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# Females and males: should nutritional recommendations be gender specific?

## Summary

Most exercise physiology research has shown that women oxidize proportionately more lipid and less carbohydrate and protein as compared to men during endurance exercise. To date, most of the sports nutrition literature has not considered the implications of gender differences in metabolism on nutritional recommendations. Consequently, most nutritional recommendations and exercise training prescriptions are based upon data collected with male subjects that were extrapolated to women. The three areas where there have been a few studies regarding gender differences in nutritional/supplement recommendations include carbohydrate (CHO) nutrition, protein requirements and creatine (CrM) supplementation. We have shown that women did not carbohydrate load in response to an increase in dietary carbohydrate intake (carbohydrate loading) when expressed as a percentage of total energy intake (i.e., 55 → 75%). However, if women consumed carbohydrate expressed relative to total (>8 g CHO·kg<sup>-1</sup>·d<sup>-1</sup>) or fat-free mass (>10 g CHO·kg<sup>-1</sup> FFM·d<sup>-1</sup>), they were able to increase their muscle glycogen content, but only to about 50% of the magnitude seen for men. In contrast, women are able to oxidize slightly more exogenous carbohydrate (i.e., glucose drinks) during endurance exercise as compared to men. The consumption of carbohydrate and protein shortly after exercise spares protein loss, enhances glycogen re-synthesis and enhances endurance exercise performance in women as well as men. Top sport male and female athletes require more dietary protein as compared to sedentary persons. The maximal requirement for elite male athletes is about 100%, and for elite female athletes is about 50–60%, above that for a sedentary person or recreational athlete. Women showed less of an increase in fat-free mass (~400 g) following acute CrM loading as compared to men (~1200 g) in spite of identical increases in intra-muscular creatine and phosphocreatine concentration. Women also did not show reductions in protein breakdown or amino acid oxidation in response to CrM loading, whereas men did. Conversely, women and men appear to derive similar improvements in high intensity exercise performance following CrM loading. Further research is needed in order to derive gender specific nutritional/supplement recommendations in all areas of sport.

## Zusammenfassung

Die Forschung im Bereiche der Sportphysiologie hat mehrheitlich gezeigt, dass Frauen während Ausdauerbelastungen proportional gesehen mehr Fette und weniger Kohlenhydrate und Protein oxidieren als Männer. Bis zum jetzigen Zeitpunkt hat aber der Grossteil der Sporternährungsliteratur die Stoffwechselunterschiede zwischen den Geschlechtern nicht berücksichtigt, und dementsprechend basieren die meisten Empfehlungen für die Ernährung und das Training von Frauen auf aus Versuchen mit Männern gewonnenen und extrapolierten Daten. Geschlechtsspezifische Unterschiede bezüglich Empfehlungen für die Ernährung bzw. Supplementzufuhr wurden bislang in drei Gebieten erforscht: Einfluss einer Ernährung reich an Kohlenhydraten (KH), Empfehlungen zur Proteinzufuhr und Creatinsupplementierung. Wir konnten zeigen, dass es bei Frauen als Reaktion auf eine kohlenhydratreiche Ernährung nicht zu einer Überfüllung der Glycogenspeicher kam, wenn das Carboloadung (die Kohlenhydratzufuhr) prozentual auf die Energiezufuhr bezogen war (d.h. von 55 auf 75 Energieprozent erhöht wurde). Wurde das Carboloadung auf die Körpermasse (d.h. mehr als 8 g KH·kg<sup>-1</sup>·d<sup>-1</sup>) oder auf die fettfreie Körpermasse (FFM) bezogen (>10 g KH·kg<sup>-1</sup> FFM·d<sup>-1</sup>), konnte zwar eine Überfüllung der Glycogenspeicher beobachtet werden, diese hatte aber nur ungefähr 50% des Ausmasses wie diejenige bei den Männern. Frauen konnten dafür während Ausdauerbelastungen etwas mehr exogene KH (d.h. Glucosegetränke) oxidieren als Männer. Der Konsum von KH und Protein unmittelbar nach Beendigung einer Belastung reduziert den Proteinverlust, erhöht die Glycogenresynthese und verbessert die Ausdauerleistungsfähigkeit sowohl bei Frauen als auch bei Männern. Eliteathletinnen und -athleten benötigen beide mehr Protein im Vergleich zu inaktiven Personen. Der höchste Bedarf für männliche Eliteathleten beträgt etwa 100% und derjenige für weibliche Eliteathletinnen 50 bis 60% mehr als der Bedarf für Personen mit ausgesprochener sitzender Tätigkeit. Nach akuter Creatinsupplementierung wiesen Frauen einen geringeren Zuwachs an FFM (~400 g) auf als Männer (~1200 g), obwohl der intramuskuläre Gehalt an Creatin und Phosphocreatin in beiden Geschlechtern auf identische Weise zunahm. Bei Frauen wurde auch keine Reduktion des Proteinabbaus oder der Aminosäureoxidation nach Creatinsupplementierung beobachtet, wogegen dies bei Männern der Fall war. Im Gegensatz dazu wurde eine ähnliche Leistungssteigerung bei Männern und Frauen nach hochintensiver Belastung und Creatinsupplementierung festgestellt. Es bedarf weiterer Forschung, um geschlechtsspezifische Empfehlungen für die Ernährung bzw. Supplementzufuhr in allen Sportarten ableiten zu können.

