Gender Differences in Metabolism; Nutrition and Supplements

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For many decades researchers did not consider that there were any differences between the genders in the metabolic response to exercise. As a result, nutritional recommendations and exercise training prescriptions have not considered the potential for gender specific responses. More recently, we and others have demonstrated that females oxidize proportionately more lipid and less carbohydrate during endurance exercise as compared to males. The oxidation of amino acids is similarly lower in females as compared to males during exercise.These gender differences are partially mediated by a higher estrogen concentration in females. Specific areas where there are gender differences in nutritional/supplement recommendations include carbohydrate (CHO) nutrition, protein requirements and creatine (CRM) supplementation. We have shown that females do not carbohydrate load in response to an increase in dietary carbohydrate when expressed as a percentage of total energy intake (i.e., 55-75%), however if they consume >8g CHO•kg^{-1}•d^{-1}, they show similar increases as compared to males. Top sport male and female athletes require somewhat more dietary protein as compared to sedentary persons. The maximal increase is ~100% for elite male athletes and ~50-60% for elite female athletes. Fortunately, most athletes habitually consume this level of protein intake. We have recently demonstrated that females show a lesser increase in lean body mass following acute CRM loading as compared to males. Females also did not show reductions in protein breakdown in response to CRM loading, whereas males did. In the future I expect that there will be further research from which gender specific nutritional/supplement recommendations can be made.

Introduction

The vast majority of the research in the areas of sports nutrition, muscle metabolism and exercise physiology has been conducted using predominantly male participants. It has been assumed that the physiologic responses to exercise are similar between males and females. Even in the 1980's major exercise physiology textbooks stated that there were no gender differences in the metabolic response to exercise. More recent, carefully controlled research has cast doubt on these conclusions which has led several researchers to reinvestigate the question of potential gender difference in metabolism (Horton, Pagliassotti, et al., 1998; Friedlander, Casazza, et al., 1998; Tarnopolsky, Bosman, et al., 1997; Tarnopolsky, Atkinson, et al., 1995, Phillips, Atkinson, et al., 1993; Tarnopolsky, MacDougall, et al., 1990). There are several probable reasons for the inherent gender bias in the literature. However, controlling for the phase of the menstrual cycle and the misperception that females are not as likely to volunteer for muscle biopsies and other invasive studies are two possibilities. It has been the experience in our laboratory that females are equally willing to volunteer for

muscle biopsy/isotope infusions and controlling for the menstrual cycle is relatively easy when testing in the follicular phase, and only moderately more time consuming when testing in the luteal phase.

This paper will outline some of the highlights from recent papers that have explored the potential for gender differences in metabolism and the implications to nutrition and dietary supplements. It is apparent that there has been a gender bias in the literature that may have resulted in discriminatory sport recommendations. One striking example where the lack of knowledge regarding the response of females to endurance exercise was the exclusion of female competitors from the Olympic marathon until 1984. More recent research has demonstrated that, in fact, females may be able to outperform males in ultra-endurance sports. Two studies from South Africa examined the capacity of equally trained males and females to perform ultra-endurance sports (Speechly, Taylor, et al., 1996; Bam, Noakes, et al., 1997). In one study, Speechly and co-workers matched males and females for their performance at the marathon distance (42 km) and had these individuals run for 90 km (Speechly, Taylor, et al., 1996). The study demonstrated that females outperformed the males at the longer distance. These researchers claim that there were no differences in aerobic capacity, training history or running economy, and made the claim that there were no difference in lipid oxidation based solely on plasma free fatty acid concentrations (which are not good indicators of lipid utilization) (Speechly, Taylor, et al., 1996). The second study matched males and females for a 56 km run time, and again females outperformed the males at the 90 km distance (Bam, Noakes, et al., 1997). This group subsequently performed regression analysis on the data and showed that the males outperformed females at distances of 5-42 km, and after 66 km the females outperformed the males (Bam, Noakes, et al., 1997). As I will discuss in this chapter, these performance differences are likely due to an enhanced capacity for females to rely on lipid oxidation during endurance exercise and better maintain plasma glucose concentrations. Some of the metabolic differences may be due to greater relative fibre area of type I/type II fibres in females as compared to males (Chorneyko & Bourgeois, 1999), and/or a lesser oxidative stress experienced by females vs. males (Tiidus, 1995).

The implications of research on gender difference in metabolism to dietary recommendations, training programs and to certain dietary supplements has not been fully explored. However, I will provide a few examples where we have started to tease out some of the differences between the genders with respect to carbohydrate nutrition, protein requirements and creatine monohydrate supplementation.

