (Malnick et al. 1983; Sömjen et al. 1989). Of particular interest for women is the cyclic nature of sex hormone regulation. Studies have shown that in the rat, CK activity

increases and decreases in synchrony with the estrous cycle, and in relation to increased and decreased production of estrogens (Sömjen et al. 1991). Whether elevated levels of serum CK correlate with the endometrial tissue breakdown that progesterone withdrawal induces during menstruation is yet to be elucidated, but can not be ruled out as contributor to these changes (Emera et al. 2012). In terms of the effectiveness of dietary creatine supplementation either for athletic performance or for other therapeutic purposes, the implications of the possible changes in creatine metabolism in line with reproductive status of women has not been thoroughly investigated. In a study of serum CK levels in 110 school-aged girls, the 21 subjects who were reported as menstruating on the day that blood was collected for assay of serum CK levels returned values at the upper end of the normal range distribution for their age group (46.6 IU/l), compared to the non-menstruating aged-matched controls (39.5 IU/l) (Bundey et al. 1979). This study did not take into consideration the diet, body composition, or exercise status of the subjects, hence the nature of this relationship between the menstrual cycle and CK activity still warrants further investigation.

Female age, sexual maturity and creatine metabolism

Studies conducted through the 1960s and 1970s investigating the usefulness of serum CK levels as a predictor of being a carrier of the duchenne muscular dystrophy (DMD) gene mutation revealed that serum CK levels vary in women of different ages and sexual maturity. Bundey et al. (1979) examined serum CK levels in women of different reproductive stages, including pre- and post-menarche, pregnancy and menopause, and found the highest serum CK values were in pre-menarche teenage girls, and decreased progressively in post-menarche teenagers and into reproductive maturity. The lowest range of CK values were present in early pregnancy (16 weeks or less), when CK values were <50 % of those reported in teenage girls (Bundey et al. 1979). The relationship between the different stages of reproductive life and CK levels is not known. We speculate that age related changes in estrogens may be associated with these CK levels, however, given the overwhelming evidence that high CK levels indicate tissue damage, further work in this area is required to tease out these associations.

Serum creatine kinase and pregnancy

A study by King et al. (1972) looked more specifically at serum CK levels across pregnancy. Findings of this study were consistent with those of Bundey et al. (1979) described above, in that maternal serum CK decreased significantly between 8 and 20 weeks of gestation. The authors discussed the potential role of hemodilution in these observations, but concluded that the degree of change was too large to be attributed solely to the normal, pregnancy-related increase in maternal blood volume (King et al. 1972). An earlier study by Konttinen et al. (1963) described serum CK levels during late pregnancy, delivery and early postpartum. They found a large range of serum CK values during late pregnancy (6.04 ± 5.77 IU/ml), compared to relatively consistent values obtained from healthy non-pregnant controls (2.1 ± 0.9 IU/ml). Interestingly, the highest CK levels were detected not at delivery, but a day later (Konttinen and Pyörälä 1963). This finding was later confirmed in another study of 80 healthy pregnancies where a significant increase in maternal serum CK levels was observed on the first day postpartum (Emery and Pascasio 1965). Strikingly, values of serum CK detected in pregnant women at term (Konttinen and Pyörälä 1963) are comparable to CK values commonly observed following myocardial infarction (Konttinen and Halonen 1963). This study also compared the CK levels in three term pregnant and three non-pregnant uterine muscle samples, to find that CK levels of pregnant uterine muscle (average, 219,500; range 199,000–234,500 units/g wet weight) was substantially higher than that of the non-pregnant uterus (average, 16,200; range 13,500–21,500 units/g wet weight) (Konttinen and Pyörälä 1963). It was therefore concluded that the physical exertion of labor and creatine kinase efflux from the contracting and then involuting uterus were the major contributors to the increase in serum CK in women for the first 1–4 days postpartum. The study of Emery and Pascasio (1965) supported these findings, showing that CK levels of the pregnant myometrium were significantly higher than the myometrium of non-pregnant females. This study also described that there appeared to be a difference (although, not quite significant) of CK values for the myometrium directly under the placenta compared to other sites of the uterus (Emery and Pascasio 1965).