## Introduction

Most of the research in the areas of sports nutrition, muscle metabolism and exercise physiology has been conducted using predominantly men. It has been assumed that the physiologic responses to exercise were similar between men and women. Even in the 1980's major exercise physiology textbooks stated that there were no gender differences in the metabolic response to exercise. Consequently, nutritional recommendations for women have essentially been extrapolations from male based research. More recent, carefully controlled research has clearly shown that there are gender differences in metabolism during endurance exercise [1–10]. Important factors that were not uniformly controlled for in earlier research included training status, gender matching criteria, menstrual cycle phase, amenorrhea and nutritional status.

Traditionally, most studies examining the adaptations to strength and power training have employed male subjects. Recent data has clearly shown that women can adapt to these types of training, although the magnitude of gains in fat-free mass are somewhat less as compared to men. As a result of the interest in power sports for women, an interest in nutrition and nutritional supplements specific to these types of activities has emerged. Creatine monohydrate has been the most popular nutraceutical compound for strength and power sports over the past decade and only recently have potential gender differences been examined.

This chapter will summarize many of the recent papers that have examined potential gender differences in metabolism during endurance exercise. Most of the chapter will focus on the potential implications of gender differences in metabolism upon nutritional recommendations.

## Substrate metabolism during endurance exercise

The major determinants of metabolic substrate selection during endurance exercise include exercise intensity [11], training history [12], nutritional state [13, 14], and the duration of exercise [15]. In general, lower intensity endurance exercise, endurance exercise training, the fasted state, and longer duration are associated with a greater proportionate lipid and lower carbohydrate oxidation. Based upon conflicting studies in the 1970's and 80's [16–19], many researchers did not consider that gender influenced metabolic fuel selection during endurance exercise. The lack of consensus regarding the effect of gender upon substrate selection is partly due to sub-optimal control over the key determinants of substrate selection mentioned above. Additionally, many of the original gender comparative studies did not control for timing or presence/absence of the menstrual cycle. The phase of the menstrual cycle is important for exercise performance and glucose production appear to be greater in the follicular phase [20], while protein catabolism appears to be greater during the luteal phase [21]. Other studies did not carefully match males and females for training history and  $VO_{2PEAK}$  expressed relative to fat-free mass. A matching process that considers training history, menstrual status, diet analysis and  $VO_{2PEAK}$  testing expressed relative to fat-free mass takes into account both genetic (genetically determined «window of  $VO_2$  potential») and environmental (state of training) factors [22]. When men and women are matched based upon these criteria, there does not appear to be a gender difference in the anaerobic or «lactate» threshold for either trained [5], or untrained [23], subjects.

Our group has completed four cross-sectional studies [3–6], and three longitudinal training studies [8, 24, 25] where we have carefully matched males and females for training history and  $VO_{2PEAK}$  relative to fat-free mass. These studies all showed that women oxidized proportionately more lipid and less carbohydrate as compared to men during sub-maximal endurance exercise. In addition, another longitudinal training study confirmed the aforementioned gender differences in metabolism during endurance exercise before and after the training program [2]. Finally, a study using 24 hour indirect calorimetry confirmed that females oxidized more

lipid and less carbohydrate during endurance exercise at two sub-maximal intensities [7].

In order to arrive at a conclusion regarding gender differences in metabolism I have extracted the data from a total of 19 studies that have examined gender differences in substrate metabolism during endurance exercise (irrespective of the conclusions) [1–10, 16–18, 24–26]. This composite analysis using a total of 178 women and 205 men clearly demonstrated that women oxidize more lipid and less carbohydrate as compared to men during sub-maximal intensity endurance exercise (Table 1). During endurance exercise women derive approximately 41% of their energy from lipid and 56% from carbohydrate, while the corresponding values for men are 29% and 65%, respectively (Table 2).