Whole Body Substrate Oxidation

In the 1970's and 80's there were a few studies which reported the metabolic response of both males and females to endurance exercise (Costill, Fink, et al., 1979; Froberg & Pedersen, 1984; Blatchford, Knowlton, et al., 1985; Friedmann & Kindermann, 1989). The results were equivocal with some researches reporting gender differences (Froberg & Pedersen, 1984; Blatchford, Knowlton, et al., 1985; Friedmann & Kindermann, 1989), and others not finding any difference (Costill, Fink, et al., 1979). One of the difficulties in interpreting these papers is the lack of many of them to control for timing of the menstrual cycle, and to not carefully match males and females for training history and $\dot{V}O_{2PEAK}$ expressed relative to

fat-free mass. As I have argued previously, a matching process with a training history, menstrual status, diet analysis and VO_{2MAX} testing expressed relative to fat-free mass takes into account both genetic (genetically determined "window of VO_2 to potential") and environmental (state of training) factors (Tarnopolsky, 1999). Since our first publication in 1990 (Tarnopolsky, MacDougall, et al., 1990), we have completed three studies where we have carefully matched males and females for training history and VO_{2MAX} relative to lean body weight (Tarnopolsky, Bosman, et al., 1997; Tarnopolsky, Atkinson, et al., 1995; Phillips, Atkinson, et al., 1993), and three longitudinal training studies taking untrained individuals and training them for 1-2 months (McKenzie, Phillips, et al., 2000; Rennie, Brose, et al., 2000; Carter, Rennie, et al., 2000). In addition to our training studies, George Brooks' group has also completed a similar study showing nearly identical results to our longitudinal studies (Friedlander, Casazza, et al., 1998). In a "meta-analysis" of most studies that have examined gender differences in metabolism, it is apparent that during endurance exercise females oxidize proportionately more lipid and less carbohydrate as compared to males (Table 1) (Horton, Pagliassotti, et al., 1998; Friedlander, Casazza, et al., 1998; Tarnopolsky, Bosman, et al., 1997;

Reference	Subjects	Exercise	RER (mean)
Costill et al., 1979	12 ♀ T 12 ♂ T	60 min run @ 70% VO_{2PEAK}	♀ = 0.83 ♂ = 0.84
Froberg and Pedersen, 1984	7 ♀ A 7 ♂ A	to exhaustion @ 80+90% VO_{2PEAK}	♀ = 0.93 ♂ = 0.97
Blatchford, et al., 1985	6 ♀ A 6 ♂ A	90 min walk @ 35% VO_{2PEAK}	♀ = 0.81 ♂ = 0.85
Tarnopolsky, et al., 1990	6 ♀ T 6 ♂ T	15.5 km run @ ~ 65% VO_{2PEAK}	♀ = 0.876 ♂ = 0.940
Phillips, et al., 1993	6 ♀ T 6 ♂ T	90 min cycle @ 65% VO_{2PEAK}	♀ = 0.820 ♂ = 0.853
Tarnopolsky, et al., 1995	8 ♀ T 7 ♂ T	60 min cycle @ 75% VO_{2PEAK}	♀ = 0.892 ♂ = 0.923
Tarnopolsky, et al., 1997	8 ♀ T 8 ♂ T	90 min cycle @ 65% VO_{2PEAK}	♀ = 0.893 ♂ = 0.918
Horton, et al., 1998	13 ♀ T+U 14 ♂ T+U	120 min cycle @ 45% VO_{2PEAK}	♀ = 0.84 ♂ = 0.86
Freidlander, et al., 1998	17 ♀ UT▶T 19 ♂ UT▶T	60 min cycle @ 45 & 65% VO_{2PEAK}	♀ = 0.885 ♂ = 0.932
McKenzie, et al., 2000	6 ♀ UT▶T 6 ♂ UT▶T	90 min cycle @ 65% VO_{2PEAK}	♀ = 0.889 ♂ = 0.914
Rennie, et al., 2000	6 ♀ UT▶T 5 ♂ UT▶T	90 min cycle @ 60% VO_{2PEAK}	♀ = 0.893 ♂ = 0.945
Carter, et al., 2000	8 ♀ UT▶T 8 ♂ UT▶T	90 min cycle @ 60% VO_{2PEAK}	♀ = 0.847 ♂ = 0.900

Values are mean (SD). ♀ = females; ♂ = males; T = trained; A = active; U = untrained; U-T = longitudinal training study: for longitudinal training studies, the pre/post rides are all collapsed across time for each gender. T+U = trained and untrained in same study. *Significant gender difference ($P<0.001$).

Table 1: Whole body substrate metabolism in males and females.

Subjects	RER	CHO (%)	FAT (%)	PRO (%)
N = 103 ♀	0.867 (0.037)	55 (10)	43 (9)	2 (2)
N = 104 ♂	0.904 (0.043)	65 (9)*	29 (8)*	6 (3)*

Values are mean (SD). *Gender difference (P<0.001).

Table 2: Summary of substrate ulitization in several studies (see Table 1) directly comparing males and females.

Reference	Subjects	Exercise	Protein (% of energy)
Tarnopolsky, et al., 1990*	6 ♀ T 6 ♂ T	15.5 km run @ ~65% VO_{2PEAK}	♀ = 0.3 ♂ = 9.1
Phillips, et al., 1993[f],*	6 ♀ T 6 ♂ T	90 min cycle @ 65% VO_{2PEAK}	♀ = 2.0 ♂ = 3.3
Tarnopolsky, et al., 1995*	8 ♀ T 7 ♂ T	60 min cycle @ 75% VO_{2PEAK}	♀ = 1.6 ♂ = 6.3
Tarnopolsky, et al., 1997*	8 ♀ T 8 ♂ T	90 min cycle @ 65% VO_{2PEAK}	♀ = 2.0 ♂ = 3.0
McKenzie, et al., 2000[f]	6 ♀ UT▶T 6 ♂ UT▶T	90 min cycle @ 65% VO_{2PEAK}	♀ = 4.8 ♂ = 8.4
		Average Values:	♀ = 2.1 (1.6) ♂ = 6.0 (2.8)

* - data based upon urinary urea excretion. [f] - data derived from L-1 9[13]C)-leucine oxidation. Mean (SD).

