THE EFFECTS OF PROTEIN AND CARBOHYDRATE SUPPLEMENTATION ON BODY COMPOSITION AND MUSCLE STRENGTH DURING RESISTANCE TRAINING

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ABSTRACT

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Introduction. The importance of body composition for health and performance are well known. Resistance training and diet are known to affect body composition. The purpose of this study was to examine the effects of different post-exercise supplementation regimens on body composition and strength development during resistance training.
Methods. A total of 78 healthy, recreational active men (mean ± SD: age 34.7 ± 1.4, height 179.9 ± 0.77 cm, weight 83.6 ± 1.4 kg) were included in this study. All subjects completed a supervised resistance training program, which included a 4-week habituation period. After the habituation period, the subjects were divided into three supplementation groups (protein, carbohydrate, and protein+carbohydrate). Within each of these three supplementation groups, the subjects were further divided into power-strength or hypertrophic-strength training groups. Subjects kept 4-day dietary records during the first month of the 12-week supplementation period. Body composition was determined using DXA at baseline, after the 4-week habituation training, and after the 12-week training period with supplementation. Cross sectional area of the knee extensor muscles were measured using ultrasound after habituation training and after 12 weeks. Strength measurements were done at baseline, after the habituation training, and after 8 and 12-weeks of actual training. Muscle strength was measured by using both dynamic and isometric leg press.
Results. The dietary intake did not differ significantly between groups relative to body weight, but total dietary intake was smaller in the protein than carbohydrate group during the recovery days ($P < 0.05$). Significant increases following the resistance training for all three groups were seen in total fat free mass ($P < 0.001$) and leg fat free mass ($P = 0.003$) without differences between the three supplementation groups. Total fat mass ($P = 0.001$) and android fat mass ($P < 0.001$) decreased with resistance training while trunk fat mass was unchanged ($P = 0.065$). A nutrition x time interaction was detected for total fat mass ($P = 0.032$), trunk fat mass ($P < 0.001$), and android fat mass ($P = 0.014$). These changes were larger in the protein group compared with the carbohydrate group ($P = 0.03$, $P < 0.001$, and $P = 0.01$, respectively). Significant increases following the resistance training for all three groups were seen for 1 RM ($P < 0.001$) and cross sectional area of quadriceps femoris ($P < 0.001$). No nutrition x time interaction effects were observed for 1RM strength ($P = 0.360$) or for cross sectional area of quadriceps femoris ($P = 0.715$).
Conclusion. The main finding for this study was that protein supplementation after resistance training session reduces total fat, trunk fat, and android fat mass more than carbohydrate or protein plus carbohydrate supplementation. These results highlight the importance of diet and resistance training in the modification of body composition and in the promotion of health.

Keywords: Resistance training, supplementation, diet, fat mass, fat free mass

TIIVISTELMÄ

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Johdanto. Lihavuudesta on tullut maailmanlaajuinen epidemia, ja sen liitännäissairaudet ovat suuri huolenaihe. Voimaharjoittelun ja ravitsemuksen tiedetään muokkaavan tehokkaasti kehon koostumusta. Tämän tutkimuksen tarkoituksena oli selvittää miten proteiini ja hiilihydraatti yhdistettynä voimaharjoitteluun vaikuttavat kehon koostumukseen sekä voimaan.
Menetelmät. Tutkimukseen osallistui 78 miestä (ikä 34.7 ± 1.4, pituus 179.9 ± 0.77 cm, paino 83.6 ± 1.4 kg), jotka eivät olleet tottuneet voimaharjoitteluun. Tutkimus sisälsi kokonaisuudessaan 16 viikkoa voimaharjoittelua, joista ensimmäiset neljä viikkoa sisälsivät kaikilla kestovoiman harjoittelua. Tämän perehdyttämisjakson jälkeen koehenkilöt jaettiin kolmeen lisäravinneryhmään (proteiini, hiilihydraatti, proteiini+hiilihydraatti). Koehenkilöt jaettiin lisäksi kahteen eri harjoitteluryhmään, nopeus-maksimivoima- ja hypertrofis-maksimivoimaryhmään. Koehenkilöt täyttivät ruokapäiväkirjan ryhmiin jakautumisen jälkeen. Kehon koostumus mitattiin DXA:lla alkutilanteessa sekä 4 ja 16 viikon jälkeen. Reiden lihasten poikkipinta-ala mitattiin ultraäänellä alkutilanteessa sekä 16 viikon jälkeen. Voimamittaukset suoritettiin alkutilanteessa sekä 4, 12 ja 16 viikon jälkeen.
Tulokset. Kehon painoon suhteutettuna ravitsemus ei eronnut merkitsevästi ryhmien kesken, mutta absoluuttisesti energiansaanti oli proteiiniryhmällä vähäisempää palautumispäivinä verrattuna hiilihydraattiryhmään ($P < 0.05$). Kehon rasvaton massa ($P < 0.001$) ja jalkojen rasvaton massa ($P = 0.003$) lisääntyivät merkitsevästi tutkimuksen aikana. Kehon kokonaisrasvan määrä ($P = 0.001$) ja vatsan alueen rasvan määrä ($P < 0.001$) vähenivät voimaharjoittelun seurauksena, kun taas ylävartalon rasvan määrä ($P = 0.065$) pysyi muuttumattomana. Ravinnon ja ajan yhdysvaikutus havaittiin kehon kokonaisrasvan massalle ($P = 0.032$), ylävartalon rasvan massalle ($P < 0.001$), ja vatsarasvalle ($P = 0.014$). Muutokset olivat merkitsevästi suurempia proteiiniryhmässä verrattuna hiilihydraattiryhmään (kehon rasva, $P = 0.03$; vartalon rasva, $P < 0.001$; vatsarasva, $P = 0.01$). Voimaharjoittelun seurauksena jokaisella ryhmällä parani merkitsevästi 1 RM ($P < 0.001$) sekä suureni reiden poikkipinta-ala ($P < 0.001$).
Johtopäätökset. Proteiini yhdistettynä voimaharjoitteluun vähentää merkitsevästi kehon kokonaisrasvan, ylävartalon rasvan sekä vatsarasvan määrää. Tulosten perusteella voimaharjoittelu yhdistettynä harjoittelun jälkeiseen proteiinin nauttimiseen vaikuttaa olevan tehokas tapa vähentää kehon rasvan määrää sekä samalla ylläpitää tai lisätä rasvatonta massaa ja näin ollen edesauttaa hyvää terveyttä.

Avainsanat: Voimaharjoittelu, ravintolisä, ravitsemus, rasvan massa, rasvaton massa

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1 INTRODUCTION

Resistance training has long been known to increase functional abilities and health status. One primary adaptation to resistance exercise is the hypertrophy of skeletal muscle. Resistance training changes body composition by increasing lean mass, which is composed mainly of muscle mass, and decreasing fat mass. Physical performance will also be enhanced. Improvements in muscular strength occur as a result of an increase in muscle cross-sectional area and the ability to effectively activate motor units. (Docherty & Sporer 2000; Westcott 2012.)

It has been well established that ingestion of dietary protein after resistance-type exercise increases post-exercise muscle protein synthesis rates and may inhibit, at least to some extent, muscle protein breakdown. This allows net muscle protein accretion during the acute post-exercise recovery period. (Burke et al. 2001; Tipton et al. 1999.) As such, a meta-analysis of protein supplementation studies suggested that dietary protein supplementation maximizes the adaptive response of skeletal muscle to at least more prolonged and heavy resistance-type exercise training (Cermak et al. 2012). Acute studies have suggested that carbohydrates do not further improve muscle protein balance acutely after single resistance exercise bout (e.g. Koopman et al. 2007; Staples et al. 2011). However, no studies have examined the effects of added carbohydrates on resistance training adaptations when compared to protein alone. In addition to just increasing protein intake, it has been suggested that nutrient supplement timing might be critically important in stimulating muscle adaptations that occur during prolonged training (Kerksick et al. 2008). However, this notion has recently been criticized (Aragon & Schoenfeld 2013).

The aim of this study was to examine the effects of different post-exercise supplementation regimens on resistance training adaptations. More specifically, the effects of protein and carbohydrate supplementation alone or in combination were investigated on body composition and strength.

2 RESISTANCE TRAINING

Resistance training has long been known to increase functional abilities and health status, primarily by changing body composition and physical performance. Improvements in muscular strength occur as a result of an increase in muscle cross-sectional area and the ability to effectively activate motor units. The increase in muscle cross-sectional area is considered to occur as a result of protein synthesis, which produces a greater number of contractile units. Motor unit activation is enhanced by a greater number of fibers being recruited, increased firing frequency, decreased co-contraction of agonists, better motor unit synchronization, and inhibition of reflexive mechanisms. (Westcott 2012.) Strength training is primarily anaerobic and results in increased muscle glycolytic enzyme activity, and intramuscular ATP and phosphocreatine stores (Tanaka & Swensen 1998). The magnitude of hypertrophy or strength improvements depend primary on the volume and intensity of the training stimulus (Docherty & Sporer 2000).

2.1 Different types of resistance training

Adaptations to exercise training and the resulting improvements in performance are highly specific to the mode of activity performed. The key components of a training program are the volume, intensity, and frequency of exercise sessions. The sum of these components can modify the training stimulus. (Hawley 2009.)

2.1.1 Strength (maximum and hypertrophic strength)

Strength is the ability of the neuromuscular system to exert force against an external resistance. Maximal strength is defined as the greatest amount of force that a muscle or muscle group can generate. Maximal strength depends on various factors; for instance, the number of recruited motor units, the motor unit firing rate, the muscle fiber type, and the degree of muscle hypertrophy.(McArdle 2013, 528–541.)

Maximum strength can be divided into hypertrophic and neural maximum strength. Some studies such as Widrich et al. (2002) have demonstrated that muscle size and absolute maximal strength have a linear relationship. For instance, according to some studies, the correlation between cross-sectional area of leg muscles and maximum strength has been about 0.5-0.7 (Aagaard et al. 2001; Masuda et al. 2003; Trappe et al. 2001). However, it is possible to increase maximal force without hypertrophy. In individuals without earlier experience with regular strength training, increases in strength are usually caused initially by neural factors with only minor hypertrophy (Häkkinen & Komi 1983; Häkkinen et al. 1998). Strength training often involves using high loads, which consequently lead to a great time under tension and low movement velocity (Burd et al. 2012).