Although carbohydrate and lipid provide the majority of the energy for muscle contraction during endurance exercise, protein oxidation can provide up to 8% of the total energy [5, 24]. Although the proportion of energy derived from protein is rather small, it is important for proteins are involved in structure and function of the human body and their oxidation is likely to have more significant consequences to an athlete as compared to carbohydrate or triacylglycerol (stored fat). Given the lower contribution of carbohydrate to metabolism for females, it would be predicted that protein oxidation should be similarly lower for women as compared to men [5, 24]. Using urea excretion [6], and amino acid oxidation methods [5, 24, 26], studies have found that women do oxidize less protein during endurance exercise as compared to men (Table 3).

## Carbohydrate metabolism

The basal levels of muscle glycogen are similar in men and women [4, 6, 24, 27]. Furthermore, the ratio of pro:macro glycogen is also similar between men and women [27]. There are no differences between men and women with respect to GLUT-4 (muscle glucose transporter) [28] or hexokinase [27]. To date I am not aware of any studies that have compared glycogen synthase activity or branching enzyme between men and women.

Although there is strong evidence that women oxidize less total carbohydrate during endurance exercise as compared to men, the exact locus of this phenomenon is not as clear. We reported that women showed significantly less glycogen depletion in the *vastus lateralis* following 15.5 km of treadmill running, as compared to men [6]. We did not replicate these findings in a subsequent study of men and women cycling for 60 min at 75% of  $VO_{2PEAK}$  [4]. In addition, we measured glycogen use in the leg with the muscle biopsy technique pre- and post-exercise before and after 31 days of endurance cycling exercise, and again found no glycogen sparing in women during cycling exercise using stable isotope methodology [8, 29]. Several studies have found that the glucose rate of appearance (a measure of liver glucose production) is lower for women during endurance exercise [2, 8, 9]. Our group has also found that the administration of 17- $\beta$ -estradiol administration to males did not attenuate skeletal muscle breakdown during endurance exercise [30]. Recently, Bente Kien's group found that glucose balance across the exercising leg was similar between men and women, yet the glucose rate of appearance was lower for women [9]. Together, the above data suggest that some of the gender differences in carbohydrate metabolism may be due to hepatic and not skeletal muscle glycogen sparing. Further study is required to explore whether there are gender differences in muscle recruitment during different modes of endurance exercise.

## Lipid metabolism

In contrast to muscle glycogen, there is a significantly higher intra-muscular triacylglycerol (IMTG) content in women as compared to men [10, 31]. We have demonstrated a higher plasma free

Reference	Subjects	Exercise	RER (mean)
Costill et al., 1979	12 F, T 12 M, T	60 min run @ 70% VO <sub>2</sub> max	F = 0.83 M = 0.84
Froberg and Pederson, 1984	7 F, T 7 M, T	to exhaustion @ 80 + 90% VO <sub>2</sub> max	F = 0.93* M = 0.97*
Blatchford et al., 1985	6 F, T 6 M, T	90 min walk @ 35% VO <sub>2</sub> max	F = 0.81 M = 0.85
Tarnopolsky et al., 1990	6 F, T 6 M, T	15.5 km run @ ~65% VO <sub>2</sub> max	F = 0.876 M = 0.940
Phillips et al., 1993	6 F, T 6 M, T	90 min cycle @ 65% VO <sub>2</sub> max	F = 0.820 M = 0.853
Tarnopolsky et al., 1995	8 F, T 7 M, T	60 min cycle @ 75% VO <sub>2</sub> max	F = 0.892 M = 0.923
Tarnopolsky et al., 1997	8 F, T 8 M, T	90 min cycle @ 65% VO <sub>2</sub> max	F = 0.893 M = 0.918
Horton et al., 1998	13 F, T + UT 14 M, T + UT	120 min cycle @ 45% VO <sub>2</sub> max	F = 0.84 M = 0.86
Freidlander et al., 1998	17 F, UT→T 19 M, UT→T	60 min cycle @ 45 & 65% VO <sub>2</sub> max	F = 0.885* M = 0.932*
Romijn et al., 2000	8 F, T 5 M, T	20–30 min cycle @ 65% VO <sub>2</sub> max	F = 0.81 M = 0.81
McKenzie et al., 2000	6 F, UT→T 6 M, UT→T	90 min cycle @ 65% VO <sub>2</sub> max	F = 0.889 M = 0.914
Davis et al., 2000	8 F, UT 8 M, UT	90 min cycle @ 50% VO <sub>2</sub> max	F = 0.92 M = 0.92
Goedecke et al., 2000	16 F, T 45 M, T	10 min @ 25,50, and 75% VO <sub>2</sub> max	F = 0.90* M = 0.92*
Rennie et al., 2000	6 F, UT→T 5, M UT→T	90 min cycle @ 60% VO <sub>2</sub> max	F = 0.893 M = 0.945
Carter et al., 2001	8 F, UT→T 8 M, UT→T	90 min cycle @ 60% VO <sub>2</sub> max	F = 0.847 M = 0.900
Lamont et al., 2001	7 F, T + UT 7 M, T + UT	60 min cycle @ 50% VO <sub>2</sub> max	F = 0.808 M = 0.868
Roepstorff et al., 2001	7 F, T 7 M, T	90 min cycle @ 58% VO <sub>2</sub> max	F = 0.886 M = 0.905
Steffensen et al., 2002	21 F, UT + T 21 M, UT + T	90 min @ 60% VO <sub>2</sub> max	F = 0.875* M = 0.895*
Melanson et al., 2002*	8 F, T 8 M, T	400 kcal @ 40 + 70% VO <sub>2</sub> max	F = 0.87* M = 0.91*
<b>Mean</b>	178 F 205 M		F = 0.868 (0.037) M = 0.899 (0.040)†