Table 3: Protein oxidation during endurance exercise.

Tarnopolsky, Atkinson, et al., 1995; Phillips, Atkinson, et al., 1993; Tarnopolsky, MacDougall, et al., 1990; McKenzie, Phillips, et al., 2000; Costill, Fink, et al., 1979; Froberg & Pedersen, 1984; Blatchford, Knowlton, et al., 1985; Rennie, Brose, et al., 2000; Carter, Rennie, et al., 2000). I feel that the consistent results from carefully controlled cross-sectional studies (Horton, Pagliassotti, et al., 1998; Tarnopolsky, Bosman, et al., 1997; Tarnopolsky, Atkinson, et al., 1995; Phillips, Atkinson, et al., 1993; Tarnopolsky, MacDougall, et al., 1990), and the results from four longitudinal studies (Friedlander, Casazza, et al., 1998; McKenzie, Phillips, et al., 2000; Carter, Rennie, Brose, et al., 2000) provide strong evidence that there are in fact gender differences in metabolism during endurance exercise. A summary of some of the studies that have specifically reported gender differences in metabolism is presented in Table 1. I have also calculated the contribution of fat, carbohydrate and protein to energy metabolism from the studies in Table 1 for 104 males and 103 females (Table 2). It would be expected that with a reduction in carbohydrate oxidation, females would oxidize less protein (amino acids) consequent to endurance exercise. A summary of the contributions of protein to energy during endurance exercises is presented in Table 1 and 3.

Carbohydrate Metabolism

In our initial study using treadmill running we found that females have significantly less glycogen depletion in the *vastus lateralis* following 15.5 km of

treadmill running, as compared to females (Tarnopolsky, MacDougall, et al., 1990). We hypothesized that this glycogen sparing was due to an enhanced oxidation of lipid by the muscles. We subsequently completed a study of glycogen utilization in males and females during 60 min of cycling and 75% of VO_{2PEAK} (Tarnopolsky, Atkinson, et al., 1995). In this study we did not find any glycogen sparing in the females. We have recently completed a training study where glycogen was measured pre- and post-exercise before and after 31 days of endurance cycling exercise (McKenzie, Phillips, et al., 2000). Again, we did not find gender difference in muscle glycogen utilization during 90 min of cycling at 60% of VO_{2PEAK} both before and after the training session (McKenzie, Phillips, et al., 2000). This latter study (McKenzie, Phillips, et al., 2000) ruled out that exercise intensity differences were responsible for the differing result in our 1st and 2nd study, but still left open the question that there may be gender differences in the pattern of activation in certain muscles during running vs. cycling.

The mechanism behind the potential glycogen sparing effect is likely the female sex hormone, 17-β-estradiol. Two studies have demonstrated that the administration of 17-β-estradiol to male (Rooney, Kendrick, et al., 1993; Kendrick & Ellis, 1991) and female oophorectomized (Kendrick, Steffen, et al., 1987) rats resulted in significant muscle glycogen sparing during exercise. Furthermore, these researchers have also shown hepatic glycogen sparing in response to 17-β-estradiol (Rooney, Kendrick, et al., 1993; Kendrick & Ellis, 1991).

In human studies, Brent Ruby and colleagues have demonstrated that 17-β-estradiol decreased glucose rate of appearance and disappearance in amenorrheic females (Ruby, Robergs, et al., 1997). We have confirmed these results in healthy males following response to 17-β-estradiol administration (Carter, Mourtzakis, et al., 1999). Furthermore, we demonstrated a better maintenance of plasma glucose during endurance exercise following 17-β-estradiol administration (Carter, Mourtzakis, et al., 1999). This finding is also consistent with the better maintenance of plasma glucose that we observed during our exhaustive treadmill exercise in the study reported in 1990 (Tarnopolsky, MacDougall, et al., 1990). A recent study by our group has also demonstrated that 17-β-estradiol administration to males did not attenuate skeletal muscle breakdown during endurance exercise (Tarnopolsky, Ettinger, et al., 1997). Together, these data suggest that some of the gender differences in carbohydrate metabolism could be due to hepatic, and not skeletal muscle glycogen sparing.

Lipid Metabolism

The mechanism behind the observed increase in lipid oxidation during endurance exercises for females is still unclear. We have demonstrated a higher plasma free fatty acid and glycerol concentration during exercise in females as compared to males in some (Rennie, Brose, et al., 2000; Carter, Rennie, et al., 2000) but not all (Tarnopolsky, Atkinson, et al., 1995; Tarnopolsky, MacDougall, et al., 1990) studies. Both our group (Carter, Mourtzakis, et al., 1999) and Brent Ruby's (Ruby, Robergs, et al., 1997) did not find that physiologic doses of 17-β-estradiol affected glycerol rate of appearance (an indicator of lipolysis) in amenorrheic males and females, respectively. However, in a recent training study, we have found that females showed a higher rate of glycerol rate of appearance as compared to males (Carter, Mourtzakis, et al., 1999). It may be that the higher percent body fat in

females contributes to the higher glycerol rate of appearance (lipolysis). This would provide for more substrate for lipid oxidation by the muscle.