Skeletal muscle hypertrophy is characterized by an increase in the size and number of myofibrils, which lead to the enlargement of muscle fibers, whole muscle cross-sectional area and volume (Ahtiainen et al. 2003; Esmarck et al. 2001). Genes, physical activity, diet, the endocrine system, the environment, and neuromuscular activity have an impact on the development and maintenance of muscle hypertrophy. In this type of training, the purpose is to achieve a high total load of training, which leads to acute fatigue of the neuromuscular system and significant muscular responses (Schoenfeld 2013).

Muscle hypertrophy results from muscle fiber deformation induced by repeated training. More specifically, it means that mechanical and metabolic stress on the components of the muscular system trigger signaling proteins to activate genes that in turn activate the translation of messenger RNA and further stimulate protein synthesis in excess of protein breakdown. (Schoenfeld 2013.) In addition, the overcompensation of protein synthesis occurs especially when the effects of insulin are combined with adequate amino acid availability, producing a net anabolic effect. Strength training also activates satellite cells, which have a regenerative function on cellular growth and impact on muscle hypertrophy. As a result, the myofibrils of the cell thicken and the amount of myofibrils and sarcomeres increases. (Phelan & Gonyea 1997.)

2.1.2 Speed-strength (power)

Maximal power is the product of velocity and force (McArdle 2013, 503). To increase muscle power, intensity of training must be maximal and loads are usually suggested to be should be between 30–60 % of 1 RM (Kraemer & Häkkinen 2002, 20–36). However, loads are dependent on the individual, the exercise type, and which muscles are involved (Bevan et al. 2010). Maximal power can be expressed with force-velocity curve, where movement velocity decreases, when an external resistance increases. A resistance training program can focus on the high-force portion or high-velocity portion of the force-velocity curve. (McArdle 2013, 499–535.)

Power training is performed using light loads and high movement velocities. Malisoux et al. (2006) reported an average of 25 % hypertrophy across fiber types using an unloaded, i.e., plyometrics, power training. Therefore, it appears that muscle hypertrophy can occur even without large external loads if the forces are high enough such as with plyometric training. Indeed, some studies have shown that high movement velocities may produce greater muscle hypertrophy than low movement velocity, when the workload is equalized between them. For example, it has been shown that high velocity eccentric muscle actions may result in greater hypertrophy than low velocity eccentric muscle actions, which may be due to greater protein remodeling (Farthing and Chilibeck 2003; Shepstone et al. 2005).

2.2 Effect of resistance training on body composition

Usually, when examining body composition, the body is divided into two components: fat mass and fat-free mass. The terms fat-free mass and lean body mass are often used synonymously; however, these two terms have different definitions. Fat-free mass refers to only all non-fat tissue while lean body mass refers to all non-fat tissue plus essential fat. With different measurements (e.g. dual-energy X-ray absorptiometry), that are commonly used to determine body composition, it is not possible to differentiate between essential fat and non-essential fat. Due to this, fat-free mass is actually what is being determined. (Fleck & Kraemer 2014, 109.)

Normally, the goals of a resistance training program are to increase fat-free mass and decrease fat mass. Usually, when fat-free mass and muscle mass increase, fat mass decreases; however, adaptations to physical exercise are highly specific. (Fleck & Kraemer 2014, 113.) Several studies report significantly greater changes in body composition with high-volume, multiple-set programs compared to low-volume, single set programs (Kraemer et al. 2000). Especially periodized heavy resistance strength training can effectively increase muscle mass and strength both in older men and women (Häkkinen et al. 2002; Kraemer et al. 2004; Sillanpää et al. 2008). Normally the largest increases in muscle mass consistently reported are a little greater than 3 kg in approximately 10 weeks resistance training. This translates into an increase in fat-free mass of 0.3 kg per week, but mean increases in training are typically much lower. (Fleck & Kraemer 2014, 114.) For instance, Westcott et al. (2009) demonstrated that 10 week resistance training typically increased lean mass on average by 1.35 kg.

Sillanpää et al. (2008) measured how a 21-week long strength and endurance training period affected body composition. According to Sillanpää et al. (2008) waist circumference decreased on average 2.9 cm in the strength training group and the decrease was already significant after 10 weeks of training. The lean mass in the legs increased significantly by strength training but not by endurance training (figure 1). (Sillanpää et al. 2008.)

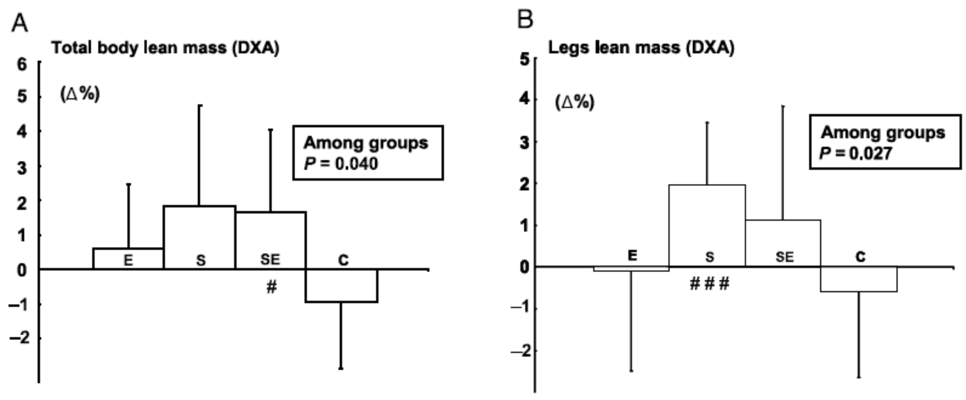

FIGURE 1. Total body lean mass (A) and legs lean mass (B) changes during the 21-week training period. E = endurance, S = strength, SE = combined strength and endurance training group, and C = control group. # # # P < 0.001, # P < 0.05 significant difference within group from week 0 to week 21. (Sillanpää et al. 2008.)

Sigal et al. (2014) compared the effects of aerobic training, resistance training, and their combination on body composition in a large sample of obese adolescents. They demonstrated that resistance training produced greater decreases in percentage body fat, waist circumference, and BMI than aerobic training alone. Moreover, Lee et al. (2012) found that when compared with controls, significant reductions in total and visceral fat were observed in both exercise groups. However, compared with controls, a significant improvement in insulin sensitivity (27 %) was observed in the resistance exercise group only. (Lee et al. 2012.) In addition, Dobrosielski et al. (2013) found that in adults with and without type 2 diabetes mellitus, after a 26-week long resistance training period, the reduction in visceral adipose tissue was attenuated in all groups (-3 to -18 %). These results suggest that resistance training promotes total body fat and abdominal fat loss.

2.3 Effect of resistance training on muscle size and fat mass regulation

2.3.1 Regulation of muscle size

Increases in skeletal muscle mass and cross-sectional area is a widely recognized adaptation to resistance training. This skeletal muscle hypertrophy has been associated with changes in the gene expression and the rate of protein synthesis (Coffey & Hawley 2007; Toigo & Boutellier 2006). Accordingly, in the recent years, the molecular mechanisms underlying muscle hypertrophy has been widely studied (Glass 2005; Welle et al. 2007). It has been recognized that a protein kinase called mechanistic target of rapamycin (mTOR) is a key regulator of muscle growth. Muscle protein synthesis can increase through an mTOR-dependent mechanism after elevations in energy status, amino acids, and growth factors. Furthermore, several studies have shown that signaling through mTOR is required for maximal mechanically induced increases in muscle protein synthesis and ultimately in muscle hypertrophy. (Drummond et al. 2009; Kubica et al. 2005.)

Muscle protein synthesis is depressed during resistance exercise. This fall in protein synthesis has been associated for instance with a decrease in mRNA translation initiation. This is due to reduced phosphorylation of a downstream effector of mTOR.

Additionally, one reason leading to a fall in protein synthesis may be a rise in phosphorylation of a negative regulator of peptide-chain elongation (Dreyer et al. 2006). However, it is widely recognized that resistance exercise results in increased muscle protein synthesis in the post-exercise recovery period (Kumar et al. 2009).

The molecular basis of the adaptations produced by different resistance training regimens, such as power and strength training, is hard to pinpoint. Recently, Lamas et al. (2010) demonstrated that both strength training and power training successfully induced transcription of genes related to the mTOR pathway although the array of pathways modulated by mechanical stimuli is largely unknown in humans. Consequently, it is crucial to identify and characterize such pathways in order to provide a better understanding of the molecular mechanisms related to the specific outcomes of different resistance training regimens. (Leal et al. 2011.)

2.3.2 Regulation of fat mass

The main role of subcutaneous and visceral fat mass is the supply and storage of energy, which is carried out by adipocytes within the tissue. Excess of ingested energy is stored within adipocytes in the form of triglycerides. During exercise, these triglycerides are broken down due to the secretion of catecholamines (adrenaline and noradrenaline). In results, fatty acids are transported to tissues such as skeletal muscle for used as energy. (Horowitz. 2003.) Adipose tissue also secretes different humoral factors called adipokines that participate in many metabolic reactions. Obesity is known to cause the dysregulated expression of adipokines. This dysregulated expression has been associated with the onset of lifestyle-related diseases such as diabetes. (Lumeng & Saltiel 2011; Rabe et al. 2008.)

Adipose tissue can expand by increasing the size and/or number of fat cells by overfeeding. Tchoukalova et al. (2010) demonstrated that after overfeeding, the size but not the number of abdominal subcutaneous adipocytes increased significantly in response to fat gain. However, they also demonstrated that lower-body subcutaneous adipocyte size remained unchanged in spite of increased leg fat, whereas overfeeding resulted in a significant increase adipocyte number. (Tchoukalova et al. 2010.) Due to this, overfeeding may result different responses in different parts of the body, but more studies are needed.