Values are mean (SD). RER = respiratory exchange ratio; F = females; M = males; T = trained; A = active; UT = untrained; U→T = longitudinal training study; for longitudinal training studies, the pre/post rides are all collapsed across time for each gender. T + UT = trained and untrained in same study.  
 \* The RER was a combination of those at both exercise intensities.  
 † Significant gender difference (P<0.001, 2 tailed independent t-test).

Table 1: Summary of studies where whole body substrate metabolism was reported in men and women.

Subjects	RER	CHO (%)	FAT (%)	PRO (%)*
N = 178 F	0.868 (0.037)	56 (10)	41 (9)	2 (2)
N = 205 M	0.899 (0.040)‡	65 (9)†	29 (8)†	5 (3)†

Values are mean (SD). \* from Tarnopolsky et al., 1989; Phillips et al., 1993; McKenzie et al., 2000; and Lamont et al., 2001. Gender difference († P < 0.01; ‡ P < 0.001).

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

fatty acid and glycerol concentration during exercise in females as compared to males in some [8, 25] but not all [4, 6] studies. A recent study has also shown that IMTG utilization during endurance exercise was also greater for women [10]. Arterial-venous balance studies across the exercising leg showed a 47% greater plasma free fatty acid uptake for women as compared to the men during endurance exercise, although this was not reported as being statistically significant [9]. We have also found that women showed a higher glycerol rate of appearance (peripheral adipocyte lipolysis) as compared to men [8]. Together the balance of the data suggests that women show a greater content and utilization of

IMTGs and possibly greater lipolysis and uptake of free fatty acids from the plasma.

From a muscle enzymatic standpoint, there is some evidence that the β-oxidation enzyme, β-hydroxy-acyl-CoA-dehydrogenase (β-HAD), activity is higher in women as compared to men [32], however, others have not found a significant gender difference in the activity of this enzyme [8, 25]. Long chain fatty acids must be transported into the mitochondrial via the carnitine palmitoyl transferase (CPT) pathway. The evidence does not support a gender difference in either total CPT activity [16], nor specific CPT-1 activity [33]. In contrast there is some evidence that women have a higher plasma membrane capacity to take up free fatty acids from the plasma due to higher fatty acid transport protein-1 mRNA in women as compared to men [34]. Given the complexity of lipid transport and oxidation, it is possible that other enzymes involved in lipid metabolism may show gender differences and subtle changes in several enzymes may account for the fact that women oxidize more lipid during endurance exercise as compared to men.

### Protein metabolism

Using 24 h urinary urea nitrogen excretion as a marker of protein catabolism we found that men, but not women, showed an