Animal data supports that 17-β-estradiol partitions lipid towards skeletal muscle. Studies have demonstrated that muscle lipoprotein lipase is increased, whereas adipocyte lipoprotein lipase is decreased following estradiol administration (Ellis, Lanza-Jacoby, et al., 1994). The administration of 17-β-estradiol also resulted in increase in intra-muscular triglyceride stores (Ellis, Lanza-Jacoby, et al., 1994). These findings are consistent with the observation that females have higher intramuscular triglyceride stores as compared to males (Forsberg, Nilsson, et al., 1991). Observations in showing that triglyceride synthesis and breakdown occur simultaneously during exercise in skeletal muscle (Dyck & Bonen 1998) and that glucose and glycerol can be taken up from the plasma and stored as triglyceride (Guo & Jensen 1999) will make the assessment of the contribution of intramuscular triglycerides and plasma free fatty acids to total energy a very technically demanding endeavour.

From a muscle enzymatic standpoint, there is some evidence that β-hydroxyacyl-CoA-dehydrogenase activity (BHAD) is higher in females as compared to males (Green, Fraser, et al., 1984). However, this effect is subtle at best. We have recently not found any differences in the total amount of long chain acyl CoA-dehydrogenase protein between males and females (Green, Fraser, et al., 1984; Rennie, Brose, et al., 2000). Others have not found gender differences in total carnitine palmitoyl transferase (CPT) activity (Costill, Fink, et al., 1979), or CPT1 activity (Berthon, Howlett, et al., 1998). It is possible, however, that other enzymes involved in intra-muscular triglyceride utilization may show gender differences. For example, we are currently examining skeletal muscle hormone sensitive lipase differences between the genders.

Protein

We initially reported that males increased 24h urinary urea nitrogen excretions following endurance exercise (compared to a rest day) whereas females did not (Tarnopolsky, MacDougall, et al., 1990). We interpreted this to indicate that males oxidize proportionately more protein (amino acids) during exercise (Tarnopolsky, MacDougall, et al., 1990). We followed up on this by using L-[1-^{13}C]-leucine tracers and nitrogen balance (NBAL) and confirmed that males oxidize proportionately more protein (NBAL) and leucine (tracers) during exercises as compared to females (Phillips, Atkinson, et al., 1993). We went on from these studies to complete a longitudinal study of the adaptations in protein metabolism for 31 days of endurance exercise training (McKenzie, Phillips, et al., 2000). Six untrained males and females completed 31 days of endurance training and performed 90 min of cycle ergometry at 65% VO_{2PEAK} before and following training. We found that females had a lower leucine oxidation as compared to males during endurance exercise, both before and after the training session. Of even more interest was the fact that both groups nearly doubled leucine oxidation from rest to exercise before training, yet there was a massive attenuation of this response following exercise training, such that leucine oxidation did not increase during exercise as compared to rest (McKenzie, Phillips, et al., 2000). Although this would seem at odds with the observation that the trained athletes required more protein, we did observe that the total activity of the rate limiting enzyme and branched chain amino acid oxidation (branched chain-2-oxo-dehydrogenase) was

increased 40% following training (McKenzie, Phillips, et al., 2000). This indicates that the total metabolic capacity to oxidize amino acids was higher in the trained athlete and individuals who might not have optimal protein and carbohydrate intakes may risk oxidizing protein during training sessions and, as a result, increase their dietary requirements for protein.

Nutritional Implications of Gender Differences in Metabolism

Carbohydrate loading

The vast majority of studies examining the response of muscle and glycogen to an increase in consumption of dietary carbohydrates (carbohydrate loading) have been conducted with predominantly male subjects (Sherman, Costill, et al., 1981; Bergstrom, Hermansen, et al., 1967; Karlsson & Saltin, 1971). Studies have shown that glycogen storage is altered by menstrual cycle phase (Nicklas, Hackney, et al., 1989) and that glycogen metabolism is markedly influenced by 17-β-estradiol (Rooney, Kendrick, et al., 1993; Kendrick & Ellis, 1991; Kendrick, Steffen, et al., 1987). Given the attenuation in glycogen utilization during exercise seen in rodents with 17-β-estradiol administration (Rooney, Kendrick, et al., 1993; Kendrick & Ellis, 1991; Kendrick, Steffen, et al., 1987), and the attenuation observed by us in male and female during a 15.4 km running trial (Tarnopolsky, MacDougall, et al., 1990), we hypothesized that the ability for females to glycogen load may be attenuated as compared to males (Tarnopolsky, Atkinson, et al., 1995).