It has been demonstrated by investigating 10 500 healthy men during a 12-year follow-up that resistance training is an effective way to reduce or prevent gain in waist circumference and, therefore, abdominal obesity (Mekary et al. 2015). One of the reasons for a resistance training-induced reduction in adipose tissue mass might be hypotrophy of mature adipocytes (Stotzer et al. 2015). This hypotrophy has been associated with greater adipocyte lipolysis in exercise-trained subjects compared to sedentary controls (Nomura et al. 2002). Resistance training appears also to suppress adipocyte differentiation, and in results, the number of adipocytes decreases (Sakurai et al. 2010). However, the effects of resistance training on the number of adipocytes contained in adipose tissue are controversial. Some other studies have shown that adipose tissue in exercise-trained rodents contains significantly fewer cells than that in sedentary controls (Oscai et al. 1974; Sakurai et al. 2010). In contrast, some studies have found no significant difference in adipocyte numbers (Bukowiecki et al. 1980). These differences may be due to the difference in the starting age for resistance training. Moreover, when resistance training was started at a very early stage of a rodent's life (0.7–1.1 weeks of age), it reduced both adipocyte number and size. However, when resistance training was started at 9–27 weeks of age, only adipocyte size was reduced in rodents. (Bukowiecki et al. 1980; Oscai et al. 1974.)

3 NUTRITION

Carbohydrate, lipid, and protein nutrients provide energy to maintain bodily functions during rest and physical activity. Aside from their role as biologic fuel, these nutrients, called macronutrients, preserve the structural and functional integrity of the organism.

3.1 Proteins

Combinations of linked amino acids form proteins. The body requires 20 different amino acids for muscle protein synthesis. The number of possible protein structures is enormous because of the tremendous number of combinations of 20 different amino acids. (McArdle et al. 2013, 30-39.)

3.1.1 Structure of proteins

Proteins contain atoms of carbon, oxygen, and hydrogen. Protein molecules also contain nitrogen along with sulfur and occasionally phosphorus, cobalt, and iron. Amino acids act as “building-blocks” for protein molecules. The protein molecule polymerizes from its amino acid constituents in numerous complex arrays. Peptide bonds link amino acids in chains that take on diverse forms and chemical combinations. A dipeptide is a molecule, which includes two amino acids that are linked by peptide bonds. Three linked amino acids are called a tripeptide. A polypeptide chain may contain even more than 1000 amino acids, but at least more than 50. A combination of more than 50 amino acids forms a protein, of which humans can synthesize an array of different kinds. Single cells contain thousands of different protein molecules meaning that, altogether, approximately 50 000 different protein-containing compounds exist in the human body. (McArdle et al. 2013, 30.)

The 20 different amino acids required by the body for the protein synthesis each have a positively charged amino group at one end of the molecule (NH2) and negatively charged organic acid group at the other end (COOH). The remainder of the amino acid, referred to as the R group, takes on a variety of forms. The R group's specific structure dictates the amino acid's particular characteristics. (McArdle et al. 2013, 30.)

3.1.2 Functions of proteins in the body

The main function of protein in the body is to provide the building blocks for synthesizing cellular material during anabolic processes. Proper energy and protein intake are recognized as important dietary factors for optimal muscle hypertrophy during strength training. (Sallinen et al. 2007.) Amino acids also contribute carbon skeletons for energy metabolism. They also incorporate nitrogen into heme components of hemoglobin and myoglobin compounds, catecholamine hormones epinephrine and norepinephrine, and the serotonin neurotransmitter. Amino acids activate also vitamins that play a key role in metabolic and physiologic regulation. (McArdle et al. 2013, 31-39.)

Proteins serve as primary constituents for plasma membranes and internal cellular material. Collagenous structural proteins compose, for example, the tendons and ligaments. Globular proteins make up the nearly 2000 different enzymes that speed up chemical reactions and regulate the catabolism of nutrients for energy release. Blood plasma also contains the specialized proteins thrombin, fibrin, and fibrinogen required for blood clotting. Within red blood cells, the oxygen-carrying compound hemoglobin contains the large globin protein molecule. Proteins help to regulate the acid-base characteristics of bodily fluids. The structural proteins actin and myosin play the predominant role in muscle action as they slide past each other during movement. (McArdle et al. 2013, 31-39; Mortimore & Pösö 1987.)

3.1.3 Supplementary protein in sports

There are many different protein sources that include animal (e.g. whey, casein, egg, and fish) and plant protein (e.g. soy and rice) sources. These sources differ from each other in numerous ways such as the presence of allergens, saturated fats, digestion rate, or the relative amount of individual amino acids. Plant protein sources are more often lower in one or more essential amino acids compared to dairy protein. Due to this, plant protein sources often fail to match the requirements of a complete protein. (Joy et al. 2013.)

In particular, whey is considered a high-quality protein fraction because it contains an abundance of essential amino acids, which are necessary for stimulating protein synthesis and supporting muscle growth (Borsheim et al. 2002; Ha & Zemel. 2003). The rate and concentration of amino acid availability may be predictive of the muscle protein synthesis response (Koopman et al. 2009). This may explain the superiority of whey versus soy, for instance, at stimulating muscle protein synthesis (Tang et al. 2009). Differences in protein digestion rates and differences in branched-chain amino acid content may impact the ability of the protein to maximize post-exercise protein synthesis (Shimomura et al. 2006).

3.2 Carbohydrates

Atoms of carbon, hydrogen, and oxygen combine to form a basic carbohydrate, sugar, molecule. Carbohydrates classify as monosaccharides, oligosaccharides, or polysaccharides. The number of simple sugars linked within each of these molecules distinguishes each carbohydrate form. (McArdle et al. 2013, 8.)

3.2.1 Structure of carbohydrates

Carbon combined with oxygen and hydrogen forms a basic carbohydrate or sugar molecules. Simple sugars consist of chains of 3 to 7 carbon atoms, with a hydrogen and oxygen ratio of 2:1. There are three groups of carbohydrates: monosaccharides, oligosaccharides, and polysaccharides. (McArdle et al. 2013, 8-18; Ivy et al. 2002.)

The monosaccharide represents the basic unit of a carbohydrate. Glucose, fructose, and galactose represent the three major monosaccharides. Glucose, also called blood sugar, consists of a 6-carbon (hexose), 12 hydrogen, and 6 oxygen atoms ($C_6H_{12}O_6$). Fructose and galactose, two other simple sugars with the same chemical formula as glucose, have a slightly different C-H-O linkage and are thus different substances with distinct biochemical characteristics. (McArdle et al. 2013, 8.)

Oligosaccharides form when 2 to 10 monosaccharides bond chemically. The major oligosaccharides, the disaccharides, form when two monosaccharide molecules combine. Monosaccharides and disaccharides collectively are called simple sugars. The three principal disaccharides include sucrose (glucose + fructose), lactose (glucose + galactose), and maltose (glucose + glucose). In addition, three or more sugar molecules linked together are called polysaccharide. There could be even thousands of sugar molecules in one polysaccharide. Polysaccharides form during the chemical process of dehydration synthesis, a water-losing reaction that forms a more complex carbohydrate molecule. For example, starch and fiber are the common forms of polysaccharides. (McArdle et al. 2013, 8-18.)

Glycogen is the storage carbohydrate within humans and other mammalian muscle and liver. It forms as a large polysaccharide polymer synthesized from glucose in the process of glycogenesis. Irregularly shaped, glycogen ranges from a few hundred to 30 000 glucose molecules linked together with branch linkages for joining additional glucose units. A well-nourished 80-kg man stores approximately 500 grams of carbohydrate. Of this, muscle glycogen accounts for the largest reserve, approximately 400 grams. The remaining glycogen, approximately 90 to 110 grams, is stored in the liver. Only 2 to 3 grams of the stored carbohydrates is in the blood as glucose. (McArdle et al. 2013, 12.)

3.2.2 Functions of carbohydrates in the body

Carbohydrates have four important functions related to energy metabolism and exercise performance. Carbohydrates serve as energy fuel, protein-sparer, metabolic primer, and fuel for the central nervous system. The most important function of carbohydrates is to act as an energy fuel, particularly during intense physical activity. Energy derived from the catabolism of blood borne glucose and muscle glycogen powers contractile elements of muscle and other forms of biologic work. Carbohydrates also preserve tissue protein. Normally, protein serves an important role in tissue maintenance, growth, and repair, and to a considerably less as a nutrient energy source. However, depletion of glycogen reserves dramatically affects the metabolic mixture of fuels of energy. Glycogen depletion triggers glucose synthesis from the labile pool of amino acids. (McArdle et al. 2013, 14-15.)

Components of carbohydrate catabolism serve as a “primer” substrate for fat oxidation. Insufficient carbohydrate or alternatively amino acid (Kainulainen et al. 2013) breakdown causes fat mobilization to exceed fat oxidation. The lack of adequate byproducts of glycogen catabolism produces incomplete fat breakdown with accumulation of ketone bodies. This could increase body fluid acidity and lead to a potentially harmful acid condition. The fourth important function of carbohydrate is to serve fuel for the central system. Under normal conditions, the brain metabolizes blood glucose almost exclusively as its fuel source. (McArdle et al. 2013, 14-15.)

3.2.3 Supplementary carbohydrates in sports

There are many different supplementary carbohydrate sources used in sports. When exercise is short, intermittent or prolonged, there are differences in which type carbohydrate should be ingested. For instance, when exercise lasts for more than a couple of hours, total carbohydrate plays a significant role in sustaining power output. According to studies, combinations of glucose, fructose, and sucrose have yielded 20–55 % greater carbohydrate availability than glucose alone (Jentjens et al. 2006; Jeukendrup et al. 2013; Roberts, et al. 2014.) Maltodextrin used as a supplementary carbohydrate is very common nowadays. Maltodextrin is a glucose polymer. For instance, many sports drink manufacturers use maltodextrins in their drinks in addition to simple sugars. In addition to maltodextrin, the major carbohydrates used in sports drinks are the monomers glucose and fructose, and the dimer sucrose as well as some other glucose polymers than maltodextrin. (Coombes & Hamilton 2000.)

