# EFFECTS OF CARBOHYDRATE CONTENT ON BODY COMPOSITION DURING WEIGHT LOSS IN FEMALE FITNESS COMPETITORS

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## CONTENT

## ABSTRACT

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**Introduction.** Bodybuilders and fitness competitors are aiming to reduce fat mass and maintain lean mass during the competition phase. Low carbohydrate diets compared to low fat diets have claimed to be more effective in weight loss, but the results are still controversial. Low carbohydrate intake and high protein intake have been reported in bodybuilders during the diet, but the evidence of its effectiveness is limited. The aim of this study was to examine the effects of carbohydrate content on body composition during weight loss in female fitness competitors.
**Methods.** A total of 50 female subjects participated in this study, 27 were competitors, and 23 were weight training stable controls. Twelve of the competitors were followed with relatively low carbohydrate (LC) to high carbohydrate (HC) diets. The HC group included six subjects (mean ± SD; age 27.5 ± 4.4 y, weight 64.8 ± 4.4 kg, and height 1.65 ± 0.03 m), whose carbohydrate intake during the weight loss was set > 145 g/d, and the proportion from the energy less than 7 % decrease from the baseline. The LC group included six subjects (mean ± SD; age 29 ± 3.3 y, weight 63.9 ± 7.7 kg and height 1.64 ± 0.06 m), and the values for carbohydrate intake during weight loss was < 120g/d, and the proportion from the energy at least 10 % decrease from the baseline. Nutritional data was analyzed based on the food diaries by nutrient analysis software (Aivodiet, Flow-team Oy, Oulu, Finland). The body composition was measured at two time points, before the diet and the day after the competition. The body composition was measured by both InBody bioimpedance and Dual Energy X-ray absorptiometry (DXA). Muscle cross sectional area of vastus lateralis (VL) muscle and thickness of triceps brachii (TB) muscle was measured using ultrasound. The energy expenditure was calculated by activity diaries as MET-hours and training frequency.
**Results.** The energy intake was significantly lower in the LC group than in the HC group during the diet (P=0.002), similarly the absolute carbohydrate intake (P=0.001), per kg body weight (P=0.002) and relative (P=0.02) was lower in LC. Protein intake was absolutely (P=0.03) and per kg body weight (P=0.04) lower in the LC group. Fat intake decreased slightly in both groups, but the relative intake decreased only in LC (P=0.05). Fat mass decreased in both groups as measured with all methods, but the loss was not significant between the groups. Fat in VL decreased more in the HC group (P=0.006) compared to the LC group (P=0.05), but was not significant between the groups (P=0.47). No significant change between the groups in fat or thickness of TB was observed. The VL CSA decreased in the LC group by 2.8 cm2 (P=0.023) and increased in the HC group by 0.9 cm2 (P=0.25), but the VL CSA was greater in the LC group at the baseline (P=0.005). Lean mass slightly increased during the diet in both groups when measured by DXA, but LBM remained unchanged, when measured by InBody bioimpedance.
**Conclusion.** The main finding of the study was that there were no consistent effects between the low and high CHO groups in the body composition and muscle or fat size changes. However, there were few differences between the groups. Fat mass decreased in both groups, but the VL CSA decreased only in the LC group compared to the HC group who even managed slightly to increase muscle size in front thighs. The findings support the fact that the energy deficit is the most important thing in weight loss rather than macronutrient content. It might be that normal carbohydrate with high protein intake would provide better outcomes in weight loss in female fitness competitors, for sparing muscle size at least in thighs, but the future investigation is needed.



**Keywords:** low carbohydrate, high carbohydrate, diet, fitness, fat mass, lean mass

# 1 INTRODUCTION

There is a lot of discussion of restricted energy intake and of repeated cycles of weight loss and regain in athletes. More specifically, women are becoming more interested in training for and competing in fitness competition (Andersen et al. 1995; Sandoval et al. 1989.) Fitness and bodybuilding competitors are judged on their physical appearance, a high level of muscularity and leanness, but not on their physical performance (Robinson et al. 2015; Halliday et al. 2016). The competitors aim is to develop an overall symmetrical physique (Van der Ploeg et al. 2001; Rossow et al. 2013; Kistler et al. 2014).

During the competition preparation, the aim is to reduce the body fat while maintaining lean mass by a combination of energy restriction, strength and aerobic training (Spendlove et al. 2015; Halliday et al. 2016). The preparation phase depends on individual athlete, but usually lasts about 12–24 weeks. The intake of low carbohydrate/low fat with high protein intake among bodybuilders and other aesthetic sport athletes are reported during this preparation phase (Spendlove et al. 2015; Helms et al. 2015b.) It seems that low carbohydrate and high protein diet is an effective method to rapid weight loss in some cases. Some authors show that the loss of body weight, and specifically, loss of fat mass and preservation of fat-free mass might be more effective in low carbohydrate and high protein diet compared to normal carbohydrate diet (Layman et al. 2005; Westman et al. 2007; Merra et al. 2016). High protein content during the weight loss may be beneficial for sparing the lean mass, and specifically, reducing fat mass (Santesso et al. 2012; Helms et al. 2015b).

On the other hand, some authors demonstrate that low carbohydrate, ketogenic diets, are connected to loss of fat-free mass, and thus reduced physical performance (Noakes et al. 2006). Hall et al. (2015) did not find any differences between low and high carbohydrate diet on body composition, and suggested that the total energy deficit is the main factor causing weight loss rather than only carbohydrate content. Johnston et al. (2008) found similar results about low and high carbohydrate diets on body composition. However, low carbohydrate diets have also reported enhanced catabolism of proteins in the body, in acutely and in long term (Lemon & Mullin 1980; Roy et al. 1997; Borsheim et al. 2004; Noakes et al. 2006). Therefore, it seems that adequate carbohydrate intake may be important for athletes. Thus, the results are controversial, and the studies about fitness and nutrition on body composition are lacking.

The aim of this study was to examine the carbohydrate content on body composition during weight loss in female fitness competitors. More specially, the effects of carbohydrate content on lean mass, fat mass and muscle size was investigated as well as possible individual differences.

# 2 NUTRITION

## 2.1 Carbohydrates

Atoms of hydrogen, carbon and oxygen combine to form a basic carbohydrate (sugar, monosaccharide) molecule in the general formula (CH20)*n*. *N* ranges from 3 to 7 carbon atoms with hydrogen and oxygen atoms, and they are attached by single bonds. Expect lactose and small amount of glycogen from animal origin, plants supply the carbohydrate source in the human diet. (McArdle et al. 2015, 8.) Carbohydrates can be divided by their structure and by the number of sugar molecules as either monosaccharides, oligosaccharides or polysaccharides (Wolinsky & Driskell, 2008, 27).

### 2.1.1 Structure of carbohydrates

Carbohydrate consist one or more monosaccharides, and are thus classified as monosaccharides, disaccharides and polysaccharides. The monosaccharides are the basic forms of carbohydrate, and the three major monosaccharides are glucose, fructose and galactose. Glucose, which is also called dextrose or blood sugar, consists of a 6-carbon, 12 hydrogen, and 6 oxygen atoms ($C_6H_{12}O_6$). Fructose and galactose are two other simple sugars with the same chemical formula as glucose.  They have a bit different C-H-O linkage, and thus are different substances with distinct biochemical characteristics. (McArdle et al. 2015, 8.) Disaccharides, such as sucrose, contain two sugar molecules, and are also characterized as simple carbohydrates. Disaccharides all contain glucose, and the three principal disaccharides include sucrose (glucose + fructose), lactose (glucose + galactose) and maltose (glucose + glucose) (Wolinsky & Driskell, 2008, 27).

Polysaccharides, with many glucose units chained together, are called complex carbohydrates (Wolinsky & Driskell, 2008, 27). Polysaccharides describes the linkage of three or more sugar molecules, and plant and animal sources both contribute to these large chains of linked monosaccharides. (McArdle et al. 2015, 8–10.) The common forms of polysaccharides are fiber, starch, dextrin and processed concentrated sugars (Wolinsky & Driskell, 2008, 27). Fiber, classified as a nonstarch, structural polysaccharides, includes, cellulose, the most abundant organic molecule on Earth (McArdle et al. 2015, 8–10).

Glycogen is the storage carbohydrate within muscle and liver in humans. It forms as a large polysaccharide polymer synthesized from glucose in the process of glycogenesis, which is catalyzed by the enzyme glycogen synthase. Glycogen varies from a few hundred to 30,000 glucose molecules linked together. (McArdle et al. 2015, 8–10.) However, our bodies have a limited capability to restore carbohydrates, and the greatest amount of glycogen usually varies from 300 to 400 grams (Wolinsky & Driskell, 2008, 29). The clucose in the blood is about 2–5 grams, and the liver contains about 75–100 grams of glycogen. (Wolinsky & Driskell, 2008, 29; McArdle et al. 2015, 8–10.) Each gram of glucose or glycogen contains approximately 4 calories (kcal) of energy. The body stores a little glycogen, and for example, fasting and low carbohydrate diet depletes the glycogen stores in 24 hours. In addition, maintaining the high carbohydrate diet for several days, could double the body`s glycogen stores compared to normal well-balanced diet. (McArdle et al. 2015, 8–10.)

### 2.1.2 Functions of carbohydrates in the body

Carbohydrate serve a few different functions in the human body, they are mainly related to the energy metabolism and exercise performance. Carbohydrates serve as an energy fuel, usually during intense physical activity (Wolinsky & Driskell, 2008). Energy received from the catabolism of blood borne glucose and muscle glycogen powers, in part, the contractile components of muscle and the other forms of biological work. Once cells reach their maximum capacity of glycogen, and when all energy for carbohydrates are not used for energy, the excess sugars convert to and store as fat. That`s why body fat can increase even though the amount of lipid for the energy intake is low. (McArdle et al. 2015, 14.)

Depletion of glycogen stores, occurring with starvation, reduced carbohydrate or energy intake and strenuous exercise, strongly affects the metabolic mixture of fuels for energy (Wolinsky & Driskell, 2008, 26). Depletion of glycogen stores usually impair power output and reduce the general work rate during training and competition, due to prolonged periods of multiple sprints or low carbohydrate diet. Adopting nutritional strategies to ensure that muscle glycogen stores are full prior to training and competition helps delaying the fatigue. The carbohydrate intake after exercise increases the repletion rate of muscle glycogen, which is essential for athletes training or competing on successive days. (Williams & Rollo 2015.) The glycogen stores can be refilled with 24 h of reduced training and adequate fuel intake

(Burke et al. 2011). The previous studies have stated that high glycogen stores are achieved without depleted phase and “carbohydrate loading” in 24-36 hours of carbohydrate intake and rest (Bussau et al. 2002).

Adequate carbohydrate intake can also help to maintain tissue protein. Furthermore, to stimulate fat catabolism, depletion of glycogen stores triggers glucose synthesis from the labile pool of amino acids. This conversion offers a metabolic possibility for augmenting carbohydrate availability even with insufficient glycogen stores. Elements of carbohydrate catabolism serve as “primer” substrate for fat oxidation. Thus, insufficient carbohydrate breakdown causes fat mobilization to exceed fat oxidation, either through limitations of glucose transport into the cell or glycogen depletion though inadequate diet or prolonged exercise. (McArdle et al. 2015, 14.) Strenuous or repeated high- intensity exercise may deplete the muscle glycogen stores (Wolinsky & Driskell, 2008). Peripheral depletion of muscle glycogen in sub-cellular compartments such as the sarcoplasmic reticulum will influence the flux of calcium and impair the contractile property of the muscle (Williams & Rollo 2015).

Carbohydrates are also fuel for the central nervous system, and it requires carbohydrate for the normal function. Normally the brain metabolizes blood glucose almost exclusively as its fuel source. The chronic low-carbohydrate, high-fat diets induce the adaptations in the muscles to spare muscle glycogen and increase fat use during low- to moderate physical activity levels. (McArdle et al. 2015, 14–15.) Exercise following a low carbohydrate diet has remarkable influence on the expression of genes that promote an increase in fat metabolism (Yeo et al. 2010).

## 2.2 Proteins

Protein and amino acids have potent biological effects that involve all tissues and extend nearly all metabolic processes in the body. (Wolinsky & Driskell, 2008, 64.) Proteins are combinations of linked amino acids, and a normal-weight adult contains between 10–12 kg of protein. The structure of proteins is more like in carbohydrates and lipids because they contain atoms of carbon, oxygen and hydrogen.

### 2.2.1 Structure of proteins

Just as glycogen forms from many simple glucoses linked together, the protein molecule polymerizes from its amino acid “building-block”. Peptide bonds link amino acids in chains constitute of chemical combinations, two joined amino acid produce a dipeptide, and linking three amino acids produce a tripeptide.

The 20 different amino acids required by the body each have a positively charged amine group and negatively charged organic acid group at the other end. The organic acid group contains 1 carbon atom and, 2 oxygen atoms and 1 hydrogen atom (COOH), whereas the amine group has two hydrogen atoms attached to nitrogen ($NH_2$). (McArdle et al. 2015, 30.) The body cannot synthesize the eight amino acids and are called essential (leucin, isoleucin, lysine, valine, methionine, phenylalaline, threonine and thryphophan). (Wolinsky & Driskell, 2008, 81; McArdle et al. 2015, 30.) Infants cannot synthesize histidine, and children have reduced capability for synthesizing arginine (McArdle et al. 2015, 30). In addition, body can synthesize cysteine from methionine and tyrosine from phenylalaline. The rest 10 of amino acids are nonessential, which means that body can synthetize them from other compounds (Wolinsky & Driskell, 2008, 81; McArdle et al. 2015, 30).

### 2.2.2 Functions of proteins in the body

Amino acids are essential components of muscle, skin, cell membranes, blood, hormones, antibodies, enzymes, genetic material, and almost all other tissue components in the body. (Wolinksy & Hickson, 1998, 12; McArdle et al. 2015, 30.) Amino acids provide the major building blocks for synthesizing tissue. They also corporate nitrogen into coenzyme electron carrier nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), heme components of hemoglobin and myoglobin compounds, catecholamine hormones epinephrine and norepipherine, and the serotonine neurotransmitter. Amino acids also activate vitamins, which play important role in metabolic and physiological regulation in the body.) Furthermore, they regulate the catabolism of nutrients for energy release. In addition, proteins help to regulate the acid-base characteristics of the fluids in the body, and play a key role in the muscle contraction. Some protein is catabolized for energy, and in well-nourished individuals at rest, protein catabolism varies between 2 to 5% of the body`s energy metabolism. (McArdle et al. 2015, 31–33.)

### 2.3 Fats

The lipids are classified usually to the three main groups, and they are simple lipids, compound lipids and derived lipids (McArdle et al. 2015, 18). The prominent lipids found in human are FFA, triglycerides (TG), steroids, phospholipids, prostaglandins, fat-soluble vitamins, provitamins and lipoproteins (Wolinsky & Hickson, 1998, 103).

#### 2.3.1 Structure of fats

The first group is simple lipids or "neutral fats". Fatty acids are bond to the glycerol molecules of the three clusters of unbranched carbon chained atoms. A carboxyl (–COOH) cluster at one end of the fatty acid chain give the molecule as its acidic characteristics. (McArdle et al. 2015, 18–19.) Fatty acids are bonded to a single glycerol molecule, and they may have one or more double bonds between carbon atoms (Wolinsky & Driskell, 2008, 52). Thus, fatty acids are divided to the two different groups, saturated and unsaturated fats. (Wolinsky & Driskell, 2008, 52; McArdle et al. 2015, 18–19.) Another type of fat is called as "trans" fat, and they are synthetic fats produced by infusing unsaturated fats with H ions in at the double bond sites, creating a newly saturated molecule (Wolinsky & Driskell, 2008, 52).

The second group is compound lipids, and they represent 10% of the body`s total fat content. One group of modified triacylglycerols, are the phospholipids. Another compound lipid includes glycolipids, and water-soluble lipoproteins. The last group is combination of simple and compound lipids, and they are called derived lipids. Cholesterol is the most known derived lipid. (McArdle et al. 2015, 19–25.)

#### 2.2.2 Functions of fats in the body

Fat provides as much as 80–90% of the energy requirement at the rest state. Fat carries a large quantity of energy per unit weight, a one gram of pure lipid contains nine kcal of energy. Fat transports and stores easily, and it provides ready source of energy. Fat protect the vital organs (e.g., heart, liver, kidneys, spleen, brain and spinal cord) against the trauma. Fat stored just below the skin permit the individuals getting extremely cold, so fat act as a thermal insulation. Fat act as a carrier of fat-soluble vitamins (e.g., A, D, E and K), and dietary lipid facilitates absorption of vitamin A precursors from nonlipid plant sources. (McArdle et al. 2015, 19–25.)

# 3 STRENGTH TRAINING

## 3.1 Definition

Strength training has become increasingly popular in last decades. Whereas strength training had been used by a few selected sports to improve their strength and size, it is now an important component in training for most sports (Wernbom et al. 2007). High-resistance strength training is one of the most practiced forms of physical activity, and it is used to improve athletic performance, augment musculoskeletal health and change body composition (Folland & William 2007). Muscle mass is a crucial factor of performance in sports that depend on high muscle strength or power. The benefits of strength training include improved physical performance and health, mostly by changing the body composition. (Walberg-Rankin 2002.) Among athletes the important goal for strength training is to prevent injuries and the concept of training specificity is also used in strength training. (Pearson et al. 2000). The maintenance of muscle mass is quite challenging for many athletes. Thus, increasing muscle mass off-season, and maintain the muscle muss during the competition season is important goal for many athletes, especially in aesthetic sports (Walberg-Rankin 2002.) Resistance training is also known as a best strategy to enhance muscle strength and size. However, it may also be effective of many other health benefits such as enhanced cardiovascular and bone health and functional capacity in daily activities (Westcott 2012).

It is well known that systemic strength training has potent stimulus improving the functionality of the neuromuscular system. These are for example, increasing size and strength of skeletal muscle due to multiple factors such as mechanical stress, neuromotor control, metabolic demands, and endocrine activities (Kraemer et al. 2002; Ahtiainen et al 2005). Depending on the specific program design, strength training can lead of improving each of the functional constituents of the neuromuscular system, strength, power, or local muscular endurance. The improvements in performance are related to the physiologic adaptations caused through prolonged resistance training. Optimal resistance training programs to improve strength or power are individualized to meet specific training goals. (Kraemer et al. 2002.

### 3.2 Classification of resistance training

The adaptations to exercise training and performance improvements are well specific to mode of the training (Ahtiainen et al. 2005). Many types of resistance training modalities (isokinetic, variable resistances, plyometric and isometric) can be used to meet the set goals. In addition, a variety of training systems or programs can produce a significant improvement in strength and muscle hypertrophy as long as an effective training stimulus is presented to neuromuscular system. The effectiveness of each specific resistance training system or program based on its efficacy and proper use in the total exercise prescription or program. (Fleck & Kraemer 2014, 1–3.) The main variables in strength training are the intensity, number of repetitions and sets, volume, length of rest periods between the sets, training velocity and frequency of exercise session (Ahtiainen et al. 2005; Salles 2009; Hawley 2009). The quantification of the dose-response relationships between the training variables (intensity, frequency and volume) and the outcome (power, strength and hypertrophy) is essential for the proper prescription of strength training. (Wernbom et al. 2007).