We examined the response of muscle glycogen to a modified carbohydrate loading protocol whereby exercise intensity was tapered for 4d and dietary carbohydrate intake was either 57 or 75% of total energy intake (Tarnopolsky, Atkinson, et al., 1995). In response to the higher carbohydrate intake, the male subjects demonstrated a 41% increase in muscle glycogen and a 45% improvement in performance during in an exhaustive exercise bout following 1 hr of cycling at 75% of VO_{2PEAK}, whereas the females showed no increase in muscle glycogen and no affect on performance (Tarnopolsky, Atkinson, et al., 1995). We hypothesized that the inability of females to carbohydrate load may have been due to an intrinsic difference in the enzymatic and/or transport capacity for glycogen resynthesis/glucose uptake, or alternatively, that the carbohydrate intake (expressed related to body weight) was not high enough to promote glycogen supercompensation for the females (Tarnopolsky, Atkinson, et al., 1995). For example, in that study (Tarnopolsky, Atkinson, et al., 1995), the energy intake was 32.4 $kcal \cdot kg^{-1} \cdot d^{-1}$ for females and 45.1 $kcal \cdot kg^{-1} \cdot d^{-1}$ for males, and carbohydrate intake was 4.8 $g \cdot kg^{-1} \cdot d^{-1}$ and 6.4 $g \cdot kg^{-1} \cdot d^{-1}$ for females and 6.6 $g \cdot kg^{-1} \cdot d^{-1}$ and 8.2 $g \cdot kg^{-1} \cdot d^{-1}$ for males, respectively, on the low and high carbohydrate diet. In most of the studies of carbohydrate loading in males (Sherman, Costill, et al., 1981; Bergstrom, Hermansen, et al., 1967; Karlsson & Saltin 1971), the dietary carbohydrate intake was greater than 8 $g \cdot kg^{-1} \cdot d^{-1}$ and review articles (Burke & Hawley, 1999) have recommended that carbohydrate be expressed per kg and that 8-10 $g \cdot kg^{-1} \cdot d^{-1}$ was required for carbohydrate loading.

We subsequently compared the glycogen resynthesis rate in males and females following endurance exercise (90 min @ 65% of VO_{2PEAK}) to placebo, carbohydrate (1 $g \cdot kg^{-1}$ CHO) and carbohydrate/protein/fat (0.7 $g \cdot kg^{-1}$ CHO/0.1 $g \cdot kg^{-1}$ PRO/0.02 $g \cdot kg^{-1} \cdot d^{-1}$ FAT) given immediately and one h after exercise. The rate of

glycogen resynthesis in the first 4h was higher in the CHO and CHO/PRO/FAT as compared to placebo and not between the genders (Tarnopolsky, Bosman, et al., 1997). This suggested that, at least in the early glycogen resynthesis phase, males and females have a similar capacity to synthesize glycogen when the substrate is delivered at the same amount relative to body mass.

We have recently gone on to further explore the original observation of an apparent inability for females to carbohydrate load and tested the hypothesis that providing extra energy, and hence increasing carbohydrate intake to greater than 8 $g \bullet kg^{-1} \bullet d^{-1}$ would allow females to carbohydrate load similarly to males (Tarnopolsky, et al, Abstract presented at this conference). Well-trained male and female (~61 athletes) were given each of three diets for 4d in random order (CHO=75% energy for carbohydrate; CHO+E=CHO + 32% of extra energy and HAB = habitual diet (~58% of CHO). We find that males increased muscle glycogen (HAB<CHO<CHO+E) whereas females did not show any increase from HAB to CHO yet did increase from HAB<CHO=E. These results suggested that a previously reported gender difference in carbohydrate loading was due to the lower energy/carbohydrate intake (32.4 $kcal \bullet kg^{-1}$/6.4 $gCHO \bullet kg^{-1}$) for females. On a practical level, a female athlete consuming 2000 $kcal \bullet d^{-1}$ is not likely to be able to CHO load by merely increasing the percentage of dietary CHO and should consume ~30% more energy for 4d to ensure that CHO intake is >8 $g \bullet kg^{-1} \bullet d^{-1}$.

Protein Requirements

As mentioned above, endurance exercise training results in a lesser amino acid (protein) oxidation, however, the metabolic capacity to oxidize amino acids increases (McKenzie, Phillips, et al., 2000). Thus, an athlete who is training very vigorously and/or is energy or carbohydrate deficient could require a higher intake of protein. To test whether the protein requirements for sedentary persons were adequate for well trained athletes we measured nitrogen balance (NBAL) in these athletes while consuming a PRO intake of 0.94g $PRO \bullet kg^{-1} \bullet d^{-1}$ and 0.8 $\bullet kg^{-1} \bullet d^{-1}$ for males and females, respectively (Phillips, Atkinson, et al., 1993). At this "safe" intake all athletes were in negative NBAL. This intake was not adequate to maintain nitrogen balance. The data suggested that for well-trained athletes protein intakes are about 1.2 $g \bullet kg^{-1} \bullet d^{-1}$ for males and 1.0 $g \bullet kg^{-1} \bullet d^{-1}$ for females, as required to maintain nitrogen balance during moderate training. We have also demonstrated that top sport male athletes required about 100% more protein as compared to sedentary individuals (Tarnopolsky, MacDougall, et al., 1988), which, based upon our gender difference studies (McKenzie, Phillips, et al., 2000; Phillips, Atkinson, et al., 1993) could amount to about a 50-60% increase in requirements for protein. Fortunately, most males and females consume enough protein habitually such that any increased requirement is easily met (McKenzie, Phillips, et al., 2000; Tarnopolsky, Bosman, et al., 1997; Tarnopolsky, Atkinson, et al., 1995; Phillips, Atkinson, et al., 1993; Tarnopolsky, MacDougall, et al., 1990). However, for energy restriction athletes, it may be important to consider these absolute protein requirements.