3.3 Fat

A lipid molecule has the identical structural elements as carbohydrate but differs in its linkage and number of atoms. Lipid is the general term for heterogeneous group of compounds. Lipid includes fats, oils, waxes, and related compounds. According to common classification, lipids belong to one of three main groups: simple, compound, and derived lipids. (McArdle et al. 2013, 18.)

3.3.1 Structure of fat

Simple lipids consist primarily of triglycerides (triacylglycerols). Triglycerides constitute the major storage form of fat in the fat cells. This molecule contains two different clusters of atoms. (McArdle et al. 2013. 18-29; Heymsfield et al. 1997.) One cluster, glycerol, consist of a 3-carbon molecule that itself does not qualify as a lipid because of its high solubility in water. Three clusters of unbranched carbon-chained atoms, termed fatty acids, bond to the glycerol molecule. A carboxyl (-COOH) cluster at one end of the fatty acid chain gives the molecule its acidic characteristics. Fatty acids have straight hydrocarbon chains with as few as 4 carbon atoms or more than 20, with the most common chain lengths of 16 and 18 carbons. The compound lipids composed of simple lipids combined with other chemicals. There are three different groups of compound lipids: phospholipids, glycolipids, and lipoproteins. The fourth group of lipids is derived lipids such as cholesterol, synthesized from simple and compound lipids. (McArdle et al. 2013. 18-29.)

Fatty acids are divided to the two groups: saturated and unsaturated fatty acids. Saturated fatty acids contain as many hydrogen atoms as chemically possible because they contain only single covalent bonds between carbon atoms and all of the remaining bonds attach to hydrogen. By contrast, unsaturated fatty acids contain fewer hydrogen atoms attached to the carbon chain. Unlike saturated fatty acids, at least a single double bond connects carbon atoms in unsaturated fatty acids. Unsaturated fatty acids are further divided to the two groups: monounsaturated and polyunsaturated with respect to the amount of double bonds. (McArdle et al. 2013, 18-29.)

3.3.2 Functions of fat in the body

The most important functions of fat in the body are to act as an energy source and reserve, protect vital organs, act as thermal insulation, and carry vitamins and suppress hunger. Fat constitutes the ideal cellular fuel because it carries a large quantity of energy per unit weight, it transports and stores easily, and it provides a ready source of energy. Fat provides the main source of the energy of an individual at rest. Fat provides also a sufficient source and transport medium for the fat-soluble vitamins A, D, E, and K. (McArdle et al. 2013, 26-27.)

4 EFFECTS OF PROTEINS AND CARBOHYDRATES ON BODY COMPOSITION ALONE OR IN COMBINATION WITH RESISTANCE TRAINING

4.1 Different body composition assessment methods

Body composition evaluations are necessary in order to monitor nutritional status, training outcomes, and general health. Sports nutrition experts can use body composition values to help develop specific dietary interventions, and strength coaches, as well as athletic trainers, can use body composition values to help create, optimize, and evaluate training programs. (Moon 2013.) One important mission in body composition research is to find the best reference method, or “gold standard.” The weighing of organs or chemical analysis of human cadavers would provide the most accurate measure of most components, but only a few complete cadaver analyses have been carried out, and this approach poses many technical problems. (Heymsfield et al. 1997.) Many methods have been used for estimating body composition and fat distribution, but less is known of their suitability for assessing the differential changes in body composition following strength training. Simple anthropometric measurements, the field techniques are used as indirect and crude estimates of adiposity and abdominal obesity. (Sillanpää et al. 2008.) Field techniques are normally simple, quick, and cheap, and they are referred for to the populations under investigation. All of these measurements rest on certain assumptions. For instance, the assumption of a constant composition of fat free mass is central to the 2-compartment model and methods based on this model. The most commonly used field techniques are skinfold thickness and bioimpedance analysis, although the body mass index (BMI) is widely used as a measure of level of fatness. (Norgan 2005.)

The ‘true’ body composition values are unmeasurable in living humans (Moon 2013). Laboratory methods are usually more difficult to perform, in terms of costs and expertise compare to field methods. Nowadays there are many different laboratory techniques, for instance hydrometry, DXA, and MRI. Imaging methods, for example

DXA, are proving useful in regional measures of body composition such as muscle mass and abdominal adipose tissue deposits. (Norgan 2005.)

Laboratory methods are inherently prone to error, though much less than field methods. Quantifying tissue mass (fat mass and fat-free mass) in living humans requires laboratory methods as well in order to include assumptions or constants, which can vary from person to person. The constants used for unknown variables in laboratory methods depend on the method. For instance, DXA assume a constant hydration of fat free mass to be 0.73. Therefore, DXA assume that fat free mass consists of 73 % water in all individuals. However, the hydration of fat free mass is known to vary from 68 to 81 %. Due to this, the constant of 0.73 is not applicable in all individuals. Therefore, multiple-compartment models that include a total body water measurement are more accurate and are used as a “gold standard” for total body fat-free mass and fat mass assessment. (Moon 2013.)

4.2 Effect of protein supplementation with or without carbohydrates on body composition

The majority of studies suggests that protein ingestion with or without carbohydrates in the context of a resistance training session can enhance individuals` skeletal muscle hypertrophy, increase lean mass, and decrease fat mass. Indeed, most of the studies have shown that protein and/or essential amino acids combined with resistance training are more effective than non-energetic placebo at increasing lean or fat-free body mass and whole muscle cross-sectional area. (Cribb et al. 2007; Hartman et al. 2007; Hulmi et al. 2009; Hulmi et al. 2010.)

There are studies that have focused on the timing, frequency, amount, and type of supplement to ingest. Especially whey is considered a high-quality protein because of its abundance of essential amino acids, which are necessary for stimulating protein synthesis and therefore supporting muscle growth. Whey protein possesses functional properties that promote fat mass reduction and lean mass preservation, including increased fat oxidation (Dougkas et al. 2011.) Moreover, whey protein as a dietary replacement compared with a carbohydrate control produced consistent, yet nonstatistically significant, beneficial effects on body fat, body weight, waist circumference, and body mass index (BMI). Overall, a statistically significant increase in lean body mass was observed among studies that included a resistance exercise component along with whey provision, indicating that the benefits of whey on lean body mass are stronger as part of resistance exercise regimen. (Miller et al. 2014.)

A recent meta-analysis of protein suggested that whey protein promoted weight loss and reduction of body fat (Sousa et al. 2012). For instance, Frestedt et al. (2008) demonstrated that supplementation with a mixture of whey protein isolate with other peptides for healthy subjects for 12 weeks, led to higher weight loss compared with control subjects who consumed glucose. The whey protein group also had greater reductions of body fat (6.1 %) and a higher maintenance of lean mass than the control group. In addition, Baer et al. (2011) demonstrated that the type of protein can influence the weight loss response. Supplementation with 56 g of whey protein for 23 weeks diminished body weight and fat mass when compared with the group that consumed just carbohydrates. Additionally, waist circumference was lower in the whey protein group when compared to the group that ingested soy protein. These results suggest that different sources of dietary protein may differentially facilitate weight loss and affect body composition.

With regard to the timing, the intake of protein with or without carbohydrate early after an exercise session may enhance protein balance. Cribb and Hayes (2006) demonstrate that after 10 weeks of training, supplementation before and after each exercise session resulted in significantly greater improvements in body composition compared with a group that consumed the same supplement at times other than pre- and post workout (figure 2). The group that consumed supplementation before and after each workout increased lean body mass and decreased body fat percentage significantly compared with a control group. (Cribb & Hayes 2006.) Moreover, according to Losse et al. (2010) consumption of fat-free fluid milk versus an isoenergetic carbohydrate drink immediately after and 1 h after resistance exercise resulted in significantly greater fat mass loss (-1.6 ± 0.4 kg vs -0.3 ± 0.4 kg, $P < 0.02$) and lean mass and strength gains in young, healthy women performing 12-week resistance training. However, the addition of carbohydrates to protein nutrition has not previously been investigated when compared to protein alone.

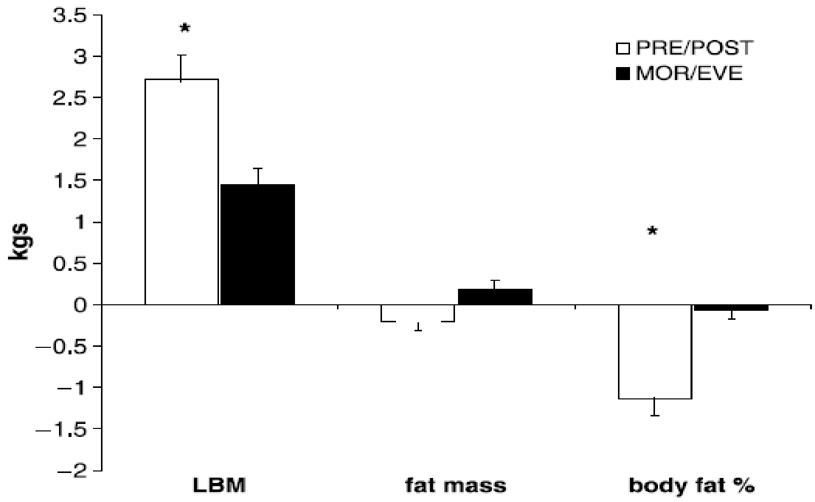

FIGURE 2. Body composition changes. * Significant difference between groups (P < 0.05). PRE/POST = supplementation before and after each exercise session, MOR/EVE = supplementation at times other than pre- and post-workout. (Cribb & Hayes. 2006.)

4.3 Effect of protein and carbohydrate consumption on muscle and fat size regulation

High-quality protein such as whey effectively stimulates the synthesis of myofibrillar and sarcoplasmic protein fractions in muscle under resting conditions and in response to resistance exercise (Moore et al. 2009). Protein supplementation and the subsequent availability of circulating amino acids, combined with resistance training, is associated with elevations in serum insulin and IGF-1, both of which may increase muscle protein synthesis (Masedu et al. 2012).