*Maximal and hypertrophy strength.* Muscle strength is determined by muscle contractile properties, such as muscle fibre composition, type and muscle fibre possession area, and muscle cross-sectional area (CSA). (Schantz et al. 1983.) The definition of maximal strength is explained by the greatest amount of force a muscle or muscle group can generate in a specific movement pattern at a specified velocity (Knuttgen & Kraemer 1987). Two types of training are typical to enhance strength in the muscles: the training of maximum force (1-5 RM series) enhance the neural adaptation, which includes improved neuromuscular coordination, recruitment of motor units and motor units firing rate or hypertrophic strength training (6-12 RM series), which increases the primary muscle cross-sectional area. Thus, the maximal strength can be divided into neural and maximal hypertrophic strength. In the neural strength training the load is 85–100% of 1RM, and 1–3 reps in the sets (3–5 sets). And the rest between the sets is 3–5 minutes. The strength enhances by the neural adaptations in the muscles. The improved strength due to neural strength training is caused primarily by the increased neuromuscular muscle coordination and the nervous system ability to activate muscles. (Zatsiorsky & Kraemer 2006, 60.)

In hypertrophy training it has been generally recommended to use multiple sets per exercise, a moderately high number of repetitions (8–12RM) per set, and short rest periods (60–120 seconds) between the sets, but also higher repetitions increase muscle size if conducted until failure (Fink et al. 2016). Short rest periods between the sets are recommended to achieve a longer duration of time under tension along with a great anabolic hormonal response to induce improvement in muscular hypertrophy (Fisher et al. 2013). The cross-sectional area of trained muscles improvement comes mainly from the increase in size of individual muscle fibers (MacDougall et al. 1977). Further improvements in strength and training-induced muscle hypertrophy are more limited in well-trained subjects. For example, like strength athletes, compared to previously untrained subjects. (Häkkinen 1994.) Increasing in muscle hypertrophy and strength is also dependent on the type and intensity of loading, such as volume of the strength training of each individual strength athlete at a given time (Ahtiainen et al. 2003).

There have been some studies of a positive relationship between muscle strength and muscle CSA (Fukunaga et al. 1992). One study supported previous investigations of the relationship between CSA and strength ($r=0.54-0.64$, $P < 0.05$) in the knee extensors/flexors and quadriceps femoris/hamstrings (Masedu et al. 2003). In addition, Trappe et al. 2001 showed a positive correlation between calf muscle strength and muscle size ($r = 0.76$; $p < 0.05$). In contrast, it seems that is possible to gain strength without hypertrophy. Specifically, among the untrained subjects, the neural adaptation occurs during the first weeks, and hypertrophy in the later phase of training. Thus, it has suggested that strength gains due to improved neural recruitment pattern rather that hypertrophy of the muscle fibers. The findings of Häkkinen & Komi (1998) study showed that the enlargements in the CSA of the trained muscles over the 6 months of training regimen were minor compared with improvements recorded in maximal strength. (Häkkinen & Komi 1998.) As mentioned earlier, most heavy resistance training programs will see improvements in muscle strength and size. But it seems to be important to use variable resistances in training for long-term progression in resistance training. Within a periodized training program, resistance ranges have been varied using light (12–15RM), moderate (8–10 RM), and heavy (3–5 RM) resistances over a training cycle. (Kraemer et al. 2004.)

*Power strength.* The definition of muscular power is explained as a force applied multiplied by the velocity of movement (Kraemer et al. 2002). Power can also be described as work completed per unit time (rate of performing work) because produced work is equal to the force multiplied by the distance moved, and velocity is the distance traveled divided by the time taken (Grahammer 1993). In other words, power can be explained by the rate of doing work or work divided by the time. Power can be increased by lifting the same weight the same vertical distance in the same period of time as a lighter resistance or same weight with larger speed (Fleck & Kraemer 2014). To improve power in the muscles, the recommended loads should be 30 – 60 % of 1RM, also the intensity of each training session should be maximal. In power lifting the loads are weight and the velocity of each movement is high. (Kraemer & Häkkinen 2002, 20–30.)

### 3.3 Effects of resistance training on body composition

Body composition can be divided in different components: fat-mass and fat-free mass. Fat-free mass is usually explained by all non-fat tissue whereas the lean mass refers to all non-fat tissue and, as well as essential fat. Dual-energy X-ray absorptiometry is typically used for measuring body composition in the studies, and it doesn`t differentiate the essential fat and non- essential fat. Because of this fat-free mass is usually being determined (Fleck & Kraemer 2014, 109.) Increase in fat-free mass (muscle CSA) and decrease in fat mass are the most remarkable changes on body composition in resistance training. (McArdle et al. 2015, 499–530.)

Häkkinen et al. (1987) showed great results on the elite Olympic weight lifters body composition after a one year of training. They exhibit an increase in fat-free body mass 1% or less, and decrease of a body-fat up to 1.7 %. These changes are much smaller than those shown by untrained or moderately trained subjects in body composition over a much shorter period of training. This indicates that causing changes in body composition in highly fit people, such as athletes (e.g., bodybuilders and weightlifters), and advanced fitness enthusiast, is more difficult than untrained or moderately trained subjects. Among untrained subjects the fat percent could decrease from 1 % to 12 % and increase of lean body mass from 0.5 kg to 3.3 kg during resistance training period even in short period of time. (Fleck & Kraemer 2014, 113.)

Additionally, when people reach essential fat levels, the high proportion of weight loss is unfortunately fat-free mass. And this is true even in well-trained weightlifters or bodybuilders who continue to strength training in weight loss period aiming to lose fat mass and body weight. (Cullinen & Caldwell 1998.) Kraemer et al. (2004) demonstrated that two heavy resistance exercise training protocols appeared to be effective in producing an increase strength, hypertrophy, and power in previously untrained women. Their findings were similar than other studies in the literature with fat-free mass increased ranging from 2 to 4%. (Kraemer et al. 2004.) Sillanpää et al. (2008) demonstrated that progressive strength and endurance training decreased the percentage of body fat similarly both separately and combined. Waist circumference decreased 2-3 cm in all training groups. Thus, these findings are consistent with previous studies showing that strength training can improve body composition and decrease abdominal obesity in the absence of changes in body weight. (Kay & Fiatorone 2006; Tsutzuku et al. 2007; Sillanpää et al. 2008; Dudgeon et al. 2016.) Strength training induces decreases in percent fat and increases of fat-free mass, and total body weight shows small increases over short training periods (Fleck & Kraemer 2014, 115).

# 4 EFFECTS OF CARBOHYDRATE INTAKE ON BODY COMPOSITION DURING WEIGHT LOSS

Carbohydrate restricted diets are popular weight loss strategies by exercising individuals, athletes and obese people.  Diets for targeted in weight loss usually recommend restriction either carbohydrates or fat (Hall et al. 2015). Many studies has suggested that low carbohydrate, and high protein diets and their variation are effective in weight loss (Samaha et al. 2003; Velderhost et al. 2005; Volek et al. 2005; Shia et al. 2008; Paoli et al. 2012). The low carbohydrate and calorie restriction might be effective to reduce weight mainly in obese people, and there is some physiological mechanism for explaining the effectiveness. (Merra et a. 2016).

Low-fat diets were popular in the 20th century, and the interest of these diets is raised in recent years. In the previous study, the authors have shown that decreased insulin secretion causes increased release of free fatty acids from adipose tissue, higher fat oxidation and energy expenditure, and greater body fat loss than restriction of dietary fat. (Westman et al. 2007.) Anyhow, the wide review studies have shown that there are no differences between low carbohydrate and low fat diets in weight loss (Hu et al. 2011). But since the food records rely on self-reporting, it is difficult to interpret the results from those studies, and accurately measure adherence to the recommended diets (Hall et al. 2015).

## 4.1 Low vs. normal carbohydrate intake in weight loss

Some authors have claimed that low carbohydrate diets are more effective in weight loss and specifically in fat loss, compared to high carbohydrate and low fat diet (Brinkworth et al. 2009; Paoli et al. 2012; Volek et al. 2005; Volek et al. 2006; Noakes et al. 2006). On the other hand, low carbohydrate diet has reported enhanced catabolism of proteins in the body, in acutely and in long term, which is not beneficial, specifically for athletes (Lemon & Mullin 1980; Roy et al. 1997; Borsheim et al. 2004; Noakes et al. 2006). In addition, it has also reported to increased amino acid oxidation in glycogen depleted state in elite athletes (Wagenmakers et al. 1991). Therefore, it seems that adequate carbohydrate intake is important for athletes for maintaining protein synthesis (Gaine et al. 2006).

There is some possible mechanism behind the weight loss in carbohydrate restricted diets, but it still seems controversial. The list includes reduced calorie intake, loss of body water, changes in energy expenditure, and different utilization of calories. Referring to latter, there is some evidence based studies that people on low carbohydrate diet can eat more calories than those on high-carbohydrate or normal carbohydrate diet, and still lose some weight. That may be explained by the thermodynamic effect. (Volek et al. 2005.) However, the effects of this phenomenon have shown to be very small.

It`s well known, that the amount of body water plays an important role in weight loss, and it might explain the rapid weight loss in the beginning of the diet compared to normal mixed diet. It´s mostly due to diuresis consequent or reduction of the body water by decreased glycogen stores, which usually occur in the first weeks (WeSterter-Plantenga et al. 2009). The explanation, why low carbohydrate diet enhances weight loss might also due to hormonal changes (Manninen 2004). The possible mechanism are following; decreased insulin levels and raised levels of glucagons, leading to activation of phosphoenolpyruvate carboxykinase, fructose 1,6-biphosphatase, and glucose 6-phosphatase and inhibition of pyruvate kinase, 6-phosphofructo-1-kinase, and hexokinase, favoring gluconeogenesis over glycolysis. (Hue 2001; Manninen 2004.) However, these effects are probably small as explained by Hall et al. (2016).

Insulin modulates the activity of several enzymes, and the role of these enzymes is to promote the uptake, retention and net storage of fat in adipose tissue. At the same time, the finding that dietary carbohydrates are the main driver of insulin secretion, have led to the hypothesis that high carbohydrate diets are mainly fattening (Hall et al. 2016). However, the carbohydrate–insulin model of obesity, assume that high carbohydrate diets enhance insulin secretion and, thus suppress the release of fatty acids from adipose tissue into the circulation. The model predicts that low carbohydrate diet and high in fat, but identical protein and calories, would reduce insulin secretion, enhance fat mobilization from adipose tissue and increase fat oxidation compared to high carbohydrate diet. The altered metabolic and hormones associated with reduced dietary carbohydrate will thus relieve the state of cellular internal starvation resulting in decreased hunger, increased body fat loss and increased energy expenditure compared with an isocaloric diet with higher carbohydrates and higher insulin secretion. (Hall et al. 2016.)

However, it may be that this phenomenon operates mainly by affecting energy intake such that low carbohydrate diets decrease hunger, reduce appetite and promote satiety rather than providing any metabolic advantage for body fat loss. Hall et al. (2015) investigated the weight loss on obese people in two different diets, restriction of carbohydrate or fat. They resulted that, carbohydrate restriction led to maintained increases in fat oxidation and loss of 53 ± 6 g/day of body fat, whereas fat oxidation didn`t change by fat restriction, leading to 89 ± 6 g/day of fat loss, and was significantly greater than carbohydrate restriction (P=0.002.) Johnston et al. (2014) examined a large meta-analysis of the effects of different diets on body composition. There wasn`t any differences between the diets (low carbohydrate vs. low-fat) on weight loss during 6-month weight loss, and all the regimens resulted about 8 kg weight reduction. The energy deficit seems to be the most important thing in weight loss with high protein intake. (Johnston et al. 2014.) In addition, it has reported that subjects with high carbohydrate diet (70 E%) have lost weight with energy deficit, and seems to be as useful method as low carbohydrate diet to lose weight. (Shintani et al 2001).

In the systematic review of Hession et al. (2008), they compared low carbohydrate/high protein diets with low fat/high-carbohydrate diets. The main finding was that weight loss was significantly greater in the low carbohydrate group after 6 and 12 months compared with low fat group. The difference was greater at 6 months resulting 4 kg greater weight loss and at that time-point there was significant heterogeneity among the studies. At time of 12 months the heterogeneity was no longer significant and the weight loss was only 1 kg greater in low carbohydrate group. Thus, the low carbohydrate diet did not provide better outcomes in long-term. (Hession et al. 2008.) Samaha et al. (2003) reported similar results on low carbohydrate diet compared to low fat diet, but they argued that the greater weight loss in the low carbohydrate group was due to lower caloric intake rather than a direct effect of macronutrient composition. Sacks et al. (2009) reported that reduced-calorie diets (low carbohydrate, low fat) resulted in weight loss regardless of which macronutrients they emphasized. Furthermore, Cardillo et al. (2006) reported that the weight loss rate did not change from baseline between low carbohydrate and low fat diets at 36 months. In conclusion, there are statistically some differences between some of the diets, anyhow these differences are so small and unimportant to many people targeting for weight loss. For example, Atkin`s diet resulted only 1.7 kg weight loss more than Zone- diet. The ideal diet is the one that is best adhered to by individuals so that they can stay on the diet as long as possible, because many diets are hardly tolerated. (Johnston et al. 2014.)

### 4.1.1 High protein and low carbohydrate diet without moderate to low fat intake

There has been much discussion surrounding the metabolic effects of weight loss diets that differ in macronutrient content (Hall et al. 2012). Some researchers believe that the weight loss is due to reduced calorie intake, increased satiety from protein rather than the low carbohydrates (WeSterter-Plantenga et al. 2009; Hall et al. 2015; Hall 2016.). In contrast, some other researchers believe that there is a metabolic gain, on the grounds of the first law of hemodynamics or law of preservation of energy. However, some studies show that the people who follow the low carbohydrate diet lose more weight compared to those who follow the balanced diets, but some authors denied allegations. (Brehm et al. 2003; Gardner et al. 2007; Shai et al. 2008: Hession et al. 2008).

Higher protein intake and low carbohydrate intake has been reported in weight loss because of its appetitive properties. Such diets reportedly have high satiety value, yield less metabolizable energy because of their high thermogenic effect, and aim in preservation of lean body mass, which also can enhance energy expenditure. Johnstone et al. (2008) reported that a high-protein, low-carbohydrate ketogenic diets reduced hunger leading to lower energy intake significantly more than high-protein, medium-carbohydrate nonketogenic diet. Dhillon et al. (2016) presented a significant correlation of protein on fullness, the findings supported that higher protein preloads have a significantly greater effect on fullness than lower protein preloads. (Dhillon et al. 2016.) There is also some evidence based studies that increased dietary protein leads to improved body composition and anthropometrics under iso-, hypo-, and hyper-caloric conditions. High protein diet had reported better results on body composition compared to high carbohydrate diet even though the subjects lost more muscle mass due to greater weight loss. (Motjahedi et al. 2011.) Diets with low carbohydrate <150g / d and high protein content (25-30% E%) seem to provide greater fat mass reduction, when compared to diets with lower protein content in obese participants (Layman et al. 2005; Stiegler & Cunliffe 2006). Motjahedi et al. (2011.) has also suggested daily protein intake of energy between 25%–30% for weight loss and maintenance of lean mass. According to the study of Helms et al. (2014) it may be that the range of 2.3–3.1g/kg of protein of FFM is the most consistently protective intake against losses of fat free mass (Helms et al. 2014). Study by Layman et al. (2005) resulted in increased weight and body fat loss with high protein intake (>1.4 g/kg/day). In contrast, Krieger et al. (2006) reported that protein intake of >1.05 g/kg/day maintained fat-free mass 0.6 kg more compared to studies in which the protein intake was <1.05 g/kg/day. It might that increased satiety and decreased energy intake are possible mechanism for improved weight loss with higher protein intake.

High protein intake increases protein synthesis by increasing the amino acid availability (Paddon-Jones et al. 2004). Also, protein such like whey proteins have potential as a functional food component to contribute to the regulation of body weight by providing satiety (Borsheim et al. 2002). Whey protein also has a blood glucose lowering effect. It is known that the effects of whey protein on metabolic mechanism seem to be in muscle protein synthesis (Merra et al. 2016). The results in studies proved the importance of protein quality as a determinant of lean body mass responses during resistance training and weight loss (Motil et al. 1981). It seems that the biological value of protein is important factor during the weight loss (Merra et al. 2016). The BCAA leucine plays multiple roles in metabolism including a key regulator of translation initiation of protein synthesis in skeletal muscle (Harper et al. 1984; Anthony et al. 2001; Layman et al. 2003). Layman & Walker (2006) suggested that the beneficial effects of a higher protein diet include the roles of leucine in sparing muscle protein loss and enhancing glycemic control in terms of low carbohydrate with high protein diet (Layman et al 2003; Layman et al. 2004; Layman & Walker 2006).

Reduction of fat mass during the weight loss usually occurs, but lean mass can also decrease if the weight loss continues for long time. Specifically, lean athletes lose more lean mass than those with higher fat mass (Huovinen et al. 2015). The meta-analysis showed a greater weight loss, decrease in BMI and a greater loss in waist circumference in high protein diet (Santesso et al. 2012). The low carbohydrate and high protein diet preserved the muscle mass during the calorie restriction and weight loss in low carbohydrate and high protein diet (Merra et al. 2016). Also, Young et al. (1971) compared the effects of 3 isoenergetic (1,800 kcal/d), isoprotein (115 g/d) diets, which contained varied carbohydrate contents (30, 60, and 104 g/d) on weight loss and body composition assessed via underwater weighing in obese men. They resulted under 9 week of intervention greater results with amount of carbohydrate <30 g/d and 16 kg of weight loss and fat accounted 95% of the weight lost, respectively. Finally, it seems that high or moderate protein intake could be more effective in weight loss compared to high fat or high carbohydrate diets (Santesso et al. 2012).

### 4.1.2 Low carbohydrate and high fat diet with high or moderate protein intake

Low carbohydrate and high fat diets became popular in 1970s. The diet promoted the weight loss, but unfortunately also includes high levels of saturated plasma fats. As the public became more conscious of the negative effects of elevated triglycerides and cholesterol, this diet program fell out of favor. Largely as the result of popularization by Atkins, low fat high carbohydrate diets have become popular again as a target to weight loss. (Veech et al. 2004.)

Volek et al. (2002) also investigated the low carbohydrate and high fat diet on body composition. Total loss of weight over 6 weeks was -2.2kg, mainly from fat and a significant increase in lean body mass in low carbohydrate group. On the other hand, during the ketogenic diets, the loss of bodyweight is primarily from the loss of water, thus it`s seems to be effective way to lose weight rapidly. The weight loss on the mix diets, tended to be loss of fat rather than water, therefore, seems to be more effective in long-term (Yang &Van Itallie 1976; Hall et al. 2015). Cornier et al. (2005) divided the subjects into the groups based on their insulin sensitivity. Insulin-sensitive women on the high carbohydrate and low fat diet lost 13.5 $\pm$1.2% of their body weight, whereas the subjects on the low carbohydrate and high in fat body weight losses was 6.8 $\pm$ 1.2%. In contrast, the insulin-resistant women, those on the low carbohydrate and high fat diet lost 13.4 $\pm$1.3% of their body weight and the loss of body weight was 8.5$\pm$1.4% by those on the high carbohydrate and low fat diet. These differences are not explained by changes in resting metabolic rate, activity, or intake. Total body insulin sensitivity is explained by mechanism of insulin to regulate glucose uptake and the metabolism. The people with insulin resistance, needs greater than normal levels of insulinemia for maintaining glycemia. In addition, it might be the state of total insulin sensitivity may have effect on individual responses to macronutrient composition of the hypocaloric diet (Yeni-Komshian et al. 2000; Cornier et al. 2005).