Creatine Monohydrate Supplementation

Creatine is a guanidino compound that is produced endogenously in the liver and pancreas and consumed exogenously in meat-containing products. Creatine is transported into skeletal muscle, heart and brain (and other tissues) by a sodium-dependent creatine transporter (Guimbal & Kilimann, 1993). In muscle, brain

and heart creatine functions predominantly as a temporal energy buffer to re-phosphorylate ADP. In addition, it has a role in "energy sensing" and "shuttling" between the cytosol and the mitochondria through the creatine-phosphocreatine shuttle (Wallimann, Wyss, et al., 1992). Studies performed in cell culture have demonstrated an increase in actin-myosin protein synthesis and an increase in myosin mRNA in response to creatine (Young & Denome, 1984).

Humans can increase the concentration of total creatine and phosphocreatine in skeletal muscle by oral supplementation with 20 gm/d for 3-5d (Harris, Soderlund, et al., 1992) or 3 gm/d for 28d (Hultman, Soderlund, et al., 1996). Numerous studies have demonstrated an enhancement of high intensity power output following creatine monohydrate supplementation and an increase in fat-free mass (Juhn & Tarnopolsky 1998; Mihic, MacDonald, et al., 2000). Several longitudinal studies have demonstrated a greater increase in strength and fat-free mass during resistance exercise training when supplementing the creatine as compared to placebo (Vandenberghe, Goris, et al., 1997; Kreider, Ferreira, et al., 1998; Volek, Duncan, et al., 1999). With a notable exception (Vandenberghe, Goris, et al., 1997), the majority of these studies have been conducted with predominantly or exclusive males.

One study had demonstrated that females have a slightly higher muscle creatine content as compared to males (Forsberg, Nilsson, et al., 1991) which, if confirmed, could indicate that the propensity for females to creatine load could be attenuated. We examined the effect of 5d of creatine loading (20 $g \bullet d^{-1}$ x 5d) in both males (N = 15) and females (N = 15) and found that females increase fat-free mass by 0.4 kg, whereas males increased by 1.4 kg. Even when considering the differences in weight, this amounted to a 1% increase for females, and a 2% increase for females (Mihic, MacDonald, et al., 2000). We had hypothesized that this could be due to gender differences in basal total and phosphocreatine, however, a subsequent study by our group found that the basal concentrations of total and phosphocreatine were similar between males and females and the response to creatine loading was identical (Parise, Mihic, et al., 2000). Using a randomized double-blind cross-over trial, we also demonstrated that both males and females had an increase in peak and mean power output on maximal cycle ergometry (30s Wingate test) (MacLennan & Tarnopolsky, 1997).

To further investigate the mechanism behind these gender differences in fat-free mass, we used stable isotope methodology to measure whole body protein kinetics and fractional synthetic rate of muscle protein in response to creatine loading in males (N = 12) and females (N = 12). As mentioned, both groups showed similar increases in total creatine and phosphocreatine, yet there was no significant increase in mixed muscle fractional synthetic rates (Parise, Mihic, et al., 2000). For the males, but not the females, we found a reduction in leucine oxidation and a reduction in whole body proteolysis in response to creatine load (Parise, Mihic, et al., 2000). The magnitude of the effect was identical to that observed in a recent study of young males, given a hypotonic saline infusion (induction of cell swelling) (Waldegger, Klingel, et al., 1999). This suggested that a positive effect of creatine on protein metabolism occurs through an attenuation of oxidation and breakdown and not a stimulation of synthesis. Given the directional similarities to the cell swelling study, it is possible that the increase in creatine led to slight cell swelling which through a signaling mechanism (i.e., h-sgk) (Waldegger, Klingel, et al., 1999) led to a reduction in proteolysis and leucine oxidation. Given

the similar increases in total and phosphocreatine between the genders and the different effect on protein metabolism, the possibility of a gender specific response in the downstream signaling pathways in response to cell swelling exists.

Overall Summary

In response to long-term endurance exercise (40-70% VO_{2PEAK}), females oxidize proportionately more lipid and less carbohydrate and leucine as compared to males.

Females are able to carbohydrate load by a similar magnitude as compared to males when consuming a diet greater than 8 g $CHO \bullet kg^{-1} \bullet d^{-1}$. Elite male and female athletes require more dietary protein and compared to sedentary individuals, however, this increase is easily met through a mixed energy sufficient diet. Following acute creatine monohydrate supplementation, females do not increase fat-free mass by the same magnitude as males, yet they show similar increases in high-intensity performance. Long-term direct gender comparative studies will be required to determine whether the interactive effect of resistance training and creatine monohydrate show similar increases in strength and fat-free mass between genders. Future studies of nutrition/metabolism should consider and examine possible gender differences.

References

Bam, J., Noakes, T.D., Juritz, J., & Dennis, S.C. (1997). Could women outrun men in ultramarathon races? **Med Sci Sports Exerc 29**: 244-7.

Bergstrom, J., Hermansen, L., Hultman, E., & Saltin, B. (1967). Diet, muscle glycogen and physical performance. **Acta Physiol Scand 71**: 140-50.