The underlying molecular mechanisms associated with enhanced stimulatory effect of feeding after exercise appear to be associated with the enhanced phosphorylation of mTOR, p70S6K1 (p70S6 kinase), and 4E-BP1, greater than that achieved by exercise alone. mTOR is a protein kinase which has been widely recognized as a key regulator of muscle growth. (Dreyer et al. 2008; Koopman et al. 2005.) Possibly the most important component of high-quality protein may be its large concentration of BCAAs. However, based upon animal and cell culture studies, it has been suggested that only leucine is required to induce protein synthesis and mTOR signaling. (Hulmi et al. 2010.) One of the mechanisms by which amino acids activate mTOR may involve increased intracellular Ca2+. Ca2+ further activates a class III PI3 kinase, which is called human vacuolar protein sorting 34 (hVps34). (Gulati et al. 2008.) In addition, recent studies suggest that a likely mechanism is that BCAAs cause the movement of proteins in the cell and mTOR is moved to the lysosome. Further, two direct activators of mTOR, RHEB and phosphatidic acid, are located in lysosome (figure 3). Additionally, TSC2 is moved away from the lysosome, so that TSC2 can no longer block RHEB. (Bar-Peled & Sabatini 2014; Demetriades et al. 2014; Menon et al. 2014.)

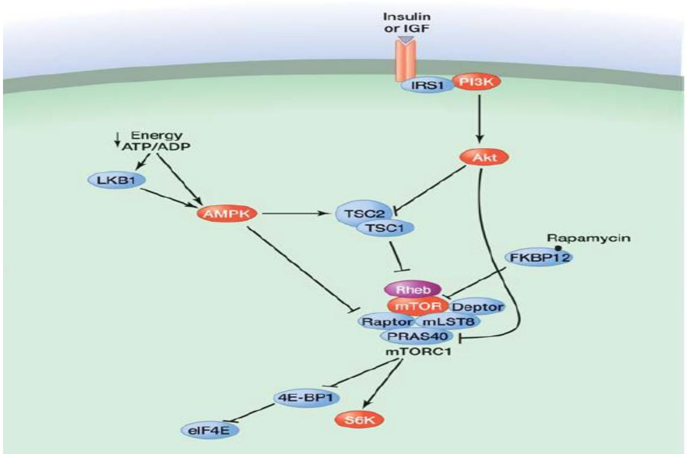

FIGURE 3. A simplified view of the mTOR pathway (Laplante and Sabatini 2009).

Glucose transport to the muscle cell is a complicated pathway. Exercise increases the energy needs of skeletal muscle. (Winder & Hardie 1999.) There have been many studies on the role of the AMP-activated protein kinase (AMPK), the metabolic sensor, in the regulation of glucose transport in skeletal muscle, and it has been demonstrated that AMPK regulates glucose transport in skeletal muscle (Ponticos et al. 1998). According to several studies, an exercise-dependent increase in the ratios of AMP/ATP and creatine/phosphocreatine leads to the activation of AMPK, both through the direct binding of AMP as well as by covalent phosphorylation catalyzed by its upstream kinase, AMPK kinase (AMPKK) (Hardie et al. 1998; Kemp et al. 1999). It has been suggested that AMPK transmits a portion of the signal by which muscle contraction increases glucose uptake, although other AMPK-independent pathways also contribute to the response (Mu et al. 2001).

Obesity is a global health epidemic and partly due to this, the cellular mechanisms of adipose tissue have recently been well investigated (Berry et al. 2014). The precise origin of adipocytes remains uncharacterized. Nonetheless, it has been demonstrated that transcription factor PPARγ2 plays an important role in adipogenesis (Tang et al. 2008). In contrast, regulation of lipolysis is better known. Nutritional regulation of lipolysis occurs at multiple levels in response to changing metabolic conditions and nutrient intakes. Acute, rapid regulation of adipose tissue lipolysis occurs in order to maintain the supply of energy substrates during the post-absorptive state and to allow for efficient storage of excess fuels following a meal. (Duncan et al. 2007.)

4.4 Acute effect of nutrient supplementation on resistance training session

Net protein balance is defined as muscle protein synthesis minus muscle protein breakdown. Therefore, a rise in skeletal muscle protein synthesis and/or reduction in muscle protein breakdown, so that the net protein balance remains positive can result in increased accretion of skeletal muscle mass. (Hulmi et al. 2010.) The synergistic effects of resistance exercise and feeding on muscle protein synthesis during the immediate one-to-four-hour post-exercise recovery period have been described thoroughly (Dreyer et al. 2008; Tipton et al. 2007). Thus, resistance exercise combined with protein supplementation provides the basis for training-mediated increases in muscle mass (Rennie et al. 2004). One reason for the timing effect may be increased skeletal muscle circulation after resistance exercise. Increased circulation enhances the uptake of amino acids and glucose into muscle after exercise. (Hulmi et al. 2010; Tipton et al. 2001.) Important factors to be taken into account are the timing, frequency, and amount of supplementation and the inclusion of carbohydrate with the supplement (Burd et al. 2011).

4.4.1 Protein supplementation

Pre- and/or post-exercise ingestion of protein or essential amino acid can increase muscle protein synthesis and result in a positive net protein balance (Hulmi et al. 2010; Moore et al. 2009). A schematic representation of skeletal muscle protein turnover and other muscle-specific metabolic fates of amino acids are shown in figure 4. The majority of studies suggest that protein and/or essential amino acid ingestion in the context of a resistance training session effectively stimulate muscle protein synthesis (Tang et al. 2007; Moore et al. 2009).

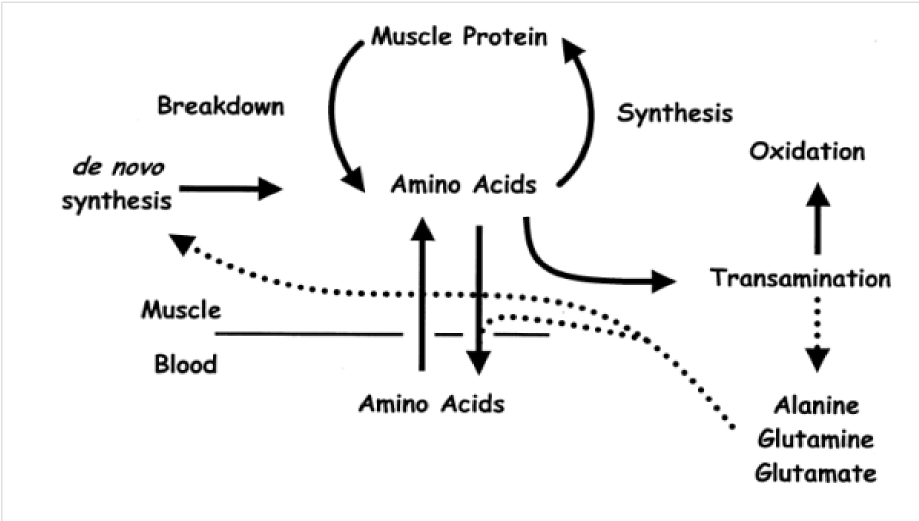

FIGURE 4. Schematic model of protein turnover and various metabolic fates of amino acids in skeletal muscle (Phillips 2004).

Even a small amount, only 10 g of whey protein has been shown to effectively stimulate muscle protein synthesis after resistance exercise (Tang et al. 2007). According to acute studies there seem to indicate a plateau in muscle protein synthesis from the ingestion of between 20-40 g of intact protein, or approximately 9-10 g of essential amino acids (Cuthbertson et al. 2005; Hulmi et al. 2010; Moore et al. 2009; Yang et al. 2012). A recent review on the role of dietary protein with resistance training on muscle mass suggests that the optimal prescription for the stimulation of muscle protein synthesis would consist of at least 25 g of high-quality protein, such as fluid milk, consumed after exercise (Lambourne et al. 2013; Phillips 2011). However, resistance training variables (e.g., intensity, volume, etc.) have to be taken into account because they may also have an effect on the optimal protein dose (Hulmi et al. 2010). Also, the type of protein consumed may affect the results of resistance training, due to variable speeds of absorption, differences in amino acid profiles, or unique hormonal response (Cribb et al. 2007; Lemon et al. 2002). For instance, studies in young individuals indicate that whey protein ingestion directly after exercise stimulates muscle protein synthesis more than other protein sources (Arnarson et al. 2013; Cribb et al. 2007; Tang et al. 2009).

Moore et al. (2009) studied the dose response of an egg protein supplement comparing 0 g, 5 g, 10 g, 20 g, and 40 g of egg protein delivered after a bout of exercise. After consumption of the supplement, muscle protein synthesis rates were monitored. Their results suggested that protein synthesis was maximally stimulated with 20 g of egg protein, which contains 1.7 g of leucine. It was also observed that when given the double that dose (40 g, 3.4 g of leucine), no significant differences in protein synthesis occurred. (Moore et al. 2009.)

4.4.2 Carbohydrate supplementation

Acute resistance exercise leads to a positive nitrogen balance. However, if exercise occurs in the fasted state, the net protein balance is still slightly negative. (Biolo et al. 1997.) The provision of carbohydrate in a fasted state can to small extent enhance net protein balance, although carbohydrates appear to work predominantly by reducing protein degradation (Borsheim et al., 2004; Tarnopolsky et al. 2008).

According to Cribb and Hayes (2006), consuming a carbohydrate supplement before and after resistance exercise enhances muscle glycogen concentrations. Muscle glycogen is considered a major contributor of energy production during resistance exercise (Haff et al. 2003). A single bout of high-intensity resistance exercise can result in a significant reduction (up to 40 %) in muscle glycogen, particularly in type II muscle fibers (Tesch et al. 1998). The type II fibers are responsible for maximum force production. Therefore, low glycogen levels in these fibers have been associated with decreased performance during resistance exercise (Volek. 2004). The consumption of carbohydrates in the context of resistance exercise is assumed to spare muscle glycogen stores as well as increased work capacity during exercise. For these reasons, it has been suggested that intake of carbohydrates before and after resistance exercise may promote more efficient recovery between bouts. Thus, consumption of carbohydrates may enhance the development of strength and hypertrophy during resistance training. (Cribb & Hayes 2006; Haff et al. 2004.) Long-term studies are, however, lacking.