Veldhorst et al. (2009) resulted in low carbohydrate, high protein, and high fat diet leading to higher EE, and 42 % of the increased EE was explained by increased gluconeogenesis. Although insulin is known to be able to influence gluconeogenesis, but insulin was not responsible for a change in glucose production or gluconeogenesis in that study. Others energy-requiring pathways in protein metabolism, such as protein synthesis, may also contribute to the increase in EE after a low carbohydrate, high-protein and fat diet. (Veldhorst et al. 2009.)

Anyhow, a greater loss of fat and preservation of lean body mass has also been shown with very low-carbohydrate diets in previous studies. (Manninen 2004.) In contrast, Noakes et al. (2006) resulted in decreased lean body mass on very low carbohydrate diet (4% CHO, 61% fat and 35 % protein) and low fat diet (70% CHO, 10% fat and 20% protein), compared to high unsaturated fat diet (50% CHO, 30% fat, 6% of high unsaturated fats, 20 % protein). Tinsley et al. (2016) examined that, carbohydrate restricted diets lead to decreases in blood glucose, thus it`s possible that increased gluconeogenic activity could promote the breakdown of muscle tissue to provide amino acid substrate. (Freeman et al. 2006; Tinsley et al. 2016.) Although this is known to occur during complete fasting, ketogenic diets promote a pseudofasted state in which oxidation of fatty acids primarily meets energy needs because of the lack of dietary carbohydrate. Decreases in resting insulin concentrations have been reported in response to 3 to 4 days of a low-carbohydrate diet high in fat. (Langfort et al. 1996) The mechanism for such a response might be in the greater reliance on fat oxidation induced by dietary carbohydrate restriction and subsequent reduced requirement for insulin to assist in glucose uptake. (Volek et al. 2002.) In contrast, some studies have shown no greater weight loss on low carbohydrate and high fat diets compared to high carbohydrate and low fat diets. (Cornier et al. 2005; Hall et al. 2015; Hu et al. 2011)

## 4.2 Effects of low vs. normal carbohydrate intake on physiological performance and health

*Health.* It has reported in some studies that low carbohydrate and high protein diet reduced the risk of cardiovascular risk in a short term, which could decrease the high frequency of DVC or mortality. (Noakes et al. 2006.) However, recent cohort studies don`t support hypothesis, and their health-related benefits in long-term and risk remains controversial (Lagiou et al. 2007; Fung et al. 2010). In addition, in low-carbohydrate diets, the intake of fiber and fruits is reduced compared to low-fat diets, and higher intake of protein from animal sources, cholesterol and saturated fat. All of those mentioned earlier are risk factors for mortality and CVD. (McCullough et al. 2002) Low carbohydrate diets are usually rich in protein, and animal-based protein foods usually contain higher levels of saturated fat and cholesterol that may adversely affect low-density lipoprotein cholesterol (LDL-C) levels, as it has reported. (Lagiou et al. 2007; Fung et al. 2010) It may lead to increase the risk for cardiovascular disease, although some studies demonstrate that lipid–lipoprotein levels improve when carbohydrate intake is decreased (Volek & Sharman 2004; Haub et al. 2005). Some authors claim that carbohydrate diets seem to enhance glycemic control and some other features of the metabolic syndrome, and might be beneficially influence risk factors for cardiovascular diseases (Noakes et al. 2006; Volek et al. 2008). Hu et al. (2011) studied of metabolic risk factors by comparing low-carbohydrate and low fat diets on their review article. The participants on low-carbohydrate diets had slightly but statistically significantly reduced total cholesterol, and low density lipoprotein cholesterol, but a greater enhance in high density lipoprotein cholesterol and a greater decrease in triglycerides. The review study reported no significant differences of the body weight reduction, waist circumference or metabolic risk factors between the diets. The findings suggest that low-carbohydrate diets are at least as useful methods as low-fat diets in weight loss and to improve metabolic risk factors. Hu et al. (2011) recommended low carbohydrate diets to obese people if they have abnormal metabolic risk factors for the aim of weight loss. Shai et al. (2008) resulted in positive effects on blood lipids, because HDL cholesterol increased during the weight loss and the greatest improvement (20%) appeared in low-carbohydrate diet group. In addition, potential metabolic strain on liver and kidney function (kidney stones) and electrolyte or pH imbalance are associated with the negative effects of LCDs. (Yancy et al. 2007; Cook & Haub 2007.)

*Performance.* Some athletes (eg.,bodybuilders) regularly need to reduce fat and/or weight before competition preferably without affecting muscle strength or muscle size and a VLCKD (very low carbohydrate ketogenic diet) is commonly used to achieve this, even though some authors disagree the effectiveness (Shepfard 1994; Noakes et al. 2006; Hu et al. 2011; Hall et al. 2015) Many coaches do not prefer the use of a ketogenic diet by their athletes, both due to the lack of knowledge of the effects of the LCKD and due to fear that the diet can have a negative effect on the physical performance of the athlete. (Paoli et al. 2012.)

Generally, it`s known, that low carbohydrate diets have a potential to deplete muscle and liver glycogen stores, leading to symptomatic side-effects of tiredness, weakness, or fatigue during high intensity exercise (McDougall et al. 1999; Helms et al. 2015b). These effects may reduce muscle performance, increase muscle fatigue, and have negative effects on physical function and exercise tolerance. That may compromise an individual’s capacity to adhere to an exercise regime and reducing the usefulness of low carbohydrate diets as part of a comprehensive weight loss program. (Bandini et al. 1994; Tinsley et al. 2016.)

It`s reported in many previous studies that low carbohydrate and high fat diet impairs the performance in long-term, and it`s not recommended, specifically for elite athletes. (Burke et al. 2016; Hawley et al. 2015.). Burke et al. resulted increased rate of whole-body fat oxidation during exercise on those who followed a ketogenic low-carbohydrate high-fat (LCHF) in elite race walkers. This adaptation results in reduced economy, which means that oxygen demand is increased for a given speed, at velocities that translate to real-life race performance. Even though there are some periods with higher carbohydrate intake, adaptation to LCHF impairs performance in elite endurance athletes despite a significant improvement in peak aerobic capacity.

# 5 WEIGHT LOSS IN ATHLETES

## 5.1 Weight loss in performance sports

Weight reduction among weight category athletes is common, and many athletes aim to change their weight to improve their competitive performance. Athletes are usually aiming to optimize performance by increasing power to weight ratio or to compete in a certain weight category, or aesthetic reasons (Fogelholm 1994; Garthe 2011c). Sports with a high proportion of athletes concerned with weight loss consist of those who must move their weight forward or up (running and jumping). Furthermore, also the athletes in different sports, as aesthetic appearance (dancing, cheerleading, figure skating, bodybuilding, gymnastics), and athletes competing in sports that have weight classes (wrestling, judo, light weight rowing, boxing (Walberg 2002).

Body weight reduction, especially from fat, improves power-to-weight ratio, and may be beneficial in weight-bearing efforts, like jumping or running (Fogelholm 1994; Huovinen et al. 2015). Athletes reduce the body weight several times per season and the magnitude of weight cycling is 5–10 % of their body weight (Fogelholm 1994; Artioli et al. 2010). In many sports the athletes decrease the body weight either before the competition season or before the major competition in the hope of improved performance (Fogelholm 1994).

### 5.1.1 Rapid weight loss

*Definition.* The rapid weight loss (3-4 kg per / 3-4 days) is a procedure, where an athlete needs to lose weight in a short time, sometimes in a few hours. It is defined as a procedure lasting under one week and the loss of weight is primarily by the loss of water. (Fogelholm 1994.) The athletes are motivated by a desire to be at a low weight and reducing body mass in a short period of time prior a competition (Brownell et al., 1987; Wilmore et al. 2000; Kiningham & Gorenflo 2001). The most common techniques are increased physical activity, dieting and eliminating a meal, starvation techniques, passive methods (e.g. sauna, hot baths), active dehydration (e.g. exercise in sweat suit), vomiting, and often in combination with excessive exercise (Garthe 2011; Farhan et al. 2014.) Some wrestlers have been reported to use pharmacological ingredients, like dietary supplements, diet pills, laxatives and diuretics to make body weight an optimal (Steen & Brownell 1990; Farhan et al. 2014). Some studies have suggested a higher frequency of eating problems in athletes than in non-athletes, particularly in athletes competing in sports that focus on physical appearance or a low body weight (Sundgot-Borgen & Torstveit 2004). The most common side effects of rapid weight loss are dizziness, irritability, poor concentration, headaches, muscle cramps, lack of awareness, confusion, heatstroke, increased heart rate, nausea and fever. Some health risks have reported in rapid weight loss such as cardiovascular dysfunctions, lowered bone density, impaired thermoregulation, negative mood state, hormonal unbalance, temporary growth impairment, poor nutritional status and increased risk of injuries (Roemmich & Sinning 1997; Choma et al. 1998; Degouette et al 2006).

*Effects on aerobic and anaerobic performance*. Because of the dehydration in the rapid weight reduction, it may have undesirable impacts on aerobic and anaerobic and strength performance (Webster et al. 1990). Walberg-Rankin et al. (1998) reported that reduction of an average of 3.3% of body weight through energy restriction (no dehydration) over 3 days caused a 7.6% decline in total work performed during a repeated upper body cranking sprint test in collegiate wrestlers. The results indicated that muscle endurance, and specifically, aerobic and anaerobic endurance work capacity impaired in combat sports and rowers during the rapid weight loss. The extent of the impairment seemed to depend on time from weigh-in to competition and strategy which was used in the recovery. (Fogelholm 1994.)

*Effects on strength and muscle power*. It`s been reported impaired maximal strength after rapid weight loss. However, it is a bit complicate to explain, because reduced blood flow should not have any effect on strength. If the muscle has all the necessary nutrients for a short maximal effort, it is not dependent on efficient nutrient exchange. (Fogelholm 1994.) However, a lot of electrolytes are lost during the rapid weight loss, on second thought, during sweating (Maughan et al. 1990). And the lack of magnesium has a potential effect on muscle performance by impairing the normal function of muscles (Fogelholm 1994). Fogelholm et al. (1993) reported no impairment in speed or vertical jump after rapid weight loss in wrestlers. Fogelholm (1994) proved, that rehydration prevented the negative effects of rapid weight loss on physical performance. On the other hand, most athletes reduce the amount of strength training in the weight loss period before the competition. Less stimuli for muscle growth combined with negative energy balance, due to reduction of muscle mass may cause impaired muscle strength and performance during the weight loss. (Koutedakis et al. 1994)

Furthermore, the aim is to lose body water over 1-7 days, so it`s impossible not to lose some fat mass and muscle mass during the extreme weight loss and fasting (Artioli et al. 2010). Timpmann et al. (2008) findings suggested that the self-selected regimen of rapid weight loss impairs muscle performance in 3-min intermittent intensity exercise.

### 5.1.2 Gradual weight loss

*Definition.* Gradual weight loss is a method lasting one week or more. The main difference between rapid and gradual weight loss is found in dehydration. Negative energy balance is the main factor causing the weight reduction during the gradual weight loss. (Fogelholm 1994.) Diet modifications are the best way to lose weight gradually. It seems that the weight reduction 0.5kg/w, may provide better outcomes than higher loss of body mass for improving body composition or minimizing hormonal alterations (Mero et al. 2010; Garthe et al. 2011; Helms et al. 2014). Gradual weight loss may cause medical complications in a long-term in lean female athletes and are mainly connected with disordered eating, specifically younger athletes. It involves the cardiovascular, endocrine, reproductive, skeletal, gastrointestinal, renal, and central nervous systems, and in addition, impair health and physical performance. (Nattiv et al. 2007.)

*Effects on strength and muscle power.* Huovinen et al. (2015) investigated the effects of high energy deficit diet (~750kcal) and a moderate one (~300kcal) in athletes just before their competition season. The athletes in the high weight reduction (HWR) group managed to preserve FFM during the study, and lost more fat mass than the low weight reduction group. (Huovinen et al. 2015.) Explosive power and sprint running performance improved ($p < 0.05$) only in HWR. Also in the Garthe et al. (2011) study, the vertical jump improved significantly by 7% in the high weight reduction group (~470kcal). Fogelholm et al. (1993) stated also significant improvements in vertical jump height (+1,4 cm) with extra load. The improved jumping height results indicated preserved capability of the neuromuscular system to produce force or more likely the effect was through decreased body weight required to be lifted during the jump (Fogelholm et al. 1993.)

*Effects on aerobic and anaerobic performance.* Walberg-Rankin et al. (1996) demonstrated a decrease in muscle strength or endurance in athletes dieting for 7 to 10 days. Thus, there is evidence that short-term energy restriction can impair physical performance of aerobic and possibly anaerobic events. (Walberg-Rankin et al. 1996.) Furthermore, Mettler et al. (2010)

reported a statistically significant decrease of 2.8% in peak jump force (P = 0.011) during the gradual weight loss. On the other hand, some studies have reported that some athletes may have even improved their performance during weight loss when using a gradual weight loss strategy (Koutedakis et al. 1994; Fogelholm et al. 1993.)

### 5.2 Weight loss in fitness

Women are becoming more interested in training and competing in bodybuilding and fitness competition (Andersen et al. 1995; Sandoval et al. 1989.) Even though the limited evidence, there is a perception that bodybuilding or fitness competing in general, have negative consequences on physiological performance and health. (Kistler et al. 2014). The studies are based largely of previous bodybuilding case studies, and the publications have mostly focused on the negative consequences of the sport, such like anabolic steroids use and performance improving drugs (Huckins & Lemons 2013; Kistler et al. 2014). Furthermore, there is a lack of evidence based studies on weight reduction in fitness. Very few studies about bodybuilding and dieting exist, but those are mostly from 90`s. (Sandoval et al. 1989; Andersen et al. 1995.)

#### 5.2.1 Changes of body composition during weight loss

It´s known that, bodybuilders, and fitness competitors regularly need to reduce fat and weight before competition preferably without the negative effects on muscle strength or muscle size (Shepfard 1994; Paoli et al. 2012). Preparing for a fitness or bodybuilding competition requires years of intensive training to increase skeletal muscle size (Kistler et al. 2014). Many bodybuilders and fitness competitors spend most of the year in a hypertrophy phase where the combination of training and diet aiming to increase muscle mass (Van der Ploeg et al. 2001). It is followed by a more acute competition preparation in which athletes lose fat mass while maintaining muscle mass (Van der Ploeg et al.2001; Maestu et al.2010; Rossow et al. 2013; Kistler et al. 2014). During this preparation phase, which is dependent on each athlete but generally lasts about 12–24 weeks, athletes usually lose weight by both reducing caloric intake and often also by increasing aerobic activity while trying to maintain resistance-training volume as a method to reduce fat mass. (Van der Ploeg et al. 2001). Losing weight too fast can lead to negative effects on lean mass (Garthe et al. 2011a). Anyhow, athletes use plenty of preparation techniques and many athletes fail to reach the extremely low levels of body fat (Rossow et al. 2013).

Most of the weight reduction studies are case studies and in males. In the case study of Kistler et al. (2014) the male bodybuilder lost 15 kg of body weight but high amount of weight loss did lead to a reduction in lean mass (–8.8%), which is reported among lean athletes (Huovinen et al. 2015). Rossow et al. (2013) demonstrated a great result on body fat reduction of 14.8 % to 4.5 % in a 12-week case study of one male bodybuilder with smaller lean mass loss (87.65 to 84.84.). Correspondingly, Van der Ploeg et al. (2001) showed changes in body composition on female bodybuilders. Their data indicated great losses of body mass of -4.4 kg, and the losses mainly coming from fat mass (76.2%). Therefore, the body fat percentage in the study decreased significantly from 18.3 % to 12.7 %. (Van der Ploeg et al. 2001.) Heyward et al. (1989) compared, body composition and nutritional profiles of 12 females and nine male bodybuilders during different phase of training, and significant weight loss was demonstrated for male (-5.4kg) and female (-6.0kg), primarily due to the fat loss.

### 5.2.2 Nutrient intake during weight loss

The low calories intake during the preparation phase is achieved primarily by reducing fat or carbohydrate and maintaining high protein intake (Kistler et al. 2014). It is recommended to 0.5 kg weight loss per week to preserve lean mass, and thus energy deficit should be about 500 kcal per day **(**Helms et al. 2014), but energy deficit of 950 kcal has reported in studies prior to competition in male bodybuilders (Mäestu et al. 2010). Energy intake ranged from 10 to 24 MJ/day for men and from 4 to 14 MJ/day for women in review study of bodybuilders (Spendlove et al. 2015). Murphy et al. (2015) have presented one recommendation of the macronutrient composition for the preparation phase (Table 1.). However, one option for the competition preparation is to follow the same diet until the competition, keeping energy intake almost at the same level including 1-2 cheat day per week (Rossow et al. 2013; Kistler et al. 2014). Another option is to continue the diet, with the same or dropped energy intake which is made based on the progression of the weight loss (Robinson et al. 2015).

TABLE 1. The recommendation of macronutrient composition for the preparation phase.

| Macronutrient | Amount |
|---|---|
| Protein (g/kg) | 1.8-2.7 |
| Carbohydrates (g/kg) | 0.69 |
| Fat (g/kg) | 0.99 |

[1](Murphy et al. 2015)

Adequate daily protein intake during contest preparation is needed to support maintenance of LBM (Helms et al. 2014a). Muscle damage increases the athlete's need for protein intake, specifically in fitness and bodybuilding when aiming to preserve lean mass. For maintaining essential amino acid availability and stimulating lean tissue preservation many athletes consume protein or amino acid supplement. The compound of high quality protein and resistance exercise is proposed to have a synergistic effect on muscle mass preservation during weight reduction. During the contest preparation, bodybuilder increase training volume and aerobic training with restricted calories leading to very lean condition. All these factors increase protein requirements, and thus, optimal protein intakes for bodybuilders may be substantially higher than existing recommendations. (Helms et al. 2014a.) Walberg et al. (1988) and Mettler et al. (2010) have presented that the higher the protein intake, might be the lower the chance for LBM loss. In contrast, Maestu et al. (2010) didn`t investigate a significant loss of LBM in among bodybuilders consuming 2.5–2.6 g/kg of protein during phase prior to competition. High protein (2.8g/ kg bw/d), low fat and moderate carbohydrate diet resulted in better outcomes in stress, fatigue and mood disturbances compared to moderate protein (1.6 g/ kg bw/d), fat and carbohydrate diet in resistance trained males, but not any differences in body composition changes (Helms et al. 2015b).

Amino acid supplements such as branched-chain amino acids (BCAA; valine, leucine, isoleucine) may increase or stimulate skeletal muscle regeneration by preventing post-exercise protein degradation. And, thus lead to greater gains in lean mass which is important in bodybuilding and fitness (Dudgeon et al. 2016.) Some studies have reported that BCAAs may delay fatigue and stimulate muscle protein synthesis resulting to post-exercise muscle recovery, enabling athletes to exercise longer at a higher intensity. (Kreider et al. 2010; Dudgeon et al. 2016.)