Reference	Subjects	Exercise	Protein (% of energy)
Tarnopolsky et al., 1990*	6 F, T 6 M, T	15.5 km run @ ~65% VO <sub>2</sub> max	F = 0.3 M = 9.1
Phillips et al., 1993 <sup>†</sup> *	6 F, T 6 M, T	90 min cycle @ 65% VO <sub>2</sub> max	F = 2.0 M = 3.3
Tarnopolsky et al., 1995*	8 F, T 7 M, T	60 min cycle @ 75% VO <sub>2</sub> max	F = 1.6 M = 6.3
Tarnopolsky et al., 1997*	8 F, T 8 M, T	90 min cycle @ 65% VO <sub>2</sub> max	F = 2.0 M = 3.0
McKenzie et al., 2000 <sup>†</sup>	6 F, UT→T 6 M, UT→T	90 min cycle @ 65% VO <sub>2</sub> max	F = 4.8 M = 8.4
Lamont et al., 2001 <sup>†</sup>	7 F, T 7 M, T	90 min cycle @ 65% VO <sub>2</sub> max	F = 2.0 M = 3.0
<b>Average Values:</b>			F = 2.1 (1.6) M = 5.5 (3.2)
* data based upon urinary urea excretion; † data derived from L-1-[ <sup>13</sup> C]-leucine oxidation. F, M, UT, T are described in the <i>table 1</i> legend. Values are mean (SD).			

Table 3: Protein oxidation during endurance exercise.

increased urea excretion in response to 15.5 km of treadmill running [6]. Subsequent studies using stable isotope tracers (L-[1-<sup>13</sup>C]-leucine) confirmed that men oxidized proportionately more leucine (an amino acid) during exercises as compared to women [5, 24, 26]. The lower leucine oxidation during endurance exercise for women was also apparent before and after 31 days of endurance exercise training [24]. What was surprising is that the total and active proportion of the rate limiting enzyme for branched chain amino acid oxidation (branched chain-2-oxo-dehydrogenase, BCOAD) in skeletal muscle was not different between men and women [24]. This latter finding implies that the lower amino acid oxidation for women is mediated at the level of the liver and/or that amino acid oxidation merely follows the content of tissue glycogen (i.e., with glycogen sparing there is amino acid sparing).

### Mechanism of gender differences

The mechanism(s) behind the gender difference in substrate selection during endurance exercise is likely due to differences in the female hormone, 17- $\beta$ -estradiol (estrogen). Two studies have demonstrated that the administration of 17- $\beta$ -estradiol to male [35, 36] and female oophorectomized [37] rats resulted in significant muscle and hepatic glycogen sparing during endurance exercise. In addition, muscle lipoprotein lipase and intra-muscular triacylglycerol content were increased, while adipocyte lipoprotein lipase was decreased following 17- $\beta$ -estradiol administration to male rats [38]. The presence of 17- $\beta$ -estradiol also appears to increase the activity of key enzymes in the fat oxidation pathway (CPT-1 and  $\beta$ -HAD) [39]. Although these types of studies are more difficult to perform in humans, 17- $\beta$ -estradiol administration to amenorrheic females [40], and males [41] resulted in a lower glucose rate of appearance and disappearance. It is unclear whether lipid is the main substrate that is oxidized and carbohydrate and protein are secondarily affected or whether carbohydrate is the main regulated substrate and lipid is secondary.

Amino acid oxidation during exercise likely responds in a passive and inverse relationship to carbohydrate availability during endurance exercise, as discussed above (i.e., when carbohydrate availability is low, protein oxidation is increased to fulfill an anapleurotic function in the tricarboxylic acid cycle). In contrast, the male hormone, testosterone, appears to have a major primary effect on the rate of skeletal muscle protein synthesis. Testosterone administration to young men results in an increase in muscle protein synthesis [42], and an increase in fat-free mass [43]. Tes-

tosterone when combined with resistance exercise results in an increase in fat-free mass and strength that is greater than that seen for either strategy in isolation [43]. Given that men have testosterone levels that are about 10–15x greater than women, it is somewhat surprising that preliminary studies did not show a gender difference in mixed muscle protein synthetic rates [44–46].

### Carbohydrate loading

The duration of endurance exercise performance during sub-maximal intensity (60–75% VO<sub>2</sub> PEAK) exercise is correlated with the initial muscle glycogen stores and with a «sparing» of glycogen stores [13, 47, 48]. Dietary manipulations that result in a higher carbohydrate intake can enhance endurance exercise performance at the aforementioned exercise intensities by increasing muscle glycogen stores [13, 47, 48]. Studies examining this dietary strategy called carbohydrate loading have been conducted with predominantly male subjects [13, 49, 50]. Given that glycogen storage is altered by menstrual cycle phase [51] and that 17- $\beta$ -estradiol can influence glycogen utilization [35–37], it is important to consider whether there are any gender differences in the ability to carbohydrate load.