Insulin secretion increases after consumption of carbohydrates. However, there is strong evidence that following resistance exercise, in the absence of an increase in amino acid concentration, an increase in insulin has only a modest effect on muscle protein synthesis. As a result, no effect of insulin is seen on muscle protein synthesis if amino acid concentrations are allowed to fall. Moreover, consumption of a carbohydrate drink after resistance exercise increased plasma insulin concentrations, but there was no increase in muscle fractional synthesis rate, presumably because of a concomitant reduction in amino acid concentrations. Due to this, protein supplement may be required to maximize protein synthesis. (Rasmussen et al. 2000.)

4.4.3 Supplementing protein with carbohydrates

Little data is available on the impact of coingesting carbohydrate with protein and the subsequent muscle protein synthetic response to food intake (Gorissen et al. 2014). Hyperinsulinemia can stimulate rates of muscle protein synthesis (Bell et al. 2005; Fujita et al. 2005) and/or inhibit protein breakdown (Miller et al. 2003), both of which could augment protein accretion (Staples et al. 2011). However, some studies suggest that hyperinsulinemia stimulated by the coingestion of carbohydrates with protein does not provide a greater stimulus for muscle protein accretion than protein ingestion alone (Glynn et al. 2010; Koopman et al. 2007; Staples et al. 2011). Gorissen et al. (2014) demonstrated that carbohydrate coingestion with protein delays protein digestion and absorption, but does not modulate the postprandial muscle protein synthetic response in healthy young and older men.

Staples et al. (2011) were studying muscle protein synthesis and breakdown. They examined if protein coingested with carbohydrates compared with protein alone would promote rates of muscle protein synthesis and/or inhibit rates of muscle protein breakdown. They demonstrated that protein coingested with carbohydrates does not further augment muscle protein synthesis or inhibition of muscle protein breakdown after resistance exercise compared with protein alone. These findings indicate that intake of protein supplementation does not require coingestion of carbohydrate to optimize muscle protein accretion. (Staples et al. 2011.)

In contrast, Levenhagen et al. (2002) demonstrated that protein synthesis rates were additive for the carbohydrate and amino acids supplement compared with the carbohydrate-only and amino acids-only supplements. However, amino acids-only supplements contained only 10 g of amino acids. In any case, Levenhagen et al. (2002) concluded that the availability of amino acids is more important than the availability of energy for post-exercise repair and synthesis of muscle protein. Moreover, Tipton et al. (2001) demonstrated that ingestion of a bolus of 6 g of amino acids combined with 35 g of carbohydrates at both 1 and 3 h post-exercise resulted in muscle protein anabolism. Tipton et al. (2001) suggested that a combination of amino acids, to increase amino acid availability, and carbohydrates, to stimulate insulin release, maximize muscle protein synthesis. That is, because amino acids availability are necessary for muscle protein synthesis and elevated insulin after resistance exercise does diminish the increase of muscle protein breakdown in response to exercise. (Tipton et al. 2001.)

4.5 Chronic effect of supplementation on resistance training

Research examining long term effects of supplementing protein and/or carbohydrates within the context of exercise is limited. Acute studies studying protein phosphorylation or muscle protein synthesis after resistance exercise and intake of protein are often used to predict chronic training outcomes. Resistance exercise combined with protein ingestion stimulates muscle protein synthesis more than either stimulus alone (Burd et al. 2012; Moore et al. 2009). The surrounding nutrient milieu would then dictate the response of the fiber in terms of muscle protein synthesis, which would ultimately accumulate to yield hypertrophy over time. As a result, an acute positive muscle protein balance would lead to eventual hypertrophy after chronic resistance training. Therefore, acute measurements of muscle protein synthesis must include some indication of training-mediated hypertrophy. (Burd et al. 2012.)

Interestingly, there has been some debate about how well acute muscle protein synthesis measurements correlate with chronic outcomes. For instance, Terzis et al. (2008) found strong correlations, but also weak correlations (Mitchell et al. 2013) and no correlations (Mitchell et al. 2012) have been found. Moreover, Pasiakos et al. (2013) demonstrated that acute changes are not always indicative of long-term changes in fat free mass. This may be because amino acids have other impacts related to metabolic pathways and immune function and before oxidation exert a regulatory influence on maintenance and growth (Millward 1989). Additionally, inconsistent results may be due to different muscle protein synthesis measurements (Wilkinson et al. 2014). Therefore, in addition to acute studies, long-term studies are also needed.

4.5.1 Protein supplementation

Results from the majority of longitudinal studies support the theory that protein supplementation in the context of resistance exercise will enhance the chronic training adaptations. These adaptations are, for instance, to enhance muscle hypertrophy and strength. (Andersen et al. 2005; Cribb et al. 2006; Cribb et al. 2007; Hartman et al. 2007; Hulmi et al. 2010; Rankin et al. 2004.) Volek et al. (2013) studied lean body mass, body composition, and amino acid responses to 9 months of resistance training. Healthy men and women were randomly assigned to supplement with whey protein, soy protein, or carbohydrate. The whey protein group increased the leanest body mass compared to the two other groups. Carbohydrate supplementation was less effective in promoting gains in lean body mass. (Volek et al. 2013.)

A recent meta-analysis examined whether protein supplementation augments muscle mass with prolonged resistance training. A total of 22 studies ranging from 6 to 24 weeks were analyzed. Carbohydrate was the placebo in most studies, and the source of protein was milk protein (whey or some combination of whey with other protein). Compared to placebo, protein supplementation resulted in an additional 0.7 kg gain in lean body mass over on average 3 months. Lean body mass increased after 3 months and plateaued at 6 and 9 months, indicating that the majority of gains in muscle mass can be achieved after 36 workouts in non-strength trained recreationally active young adults. However, the greater gains in lean body mass observed in the groups who consumed protein supplementation, translated into greater gains in maximal strength in some (leg press), but not in other maximal strength variables. (Cermak et al. 2012.) Maximal strength is only partially related to muscle size, and there is often a disconnect between resistance training-induced adaptations in muscle mass and maximal strength (Kirk et al. 2007).

In addition, in young men, protein supplementation in combination with resistance training has been shown to significantly increase muscle cross-sectional area more than carbohydrate supplementation (Cribb et al. 2007). Additionally, Andersen et al. (2005) examined the effects of intake whey protein supplementation versus carbohydrate supplementation before and after resistance exercise for 14 weeks, in previously untrained young men. After the resistance training period, only the protein group showed myofiber hypertrophy. Moreover, according to Hulmi et al. (2009), 15 g of whey protein consumption in the context of resistance exercise by previously untrained young men further increased muscle hypertrophy. These results, therefore, suggest the importance of intake of high-quality protein within the context of resistance exercise. (Hulmi et al. 2009.) However, some studies have demonstrated that intake of protein and/or essential amino acid does not provide significant effect on myofiber cross-sectional area or lean body mass during resistance training (Hulmi et al. 2010; Olsen et al. 2006; Verdjik et al. 2009).

Additionally, the type of protein consumed may affect the results of resistance training due to variable speeds of absorption, differences in amino acid profiles, or unique hormonal response (Cribb et al. 2007; Lemon et al. 2002). For instance, studies in young individuals indicate that whey protein ingestion directly after exercise stimulates muscle protein accretion more than other protein sources (Arnarson et al. 2013; Cribb et al. 2007; Tang et al. 2009). Phillips et al. (2009) also discovered that whey protein elicited significantly greater gains in lean body mass than soy or carbohydrate. In addition, Hartman et al. (2007) studied differences between milk protein, soy protein, and maltodextrin in untrained individuals. Milk protein and soy protein group received a total of 17.5 g of protein immediately and one hour following exercise, while the control group consumed an isocaloric maltodextrin beverage. 17.5 g of milk protein contains approximately 1.7 g of leucine, whereas the same amount of soymilk contains only 1.4 g of leucine. After a 12-week resistance training program, the milk protein group showed greater increases in myofiber cross-sectional area. Thus, this study suggested that a moderate dose of milk protein in the context of resistance training increases lean mass more, when compared to soy or a maltodextrin. (Joy et al. 2013.)

Joy et al. (2013) investigated the effects of high doses of rice protein compared to equal doses of whey protein on skeletal muscle hypertrophy, lean body mass, strength and power, when given after eight weeks of resistance training. Findings from the study supported the fact that higher doses of rice protein (48 g) are comparable to an equally high dose of whey protein in their effect on body composition and exercise performance after resistance training. At these doses, the rice protein supplement contains approximately 3.8 g of leucine, whereas the whey protein supplement contains 5.5 g of leucine. Furthermore, at these doses, it is predicted that both supplements will reach the levels required for optimization of muscle protein accretion. (Joy et al. 2013.)

4.5.2 Carbohydrates supplementation

There are very few studies that examine the chronic effect of carbohydrate supplement. Skeletal muscle growth is influenced by a number of physiological factors such as hormonal action, nutrient supply, and level of contractile activity. Tarpenning et al. (1998) examined the effects of a carbohydrate supplement versus a non-caloric placebo beverage on skeletal muscle hypertrophy throughout 12 weeks of progressive resistance training. Specifically, they investigated whether the chronic alteration of the acute hormonal response associated with carbohydrate ingestion would have a positive effect on skeletal muscle hypertrophic adaptation. After the resistance-training period, there was a non-significant change in cortisol concentration in the resistance exercise plus carbohydrate supplementation group. On the contrary, in the exercise plus placebo group, cortisol concentration increased significantly. Moreover, resistance training with carbohydrate consumption resulted in significantly greater gains in muscle fiber cross-sectional area compared with resistance training alone. These findings suggest that the modification of the hormonal response associated with carbohydrate ingestion can have a positive impact on chronic skeletal muscle hypertrophic adaptation. (Tarpenning et al. 1998.)