High carbohydrate consumption on diet is taught to be the standard for optimal athletic performance. Carbohydrate content needs to be customized for each athlete based on sport and different training phase (Leveritt & Abernethy 1999). Heyward et al. (1989) compared female and male bodybuilders and they showed that during the competition stage, male bodybuilders consumed relatively larger amounts of carbohydrate and protein, but smaller amounts of fat. Female bodybuilders showed similar patterns for carbohydrate and fat, but their relative intake of protein was less in the competitive state. (Heyward et al. 1989.) Also, Spendlove et al. (2015) have reported low carbohydrate and high protein intakes than, the current recommendations for strength athletes during the preparation phase.

It`s well known that resistance training utilizes glycogen as its main fuel source, specifically in high intense exercises. The energy expenditure in strength athletes is lower than of mixed sport or endurance athletes and therefore, some review studies recommend that carbohydrate intakes for strength sports, including bodybuilding, be between 4–7 g/kg/d depending on the phase of training. (Slater & Phillips 2011; Helms et al. 2015b.) It has reported that low carbohydrate intake can impair strength training and inadequate carbohydrate intake prior to training could delay glycogen refilling (Leveritt & Abernethy 1999; Haff et al. 2000). The diet with adequate carbohydrate at the expense of protein (1 g/kg) showed greater LBM losses compared to a diet that increased protein (1.6 g/kg) through a carbohydrate restriction in male bodybuilders. (Helms et al. 2014b; Helms et al. 2015b.) Fat intake during the diet is also important for athlete to maintaining hormonal levels, such as testosterone and other anabolic hormones needed to preserve LBM (Helms et al. 2014b).

# 6 PURPOSE OF THE STUDY AND RESEARCH QUESTIONS

The purpose of this thesis was to investigate the effects of the carbohydrate content on body composition during weight loss in female fitness competitors.

**The study was based on the following study problems and hypotheses:**

1. Are there any differences between diets of containing low carbohydrate and high protein or high carbohydrate and moderate/high protein on lean body mass (LBM) or muscle CSA in female fitness competitors measured by Ultrasound, InBody and DXA?

   **Hypothesis 1.** Low carbohydrate with high protein intake has reported to maintain or even increase LM due to higher satiety and amino acid availability during the weight loss in obese and active individuals, but more specifically in untrained subjects. (Leveritt & Abernethy 1999; Paddon-Jones et al. 2004; Layman et al. 2005; Heisson et al. 2008; Motjahedi et al. 2011; Helms et al. 2014b; Merra et al. 2016) On the other hand, low carbohydrate diets have reported to enhance catabolism of proteins in the body, in acutely and in long term. It has also reported to increase amino acid oxidation in glycogen depleted state. Therefore, it seems that adequate carbohydrate intake is important for athletes for maintaining protein synthesis and lean mass (Lemon & Mullin 1980; Wagenmakers et al. 1991; Roy et al. 1997; Borsheim et al. 2004; Noakes et al. 2006; Gaine et al. 2006) Therefore the hypothesis is that there are no differences on body composition between the groups when protein intake is high.

2. Are there any differences between the effects of containing low carbohydrate high protein or high carbohydrate moderate/high protein on fat mass (FM) in female fitness competitors measured by Ultrasound, InBody and DXA?

   **Hypothesis 1.** Low carbohydrate with high protein intake has reported to effect on FM reduction if protein intake is high with energy deficit (Motjahedi et al. 2011; Helms et al. 2014b; Merra et al. 2016) On the other hand, when the protein content is equal, usually low carbohydrate/high protein diet had no advantage on fat mass reduction compared to medium or high carbohydrate/high protein with energy deficit. (Motjahedi et al. 2011; Helms et al. 2014b; Hall et al. 2015; Heisson et al. 2008; Merra et al. 2016)

# 7 METHODS

The study was part of the projected led by University of Jyväskylä, Biology and Physical Activity and collaborated by the units of Health Sciences and Physical Education, and the University of Helsinki and the National institute of Health and Welfare. The study was conducted during the years 2015 and 2016, and the subjects were recruited during the spring of 2015 Finnish Fitness Sports Association (IFBB) and the University of Jyväskylä information channels. The study was approved by the Ethics Committee of the University of Jyväskylä, Finland.

## 7.1 Subjects

60 women were selected to the study, and 30 subjects were chosen to the diet group and 30 subjects to the control group, in total 50 subjects continued the study to the end. 27 female competitors were 21–36 years old (mean 27.5 ± SD 4.2), from 157 to 175 cm long (165.7 ± 4.3) and from 52.3 to 78.3 kg in weight (64.3 ± 6.9) (Table 2). The basic background of the competitors is presented in table 1. 23 subjects in the control group managed to finish the study. They were used to do strength training 3 to 4 times per week, and had fitness training background of approximately 3.1 years and four of the control group had previously competed in fitness competitions. The length of the diet and between the diet and measurements is presented in table 2. Competition and the control group had no differences in background variables at the beginning of the study.

Three competitors of the diet group won the Finnish Championships in their own divisions and managed to get to the Fitness World Championships in Ukraine. The competition group consisted of 17 bikini fitness athletes, 9 body fitness athletes and one fitness athlete. 16 of the athletes were competing for the first time, and 11 were competed before. In this study, the one fitness athlete was in the body fitness group. The subjects were expected to have a background of at least two years of fitness training on entering the study. All the subjects of the diet group were IFBB amateur fitness competitors and drug-tested.

TABLE 2. Baseline characteristics of subjects before the diet. (MEAN ± SE)

| | **Diet group (n=27)** | **Control group (n=23)** | **P-value [1]** |
|---|---|---|---|
| **Age (y)** | 27.5 ± 4.2 | 27.2 ± 3.5 | 0.72 |
| **Weight (kg)** | 64.3 ± 6.9 | 64.3 ± 5.3 | 0.99 |
| **Height (m)** | 1.66 ± 0.1 | 1.65 ± 0.0 | 0.69 |
| **Training years (y)** | 3.5 ± 1.4 | 3.1 ± 1.1 | 0.35 |

[1] Differences between the groups are tested with independent samples t-test.

The subjects were required to attend the measurements in three times in Jyväskylä. The subjects needed to carry out all the measurements properly and to report physical activity, food diaries and training programs to the investigators. Smokers, three-shift workers and those with mental diseases (depression or eating disorders) or prescribed medications were excluded from the study. In addition, the participation in Spring 2015 fitness competitions were forbidden.

From the food diaries (see below 7.3.1), the competitors were retrospectively divided in two groups based on their carbohydrate intake. The low and high carbohydrate groups included 6 subjects per group, in total n=12. These two groups were chosen based on the carbohydrate intake during the diet. The criteria of low carbohydrate group were following: The values for absolute carbohydrate intake were <120g/d and relative carbohydrate intake at least 10% proportion from the energy from the baseline. The criteria of high carbohydrate group were following: The values for absolute carbohydrate intake were >145g/d and relative carbohydrate reduction less than 7 E% of carbohydrate from the baseline. This group is called high carbohydrates even though as the subjects were on a diet, the absolute carbohydrate intake was rather low.

TABLE 3. The differences between diets and measurements (w=week).

| | **Competitors** | **Controls** |
|---|---|---|
| **Diet length (w)** | 22 ± 4.4 | |
| **Recovery period length (w)** | 17.5 ± 2.6 | |
| **Time between pre and mid-term measurements (w)** | 19.8 ± 3.6 | 22.4 ± 2.1 |
| **Time between mid-term and post measurements (w)** | 17.5 ± 2.6 | 19.2 ± 3.6 |
| **Time between pre and post measurements (w)** | 37.3 ± 4.9 | 41.6 ± 3.6 |

### 7.2 Study design

The study included three test days at the laboratory (pre, mid and post). The pre–measurements were carried out in April-May 2015, when all the subjects were measured for the first time. 30 women in the competing group and 29 in the control group attended in the pre-measurements eventually. The mid-term measurements were carried out in September and October, and the competition group was measured the day after the competition. The mid-term measurements were carried out either after the qualification of Finnish Championship competitions or championship competitions. By the mid-term measurements two competitors and five controls were dropped out. Competitors dropped out of the study because their regimen was not followed to the plan, and they did not participate in competitions.

The control group was advised to continue their normal lifestyle and training over the study. The controls reported major changes in their life, such as barriers to practice as belonging to the control group was instructed. One control dropped out of the study, because she became pregnant. 10 competitors ended up to National Championship competitions, and thus continued their regimens for another 4 weeks after the mid-term measurements. The post measurements were carried out during the spring of 2016, in January, February, March and April. In total 50 subjects carried out the study properly.

Three test days included first InBody- and DXA-, measurements and were taken at the fasting state early in the morning. All subject of the study came to the laboratory after at least 8 hours of fasting. The subjects ate standardized breakfast (protein bar and drink, and apple or banana: about 72–80 g carbohydrates, 47–48 g proteins and 6 g fat, in total 517–553 kcal) before they conducted to ultrasound measurements.  The subjects in the control group were measured on the same days like the competitors. The same researcher was always responsible for conducting the measurements and analysis to make sure the good reliability and validity.

### 7.3 Data collection and analysis

#### 7.3.1 Nutrient intake

The data was collected for this thesis at three time-points during the study, before, in the middle and the last week of the diet. Competitors continued their normal diet regimen during the study, and about 50 % of the subjects reported all their meals to the investigators. The rest of the subjects reported representative days from diet, 12 subjects reported their diet from the competition day. The controls kept their food diaries at three time points as well, before and during the diet. They kept food diary over 3 weekdays and one day at the weekend. All the nutrient supplements were included in the analysis. All the food diaries were analyzed by nutrient analysis software (Aivodiet, Flow-team Oy, Oulu, Finland)

#### 7.3.2 Dual energy X-ray absorptiometry (DXA)

Lunar Prodigy dual-energy X-ray absorptiometry (DXA) densitometer (Lunar Prodigy Advance, GE Medical Systems – Lunar, Madison WI USA) was used in this study to measure subject`s body composition: fat mass and boneless fat-free mass designated “lean mass” from now on in this thesis. Before the measurements, the subjects were positioned supine in the center of the DXA table with their arms at their sides and feet together, wearing a pair of shorts and sport bra. They were scanned using the default scan mode for total body scanning automatically selected by the Prodigy software (enCORE 2005, version 9.30 and Advance 12.30). DXA measurements were conducted following a 10-hour fasting, and avoiding alcohol, nicotine products and strenuous exercise. For improving the reliability of the results the line of shoulders- and pubic-measuring points were repaired manually, if the device was automatically analyzed these measurement points the wrong points. Example DXA body composition report to the body of the image is shown in Figure 1. DXA was chosen as it has been shown to be a suitable, reliable and accurate method to measure short-term changes in body composition. (Kiebzak et al. 2000; Aasen et al. 2006).

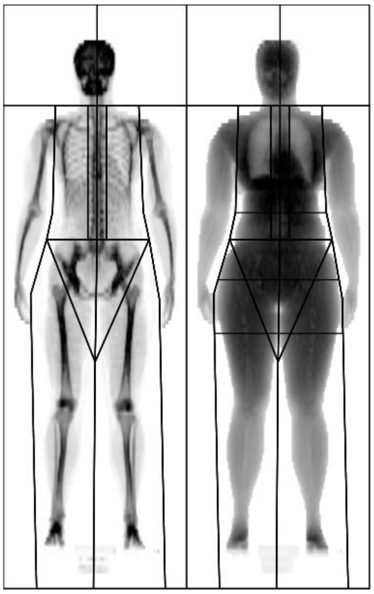


FIGURE 1. The body image with DXA. Torso-image area is determined between the top horizontal line (shoulder) and an upside-down triangle base line (christa iliaca) area. (Koukkari 2016.)

*InBody: Biompedance.* The body weight, fat and lean mass were measured by bioimpedance InBody720 in the fasted state (Inbody 3.3, Biospace Co. Soul, Korean). The subjects were asked to take off the extra clothes, and they wore only a pair of shorts and sport bra to minimize the extra weight on analysis.

### 7.3.4 Ultrasound

Cross sectional (CSA) area of vastus lateralis muscle were measured at the mid-thigh (Figure 2.) using ultrasound (model SSD-2000, Aloka, Tokyo). The linear array probe was 10 MHz

(60mm width). Imaging was done with an ultrasonic device sarcopenia, which is suitable using for imaging of muscle function. The CSA of the vastus lateralis muscle was examined in two levels. The first was 40 % from the superior point of patella towards anterior superior spina iliaca, and another one was 2 cm distally from the first level. Furthermore, the thickness of triceps brachii muscle and subcutaneous fat were measured at the midpoint between medial epicondyle and acromion.

The ultrasound measurements were taken from the skin without any pressure, and all measurement sites was marked with a marker pen. The measurements were taken while the subjects laid on the bed and were quietly relaxed. The transducers were used with technician and the measurements were taken three times, to make sure the good quality of the images. The scanning head was coated with water-soluble transmission gel to provide acoustic contact and reduce pressure in the skin by the scanning head to make a clear image. The images of vastus lateralis CSA and triceps thicknesses were analyzed manually using ImageJ software (version 1.44p; National Institutes of Health, Bethesda, MD). US was chosen as a direct measure of muscle size and for fat thickness.

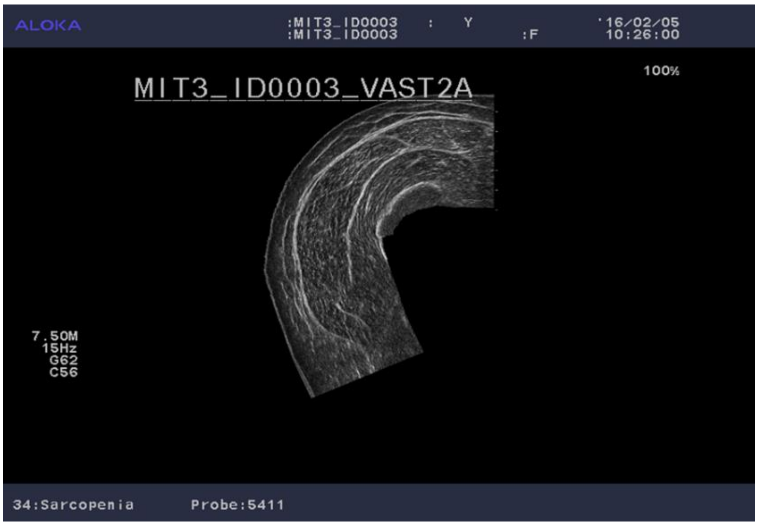



FIGURE 2. Ultrasound image of vastus lateralis (panorama). (Järvinen 2016.)

### 7.3.5 Resistance and aerobic training

Total exercise metabolic equivalents in hours (MET-hours) were analyzed and calculated based on the diaries including physical activity, training frequency and volume. (Suonpää, Thesis 2016). All of the participants used split routines for resistance training in the diet group, in other words, they trained single muscle group per session, which is seen among bodybuilders (Hackett et al. 2013). The major muscle groups included thighs, hamstrings, buttocks, chest, shoulders, arms, upper and lower back, calves, and abdominals. Aerobic training included mainly of high-intensity interval training (HIT) or both HIT and low-to medium intensity aerobic such like running or walking. Aerobic training was implemented mainly together with resistance training, but part of participants completed also separate aerobic workouts. One week prior to competition, the competitors have tapering period leading to replenish the muscle glycogen stores with high carbohydrate intake with decreased total training load.

### 7.4 Statistical analysis

Statistical evaluation of the data was accomplished by two-way repeated-measures analyses of variance (ANOVA) with group (low or high carbohydrate) and time as the factors and pre as an cofactor when appropriate using SPSS statistical analysis software (SPSS version 24; Chicago, IL). Correlations were analyzed using Pearson's Product Moment Coefficient. Statistical significance was set at $P < 0.05$. Microsoft Office 2010 Excel-program was used to calculate averages and statistical errors and independent samples t-test, when analyzing differences between pre and post time-points in both groups and differences between the groups. Values are presented as means ± statistical error of the mean (SE).

## 8 RESULTS

### 8.1 Nutrient intake

The mean energy intake during the diet was 1798 ± 371 kcal/day (baseline 2360 ± 426 kcal / day), and the energy intake in control group was 2341 ± 424 kcal/day and remained unaltered (see kJ values in Table 4). The caloric restriction in the diet was mainly from reduced carbohydrate intake, whereas fat intake remained unaltered and protein intake increased (Table 4). The absolute protein and fat intake decreased in the diet together with weight loss but the values per kg of body weight didn`t alter in protein but fat values slightly decreased (Table 4).

TABLE 4. Macronutrient consumption in diet and control groups.

| | Pre | Mid-diet | Diet average | Competition-week | Recovery | Diet x group (p) |
|---|---|---|---|---|---|---|
| **Energy (kJ)** | | | | | | |
| Diet | 9903.7 ± 1785.9 | 7887.6 ± 1440.1*** | 7524.2 ± 1556.2*** | 9789.7 ± 2553.9 | 9273.4 ± 2186.6 | < 0.01 |
| Cont | 10446 ± 2307.5 | | | 9795 ± 1774.0 | 10425.5 ± 1549.8 | |
| **Energy (kJ/kg bw)** | | | | | | |
| Diet | 155.0 ± 27.8 | 131.4 ± 24.4*** | 125.4 ± 26.2*** | 166.0 ± 61.1 | 158.8 ± 41.4 | 0.067 |
| Cont | 162.6 ± 34.7 | | | 151.9 ± 24.7 | 163.6 ± 24.5 | |
| **Carbohydrates (g)** | | | | | | |
| Diet | 215.6 ± 67.7 | 126.1 ± 49.1*** | 127.8 ± 39.7*** | 229.9 ± 199.0 | 188.5 ± 72.5 | < 0.01 |
| Cont | 218.8 ± 50.3 | | | 216.7 ± 41.4 | 224.0 ± 49.8 | |
| **CHO (g/kg bw)** | | | | | | < 0.01 |
| Diet | 3.4 ± 0.99 | 2.10 ± 0.84*** | 2.12 ± 0.66*** | 4.10 ± 1.65 | 3.24 ± 1.34 | |
| Cont | 3.4 ± 0.7 | | | 3.4 ± 0.6 | 3.5 ± 0.8 | |
| **Proteins (g)** | | | | | | |
| Diet | 202.5 ± 44.1 | 189.7 ± 39.4* | 184.8 ± 40.5* | 160.6 ± 32.5** | 195.4 ± 41.5 | < 0.05 |
| Cont | 172.3 ± 37.6 | | | 181.9 ± 37.9 | 184.7 ± 39.5 | |
| **Prot (g/kg bw)** | | | | | | 0.282 |
| Diet | 3.2 ± 0.61 | 3.14 ± 0.63 | 3.07 ± 0.64 | 2.84 ± 0.52 | 3.3 ± 0.81 | |
| Cont | 2.7 ± 0.5 | | | 2.8 ± 0.5 | 2.9 ± 0.5 | |
| **Fat (g)** | | | | | | 0.184 |
| Diet | 64.4 ± 16.3 | 56.8 ± 16.4* | 52.8 ± 16.4*** | 63.9 ± 25.3 | 59.7 ± 13.0 | |
| Cont | 82.2 ± 23.3 | | | 73.8 ± 28.8 | 84.9 ± 29.4 | |
| **Fat (g/kg bw)** | | | | | | |
| Diet | 1.0 ± 0.29 | 0.95 ± 0.29 | 0.88 ± 0.29* | 1.07 ± 0.49 | 1.02 ± 0.23 | 0.692 |
| Cont | 1.3 ± 0.4 | | | 1.1 ± 0.4 | 1.3 ± 0.5 | |

Data was collected at 5 timepoints, n=27 diet and n=18 in control subjects at pre and mid/average. N=21 in competition week, n= 18 diet and n= 16 control participants in recovery timepoint. Diet x group interaction includes pre and diet average time-points and the values are daily averaged. The p-values (*, **, ***) are between P< 0.05-0.001, where change vs. pre. bw, body weight; CHO, carbohydrates. The nutrients diaries were collected in the middle of recovery in the recovery time-point.