In addition to changes in maternal serum CK levels due to postpartum muscle breakdown, it is also likely that changes in female sex hormone expression during pregnancy, labor, delivery, and postpartum contribute significantly to changes observed in serum and tissue CK levels. In addition to estrogens, progesterone has been linked to CK activity in the myometrium (Lanza 1974). In non-gravid women injected with various concentrations of progesterone prior to hysterectomy, CK levels measured in fundus biopsies were lower than for biopsies from untreated women; exposure of uterine tissue to 500 mg of progesterone for 24 h before biopsy was associated with a decrease of CK levels in the myometrium by ~30 %. Significantly, progesterone had no effect on CK levels in the rectus muscle, indicating a specific effect of progesterone on CK in uterine muscle fibres (Lanza 1974).

A limitation in evaluating the above studies is that data collection was usually cross-sectional, and the relative expression of CK isoforms were not examined in detail. As many of the studies discussed have used CK as a biomarker for other conditions (e.g., serum CK to indicate carriers of the gene mutation associated with DMD) there has been limited discussion of the physiological relevance of changes to serum CK levels during different phases of the reproductive cycle. To the knowledge of the authors there are no studies (in any species) that provide longitudinal data of changes to serum CK levels over the life course of male, or female, individuals. With respect to the findings associated with changes to serum CK levels throughout a female's reproductive life, how increased CK levels (serum/tissue) influence creatine/phosphocreatine utilization; what increased CK levels tell us about the metabolic demands of those cells at that particular time; whether these changes affect creatine synthesis; and what the consequences are if these shifts do not occur as required, are questions that should be explored in more detail. Nevertheless, the age and stage of reproductive cycle of females should be taken into consideration whenever studies of creatine metabolism in females are undertaken; as it is clear that creatine kinase activity (and perhaps creatine metabolism) is intrinsically connected to changes in the female reproductive cycle.

Creatine metabolism and pregnancy

Is creatine an essential dietary metabolite of pregnancy?

The link between creatine and the feto-placental unit was established in 1974, with studies by Miller et al. describing the active transport of creatine into the human placenta from the maternal circulation, where it appears to pool and then diffuse down a concentration gradient into the fetal circulation (Miller et al. 1974). Similar observations have also been made in the pregnant rat and spiny mouse (Miller et al. 1977; Ireland et al. 2008). The potential role of the placenta in fetal creatine supply from early in gestation is supported by identification of *SLC6A8* mRNA in the human placenta from 13 weeks gestation (Miller et al. 1974; Nash et al. 1994). Hormones known to be up-regulated during pregnancy (IGF-1, triiodothyronine) are known mediators of increased *SLC6A8* expression (Osathanondh et al. 1976; Furlanetto et al. 1978) and may induce increased expression of *SLC6A8* in the placenta and increase creatine uptake by virtue of their effects on the Na^+ transmembrane potential, as has been shown for skeletal myoblast cells (Odoom et al. 1996).

In addition to transfer of creatine from the placenta to the fetus, the high metabolic activity of the placenta itself raises questions about its direct requirement for creatine/phosphocreatine. For the creatine/phosphocreatine/CK circuit to operate as an effective shuttle of ATP from the site of synthesis at the mitochondria to areas of demand in the cytosol, there needs to be coordinated expression of ubiquitous mitochondrial CK (uMITCK) and ubiquitous brain-type CK (BCK), as genes for these two isoforms of CK are located on different chromosomes (Stallings et al. 1988; Haas et al. 1989). Thomure et al. (1996) studied CK mRNA expression in the human placenta across gestation and concluded that the expression of uMITCK and BCK was indeed highly coordinated. Both uMITCK and BCK were expressed, albeit at low levels in the first and second trimesters of pregnancy, before a substantial increase in the expression of both enzymes in the third trimester (Thomure 1996). This pattern of expression parallels the increased metabolic activity of the placenta in late gestation in the human and many other species, and suggests that the CK pathway has an integral role in placental metabolism, an assumption consistent with the evolutionary development of CK in other tissues of high and fluctuating energy demands, such as skeletal muscle (Thomure 1996; Wyss and Kaddurah-Daouk 2000). It is likely that rising concentrations of serum estrogens during pregnancy may regulate the increases in both uMITCK and BCK expression across gestation in the human placenta, as response elements to estrogens have been identified on both the uMITCK and BCK genes (Payne et al. 1993).