Berthon, P. M., Howlett, R. A., Heigenhauser, G. J., & Spriet, L. L. (1998). Human skeletal muscle carnitine palmitoyltransferase I activity determined in isolated intact mitochondria. **J Appl Physiol 85**: 148-53.

Blatchford, F. K., Knowlton, R. G., & Schneider, D. A. (1985). Plasma FFA responses to prolonged walking in untrained men and women. **Eur J Appl Physiol 53**: 343-7.

Burke, L. M., & Hawley, J.A. (1999). Carbohydrate and exercise. **Curr Opin Clin Nutr Metab Care 2**: 515-20.

Carter, S. L., Mourtzakis, M., McKenzie, S., Lowther, S., & Tarnopolsky, M. A. 17-estradiol reduces glucose Ra and Rd, but not substrate oxidation in males. **FASEB J. 3** (5), A878. 99.

Carter, S. L., Rennie, C. D., & Tarnopolsky, M. A. (2000). Endurance training results in a decrease in glucose Ra/Rd during endurance exercise at both absolute and relative intensities. **Med Sci Sports Exerc 32** (5): S261.

Chorneyko, K., & Bourgeois, J. M. (1999). Gender differences in skeletal muscle histology and ultrastructure. In: **Gender Differences in Metabolism**, M. Tarnopolsky (Ed)., pp.37-59.

Costill, D. L., Fink, W.J., Getchell, L.H., Ivy, J.L., & Witzmann, F.A. (1979). Lipid metabolism in skeletal muscle of endurance-trained males and females. **J Appl Physiol 47**: 787-91.

Dyck, D. J. & Bonen, A. (1998). Muscle contraction increases palmitate esterification and oxidation and triacylglycerol oxidation. **Am J Physiol 275**: E888-96.

Ellis, G. S., Lanza-Jacoby, S. Gow, A. & Kendrick, Z. V. (1994). Effects of estradiol on lipoprotein lipase activity and lipid availability in exercised male rats. **J Appl Physiol 77**: 209-15.

Forsberg, A. M., Nilsson, E., Werneman, J. Bergstrom, J., & Hultman, E. (1991). Muscle composition in relation to age and sex. **Clin Sci (Colch) 81**: 249-56.

Friedlander, A. L., Casazza, G. A., Horning, M. A., Huie, M. J., Piacentini, M. F., Trimmer, J. K., & Brooks, G.A. (1998). Training-induced alterations of carbohydrate metabolism in women: women respond differently from men. **J Appl Physiol 85:** 1175-86.

Friedmann, B., & Kindermann, W. (1989). Energy metabolism and regulatory hormones in women and men during endurance exercise. **Eur J Appl Physiol 59**: 1-9.

Froberg, K., & Pedersen, P.K. (1984). Sex differences in endurance capacity and metabolic response to prolonged, heavy exercise. **Eur J Appl Physiol 52**: 446-50.

Green, H. J., Fraser, I. G., & Ranney, D.A. (1984). Male and female differences in enzyme activities of energy metabolism in vastus lateralis muscle. **J Neurol Sci 65**: 323-31.

Guimbal, C., & Kilimann, M.W. (1993). A Na(+)-dependent creatine transporter in rabbit brain, muscle, heart, and kidney. cDNA cloning and functional expression. **J Biol Chem 268**: 8418-21.

Guo, Z., & Jensen, M. D. (1999). Blood glycerol is an important precursor for intramuscular triacylglycerol synthesis. **J Biol Chem 274**: 23702-6.

Harris, R. C., Soderlund, K., & Hultman, E. (1992). Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. **Clin Sci (Colch) 83**: 367-74.

Horton, T. J., Pagliassotti, M. J., Hobbs, K., & Hill, J. O. (1998). Fuel metabolism in men and women during and after long-duration exercise. **J Appl Physiol 85**: 1823-32, 1998.

Hultman, E., Soderlund, K. Timmons, J. A., Cederblad, G., & Greenhaff, P.L. (1996). Muscle creatine loading in men. **J Appl Physiol 81**: 232-7.

Juhn, M. S., & Tarnopolsky, M. (1998). Oral creatine supplementation and athletic performance: a critical review. **Clin J Sport Med 8**: 286-97.

Karlsson, J., & Saltin, B. (1971). Diet, muscle glycogen, and endurance performance. **J Appl Physiol 31**: 203-6.

Kendrick, Z. V., & Ellis, G.S. (1991). Effect of estradiol on tissue glycogen metabolism and lipid availability in exercised male rats. **J Appl Physiol 71**: 1694-9.

Kendrick, Z. V., Steffen, C.A. Rumsey, W.L., & Goldberg, D. I. (1987). Effect of estradiol on tissue glycogen metabolism in exercised oophorectomized rats. **J Appl Physiol 63**: 492-6.

Kreider, R. B., Ferreira, M. Wilson, M., Grindstaff, P., Plisk, S., Reinardy, J., Cantler, E., & Almada, A.L. (1998). Effects of creatine supplementation on body composition, strength, and sprint performance. **Med Sci Sports Exerc 30**: 73-82.

MacLennan, D. P., & Tarnopolsky, M. A. (1997). Creatine supplementation and high intensity performance in males and females. **Can. J. Appl. Physiol. 22**: S192.