**8.2 Nutrient intake and in high and low carbohydrate groups**

The subjects were divided in two groups, in low (n=6) and in high (n=6) carbohydrate groups based on their dietary diaries when the study had been completed. The baseline characteristics of low and high carbohydrate groups are presented in table 5.

TABLE 5. Baseline characteristics of low and high carbohydrate groups before the diet. (MEAN ± SE)

| | **Low CHO group (n=6)** | **High CHO group (n=6)** | **P-value** [1] |
|---|---|---|---|
| **Age (yr)** | 29 ± 3.3 | 27.5 ± 4.4 | 0.52 |
| **Weight (kg)** | 63.9 ± 7.7 | 64.8 ± 4.4 | 0.92 |
| **Height (m)** | 1.64 ± 5.7 | 1.65 ± 3.0 | 0.67 |
| **Training years (yr)** | 3.1 ± 1.5 | 3.8 ± 2.1 | 0.54 |
| **The length of diet (w)** | 24.5 ± 3.0 | 22.6 ± 2.1 | 0.25 |

[11] Differences between the groups are tested with independent samples t-test.

There was a group x time interaction ($P < 0.03$) in total energy intake (Figure 3, –A) total energy intake per kg bodyweight ($P < 0.01$) in low and high carbohydrate groups (Figure 3, –B). The absolute energy intake reduced in both groups, in HighCHO group (P=0.005), and in LowCHO group (P=0.0004). The energy intake in LowCHO group was lower than in HighCHO group during the diet (P=0.002). The energy intake per kg bodyweight decreased in both groups, in HighCHO (P=0.007) and in LowCHO group (P=0.005), but it was lower in LowCHO group compared to HighCHO group (P= 0.002).

There was a group x time interaction ($P < 0.01$) in relative protein intake (Figure 4, –C). The absolute protein intake (Figure 4, –A) was lower in LowCHO group before (P=0.036) and during the diet (P=0.030). Protein intake per kg bodyweight (Figure 4, –B) was lower in LowCHO group during the diet (P=0.041), but relative protein intake was higher in LowCHO group (P=0.004) than in HighCHO group (P=0.020). There was a group x time interaction

(Figure 5) in carbohydrate intake (p< 0.009), in carbohydrate intake per kg bodyweight (p< 0.006) and relative carbohydrate intake (p< 0.001) in between the groups. The absolute (P=0.005), per kg bodyweight (P=0.004) and relative carbohydrate intake (P=0.002) were lower in LowCHO group. Carbohydrate intake did not alter in HighCHO group, and absolute (P=0.01), per kg bodyweight (P=0.05) and relative (P=0.49) carbohydrate intake were thus higher. The absolute (P=0.000) and per kg bodyweight (P=0.002) carbohydrate intake was lower in LowCHO group during the diet and relative carbohydrate intake was lower before (P=0.028) and during the diet (P=0.027) in LowCHO group.

Absolute fat intake (Figure 6, –A) decreased more in LowCHO group (P=0.05) than in HighCHO group (P=0.068). Fat intake didn`t change statistically per kg bodyweight in the groups, but relative fat intake increased in LowCHO group (P=0.049), due to the lower carbohydrate intake. Relative protein and fat intake was greater during the diet in LowCHO group (Figure 7, C & D), whereas relative protein intake remained to be greater in HighCHO group (Figure 7, –B) Relative protein and fat intake increased in both groups and relative carbohydrate intake decrease by 20 E% in LowCHO group (Figure 8.).

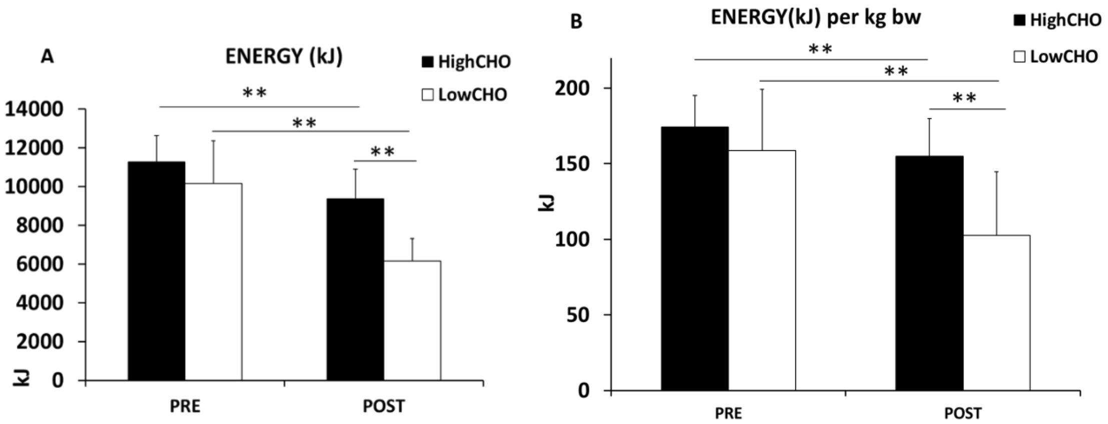



FIGURE 3. Changes in absolute energy intake (A) and energy intake per kg bodyweight (B) in high and low carbohydrate groups from the baseline to the end of study. A and B figures depict statistical changes between pre and post time-points, and also differences between the groups at pre and post. * P < 0.05, ** P < 0.01, *** P < 0.001.

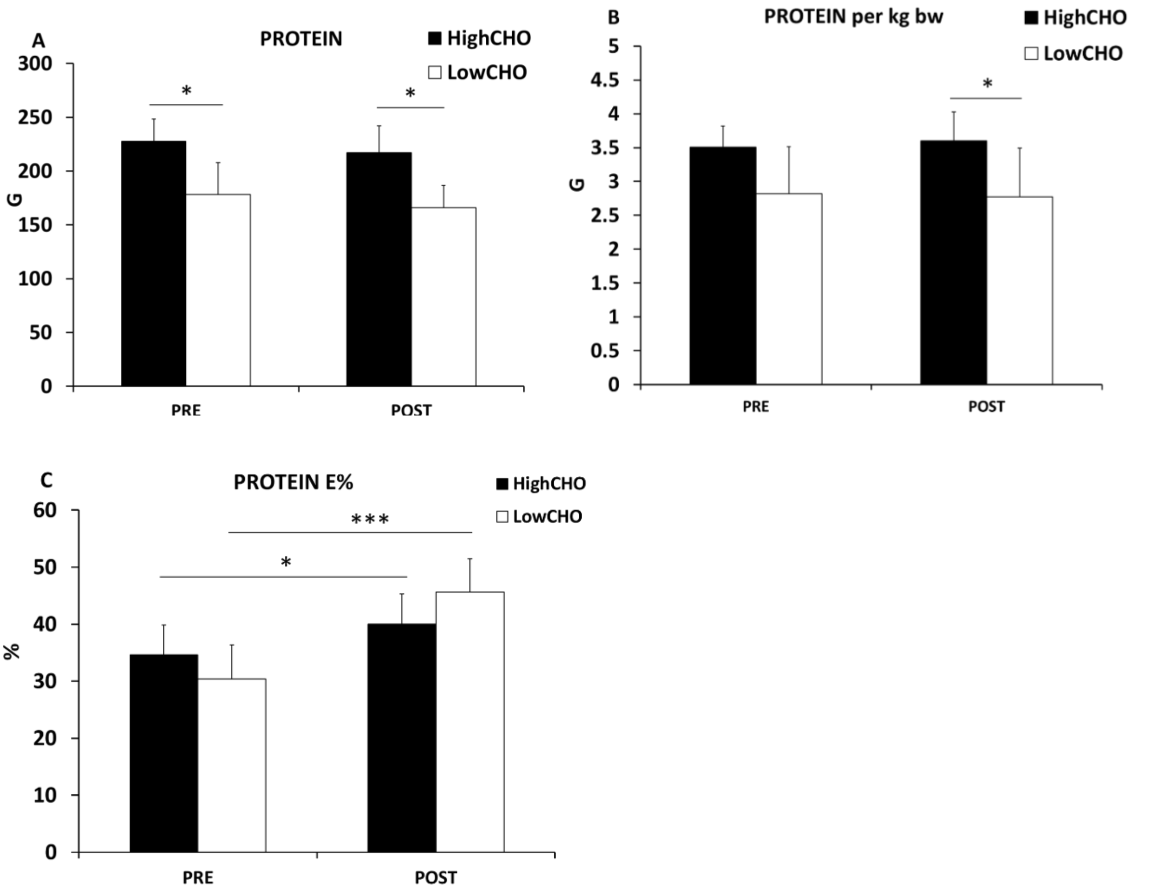
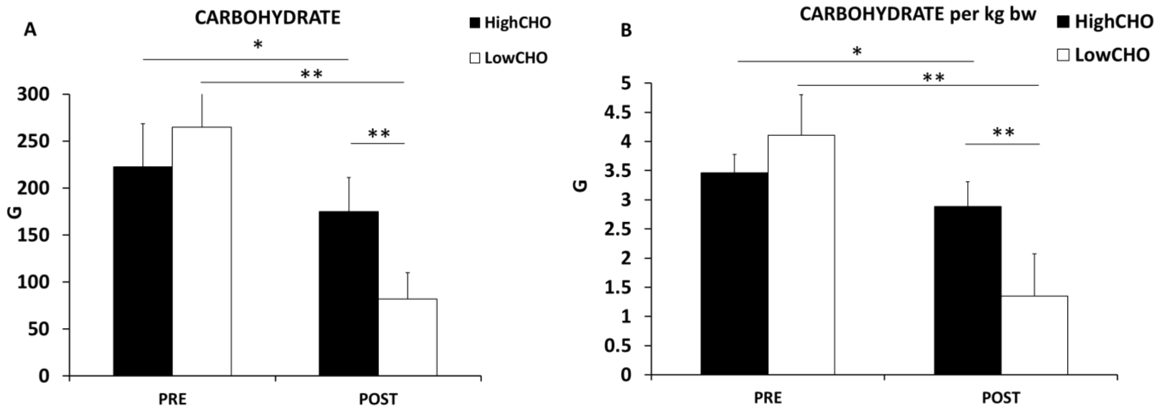



FIGURE 4. Changes in absolute protein intake (A), protein intake per kg bodyweight (B) and relative protein intake (C) from the beginning to the end of study in high and low carbohydrate groups. A, B and C figures depict statistical changes between pre and post time-points and also differences between the groups at pre and post. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



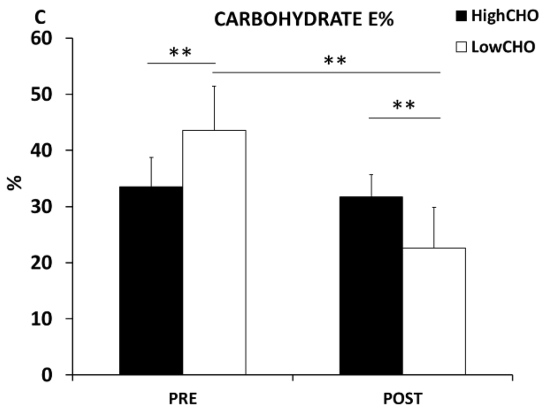



FIGURE 5. Changes in absolute carbohydrate intake (A), carbohydrate intake per kg bodyweight (B) and relative carbohydrate intake (C) from the beginning to the end of study in high and low carbohydrate groups. A, B and C figures depict statistical changes between pre and post time-points and also differences between the groups at pre and post. * P < 0.05, ** P < 0.01, *** P < 0.001.

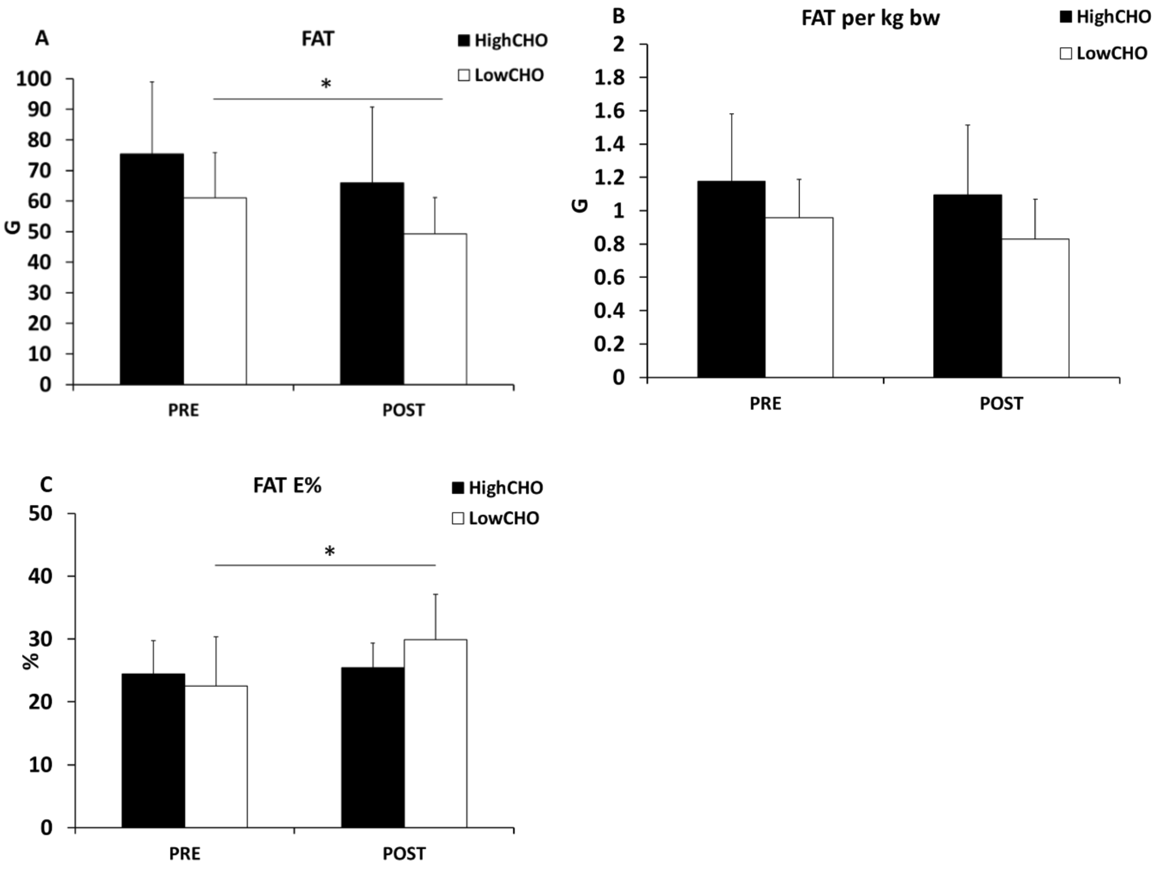



FIGURE 6. Changes in absolute fat intake (A), fat intake per kg bodyweight (B) and relative fat intake (C) from the beginning to the end of study in high and low carbohydrate groups. A,

B and C figures depict statistical changes between pre and post time-points. * P < 0.05, ** P < 0.01, *** P < 0.001.

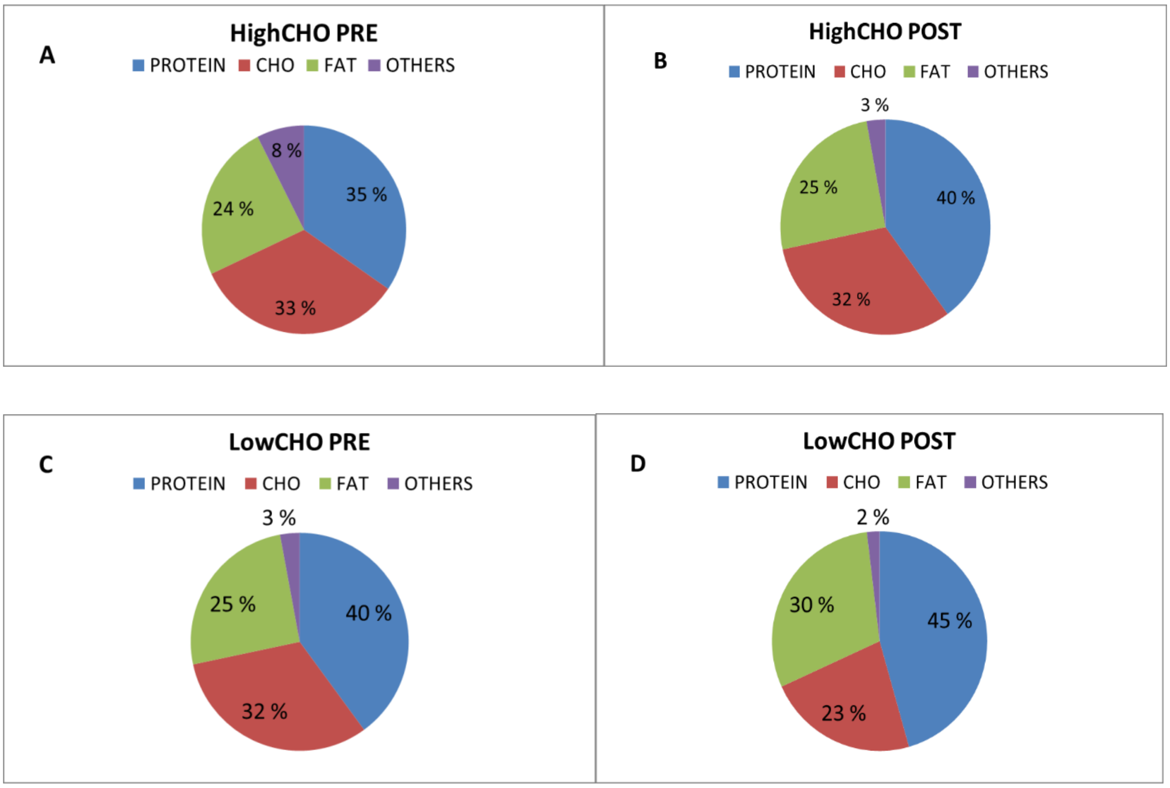



FIGURE 7. Relative protein, carbohydrate and fat intake before (A) and during (B) the diet in HighCHO group, in LowCHO group (C & D). Others means alcohol and measurement errors.

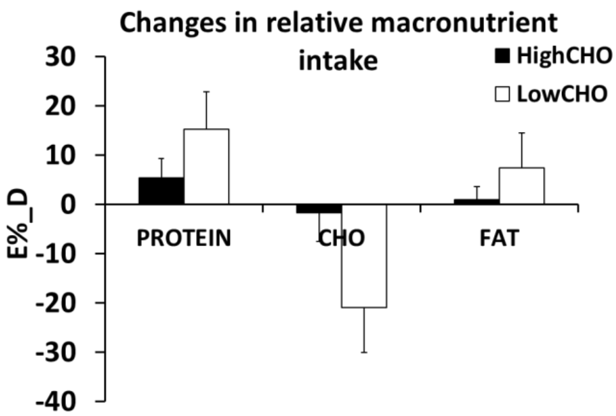



FIGURE 8. Changes in relative protein, carbohydrate and fat intake during the diet in HighCHO and LowCHO groups.