Whilst not considered an essential metabolite to support fetal growth and development at this point in time, consideration should be given to the effect that low maternal creatine levels might have on fetal growth and development. Dietary preferences that avoid consumption of animal products, or variations of the de novo synthesis of creatine in the mother, might affect the provision of creatine to the fetus and placenta. It is not yet known when the reno-hepatic axis in the human fetus is developmentally mature enough to be able to synthesize creatine from arginine, glycine, and methionine, and presumably until this time there is an absolute requirement for transfer of creatine from the maternal and placental creatine pools. A study of creatine homeostasis in the pregnant spiny mouse showed that maternal creatine synthesis, excretion, transport and storage were all fundamentally changed by pregnancy (Ellery et al. 2015b), suggesting that pregnancy provokes substantial and far-reaching adaptations to creatine balance in the mother. These changes include a decrease in maternal plasma creatine concentration, decreased renal excretion of creatine between mid and late gestation, increased renal AGAT mRNA and protion expression, and increased CrT1 mRNA expression in the heart and gastrocnemius muscle just prior to parturition, raising the possibility that alterations to maternal creatine homeostasis might be a necessary

adjustment of maternal physiology with pregnancy to meet the metabolic demands of the placenta and developing fetus (Ellery et al. 2015b). This notion is also supported by studies conducted by Braissant et al. (2005), describing embryonic expression of AGAT, GAMT and CrT1 in numerous tissue types in the rat, particularly the central nervous system, from early in gestation. This study placed emphasis on the need for creatine for adequate growth and development in utero (Braissant et al. 2005). These studies are yet to elucidate whether placental and fetal creatine homeostasis is different for male and female fetuses. There is increasing evidence that placental function, especially metabolic function, is modified by the sex of the fetus (O'Connell et al. 2013). This is an area of research that needs further attention, and may provide useful insights into the role creatine might have in obstetric conditions where placental cellular energy failure may be a factor in major pathologies such as birth asphyxia, intrauterine growth restriction or stillbirth.

A recent retrospective study in pregnant women identified changes to creatine homeostasis, in terms of plasma and urinary creatine concentrations with advancing gestation (Dickinson et al. unpublished observations). This study provides data to suggest that plasma and urinary creatine concentrations are higher in pregnant women compared to non-pregnant women, and that placental and newborn weight at birth are related to maternal creatine excretion. These findings indicate that creatine may be an important determinant of fetal growth and development, and that maintenance of maternal creatine homeostasis across pregnancy may be vital for the health of the newborn. This notion is supported by a human study dating back to 1913, where increases in body weight of a newborn was shown to be roughly proportional to the creatine excreted in the urine by the mother (indicative of more than adequate circulating levels of creatine) (Mellanby 1913). Indeed, there is a great need to know when the human fetus can synthesize creatine. While important for understanding in utero development and placental supply of creatine, the increased numbers of preterm infants, and their subsequent neurological decline, raise the possibility that cerebral creatine deficiency is a consequence of preterm birth not yet fully recognized, clinically.