McKenzie, S., Phillips, S.M., Carter, S. L., Lowther, S., Gibala, M. J., & Tarnopolsky, M. A. (2000). Endurance exercise training attenuates leucine oxidation and BCOAD activation during exercise in humans. **Am J Physiol Endocrinol Metab 278**: E580-7.

Mihic, S., MacDonald, J.R., McKenzie, S., & Tarnopolsky, M.A. (2000). Acute creatine loading increases fat-free mass, but does not affect blood pressure, plasma creatinine, or CK activity in men and women. **Med Sci Sports Exerc 32**: 291-6.

Nicklas, B. J., Hackney, A.C., & Sharp, R.L. (1989). The menstrual cycle and exercise: performance, muscle glycogen, and substrate responses. **Int J Sports Med 10**: 264-9.

Parise, G., Mihic, S., MacLellan, D., Yarasheski, K., & Tarnopolsky, M. (2000). Creatine monohydrate does not increase whole body or mixed muscle fractional protein synthetic rates in males and females. **Med Sci Sports Exerc. 32** (5): S289.

Phillips, S. M., Atkinson, S.A., Tarnopolsky, M.A., & MacDougall, J.D. (1993). Gender differences in leucine kinetics and nitrogen balance in endurance athletes. **J Appl Physiol 75**: 2134-41.

Rennie, C. D., Brose, A. N., Carter, S. L., & Tarnopolsky, M. A. (2000). Muscle enzyme changes following endurance exercise in males and females. **Med Sci Sports Exerc 32** (5): S363.

Rooney, T. P., Kendrick, Z. V., Carlson, J., Ellis, G. S., Matakevich, B., Lorusso, S. M., & McCall, J.A.(1993). Effect of estradiol on the temporal pattern of exercise-induced tissue glycogen depletion in male rats. **J Appl Physiol 75**: 1502-6.

Ruby, B. C., Robergs, R.A., Waters, D.L., Burge, M., Mermier, C., & Stolarczyk, L. (1997). Effects of estradiol on substrate turnover during exercise in amenorrheic females. **Med Sci Sports Exerc 29**: 1160-9.

Sherman, W. M., Costill, D. L., Fink, W. J., & Miller, J.M. (1981). Effect of exercise-diet manipulation on muscle glycogen and its subsequent utilization during performance. **Int J Sports Med 2**: 114-8.

Speechly, D. P., Taylor, S. R., & Rogers, G.G. (1996). Differences in ultra-endurance exercise in performance-matched male and female runners. **Med Sci Sports Exerc 28**: 359-65.

Tarnopolsky, L. J., MacDougall, J.D., Atkinson, S.A., Tarnopolsky, M.A., & Sutton, J.R. (1990). Gender differences in substrate for endurance exercise. **J Appl Physiol 68**: 302-8.

Tarnopolsky, M. A., Atkinson, S. A., Phillips, S. M., & MacDougall, J.D. (1995). Carbohydrate loading and metabolism during exercise in men and women. **J Appl Physiol 78**: 1360-8.

Tarnopolsky, M. A., Bosman, M., Macdonald, J.R., Vandeputte, D., Martin, J., & Roy, B.D. (1997). Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. **J Appl Physiol 83**: 1877-83.

Tarnopolsky, M. A., Ettinger, S., MacDonald, J. R., Roy, B. D., & MacKenzie, S. (1997). 17-estradiol (E2) does not affect muscle metabolism in males. **Med. Sci. Sports Exerc. 29** (5): S93.

Tarnopolsky, M. A., MacDougall, J.D., & Atkinson. S.A. (1988). Influence of protein intake and training status on nitrogen balance and lean body mass. **J Appl Physiol 64**: 187-93.

Tarnopolsky, M.A. (1999). **Introduction. Gender differences in metabolism**. (Ed.) M.A. Tarnopolsky. pp.1-14.

Tiidus, P. M. (1995). Can estrogens diminish exercise induced muscle damage? **Can J Appl Physiol 20**: 26-38.

Vandenberghe, K., Goris, M., van Hecke, P., Van Leemputte, M., Vangerven, L., & Hespel, P. (1997). Long-term creatine intake is beneficial to muscle performance during resistance training. **J Appl Physiol 83**: 2055-63.

Volek, J. S., Duncan, N.D., Mazzetti, S.A., Staron, R.S., Putukian, M., Gomez, A.L., Pearson, D.R., Fink, W. J., & Kraemer, W. J. (1999). Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. **Med Sci Sports Exerc 31**: 1147-56.

Waldegger, S., Klingel, K., Barth, P., Sauter, M., Rfer, M.L., Kandolf, R., & Lang, F. (1999). h-sgk serine-threonine protein kinase gene as transcriptional target of transforming growth factor beta in human intestine. **Gastroenterology 116**: 1081-8.

Wallimann, T., Wyss, M. Brdiczka, D. Nicolay, K., & Eppenberger, H.M. (1992). Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. **Biochem J 281** (Pt 1): 21-40.

Young, R. B., & Denome, R.M. (1984). Effect of creatine on contents of myosin heavy chain and myosin-heavy- chain mRNA in steady-state chicken muscle-cell cultures. **Biochem J 218**: 871-6.