### 8.3 Physical activity in high and low carbohydrate groups

Strength training frequencies decreased in both groups from the pre-state to diet, and aerobic exercises increased in both groups but a slightly more in HighCHO group (Table 6). In HighCHO group aerobic training was 4.0 ± 1.4 times per week and during the diet 5.1 ± 1.9, and remained unaltered in LowCHO group (Table 7.)

TABLE 6. Physical activity at the baseline in HighCHO and LowCHO groups.

| **Physical Activity (times per week)** | **High CHO Pre** | **Low CHO Pre** | **P-value[1]** |
|---|---|---|---|
| **Strength training** | 4.8 ± 0.4 | 5.2 ± 0.4 | 0.18 |
| **Aerobic exercise** | 4.0 ± 1.4 | 5.6 ± 2.7 | 0.27 |
| **Other (Posing)** | 0 | 0 | - |
| **In total** | 8.8 ± 1.2 | 9.8 ± 3.4 | 0.91 |

[1] Differences between the groups tested with independent sample t-test.

TABLE 7. Physical activity during the diet in HighCHO and LowCHO groups.

| **Physical Activity (times per week)** | **HighCHO Post** | **LowCHO Post** | **P-value[1]** |
|---|---|---|---|
| **Strength training** | 4.3 ± 0.7 | 4.7 ± 0.7 | 0.31 |
| **Aerobic exercise** | 5.1 ± 1.9 | 5.6 ± 5.2 | 0.71 |
| **Other (Posing)** | 4.6 ± 1.9 | 4.2 ± 1.2 | 0.64 |
| **In total** | 11.9 ± 2.4 | 12.0 ± 3.2 | 0.98 |

[1] Differences between the groups tested with independent sample t-test.

### 8.4 Body composition in high and low carbohydrate groups

The body weight was 64.8 ± 4.4 kg in HighCHO group at the baseline and 63.9 ± 7.6 kg in LowCHO group. HighCHO group lost 8.6 ± 2.6 kg (P=0.001) of body weight during the diet and LowCHO group 7.4 ± 2.5 kg (P=0.001) of their total body weight.

### 8.4.1 Ultrasound

There was a group x time interaction (P < 0.002) in CSA of vastus lateralis and in thickness of triceps brachii (P < 0.01) (Figure 9). VL CSA decreased in LowCHO group (P=0.02) but not in HighCHO group (P=0.25). It needs to take into an account that VL CSA was greater in LowCHO group before the diet (P=0.005). Triceps brachii thickness remained unchanged in both groups (Figure 9, –B). There was a group x time interaction (P < 0.001) in fat of vastus lateralis (Figure 10, –A). VL fat decreased in LowCHO (P=0.04) and in high CHO group (P=0.006). Fat of triceps brachii decreased slightly in HighCHO group and remain unchanged in LowCHO group, but the change in HighCHO group wasn`t significant.

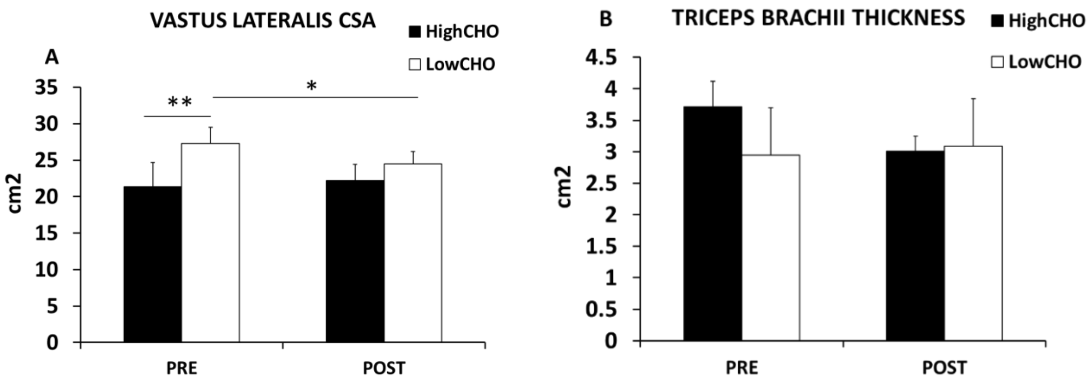



FIGURE 9. Changes in vastus lateralis CSA (A) and triceps brachii thickness (B) from the beginning to the end of study in high and low carbohydrate groups. A and B figures depict statistical changes between pre and mid time-points. * P < 0.05, ** P < 0.01, *** P < 0.001.

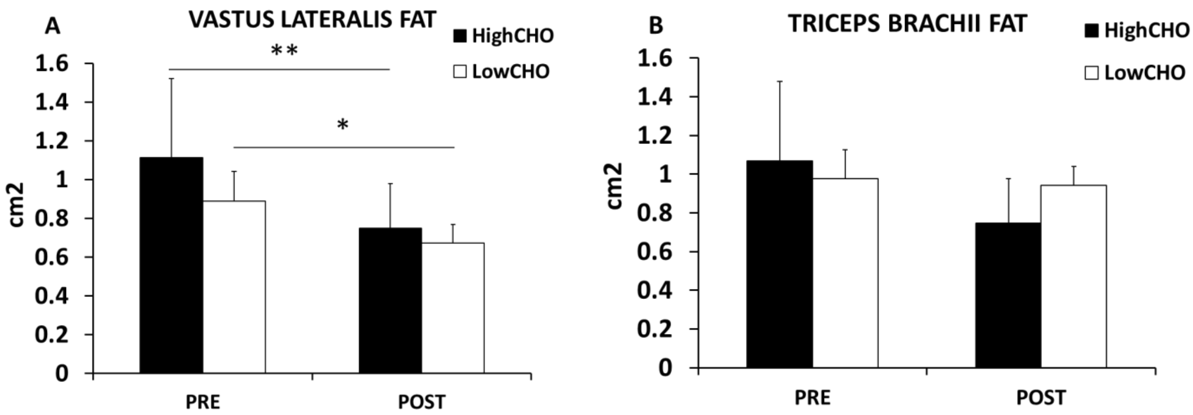


FIGURE 10. Changes in vastus lateralis fat (A) and triceps brachii fat (B) from the beginning to the end of study in high and low carbohydrate groups. A and B figures depict statistical changes between pre and mid time-points. * P < 0.05, ** P < 0.01, *** P < 0.001.

### 8.4.2 Bioimpedance (InBody) and Dual Energy X-ray absorptiometry (DXA)

Lean mass remained unchanged in LowCHO group (P=0.47) and in HighCHO group (P=0.23) measured by InBody during the diet. In contrast, lean mass increased in LowCHO group (P=0.006) and tended to increase also in HighCHO group (P=0.08) during the intervention measured by DXA. There was group x time interaction in fat mass measured by InBody (P < 0.009) and in fat mass measured by DXA (P < 0.003) (Figure 11). Fat mass decreased in LowCHO group (P=0.001) and in HighCHO group (P=0.01) measured by InBody. Fat mass also decreased in LowCHO group (P=0.001) and in HighCHO group (P=0.001) (Figure 12).

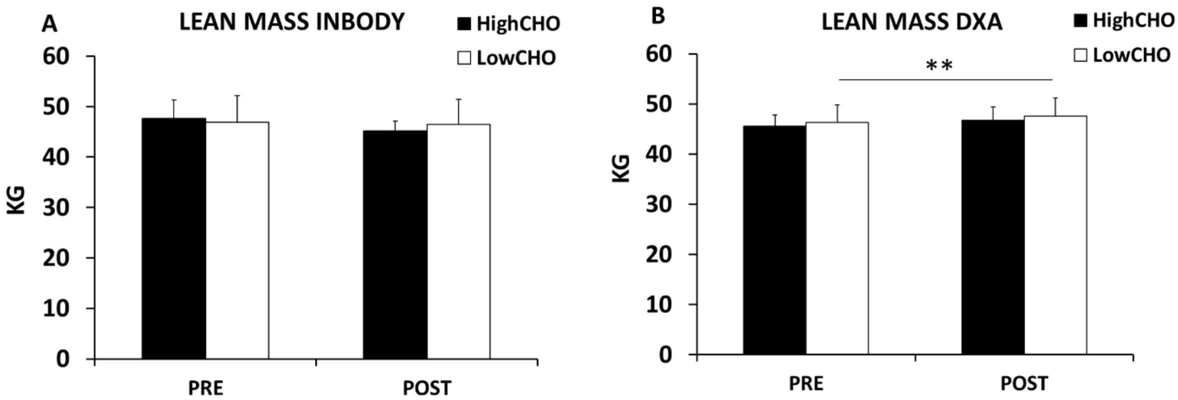



FIGURE 11. Changes in lean mass measured by InBody (A) and by DXA (B) from the beginning to the end of study in high and low carbohydrate groups. A and B figures depict statistical changes between pre and mid time-points. * P < 0.05, ** P < 0.01, *** P < 0.001.

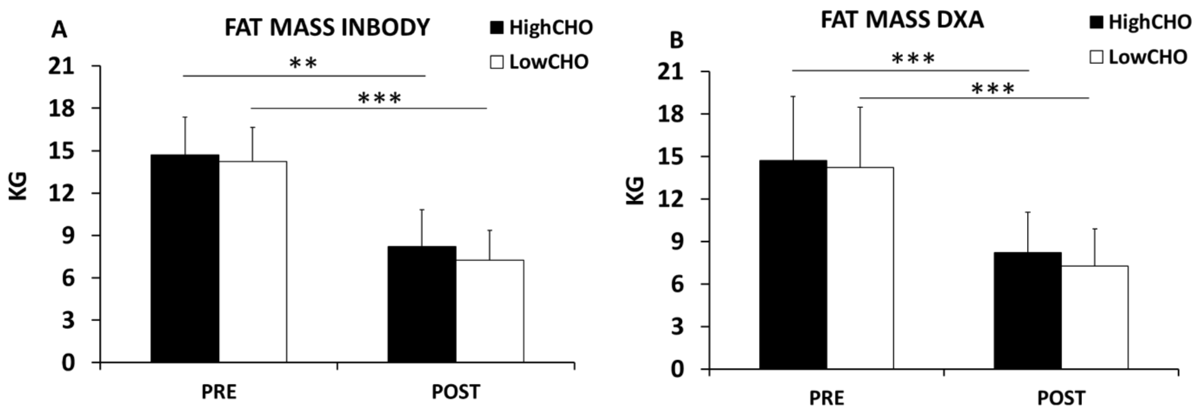



FIGURE 12. Changes in fat mass measured by InBody (A) and by DXA (B) from the beginning to the end of study in high and low carbohydrate groups A and B figures depict statistical changes between pre and mid time-points. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

### 8.5 Comparison of the lowest CHO and highest CHO individuals

To analyze extremes of the subjects, 2 subjects with highest and lowest CHO-intake were further analyzed. Due to the low subject numbers, these subjects are not compared statistically.

#### 8.5.1 Nutrient intake

The absolute and per kg bodyweight energy intake was slightly less in LowCHO individuals before and during the diet (Figure 13). The absolute and per kg bodyweight protein intake remained unaltered, thus relative protein intake was greater in LowCHO individuals (Figure 14, –C), due to lower carbohydrate intake during the diet (Figure 15). The absolute, per kg bodyweight and relative carbohydrate intake reduced in both groups, but was remarkably lower in LowCHO individuals during the diet (Figure 15). The absolute and per kg bodyweight fat intake decreased in both groups, but relative fat intake increased during the diet in LowCHO individuals.

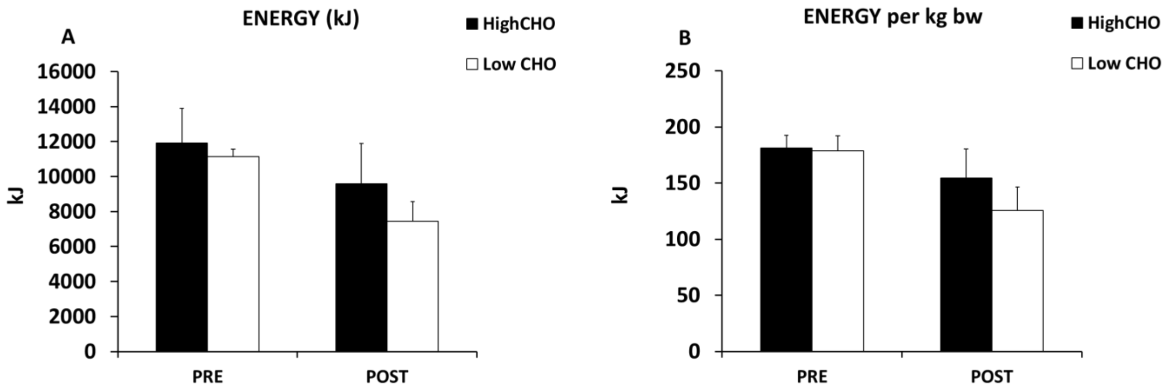



FIGURE 13. Changes in absolute energy intake (A) and per kg bodyweight (B) in high (n=2) and low (n=2) CHO individuals from the beginning to the end of the diet. (MEAN ± SE)

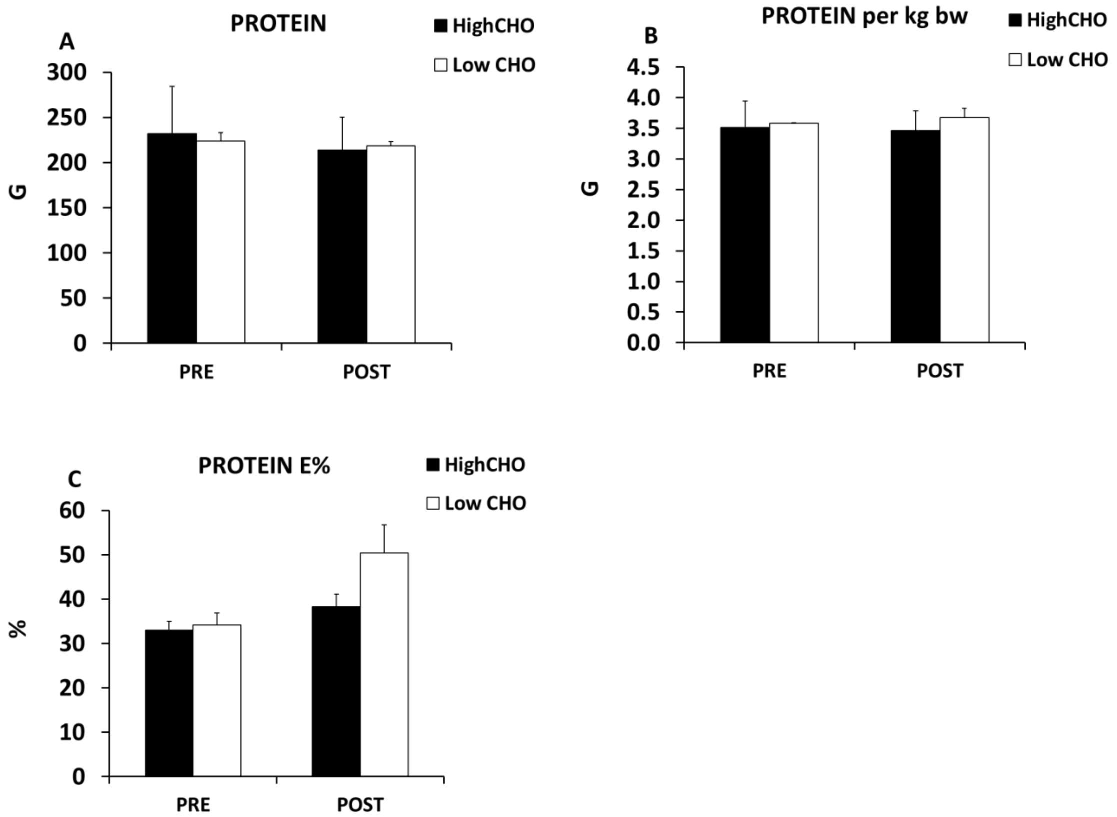



FIGURE 14. Changes in absolute (A), per kg bodyweight (B) and relative (C) protein intake in high (n=2) and low (n=2) CHO individuals from the beginning to the end of the diet. (MEAN ± SE)

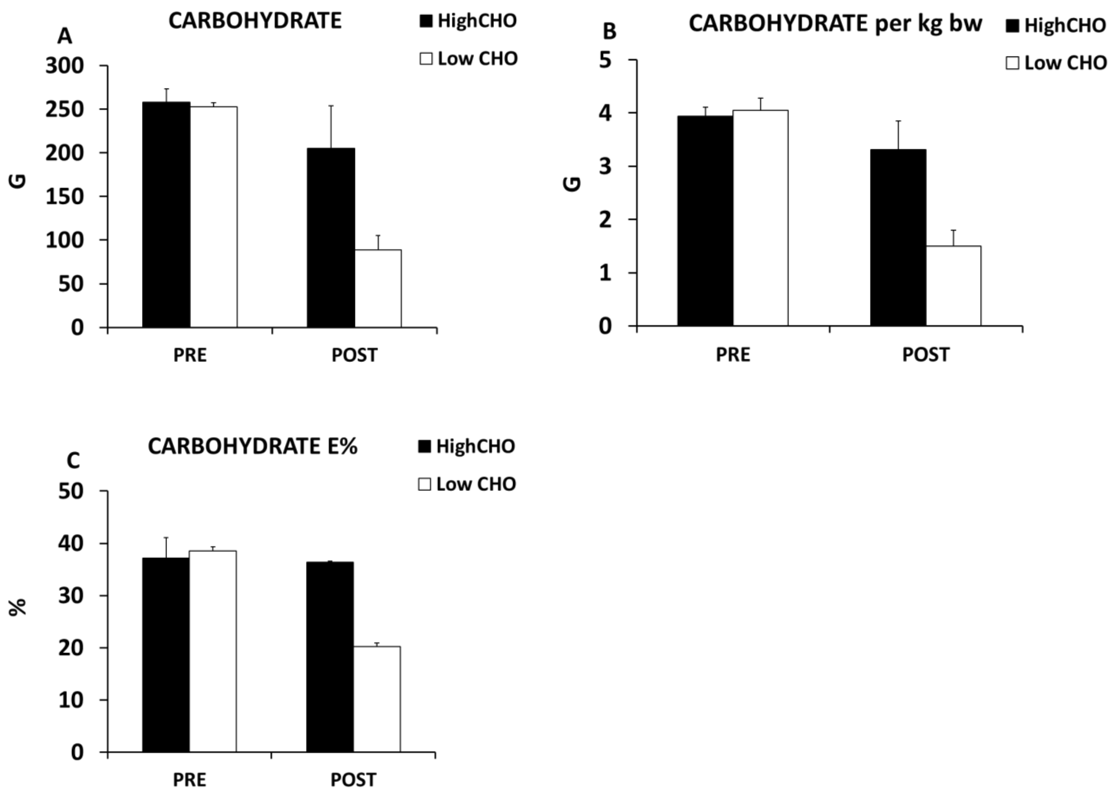



FIGURE 15. Changes in absolute (A), per kg bodyweight (B) and relative (C) carbohydrate intake in high (n=2) and low (n=2) CHO individuals from the beginning to the end of the diet. (MEAN ± SE)