It is also of interest to note that, in addition to late gestational and postpartum pregnant women, newborn babies are reported to have very high levels of serum CK—up to 10 times higher than normal healthy adult levels (Gilboa and Swanson 1976). These increased levels begin to decline by 4 days after birth and reach average population levels by 6–10 weeks of age. These high serum CK values are thought to arise from the physical stress placed on the newborn during labor and delivery (Rudolph and Gross 1966), although Gilboa et al. (1976) reported that mean CK levels in the cord blood of babies delivered via caesarean section were higher than the cord blood levels detected in vaginally delivered babies (Gilboa and Swanson 1976). Conversely, mean capillary CK levels were higher in vaginally delivered newborns compared to caesarean delivered babies. Hence, there is no clear association with birth trauma and newborn serum CK levels. Also unique to newborns in the first few days of life is that venous and capillary levels of CK are similar, unlike the adult where CK levels are slightly but significantly lower in capillary compared to venous blood (Gilboa and Swanson 1976). The physiological significance of increased serum CK levels in newborns is not known. Again, as these studies were conducted purely to assess the potential of CK as a biomarker for DMD, the actual physiological relevance of serum CK levels in the newborn has not been thoroughly investigated. Whether pregnancy complications also affect CK measures in neonates is yet to be established.

Creatine supplementation as a therapeutic to alleviate poor pregnancy outcomes

The ability of creatine to maintain ATP turnover, acid–base balance, mitochondrial function, together with its antioxidant, vasodilator, and anti-excitotoxic properties (Wallimann et al. 2011), make it a candidate for use to treat ischemic/reperfusion injuries, particular when these occur in the brain. Whether these properties of creatine could be exploited to the advantage of the neonate were first assessed by Wilken et al. (1998) in mouse brain slices, and by Berger et al. (2004) in brain slices of fetal guinea pigs, both of which described sustained ATP turnover and a reduction in neuronal cell injury when brain slices were exposed to creatine (Wilken et al. 1998; Berger et al. 2004). Similar benefits were observed in vivo with rat pups (Adcock et al. 2002). The ability of creatine to easily load into neuronal cells prior to birth may mean that creatine is more effective in neonatal conditions of acquired brain injury than for adult brain injury [reviewed by (Dickinson et al. 2014)]. These results lead to suggestions that creatine may act to protect the neonatal brain from injury induced by intrapartum asphyxia. Studies conducted by Ireland et al. (2011) identified the neuroprotective capacity of creatine, administered antenatally by supplementation of the maternal diet, to protect the spiny mouse pup from the effects of birth asphyxia (Ireland et al. 2011). Specifically, the amelioration of neuronal cell death and maintenance of mitochondrial integrity in the presence of creatine was described. These were promising results for the treatment of neonatal HIE, but the overall improvement to survival rate of offspring of creatine-fed dams lead to thoughts that creatine, when

administered and loaded into fetal organs in utero, might provide protection to other peripheral organs known to be highly susceptible to the global oxygen deprivation associated with an asphyxic episode at birth (Ireland et al. 2008). Exploration of this hypothesis to date has included characterisation of the diaphragm muscle and kidney following birth asphyxia. The benefits of creatine loading for the diaphragm included attenuation of muscle atrophy and improvement of contractile function, such that function of this important muscle did not differ from diaphragm samples obtained from pups from a control birth (Cannata et al. 2010). Analysis of the kidney showed birth asphyxia caused structural damage to the neonatal renal cortex, medulla and renal papillae in the spiny mouse offspring (Ellery et al. 2012), and the presence of changes in young adult male spiny mice suggest the possibility that neonatal acute kidney injury has the longer term risk of developing into chronic kidney injury, in males at least, where reduced nephron endowment and GFR were detected (Ellery et al. unpublished observation). As these studies progress into higher order animal models and human-based analyses, careful consideration of fetal sex should be given when reporting outcomes. In our own animal experiments, only 52 % of male spiny mouse offspring survive the birth asphyxia insult, compared to 69 % of females, suggesting that male fetuses are more vulnerable to this type of insult (LaRosa et al. 2016). The male vulnerability to prenatal insults is believed to be a result of the faster in utero growth rate of males, compared to females (Eriksson et al. 2010). When we supplement the diet of the mother with creatine before birth asphyxia, survival of females is improved by 12 % and male survival is improved by 19 %, suggesting that creatine is beneficial to fetuses of both sexes, but perhaps slightly more so for males. Importantly for the progression of these findings into the clinic, studies on the safety of supplementary creatine in the pregnant spiny mouse have shown that a high and prolonged oral dose of creatine does not result in any adverse outcomes for the mother (Ellery et al. 2015a). It has also been shown that high exposure to creatine in utero does not affect the expression levels of the enzymes required for creatine synthesis, 24 h after birth (Dickinson et al. 2013). A recent study of maternal dietary creatine supplementation during pregnancy in rats concluded that creatine exposure in utero had a positive effect on morphological and electrophysiological development of CA1 neurons. However, this affect persisted beyond the half-life of creatine and may have the potential to increase epileptogenic focus (Sartini et al. 2016). Studies are now underway to determine the safety and efficacy creatine supplementation during pregnancy, using non-human primates.