Also Bird et al. (2006a) studied the chronic alteration of the acute hormonal response associated with carbohydrate ingestion on hormonal and muscular adaptations following resistance training. They got results that were consistent with the findings of Tarpenning et al. (1998). Nevertheless, it should be noted that blood hormonal responses are not often directly related to muscular responses, but instead are a measure of a homeostatic regulation (West & Phillips 2012). Therefore, carbohydrates or energy alone may affect on the resistance training adaptations. Nevertheless, most of the studies comparing protein supplementation with carbohydrate, not just non-energic placebo within the context of resistance training, have demonstrated that protein is more effective to increase muscle mass than energy/carbohydrates alone (Cermak et al. 2012).

4.5.3 Supplementing protein / amino acids with carbohydrates

Research examining long term effects of supplementing protein and carbohydrates within the context of exercise is limited. Nonetheless, few studies investigating amino acids with carbohydrates vs. amino acids alone exist. Bird et al. (2006a) studied the combination of carbohydrate and essential amino acids on changes in body composition, muscle fiber cross-sectional area, and muscle strength, measured throughout 12 weeks of resistance training. Supplementation with carbohydrate, essential amino acids, carbohydrate plus essential amino acids, or placebo was provided throughout the training sessions. Muscle isokinetic leg strength increased similarly for all groups in response to training. Lean mass and muscle fiber type I and II cross-sectional area also increased in all groups. However, gains in muscle mass and cross-sectional area were the greatest in those consuming the combined carbohydrate and essential amino acids supplement. (Bird et al. 2006a.)

4.6 Timing

Nutrient intake immediately after resistance exercise session may be more beneficial in terms of muscle protein anabolism than nutrient ingestion at other times such as in the morning and late evening (Cribb & Hayes 2006) or even 2 h after the workout (Esmarck et al. 2001). Especially milk protein supplementation may be advantageous for gaining muscle size (Hartman et al. 2007) and improving muscle protein balance after a resistance exercise (Hulmi et al. 2009). Some studies have suggested that intake of protein immediately after resistance exercise promotes lean mass accretion and muscle fiber hypertrophy more than some hours later (Esmarck 2001; Cribb 2001; Levenhagen et al. 2001). However, not all studies agree with this (Hoffman et al. 2009; Verdijk et al. 2009).

4.7 Recovery

Several nutritional strategies can optimize muscle bulk and strength adaptations and enhance recovery from heavy training sessions. Muscle glycogen is an essential fuel source for moderate- to high-intensity exercise. If glycogen stores are depleted, the capacity to perform at these exercise intensities is severely limited. (Coyle et al. 1986; Ivy et al. 2002.) Therefore, the faster the muscle glycogen stores can be replenished after exercise the faster the recovery process and theoretically the greater the return of performance capacity. Due to this, adequate energy intake to meet the needs of training and carbohydrate intake sufficient to maintain glycogen stores are important. (Burke et al. 2004; Tarnopolsky et al. 2008.)

Research studies addressing recovery have focused on the timing (Levenhagen et al. 2001), frequency (Doyle et al. 1993), amount of supplementation (Jentjens et al. 2002), as well as type of supplement to ingest (Piehl et al. 2000). With regard to the type of supplement, Zawadzki et al. (1992) found that the combination of carbohydrate and protein was more effective than carbohydrate alone in the replenishment of muscle glycogen during the 4 h immediately after exercise. Ivy et al. (2002) also concluded that a distinct advantage in muscle glycogen storage can be achieved after exercise with the addition of protein to a carbohydrate supplement. The effectiveness of protein to enhance muscle glycogen storage appears limited to the first hour after supplementation. (Ivy et al. 2002.) Results from several studies have suggested that the rate of muscle glycogen storage after carbohydrate supplementation is related in part to the plasma insulin response (Van Loon et al. 2000; Zawadzki et al. 1992).

With regards to resistance training, several studies support the use of essential amino acids or protein, when supplemented within the context of exercise, to enhance recovery of muscle function (Buckley et al. 2010; Etheridge et al. 2008; Hoffman et al. 2010; Hulmi et al. 2010), but the evidence is not clear (Pasiakos et al. 2014). The availability of increased essential amino acids may simply provide a more robust anabolic environment and thus, enhance recovery of muscle function. This improved recovery may also be partly due to the possible anti-catabolic effect of essential amino acids to decrease whole body myofibrillar protein degradation following strenuous exercise. (Bird et al. 2006b; Hulmi et al. 2010.) In the case of whey protein, whether consumed in the absence or presence of carbohydrate, one explanation may be that whey provides a post-exercise insulin response and in results, glycogen resynthesis occurs more rapidly (Power et al. 2009). As a result, the recovery from exercise may be enhanced (Hulmi et al. 2010). Recent investigations have demonstrated a reduction in muscle damage, attenuation of force decrements, and enhanced recovery from resistance exercise in individuals using protein or amino acid supplements (Hoffman et al. 2009; Kraemer et al. 2006; Ratamess et al. 2003).

5 PURPOSE OF THE STUDY AND RESEARCH QUESTIONS

The aim of this study was to examine the effects of different post-exercise supplementation regimens on resistance training adaptation. The empirical part of the thesis is based on a sixteen-week resistance training period in which the research group consisted of totally 78 recreationally active, normal weight men. More specifically, the purpose of this study was to find out the effects of protein and carbohydrate supplementation on lean mass, fat mass and muscular performance. Research questions and hypotheses are as follows:

Research questions and hypotheses

1. Are there differences between the effects of taking a protein and carbohydrate supplement compared to protein-only or carbohydrate-only supplements immediately after resistance exercise session on lean mass and/or fat mass?

   Hypothesis 1. Protein has been consistently more effective than isoenergic placebo. Therefore, protein plus carbohydrate supplement and protein alone will increase lean mass more than carbohydrate-only supplement. (Candow et al. 2004; Cribb et al. 2007.)

   Hypothesis 2. Protein net balance has not been consistently elevated with co-ingested carbohydrates when compared to protein alone. Therefore, in the protein plus carbohydrate supplement group, lean mass will increase at a similar rate as the protein-only supplement group. (Koopman et al. 2007; Staples et al. 2011.)

   Hypothesis 3. There is evidence that protein, but not carbohydrates, promote fat mass reduction especially when combined with resistance training. Therefore, protein alone will decrease fat mass more than carbohydrate-only supplement. (Arciero et al. 2014; Miller et al. 2014; Sousa et al. 2012.)

2. Are there differences between the effects of taking a protein and carbohydrate supplement compared to protein-only or carbohydrate-only supplements immediately after resistance exercise session on muscle strength?

   Hypothesis. The combination of carbohydrate and protein is more effective than carbohydrate or protein alone in the replenishment of muscle glycogen during immediately after exercise and increases muscle hypertrophy leading to increased muscle strength (Esmarck et al. 2001; Ivy et al. 2002; Zawadzki et al. 1992, Cermak).

6 METHODS

6.1 Subjects

A total of 86 healthy, recreational active men were recruited by newspaper advertisements. Before randomization, 8 subjects declined to continue with the study, resulting in 78 subjects (age 34.4 ± 1.3, height 179.9 ± 0.8 cm, weight 83.6 ± 1.4 kg) who actually started the resistance training program (table 1). The inclusion criteria required that the subjects be between the ages of 18-45. Smokers and those with chronic diseases or prescribed medications were excluded from the study. Moreover, the subjects were expected to have a background of not more than one year of systematic resistance training upon entering the study. The subjects were not allowed to ingest any other nutritional supplements during the study other than what was provided except for vitamins and minerals. Before participation, all subjects underwent medical screening including examination of a resting ECG. After comprehensive verbal and written explanations of the study, all subjects gave their written informed consent to participate.

TABLE 1. Characteristics of subjects (mean ± SE).

	Carbohydrate (n = 25)	Protein (n = 25)	Protein + Carbohydrate (n = 28)	All (n = 78)
Age (y)	36.0 ± 3.7	31.5 ± 1.3	35.4 ± 1.3	34.4 ± 1.3
Height (cm)	179.3 ± 1.5	180.5 ± 1.2	180.0 ± 1.3	179.9 ± 0.8
Weight (kg)	82.9 ± 2.2	83.9 ± 2.4	84.0 ± 2.6	83.6 ± 1.4
BMI (kg/m^2)	25.9 ± 0.7	25.7 ± 0.6	25.9 ± 0.7	25.8 ± 0.4

6.2 Study protocol

The study protocol included a total of 16 weeks of resistance training, with pre-, mid- and post-measurements completed between each period (on weeks 0, 4, 12 and 16, respectively). The first phase of the study was a four-week long habituation period, during which subjects were familiarized with resistance training that can be considered as strength-endurance training. In this period, subjects were exercising two times per week. During this habituation period, subjects used approximately 9 exercises in each training session, 2-3 sets of every exercise, and 10-15 repetition in every set. Recovery time between the sets lasted 2 minutes. Training loads were 50–70 % of one repetition maximum (1 RM).

After the habituation period, the subjects we further divided into three supplementation groups so that every group included subjects that were, on average, the same height and the same body mass. A double-blind protocol was used in this case. One group received protein, one group carbohydrate, and one group protein plus carbohydrate immediately after every training session. Protein and carbohydrates were provided by Northforce (Kuusamon Juusto Oy, Kuusamo). The protein group received 37.5 grams of whey concentrate (30 g of whey protein, 5 g of lactose < 1 g of fat) and the carbohydrate group received 34.5 grams of maltodextrin isocaloric to the whey protein. In contrast, the protein plus carbohydrate group received 37.5 grams of whey plus 34.5 grams of maltodextrin. The supplements were mixed with non-caloric concentrate (provided by Orkla Foods: FUN Light) to ensure that supplements were similar in taste, mixture, and appearance. The subjects ingested the supplements immediately after the training bout in the gym. A serving of the supplement was mixed with 0.5 L of pre-sweetened water (depending on the week and subject’s preference either strawberry, forest fruit, pomegranate-strawberry apple-pear or raspberry-lemon). The drinks were provided for the subjects in a double-blind fashion.

Within each of the three supplementation groups, the subjects were further divided into power-strength (PS) or hypertrophic-strength (HS) training groups (figure 5). PS included both power and maximal strength training and HS both hypertrophic and maximal strength training of 12 weeks for a total of 32 sessions. Each training session was supervised by an experienced student from the Department of Biology of Physical Activity. The intensity of training increased throughout the training program. The training program was especially focused on muscles of the lower body. The following exercises were used in each training session: bilateral leg press and bilateral knee extension, and bilateral knee flexion. The training program also included exercises for the other main muscle groups of the body: chest and shoulders, upper back, trunk extensors and flexors, and upper arms.