Our group examined the response of muscle glycogen to a modified carbohydrate loading protocol where exercise intensity was tapered for four days and dietary carbohydrate intake was manipulated to 57 and 75% of total energy intake [4]. In response to the higher carbohydrate intake, the men showed a 41% increase in muscle glycogen and a 45% improvement in performance time in an exhaustive exercise bout following 1 h of cycling at 75% of VO<sub>2</sub>PEAK, whereas the women showed no increase in muscle glycogen and no performance enhancement [4]. Given that there is no evidence to support a gender difference in either the enzymatic and/or transport capacity for glycogen synthesis/glucose uptake (see above), our hypothesis was that the carbohydrate intake (expressed related to body weight) was not high enough to promote glycogen super-compensation for the women athletes in our study [4]. In our initial carbohydrate loading study, the carbohydrate intake was 4.8 g·kg<sup>-1</sup>·d<sup>-1</sup> and 6.4 g·kg<sup>-1</sup>·d<sup>-1</sup> for women and 6.6 g·kg<sup>-1</sup>·d<sup>-1</sup> and 8.2 g·kg<sup>-1</sup>·d<sup>-1</sup> for men, respectively, on the low and high carbohydrate diet [4]. In most of the studies of carbohydrate loading in men [13, 49, 50], the dietary carbohydrate intake was greater than 8 g·kg<sup>-1</sup>·d<sup>-1</sup> and review articles [52] have recommended that carbohydrate be expressed per kg and that 8–10 g·kg<sup>-1</sup>·d<sup>-1</sup> is required for carbohydrate loading.

There have now been three studies that have shown that women can carbohydrate load provided that they consume at least 8 g·kg<sup>-1</sup>·d<sup>-1</sup> [27, 53, 54]. In order to achieve this level of carbohydrate intake the women had to increase their total energy intake by about 34 percent [27]. In spite of the ability of females to carbohydrate load, we found that the magnitude of the increase in glycogen was about 50% less for women, and the study from Lawrence Spreit's laboratory concluded that, «...the magnitude [of glycogen loading] was smaller than previously observed in men.» [54]. In contrast, one other study found similar levels of glycogen supra-compensation for men and women after a carbohydrate loading protocol that provided 12 g·kg<sup>-1</sup> FFM·d<sup>-1</sup> and no exercise was performed for three days before the trial [53]. From a practical standpoint, a female athlete consuming 8.2 MJ·d<sup>-1</sup> (2000 kcal·d<sup>-1</sup>) is not likely to be able to carbohydrate load by solely increasing the percentage of dietary carbohydrate in the diet and must consume ~30% more energy for four days to ensure that carbohydrate intake is >8 g·kg<sup>-1</sup>·d<sup>-1</sup> [27].

### Carbohydrate consumption in the immediate post-exercise period

Several studies have demonstrated that the rate of glycogen re-synthesis is greater if carbohydrate (and carbohydrate-protein) are consumed in the early post-exercise period as compared to hours later [55–57]. Our group compared the rate of glycogen re-synthesis in men and women following endurance exercise (90 min at 65% of VO<sub>2PEAK</sub>) in response to a placebo, carbohydrate (1 g CHO·kg<sup>-1</sup>) and carbohydrate/protein/fat (0.7 g CHO·kg<sup>-1</sup>/0.1 g PRO·kg<sup>-1</sup>/0.02 g FAT·kg<sup>-1</sup>) given immediately and one hour after exercise [3]. The rate of glycogen re-synthesis in the first 4 h was higher in the CHO and CHO/PRO/FAT as compared to placebo and was the same for both men and women [3]. These results suggested that men and women have a similar capacity to synthesize glycogen when the substrate has delivered in the early post-exercise period (and at the same amount relative to body mass).

To study the practical implications of the above findings, we designed a study where females were exposed to two periods of increased training volume (identical between periods) and were given identical diets with the exception that on one trial (POST) they consumed a defined formula diet immediately after every work-out (1.2 g CHO·kg<sup>-1</sup>/0.1 g PRO·kg<sup>-1</sup>/0.02 g FAT·kg<sup>-1</sup>), and on the other trial (PRE) the same supplement was consumed with breakfast and a placebo was taken post-exercise. After one week a performance trial demonstrated improvements and there was a strong trend towards a reduction in protein oxidation in the POST trial [58].