Creatine as a therapeutic for women

As discussed earlier, the effectiveness of creatine as an ergogenic aid for female athletes is less than that for men. The possibility that this reflects the cyclic nature of female sex hormones, and/or the presence or absence of testosterone, requires further studies to characterize the differences of creatine uptake and utilization between men and women, and how these might change across the menstrual cycle, with conception and menopause. Despite the paucity of data around this, there are a number of conditions for which treatment of women with dietary creatine is proving highly effective.

Mental illness

Metabolic impairment within the brain initiates the cellular injury-death cascade, driven by the production of reactive oxygen species, lipid peroxidation, DNA damage and apoptosis. This pathophysiology has been shown to hinder cellular resilience and contribute to depressive disorders (Fuchs et al. 2004; Seifried 2007). It has been hypothesized that damage to mitochondria in the hippocampus and prefrontal lobe could compromise the creatine/phosphocreatine circuit and instigate depression-like behavior (Allen 2012); indeed, CK activity is inversely related to the severity of a depressive episode (Dager et al. 2004; Segal et al. 2007). Healthy females have less phosphocreatine in the frontal lobe than healthy males (Riehemann et al. 1999). This may suggest that females are more susceptible to depressive disorders associated with shifts in brain creatine metabolism. Indeed, depression occurs twice as often in females than males (Bebbington et al. 2003), with episodes being more severe, frequent and prolonged (Kornstein et al. 2000).

Dietary creatine supplementation has been considered as a potential therapy to counter the reductions in brain metabolism associated with depression. Encouragingly for women, the associations of estrogens and increased CK activity have lead to suggestions that creatine supplementation may be more beneficial in treating depression in females over males (Allen et al. 2010). In studies conducted in rats, increasing dietary intake to 4 % of daily food consumption for 5 weeks prior to assessment, significantly improved performance on tests such as the forced swim test, known to identify depressive-like behaviours (Allen et al. 2010). This result was not present in male rats. A recent extension of these studies showed that the presence of sex hormones was essential to the protective effects afforded by creatine to depressed rats (Allen et al. 2015). In human studies, the use of dietary creatine as an adjunct therapy accelerated treatment response in depressed adolescent (Kondo et al. 2011) and adult females (Lyoo et al.

2003). In a recent pilot study by Hellem et al. (2015), six females with a major depressive disorder, in addition to methamphetamine dependence, received dietary creatine supplementation for a period of 8 weeks. During this time, the subjects displayed lower levels of depression and anxiety, as evaluated by the Hamilton Depression Rating Scale and Beck Anxiety Inventory Scales (Hellem and Renshaw 2015).