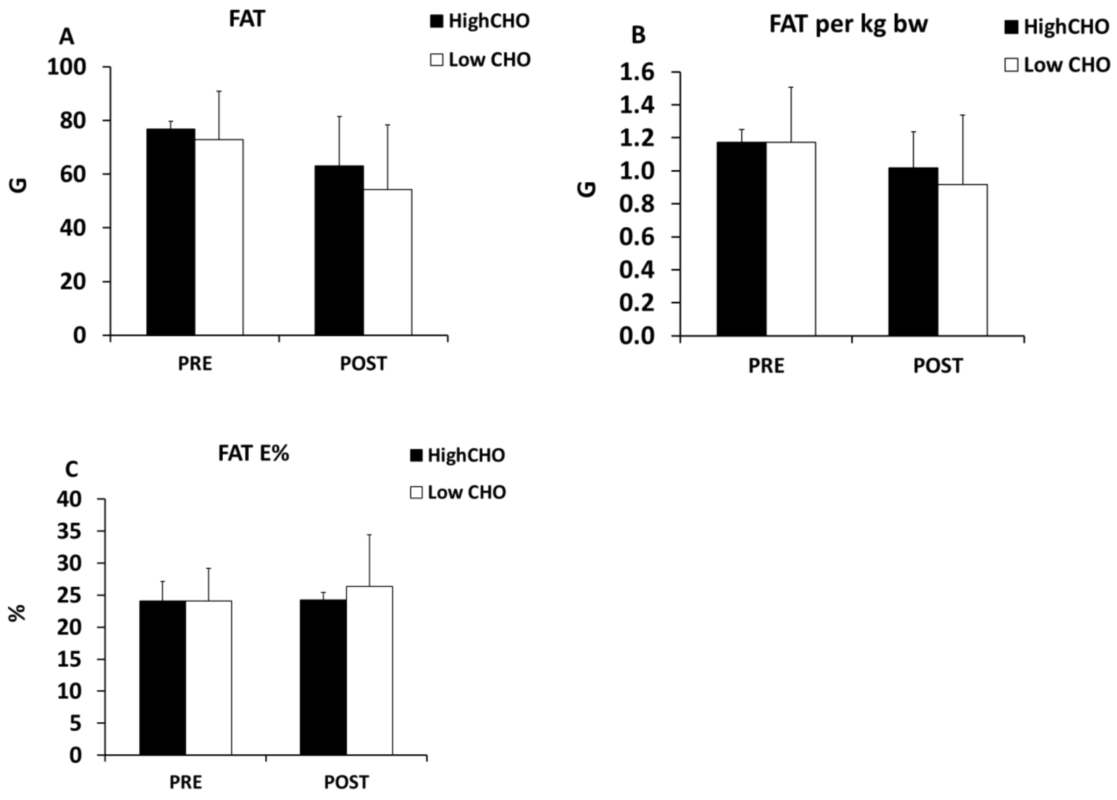


FIGURE 16. Changes in absolute (A), per kg bodyweight (B) and relative (C) fat intake in high (n=2) and low CHO (n=2) individuals from the beginning to the end of the diet. (MEAN ± SE)

### 8.5.2 Body composition

Vastus lateralis CSA was greater before the diet in LowCHO group, and was reduced in both groups during the diet. Triceps brachii thickness increased in LowCHO individuals but decreased in HighCHO individuals (Figure 17). Vastus lateralis and triceps brachii fat reduced in both groups but more in HighCHO individuals (Figure 18). Lean mass remained unaltered in both groups, but slightly increased in HighCHO individuals measured by DXA (Figure 19, –B). Fat mass reduced in both groups measured by InBody and DXA (Figure 20), but more in LowCHO individuals. It needs to take into consideration that the fat mass was lower in LowCHO group before the diet.

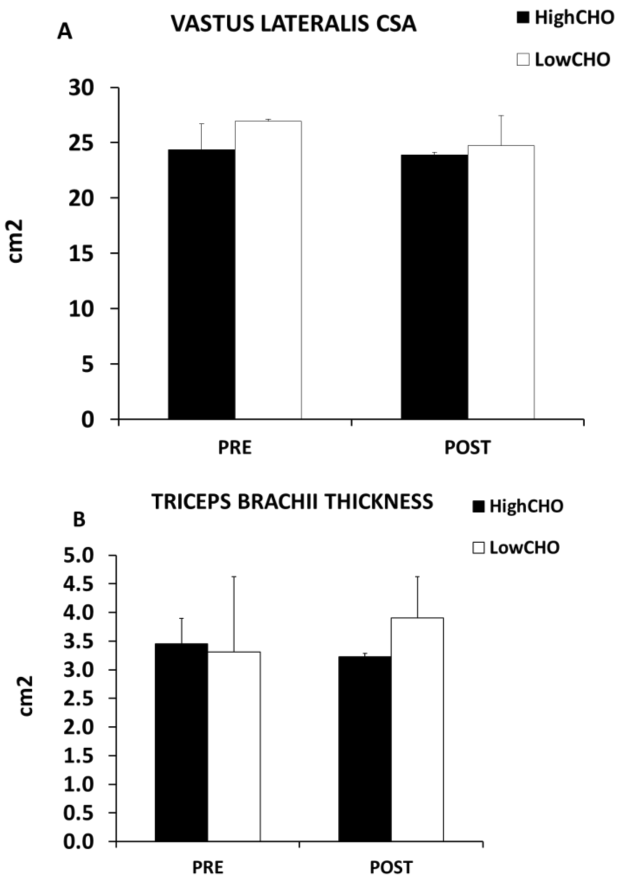


FIGURE 17. Changes in vastus lateralis CSA (A) and triceps brachii thickness (B) in high (n=2) and low (n=2) CHO individuals from the beginning to the end of the diet. (MEAN ± SE)

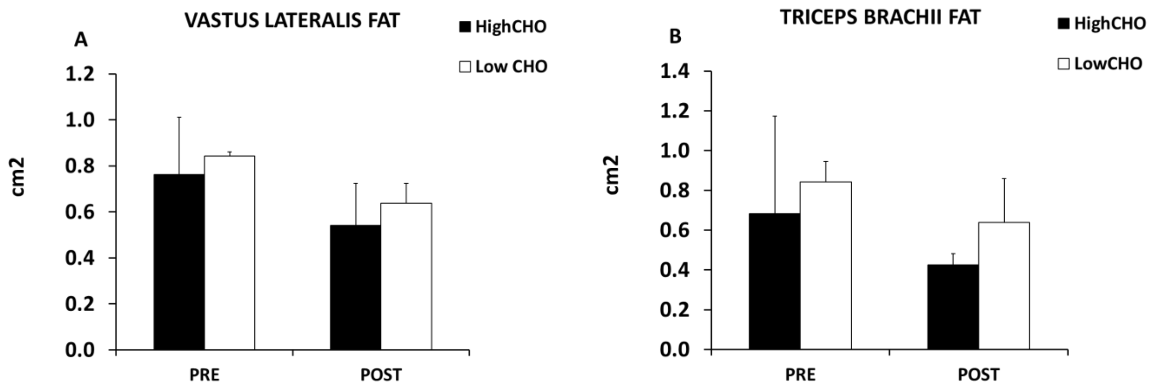



FIGURE 18. Changes in vastus lateralis (A) and triceps brachii fat (B) in high (n=2) and low (n=2) CHO individuals from the beginning to the end of the diet. (MEAN ± SE)

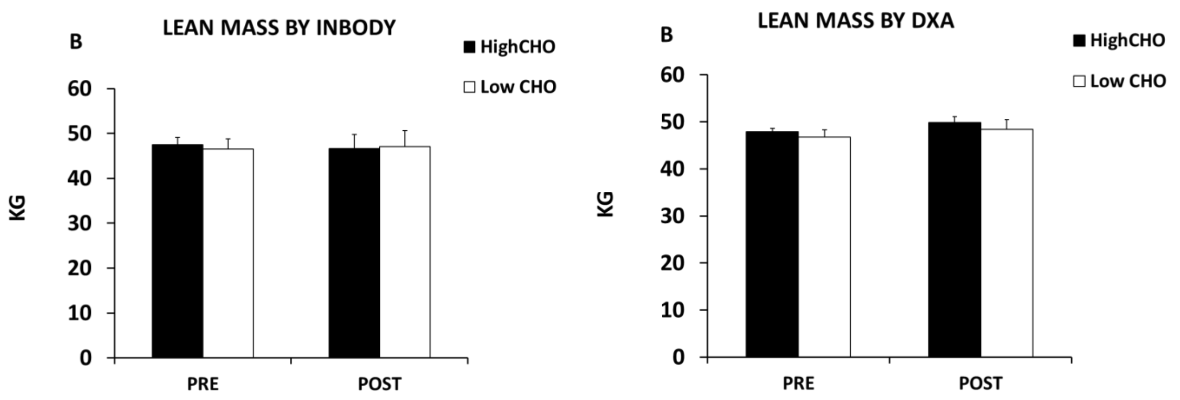



FIGURE 19. Changes in lean mass measured by InBody (A) and DXA (B) in high (n=2) and low (n=2) CHO individuals from the beginning to the end of the diet. (MEAN ± SE)

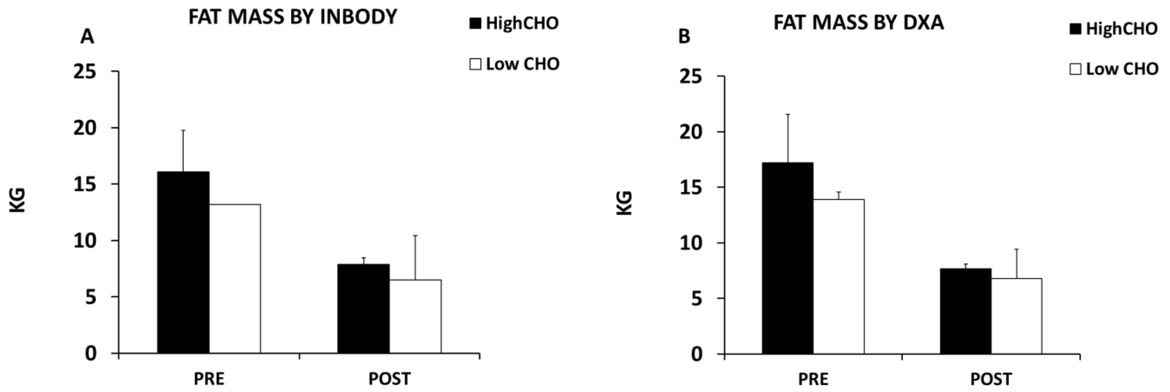



FIGURE 20. Changes in fat mass measured by InBody (A) and DXA (B) in high (n=2) and low CHO (n=2) individuals from the beginning to the end of the diet. (MEAN ± SE)

# 9 DISCUSSION

The aim of this study was to examine the carbohydrate content on body composition during weight loss in female fitness competitors. More specially, the effects of carbohydrate content on lean and fat mass, a muscle size was investigated as well as possible individual differences. The competitors were further divided in two groups based on their carbohydrate intake during the diet, in LC (n=6) and HC (n=6) groups. In addition, 2 participants from the LC group and 2 participants from HC group were chosen in observation based on their similar baseline characteristics. Competition preparation for the competitors mainly involved reducing energy intake while increasing aerobic exercise and strength training targeting to reduce fat and preserve lean mass. The main finding of the study was that there were no consistent effects between the low and high CHO groups in the body composition and muscle/fat size changes. However, there were few differences between the groups. Fat mass decreased in both groups but VL CSA decreased only in LC (lowCHO) group compared to HC (highCHO) group who even managed to slightly increase VL CSA. However, in triceps brachii and in lean mass similar changes were not observed and thus it can´t be strongly stated based on this present data that low carbohydrate intake would spare the lean mass during weight loss in female fitness competitors.

## 9.1 Energy and macronutrient intake

Nutritional energy intake was assessed based on the food diaries which have been reported to underestimates the energy intake in athletes in terms of under-eating or underreporting (Magkos & Yannakoulia 2003; Slater & Phillips 2011). In contrast, fitness and bodybuilders competitors tended to have extremely good self-control therefore, the food diaries might be more valid than usual. The competitors in bodybuilding and fitness aiming to preserve lean mass and reduce fat mass on the competition phase, and this is commonly implemented by caloric restriction and increased aerobic training and strength training (Van der Ploeg et al. 2001; Kistler et al. 2014; Spenlove et al. 2015). Increased aerobic training is reported in previous studies among bodybuilders and fitness competitors. (Van der Ploeg et al. 2001; Mäestu et al. 2010; Kistler et al. 2014; Robinson et al. 2015.)

The energy intake in this study was absolutely (P < 0.03) and per kg bodyweight (P <0.01) significantly lower in LC group (P=0.002) when compared to HC group. The total energy intake was 9374.0 ± 1521.0 kJ and 155 ± 24.7 kJ/kg/d in HC group, and 6144.8 ± 1165.8 kJ and 102.7 ± 20.9 kJ/kg/d for the LC group. Spendlove et al. (2015) presented the results in a wide review of nutrient intake for bodybuilders and energy intake in women ranged from 5081 ± 1697 kJ/day and 91 kJ/kg/d to 7278 ± 4027 kJ/d and 135 kJ/kg/d in the preparation phase. The results were bit higher in HC group than previously reported in female bodybuilders, but the energy intake of LC group was similar than previously reported (Walber-Ranking et al. 1989; Kleiner et al. 1990; Spendlove et al. 2015).

The diets with low carbohydrate and high protein intake reportedly have high satiety value, and yield less metabolizable energy because of their high thermogenic effect, and aim in preservation of lean body mass, which also can enhance energy expenditure. But these changes have shown to be very small (Hall 2016.). Dhillon et al. (2016) presented a significant correlation of protein on fullness, the findings supported that higher protein preloads have a significantly greater effect on fullness than lower protein preloads. Thus, it might be one explanation why the energy intake in LC group was lower than, because the relative protein intake was higher compared to HC group. Total energy intake in LC group was lower already at the baseline, thus the energy intake was lower also during the diet compared to HC group.

Some authors recommend the energy deficit for athletes about 500 kcal to 1000 kcal in terms to maintain lean body mass and reduce fat mass without hampering the physical performance (Fogelholm 1994; Mero et al. 2010; Garthe et al. 2011; Huovinen et al. 2015). The energy expenditure wasn`t measured in this study, therefore, it`s difficult to estimate the amount of energy deficit and it`s possible effects on body composition. The protein is important macronutrient for sparing the lean mass, even if the energy deficit is high, as it is the case in fitness. In the study of Mettler et al. (2010) over 2 weeks of weight loss with energy deficit of 1300 kcal did not reduce lean mass in high protein group. Thus, it seems that even the energy deficit is great the protein-rich diet would spare the lean mass during the weight loss. Kyröläinen et al. (2008) investigated the effects of energy deficit on hormonal concentrations, and they reported that energy deficit of 4000 kcal lead to higher concentration of cortisol and growth hormone during weight loss in military subjects. The hormonal concentrations increased to pre-exercise level during the second (< 450 kcal of ED) and third week (<

1000kcal of ED). It seems that higher than 1000 of energy deficit might influence negatively on hormonal concentrations and, also to weight loss in long-term (Kyröläinen et al. 2008.)

Total physical activity increased in both groups from the pre-state but there were no differences between the groups in total. It´s know that the frequencies of aerobic workouts are increased during the competition phase while strength training might be decreased targeting to maximize fat mass losses (Kistler et al. 2013; Spendlove et al. 2015). This phenomenon was also seen in this study leading to higher frequencies of aerobic workouts and decreased or unaltered frequencies of strength training during the competition phase. HC group did 4.3 ± 0.7 / times per week strength training while LC group did 4.7 ± 0.7 / times per week during the diet.

*Macronutrient intake*. The low energy intake in LC group might be explained by high satiety of protein intake, when the relative protein intake was 45.6 E% and thus higher than in HC group. In contrast, the protein intake absolutely and per kg bw was higher in HC group, but in 2 vs. 2 observation tended to be higher also in LC group in terms of protein intake per kg bodyweight. The protein intake was high in this study in both groups and ranged from 2.7 to 3.9 g/kg even during the energy deficit. Protein intake was significantly higher in HC group when compared to LC group and this could also be explained by higher energy intake in HC group. The high protein intake in strength athletes and during the weight loss has reported in studies of bodybuilders, and Spendlove et al. (2015) reported for protein intake in female bodybuilder about 2.8 g/kg/day.

The carbohydrate content was lower during the diet in diet group compared to control group in general. Carbohydrate intake during the diet in LC group was 81.7 ± 28.0 g/d, and 1.3 ± 0.5 g/kg/bw and in relatively 23 E% and in HC group 174.9 ± 36.3 g/d, and 2.8 ± 0.5 g/kg/bw and in relatively 31 E%. Carbohydrate intake in female bodybuilders was in competition phase about 160 g/day (2.8 g/kg/day) and the proportion of energy from carbohydrate was 48% (Spendlove et al. 2015.). The carbohydrate intake in HC group remained similar than previously reported, except the lower proportion of carbohydrate from energy. Carbohydrate intake in LC group was remarkably lower than in the previous studies. Carbohydrate intake tended to be higher at the baseline, where competitors typically aim to increase lean mass, and lower during the competition phase, like was reported in this present study. (Richardson et al. 2013.)

The fat intake remained decreased in both groups absolutely and per kg bodyweight, but increased relatively in HC group, this is explained by decreased carbohydrate content. The absolute fat intake in LC group was 49.2 ± 11.9 g/d and 65.9 ± 24.8 g/d in HC group, lower amount of fat (24g/d) is reported by Spendlove et al. (2015) in female bodybuilders. The fat intake per kg bodyweight decreased from the baseline in both groups resulting 1.1 ± 0.4 g/kg in HC group and 0.8 ± 0.2 g/kg/bw in LC group. The proportion of fat from the energy was 25.5 E% in HC group and 30 E% in LC group. Spendlove et al. (2015) published lower proportion of fat from the energy compared the results from this study, but the carbohydrate content tended to be higher and explaining the higher fat intake.

### 9.2 Changes in lean mass and muscle CSA

High protein and low carbohydrate diets have been reported to be effective in weight loss for providing lean mass sparing effect, with high satiety and increased energy expenditure. (Walberg et al. 1988; Mettler et al. 2010; Mero et al. 2010; Helms et al. 2014b.) Therefore, such diets have seen among athletes aiming to lose fat mass and maintaining lean mass. In bodybuilding and fitness, the competitors are judged on their muscularity, leanness and physical appearance, and thus the high protein intake and usually low carbohydrate intake has been reported, specifically on contest preparation phase (Van der Ploeg et al. 2001; Kistler et al. 2013; Spendlove et al. 2015).