### Carbohydrate consumption during exercise

A number of studies have found that the consumption of exogenous glucose and other sugar solutions during exercise improves endurance exercise performance [59]. Based upon the fact that there is a lower rate of glucose disposal (uptake from the plasma in to tissues) in the fasted state, and that women use more lipid during endurance exercise (see above), it may be predicted that women would have an attenuated ability to utilize exogenous glucose. However, three studies have shown that women are at least as capable as men in responding favorably to the consumption of exogenous glucose solutions [20, 60, 61]. One study found a 14% improvement in performance during the follicular phase and an 11% improvement during the luteal phase of the menstrual cycle in response to a 0.6 g·kg<sup>-1</sup>·h<sup>-1</sup> glucose solution [60]. A second study found an even greater performance enhancement (follicular: + 19%; luteal: + 26%) during a two hour cycle in young women in response to a glucose ingestion of ~1.1 g·kg<sup>-1</sup>·h<sup>-1</sup> [20]. The latter study suggested that the improvement in performance for the women was slightly greater than that reported for men in other studies. We recently used a labeled glucose drink (stable isotope) and demonstrated that women derived more of their energy from

the glucose drink as compared to men and that they spared more glycogen in response to the drink [61].

### Protein requirements for endurance exercise

Endurance exercise training results in a reduced amino acid (protein) oxidation, however, the metabolic capacity to oxidize amino acids increases due to an increase in the rate limiting enzyme for branched chain amino acid oxidation (BCOAD) [24]. Thus, a top sport athlete who is training very vigorously and/or is energy or carbohydrate deficient could require a higher intake of protein. The maximal increase in protein requirements for top sport male athletes appears to be around 1.7 g PRO·kg<sup>-1</sup>·d<sup>-1</sup> [62]. As discussed above, men have a higher amino acid oxidation during endurance exercise as compared to women and this may influence their protein requirements. To test whether the protein requirements for sedentary persons were adequate for well trained (but not top sport) athletes we measured nitrogen balance (NBAL) in athletes while consuming a protein intake of 0.94 g PRO·kg<sup>-1</sup>·d<sup>-1</sup> and 0.8 g PRO·kg<sup>-1</sup>·d<sup>-1</sup> for men and women, respectively [5]. We found that both the men and women were in negative nitrogen balance (although men were in greater negative balance) at these «safe» intake levels even after a 10 day adaptation period. Estimates of the «safe» intake for these moderately trained athletes were approximately 1.2 g·kg<sup>-1</sup>·d<sup>-1</sup> for men and 1.0 g·kg<sup>-1</sup>·d<sup>-1</sup> for women to maintain nitrogen balance. In a similar study in moderately trained athletes consuming 1.0 g PRO·kg<sup>-1</sup>·d<sup>-1</sup>, Lamont and colleagues found that the women were in slightly negative NBAL (-0.22 g·d<sup>-1</sup>) and the men were in more negative NBAL (-3.95 g·d<sup>-1</sup>) [26]. To date there have been no studies that have looked at the protein requirements for top sport women athletes, however, the above data would suggest that their requirements would likely be less than men at around 1.2–1.4 g·kg<sup>-1</sup>·d<sup>-1</sup>. Fortunately, most men and women consume enough protein habitually such that any increased requirement is easily met [3–6, 24]. However, given that top sport female athletes are more prone to excessive energy restriction than many other athletes (see chapter by Dr. Sundgot-Borgen), it is important to consider these absolute protein requirements in the assessment of dietary adequacy.

### Creatine monohydrate supplementation

Creatine is a guanidino compound produced endogenously in the liver and pancreas and consumed in the diet via meat and fish foods. Creatine is transported into skeletal muscle, heart and brain (and other tissues) by a sodium-dependent creatine transporter [63]. In muscle, brain and heart, creatine functions as a temporal energy buffer to re-phosphorylate ADP and also has a role in «energy sensing» and «shuttling» between the cytosol and the mitochondria through the creatine-phosphocreatine shuttle [64]. Creatine may also function to increase myofibrillar protein synthesis either directly [65], or indirectly by allowing a person to perform more muscle contractions over a period of time [65–67]. There has been much interest in the use of creatine as a nutritional agent following several reports that creatine monohydrate ingestion could enhance high intensity exercise performance [68, 69].

The concentration of total creatine and phosphocreatine can increase in skeletal muscle following oral creatine monohydrate supplementation with 20 g·d<sup>-1</sup> for three to five days [70] or 3 g·d<sup>-1</sup> for 28 days [71]. Several studies have shown an enhancement of high intensity power output following creatine monohydrate supplementation and an increase in fat-free mass [72, 73]. Several longitudinal studies have demonstrated a greater increase in strength and fat-free mass during resistance exercise training with creatine monohydrate supplementation as compared to placebo [65, 66, 74, 75]. With a notable exception [66], the majority of the acute and longer term studies have been conducted with predominantly or exclusively males. In the latter study, women showed a

greater increase in muscle strength and fat-free mass following a 10 week resistance exercise program with a CrM compared to placebo supplement [66].