Another interesting aspect of female mental health and wellbeing associated with levels of estrogens are episodes of premenstrual tension (PMT). Low levels of estrogens can characterize this syndrome, which affects women from the mid-luteal phase of the menstrual cycle until menstruation. Whether there is a link between low circulating estrogens associated with PMT and creatine metabolism is yet to be established. However, administration of estrogens at this time has been shown to reduce cyclic bouts of worsening mood (Hammarbäck et al. 1985). Such treatment would be most important for those women who suffer from a rare form of PMT who undergo severe episodes of depression, insomnia, forgetfulness and confusion during the mid-luteal phase of their menstrual cycle (Hammarbäck et al. 1985). Characterization of the hormone profile of these women show disproportionately low estrogens compared to progesterone during these depressive episodes (Abraham 1983). As with other depressive conditions, increasing CK activity through enhanced creatine dietary consumption may be a safe and beneficial treatment to reduce the symptoms of PMT.

Morbidities associated with ageing

Due to the changes in sex hormone production during and after menopause, females are particularly susceptible in their older years to bone and muscle degeneration (Evans 2004). Age-related bone loss is accelerated during menopause, leading to osteoporosis and contributing to osteoarthritis (Hernandez et al. 2003). Creatine is showing promise in targeting the progression of these ailments. The differentiation of bone and cartilage cells is a highly energy dependent process, which has been previously shown to utilize the creatine/phosphocreatine/CK circuit (Wallimann and Hemmer 1994). Whether dietary creatine supplementation could be used to aid bone regeneration was first assessed in cultured osteoblast-like cells (Gerber et al. 2005). This study found that the addition of creatine to culture media promoted differentiation of primary osteoblast-like cells by increasing alkaline phosphatase activity, and concluded that dietary creatine may be beneficially to aid fracture healing or prevent the progression of osteoporosis (Gerber et al. 2005). In addition to frail bones, ageing is associated with sarcopenia or reduced muscle mass. Together, these conditions reduce the capacity for rapid muscle contraction, and can lead to loss of balance, increased falls and injury (Schneider and Guralnik 1990). Dietary creatine supplementation has thus been trialed in aged individuals to assess its capacity for rebuilding and/or maintaining lean muscle and bone mass. When creatine was given in conjunction with moderate exercise, significant improvements in physical function and lower limb lean mass were observed in aged men and women (Gotshalk et al. 2008). Preliminary studies in postmenopausal women aged 50–65 years suffering from osteoarthritis have also shown that introducing a standard dietary creatine supplementation regime in conjunction with resistance training improved physical function, lower limb lean mass and overall, improved quality of life for these women (Neves et al. 2011).

With an ageing population in the western world, simple nutritional interventions with the capacity to reduce the burden of fall related injuries on our healthcare system, prolong functional independence, and overall improve the quality of life should be held in the highest regard. Whilst manipulation of the creatine/phosphocreatine/CK circuit to target these aliments is somewhat in its infancy, compared to studies of exercise performance in younger individuals, it shows much promise, particularly for women where changes to hormone production rates underpin tissue loss and reduced tissue regeneration.

Conclusions and recommendations for future research

Assessment of the literature as a whole clearly suggests that males and females store, metabolize and utilize creatine in a sex-specific manner. However, this remains an incomplete story, with evidence spread across an array of studies, mainly conducted in the 1960s–1990s, where sex-dependent effects were not the primary outcome. As a whole, this topic of sex-specific differences in creatine metabolisms is deserving of new investigations using modern technologies, including in vivo tracer and imaging techniques and high throughput genomics, to establish which aspects of the creatine/phosphocreatine/creatine kinase circuit are most influenced by gender, and which of the isoforms of creatine kinase are affecting serum CK levels throughout a female's reproductive life. There also remains the need to conduct population-based studies that characterize creatine homeostasis in women, taking into consideration age, body composition and stage of the reproductive cycle, and the further effects of conception, pregnancy, and parturition. It is probably essential that these studies should be conducted on cohorts of women followed through the menstrual cycle, or followed from conception to birth, and then post-partum. Such longitudinal studies would provide data integral

to understanding the adaptability of this phosphagen system for females, and how this can be used to optimize and enhance health outcomes for women.

Acknowledgments HD is an NHMRC Career Development Fellow & DWW is a Distinguished Researcher of the Cerebral Palsy Alliance.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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