Lean mass did not change significantly when measured by Inbody and DXA in both groups, but VL CSA decreased in LC group (P=0.02) and remained unchanged or even slightly increased in HC group (P=0.25). Maintained lean mass during competition phase in fitness competitors is investigated in one study (Halliday et al. 2016). In addition, the study by Mäestu et al. (2010) reported similar results on LBM with male bodybuilders. Low carbohydrate, more specifically, ketogenic diets have stated to increase energy expenditure and loss of lean body mass, but the results have shown to be very small. Such diets with high energy expenditure are often connected to losses of lean mass and would enhance protein breakdown in some cases. Thus, it might be one explanation with LC group for losing front thigh muscle size. Robinson et al. (2015) reported similar results of low carbohydrate and high fat diet in male bodybuilder of losing 43% of LBM during preparation period. Garthe et al. (2011) recommended adequate carbohydrate intake with moderate energy deficit and strength training for sparing LBM during weight loss. But, it`s needed to notice that VL CSA was greater at the baseline in LC group compared to HC group. On the other hand, thickness of triceps brachii slightly decreased in HC group (non-significant), but remained unchanged in LC group. Maybe the LC group did less strength training for the lower body and therefore was incapable to maintain VL CSA. It seems that LC group lost more muscle size from the thighs and managed to maintain the thickness of biceps brachii in the upper body, therefore the total LBM tended to be mainly unchanged. The results of the extreme of lowest and highest individuals were bit different, thickness of triceps brachii decreased in HC group individuals, but increased in LC group. It might be that they did different resistance training for different parts of the body, which could explain the changes between the groups. But, even though LC group did more resistance training on the diet the front thigh muscle size decreased by 2.83 cm2 during the weight loss. On the other hand, by InBody and DXA the total lean mass did not reduce in the low and high carbohydrate groups. In contrast, HC group had 0.9 cm2 increase in their vastus lateralis CSA during the diet, and it has reported that lean mass could be increased when combined with strength training and well planned diet, specifically in untrained subjects (Garthe et al. 2011; Helms et al. 2015a). More specifically hypertrophy training is usually connected with improved muscle CSA, and leading to improved strength (Frontera et al. 2000; Fonseca et al. 2014).

The subjects did not have many years of training background and strength training with high protein intake has reported leading to increase of lean mass during the energy deficit. (Josse et al. 2011; Areta et al. 2014; Hulmi et al. 2015; Longland et al. 2016). Strength training attenuates the loss of lean body mass during an energy deficit presumably by stimulating muscle protein synthesis (Pasiakos et al. 2013; Areta et al. 2014). High protein intake with strength training during energy deficit would act synergistically on the rates of muscle protein synthesis, leading in a greater ratio of fat to LBM lost during weight loss, which may be advantageous for physical performance (Mettler et al. 2010; Josse et al. 2011). In terms of this, training background in HC group was 3.1 ± 1.5 years and in 3.8 ± 2.1 years in LC group, and might explain the possible increase in VS CSA. It is stated that isometric force is decreased during the weight loss even though the protein intake is high, specifically in lean athletes. CSA and isometric force have been reported to have connection, thus it might lead to losses of CSA in lean athletes during the weight loss (Ahtiainen et al. 2005; Spendlove et al. 2015; Degouette et al. 2007). The losses of muscle CSA during the weight loss is reported, when fat mass reduces, specifically in lean athletes, if the proportion of initial fat is low

(Huovinen et al. 2015). Also, Forbes et al. (2000) reported that athletes with higher fat% indeed lose less fat-free mass compared to leaner athletes during gradual weight reduction. The fat mass was higher in HC group than LC group, and might explain why they maintained VL CSA better. In contrast, Wilson et al. (2012) argued that aerobic exercise might suppress the gains in lean mass. Aerobic training was increased more in LC group, as mentioned earlier and might also explain the losses of VL CSA. Also, Kistler et al. (2014) resulted reduction in lean mass by 8.8% of one male bodybuilder during the preparation phase. In contrast, Rossow et al. (2013) demonstrated under 12-week case study of one male bodybuilder with smaller lean mass loss (87.65 to 84.84.). So, there are individual differences in maintaining and losing LBM during weight loss, and not one explanation for clarifying the results.

High protein intake > 2.3 g/kg/d is better for maintaining lean mass compared to low protein diet < 1.0 g/kg/d with energy deficit (Mettler et al. 2010). During the contest preparation, bodybuilder increase training volume and aerobic training with restricted calories leading to very lean condition. All these factors increase protein requirements, and thus, optimal protein intakes for bodybuilders may be substantially higher than existing recommendations for maintaining LBM. (Helms et al. 2014a.) It has reported in several weight loss studies that the lean mass is preserved with high protein diet in athletes and active normal weighted people (Walberg et al. 1988; Mero et al. 2010; Garthe et al. 2011; Huovinen et al. 2015). Also, in this study the protein intake was high, about 3 g/kg/bw per day in both groups. It`s well known that high protein > 1.7 g/kg diet with energy deficit could spare lean mass in relation to carbohydrate, as mentioned earlier (Roy 1997; Mero et al. 2010; Pasiakos et al. 2013; Sassatelli et al. 2014; Huovinen et al. 2015). The mechanism for FFM-sparing effect may be related to dietary protein-induced alterations in protein turnover, specifically muscle protein synthesis (Phillips 2008). However, low carbohydrate diet has investigated to enhance catabolism of proteins in the body in acutely and in long term (Lemon & Mullin 1980; Roy et al. 1997; Borsheim et al. 2004; Noakes et al. 2006). In addition, it has also reported to increase amino acid oxidation in glycogen depleted state (Wagenmakers et al. 1991). Therefore, it seems that adequate carbohydrate intake is important for athletes for maintaining protein synthesis (Gaine et al. 2006; Pasiakos et al. 2013). HC group had higher content of carbohydrate, protein (absolutely and per kg bw) and total energy intake during the diet, and could perhaps explain the minor losses of lean mass.

The magnitude of caloric deficit is one of the most important variables that effects on FFM loss. On the other hand, energy deficit and regular exercise training, specifically aerobic and strength training, can independently affect the degree of fat mass reduction and lean mass loss during weight loss (Chaston et al. 2007; Miller et al. 2013). It might be that aerobic or resistance training with energy deficit results in lower LBM loss than energy deficit alone, and training hamper the negative losses of LBM (Miller et al. 2013). Both aerobic and resistance training were used in this study, as mentioned earlier, and need to take into account in the results.

It is also taken into an account that the competition week, which is also named as "peak week" is preparation for competition, and includes tapering of training and carbohydrate loading. This makes muscle mass better comparable in the competition. Gains of glycogen are stated to be associated with an extra three to four parts of water (Olsson & Saltin 1970). One gram of glycogen bound about 2.7 g of water (McArdle et al. 2014, 580). Thus, increased lean mass in this study, when measured by DXA might be related to carbohydrate loading and full glycogen stores. Bone et al. (2016) investigated the validity of DXA after glycogen loading, and reported that carbohydrate loading resulted in overestimated the lean body mass and leg lean mass.

**9.3 Changes in fat mass**

Many studies have suggested that low carbohydrate, and high protein diets and their combination are effective in weight loss, and more specifically for reducing fat mass. (Samaha et al. 2003; Velderhost et al. 2005; Volek et al. 2005; Shia et al. 2008; Paoli et al. 2012.) Fat mass decreased in both groups when measured with all methods (Ultrasound, DXA, Bioimpedance). There was group x time interaction in fat mass measured by InBody ($P < 0.009$), DXA ($P < 0.003$), and ultrasound in VL ($P < 0.001$) and thickness of triceps brachii ($P < 0.01$). In this study, the fat mass decreased by -6.5 kg in HC group and by -6.9 kg in LC group as measured by bioimpedance, and -8.9 kg in HC group and -7.9 kg in LC group as measured by DXA. VL fat decreased in LC ($P=0.04$) and HC group ($P=0.006$), in addition, the fat of triceps brachii remained unchanged in LC group but decreased in HC group. On the other hand, fat of vastus lateralis and triceps brachii decreased more in some high carbohydrate group`s subjects compared to individuals in LC group.

The body weight was 64.8 ± 4.4 kg in HC group at the baseline and 63.9 ± 7.6 kg in LC group. HC group lost more body weight (8.6 ± 2.6 kg) during the diet compared to LC group (7.4 ± 2.5 kg), but the difference wasn`t significant. Similar results on female bodybuilders have reported in previous studies (Walberg-Rankin et al. 1993; Andersen et al. 1995; Van de Ploeg et al. 2001). Heyward et al. (1989) compared, body composition and nutritional profiles of 12 females and nine male bodybuilders during different phase of training, and significant weight loss was demonstrated ($P<0.001$) for male (-5.4kg) and female (-6.0kg), primarily due to the fat loss. However, HC group lost in absolutely more fat mass than LC group but their amount of fat mass was greater at the baseline. Thus, they did not lose fat mass significantly more than LC group. So, LC group had less fat at the baseline, and they lost nearly same amount of fat than HC group leading to lower fat mass after the diet. The findings support the fact, that low carbohydrate and high fat operates mainly by affecting energy intake such that low carbohydrate diets decrease hunger, reduce appetite and promote satiety rather than providing any metabolic advantage for body fat loss (Hall et al. 2015). However, Hall et al. (2015) predicted a model which claims, that low carbohydrate diet and high in fat, but identical protein and calories, would reduce insulin secretion, enhance fat mobilization from adipose tissue and increase fat oxidation compared to high carbohydrate diet. They resulted that, carbohydrate restriction led to maintained increases in fat oxidation and loss of 53 ± 6 g/day of body fat, whereas fat oxidation didn`t change by fat restriction, leading to 89 ± 6 g/day of fat loss, and was significantly greater than carbohydrate restriction ($P=0.002$.) These findings support the results of this present study, where the differences of losing fat mass wasn`t significant between the groups. These findings also suggest that too high energy deficit >1100 kcal is not beneficial anymore aiming to lose fat as much as possible. It needs to notice that higher fat mass at the baseline in HC group might have influenced the great losses of FM during the intervention such it has reported (Huovinen et al. 2015).

The weight loss by low carbohydrate diet is primarily by loss of water, at least in a short-term, and thus it might be that normal or high carbohydrate and high protein intake during the diet would provide better outcomes in long-term, specifically for athletes. Also, Johnston et al. (2014) examined a large meta-analysis of the effects of different diets on body composition, and they did not found any differences between the diets (low carbohydrate and high fat, or high carbohydrate and low fat) on weight loss during 6-month weight loss. The results from this study support the previous findings, that the carbohydrate content is not the key factor in terms of reducing fat mass. The findings also support the fact that the most important thing in weight loss is reduced energy intake rather than macronutrient composition, but it seems to be important to consume high amount of protein during the weight loss aiming to maximize lean mass preservation. (Johnston et al. 2014; Hall et al. 2015; Heisson et al. 2008: Huovinen et al. 2015.) Thus, it might be that the ketogenic diet is more preferable way to lose weight if compared the results of low carbohydrate diet in this study. It seems that either ketogenic diet or low-fat/high or moderate carbohydrate diet might be better to lose weight and preserve muscle mass than low carbohydrate diet, as it was the case in this present study. Merra et al. (2016) investigated the effects of VLCKD on body composition and, the results were good in weight loss without losses of lean mass.

The differences between the high and low carbohydrate groups were minimal in terms of fat reduction, and therefore it needs to take into an account that carbohydrate content is important for athletes, also for strength athletes for providing energy during the exercise, and sparing the lean tissue. If there is not any advantage in terms of greater fat mass reduction during the competition phase on low carbohydrate group, the higher carbohydrate content might be better for fitness athletes. Depletion of glycogen stores, occurring with reduced carbohydrate or energy intake and strenuous exercise, strongly affects the metabolic mixture of fuels for energy. Thus, it`s well known that, depleted glycogen stores impair power output and reduce the general work rate during training and competition, due to prolonged periods of multiple sprints or low carbohydrate diet (Wolinsky & Driskell, 2008, 26). The results from the study support the fact that low carbohydrate diet is not more effective for fat reduction than high or moderate carbohydrate diet with high protein intake. On the other hand, high protein content is beneficial for fitness athletes for maintaining lean mass and decrease of fat mass, but it might be ineffective to consume low carbohydrate on competition phase in terms of the fact that low carbohydrate impairs physical performance.

Macdougall et al. (1999b) demonstrated the importance of muscle glycogen for strength training resulting three sets of bicep curls (8–10 reps per set) at 80 % of 1RM decreased local muscle glycogen content by around 35 %. Thus, it might be important for fitness competitors to consume carbohydrate for maintaining glycogen stores aiming to do strength training and spare lean mass during the competition phase. Some review studies recommend that carbohydrate intakes for strength sports, including bodybuilding, be between 4–7 g/kg/d depending on the phase of training (Slater & Phillips 2011; Helms et al. 2015b). Based on the results of this study, it seems that at least 3 g/kg/bw of carbohydrate provide better results for maintaining LBM or muscle CSA and still reduce fat mass as much as low carbohydrate group during energy deficit. It has reported that carbohydrate supplement give a significant benefit for strength training compared to placebo. Subjects consuming carbohydrate supplement showed better results with a greater number of sets and repetitions during workouts, which is important for bodybuilders and fitness competitors targeting to maximize training adaptations and reduce fat (Haff et al. 1999).

**9.4 Study strength and limitations**

There were several advantages and strengths in this present study. First, the length of the diet was 20 weeks or more, and this was strength of this study comparing the weight loss studies of athletes lasting one month. Secondly, the good comprehensive of analysis in direct body composition measurements was strength in this study. The same technicians were measuring at every timepoint and this could increase the reliability and validity of the results. However, in the light of the results from the present study, the body composition measurements were taken one day after a competition when the glycogen stores were filled after the competition and thus providing more valuable data of changes in lean body mass. In previous studies, the measurements have done after the competition.  In addition, the ultrasound, DXA and InBody together make the data quite validity, if comparing the results only from DXA and InBody, which showed that LBM did not decrease. Thirdly, the scientific studies of female fitness competitors are limited, and this was the first study including many physiological characteristics. It has reported that food diaries are not so valid for assessing nutritional profiles in general, but in this study the extremely good self-control in female fitness competitors was an advantage. In addition, all the analyzed food was weighted and it has investigated to be more valid for assessing nutrient intake. The competitors reported all the food items during the diet, but the under reporting and undereating is always possible, and need to take into an account when analyzing the results.

On the other hand, small sample size (n=6+6) in high and low carbohydrate groups was a limitation in this study. In addition, the high carbohydrate group was not very high compared to previous studies comparing high and low carbohydrate intake, and need to take into an account and could be seen limitation in this thesis. In fact, the carbohydrate intake in high carbohydrate group was lower than previously reported in bodybuilders (Spendlove et al. 2015). Also, the huge differences at the baseline in nutrient intake, muscle CSA and fat and lean mass was limiting the validity and reliability. The energy expenditure was not studied and could not been in a valid way calculated. Thus, the energy deficit could not been calculated.

*Validity.* The assessments of nutrition by the food diaries and/or food records have been considered accurate enough for groups or individuals. In addition, it seems that a 3–4-day estimated diet record has been recognized as the most generally used approach. Weighed diet records are certainly more accurate than estimated ones, and in this study the nutritional data was assessed by properly planned diet with only weighed food items (Dwyer 1999; Magkos & Yannakoulia (2003). However, Magkos & Yannakoulia (2003) suggested that dietary assessment tools still need validation among athletic population. The possible errors or limitation due to the food records could be minimized with motivated subjects, and the subjects who were chosen to this study were motivated, and they have extremely good self-control increasing the validity of the nutritional data. But as long as the food diaries rely on self-reporting, it is difficult to interpret the results from the studies, and accurately measure adherence to the recommended diets (Hall et al. 2015).

The different results on LBM and muscle CSA changes might conducted to the validity and reliability of different methods to measure changes in body composition (Pateyjohns et al. 2006; Bosy-Westphal et al. 2008; Ackland et al. 2012) It might be that bioimpedance overrate the lean mass and underrate the fat mass against to DXA (Sillanpää et al. 2014). But on the other hand, Bone et al. (2016) resulted greater lean mass after glycogen loading, and thus might overrate the lean body mass. The changes in LBM and FM were investigated during weight loss and the results in differed of 4-component model: 1.9% DXA, 2.1% bioimpedance and 2.0% skinfolds (Evans et al. 1999). Kiebzak et al. (2000) investigated the validity of DXA and resulted of the precision (CV %) 1.1% for lean tissue and 2.0% for FM. In the previous study, it has stated that ultrasound is more valid method to measure changes in muscle mass or fat mass when compared to DXA or bioimpedance. In general, DXA has shown to be a quite suitable, reliable and mostly very accurate method to measure short-term changes in body composition (Kiebzak et al. 2000; Aasen et al. 2006).

Ultrasound has shown to be a reliable and valid method to estimate body composition, specifically adipose tissue and muscle cross-sectional area, and thus the results of changes in muscle CSA could be the most reliable in this study. (Ahtiainen et al. 2010; Mendis et al.

2010; Müller et al. 2010; Schlecht et al. 2014.). Ultrasound is used to measure bones, tendons, muscle cross-sectional area (CSA), muscle size, adipose tissues and veins. It has reported to be a reliable and valid method to measure body composition, and more specifically adipose tissues (subcutaneous and visceral adipose tissue) and muscle CSA in many studies. (Ahtiainen et al. 2010; Mendis et al. 2010; Müller et al. 2010; Schlecht et al. 2014.) The gold standard method for assessing skeletal muscle CSA is MRI (coefficient variation <1%) (Reeves et al. 2004). MRI is very expensive method, so ultrasound has been used as a valid and low-cost method for skeletal muscle assessment. Ultrasound provides high-quality images of muscle CSA, and it shows the differences between the tissues, but the limitation of B-mode ultrasound is that the US probes don`t show the whole muscle CSA in the images (Lixandrao et al. 2014). Ultrasound is compared against different techniques such as MRI, CT and DXA and it correlated strongly in many studies, for example MRI and US correlation was 0.905 in one study when measuring muscle CSA. (Ribeiro-Filho et al. 2003; Ahtiainen et al 2010; Kim et al. 2016).

In the future, the energy intake should be set at the same level, and thus providing better results on carbohydrate content on body composition. Would be interesting to investigate the high and low carbohydrate groups with same protein intake in weight loss, because it`s known that high protein intake would result in greater weight loss than moderate protein intake. It would be beneficial for fitness competitors to know the limit for adequate protein intake leading to provide the best results in terms of sparing lean mass and reducing fat mass during the weight loss. In addition, the energy expenditure should be measured with direct calorie method without the possible errors in estimating MET-hours and physical activity. In addition, the effect of different diets on body composition, hormonal changes and recovery should be investigated in the future for providing better information of weight loss in female fitness competitors. The sample size should be larger in the future, specifically when investigating the nutrition and its effectiveness on weight loss.

### 9.5 Conclusion

The competition phase mainly involved reduced energy intake while increased aerobic exercise and strength training targeting to reduce fat and preserve lean mass. The main finding of the study was that there were no consistent effects between the low and high CHO groups in the body composition and muscle/fat size changes. However, there were few differences between the groups. Fat mass decreased in both groups but VL CSA decreased only in LC group compared to HC group who even managed slightly to increase the VL CSA.  It needs to notice that VL CSA was greater in LC group at the baseline compared to HC group, and might have affected loss of VL CSA. However, in triceps brachii and in lean mass similar changes were not observed and thus it cannot be strongly stated based on this present data that low carbohydrate intake would spare the lean mass during weight loss in female fitness competitors. In addition, the lower energy and protein intake (g/kg) with lower carbohydrate intake and greater frequency of aerobic exercises might have affected losses in VL CSA. The total lean body mass did not decrease in either group when measured by InBody and DXA, but in the light of this study seems that the total outcomes of HC group were a slightly better, and might have protected the possible losses of muscle size at least in front thighs. The sample size was quite small and, thus the future investigations with greater sample size and direct energy expenditure measurements are needed.

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