Although one study reported a slightly higher muscle creatine content in women as compared to men [31], our group [76] and a recent study from Dr. R. Snow's laboratory [Snow R., personal communication, 2002] found no gender difference in total or phospho-creatine nor in the amount of the creatine transporter. We examined the effect of five days of creatine loading (20 g·d<sup>-1</sup> for 5 days) in both men (N = 15) and women (N = 15) and found a greater increase in fat-free mass for the men (1.4 kg, 2% of body mass) as compared to the women (0.4 kg, 1% of body mass) [73]. To investigate the potential mechanism(s) behind the gender differences in fat-free mass accumulation we used stable isotopes to measure whole body protein kinetics and muscle fractional synthetic rate following creatine loading in men (N = 12) and women (N = 12). We found similar increases in total and phospho-creatine for the men and women and there was no effect mixed muscle fractional synthetic rate [76]. Two other studies have subsequently confirmed that creatine supplementation did not influence muscle protein synthesis in the fed state in men [77, 78]. For the men, but not the women, there was a reduction in leucine oxidation and whole body proteolysis following the creatine load [76]. Interestingly, the magnitude and direction of the creatine effect was identical to that observed in a recent study of young males given a hypotonic saline infusion to induce cell swelling [79]. These results suggested that the positive effect of creatine on protein metabolism occurs through an attenuation of protein oxidation and breakdown and not a by a stimulation of whole body or muscle protein synthesis. Given the recent demonstration that creatine loading results in an increase in intra-cellular water [80], it is likely that cell swelling is involved in some aspects of protein turnover via some sensing/messenger system. With similar increases in total and phospho-creatine between the genders and the different effect on protein metabolism, it is likely that there are gender specific responses in the downstream signaling pathways that respond to cell swelling.

Given that the increase in muscle total and phospho-creatine are similar between men and women, it would be predicted that the enhancement of high intensity performance would be similar. Using a randomized double-blind cross-over trial, we have shown that men and women had an increase in peak and mean power output on maximal cycle ergometry (30 s Wingate test) and in an isometric fatigue protocol following creatine monohydrate supplementation [81]. A recent «field study» found that elite female soccer players showed improved performance in agility and speed testing after creatine supplementation [82].

### Summary and practical recommendations for athletes

- In response to long-term endurance exercise (55–70% VO<sub>2PEAK</sub>), women oxidize proportionately more lipid and less carbohydrate and protein as compared to men.
- Women are able to carbohydrate load when consuming a diet containing more than 8 g CHO·kg<sup>-1</sup>·d<sup>-1</sup>. In order to attain this level of carbohydrate, some women will have to consume extra energy for the duration of the loading period (3–4 days). The magnitude of the increase in muscle glycogen may be slightly attenuated for women.
- Women show improvements in endurance exercise performance following the ingestion of glucose solutions containing 0.6–1.2 g CHO·kg<sup>-1</sup>·h<sup>-1</sup>. There does not appear to be any influence of menstrual cycle on these effects in women.
- Men and women show similar rates of glycogen re-synthesis when carbohydrate (~0.6–1.0 g·kg<sup>-1</sup>) and protein (~10 g) are consumed in the minutes following exercise. The consumption of similar amounts of carbohydrate and protein immediately after a work-out during a period of intensive training will result in improved nitrogen balance and performance for female athletes.

- Top sport or elite male and female athletes require more dietary protein and compared to sedentary individuals, however, this increase is usually met through an energy sufficient mixed diet. The suggested «safe» dietary protein intake for top sport male athletes is about 1.7 g·kg<sup>-1</sup>·d<sup>-1</sup> and for females is about 1.2–1.4 g·kg<sup>-1</sup>·d<sup>-1</sup>.
- Women are at greater risk for energy and protein insufficiency as compared to men due to the greater incidence of energy restriction. Strategies such as the consumption of carbohydrate and protein immediately after exercise (see above) can minimize the negative effects of energy insufficiency but may not be sufficient to allow for optimal carbohydrate loading.
- Following acute creatine monohydrate supplementation, women do not increase fat-free mass to the same extent as men. Women do show similar increases in muscle total and phospho-creatine after a creatine load and they show similar increases in high-intensity performance. Creatine supplementation will result in greater gains in fat-free mass and in some measures of strength following a period of weight training in men and women, however, the magnitude of the increase in fat-free mass is less for women (likely due to differences in testosterone concentration).

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