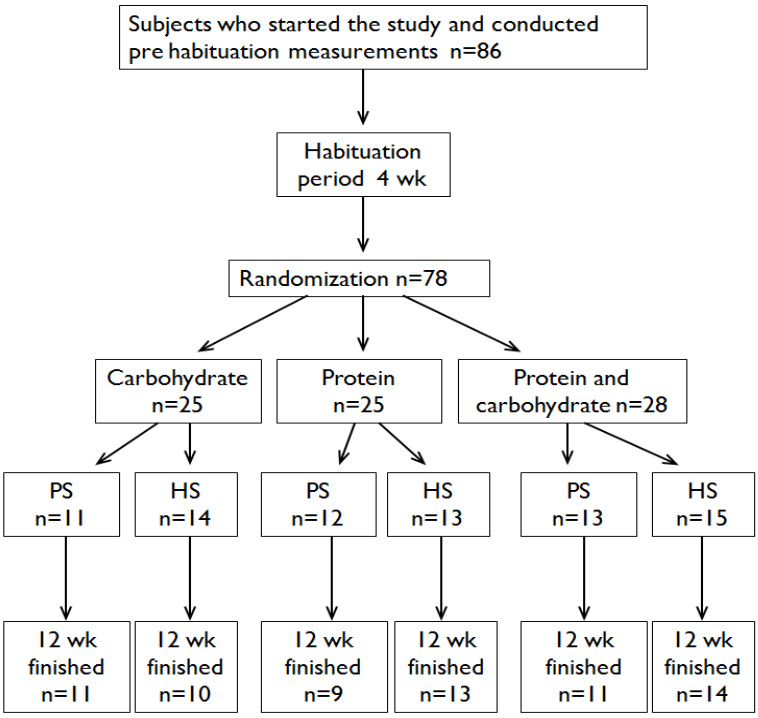

FIGURE 5. Study protocol.

The 12-week training phase was divided further into three different blocks. Every part consisted of four weeks of strength training, and five to seven exercises in each training session. In the first part of the 12-week training phase, the PS group had 25 % power-strength and 75 % maximal-strength training sessions. In the second part, the PS group had 75 % power-strength and 25 % maximal-strength training sessions. The last part consisted of 87.5 % power-strength and 12.5 % maximal-strength training sessions. During the training sessions, subjects used 9 movements in one practice, 2-5 sets of each movement, and 5–15 repetition in every set. Recovery time lasted 2-3 minutes depending on repetitions and the machines used. During the power-strength training sessions, training loads were 50–70 % of 1 RM while during the maximal-strength training sessions, training loads were 70–90 % of 1 RM.

In contrast, in the first part of the 12-week training phase, the HS group had 100 % hypertrophic-strength training sessions. In the second part, the HS group had 75 % hypertrophic-strength and 25 % maximal-strength training sessions. The last part consisted of 25 % hypertrophic-strength and 75 % maximal-strength training sessions. During the hypertrophic-strength training sessions, subjects used 9 movements in each training session, 2–4 sets of every movement, and 6–15 repetition in every set.

Recovery time lasted 1–2 minutes depending on repetitions and machines used. Training loads were 70–85 % of 1 RM. During the maximal-strength training session, subjects used 9 movements in one training session, 2–4 sets of every movement, and 5–12 repetition in every set. Recovery time lasted 2–3 minutes depending on repetitions and machines. Training loads were 70–90 % of 1 RM.

6.2.1 Dietary intake

Subjects kept a 4-day dietary record at the beginning of the 12-week supplementation and training period. Dietary intake was recorded over three weekdays and one weekend day. Subjects received both verbal and written nutritional recommendations (Finnish Nutrition Recommendations 2014). These, as a rule, follow the Nordic recommendations published in autumn 2013 (NNR2012). The subjects were instructed on how to report nutritional intake in the diaries. Subjects were asked to maintain a consistent dietary intake throughout the study period. Nutrients provided by the supplements were included in the analysis. The food diaries were analyzed by nutrient analysis software (Nutri-Flow; Flow-team Oy, Oulu, Finland).

6.2.2 Dual energy X-ray absorptiometry (DXA)

Body composition was estimated by DXA (Lunar Prodigy Advance, GE Medical Systems – Lunar, Madison WI USA). At the beginning of 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. 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). The system software provides the mass of lean soft tissue, fat, fat free mass, and bone minerals for the whole body and specific regions (trunk, android and both arms and legs). Lean soft tissue mass is bone free and used in this study as fat free mass. The trunk region includes the neck, chest, abdominal, and pelvic areas. The android region includes the abdominal area from the iliac crest to the ribs (figure 6). Automatic generated regions of the legs were manually adjusted to include the hamstrings and gluteus muscles. DXA measurements were conducted following a 12-hour overnight fast and 24-hour abstinence of alcohol and strenuous exercise.

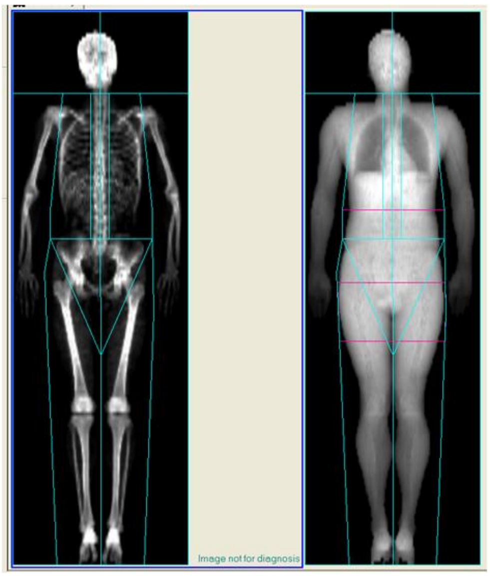

FIGURE 6. Body images of the DXA. The android region includes the abdominal area from the iliac crest to the ribs. In the figure, the android region is between the triangular base of the upside down triangle and the next, upward line.

6.2.3 Ultrasound

Cross sectional area of the knee extensor muscles (vastus lateralis, rectus femoris, and vastus intermedius) were measured using ultrasound (model SSD-2000, Aloka, Tokyo). Cross sectional images were obtained at 50 % of the femur length. Three clear images from this measurement point were saved for analysis. Images were analyzed with ImageJ (National Institute of Health, USA, version 1.42) program, and a cross sectional area was measured by marking manually the lines of the muscles to the image. The two closest values of vastus lateralis, rectus femoris, and vastus intermedius muscles were taken to account and the averages were calculated. The method has been validated earlier in our laboratory against MRI (Ahtiainen et al. 2010).

6.2.4 Strength tests

Isometric strength. Maximal isometric voluntary strength was measured with the dynamometer and the analyses were done with the Signal 2.15, Cambridge Electronic Design Ltd. 1997–2004. Before starting, subjects were instructed to press with their whole foot, to push as fast and as hard as possible and to hold the maximal force for approximately 3 seconds. Subjects were also instructed to keep their back and pelvis in contact with the bench throughout the movement. Subjects gripped the handles tightly throughout the measurement. Subjects performed 2–3 warm up trials before the actual measurement, where they were instructed to push with sub-maximal effort. After that, 3 maximal trials with one-minute rest were performed. Subjects performed familiarization measurements one week before the actual measurements.

1RM leg press. One repetition maximum was assessed using the leg press (David 210, David Fitness and Medical LTD., Helsinki, Finland). Before starting, the correct technique was explained to subjects and it was controlled during the measurements. To determine 1 RM, subjects started with warm up which included 5 repetitions with approximately 70 % of perceived capacity. After that, subjects did 2 repetitions with 80–85 %, one repetition with 90–95 % and finally 1 RM. 50 % from isometric maximal force was determined as the reference value for dynamic 1 RM. After that the increase of the weight was either 5 or 2.5 kg. Subjects had as many trials as 1 RM was found. Between the trials, subjects were allowed to rest for one minute.

6.3 Statistical analysis

Statistical evaluation of the data was accomplished by two-way repeated-measures analyses of variance (ANOVA) with nutrition (supplement) and time (training) as the factors with training type as a covariate when appropriate using SPSS statistical analysis software (SPSS version 20; Chicago, IL). Any violations of the assumptions of sphericity were explored and, if needed, corrected with a Greenhouse-Geisser or Huynh-Feldt estimator. The differences in the chances from pre-to post measurements between different supplement groups were analysed using univariate ANOVA and training type as a covariate. Bonferroni post hoc tests were performed to localize these changes. Correlations were analyzed using Pearson's Product Moment Coefficient. Statistical significance was set at $P < 0.05$. Values are presented as means ± statistical error of the mean (SE). Microsoft Office 2010 Excel-program was used to calculate averages and statistical errors.

7 RESULTS

7.1 Participants and compliance

Of the 78 subjects who completed the habituation period, 10 were not included in the data analysis, due to noncompliance with the exercise training sessions or inaccurate DXA measurements. There were no differences among the groups in the rate of noncompliance or drop-outs (CHO, n = 4, PRO, n = 3, PRO+CHO, n = 3). Thus baseline physical characteristics of the n = 68 subjects who completed the study are presented in table 2.

TABLE 2. Baseline characteristics of subjects before training habituation (mean ± SE).

	Carbohydrate (n = 21)	Protein (n = 22)	Protein + Carbohydrate (n = 25)	All (n = 68)
Age (y)	36.4 ± 4.2	31.4 ± 1.4	36.2 ± 1.2	34.7 ± 1.4
Height (cm)	179.1 ± 1.5	180.8 ± 1.9	180.0 ± 1.5	180.0 ± 0.8
Weight (kg)	81.4 ± 2.5	83.8 ± 2.4	85.1 ± 2.3	83.6 ± 1.4
BMI (kg/m^2)	24.5 ± 1.6	25.4 ± 0.7	26.4 ± 0.8	25.5 ± 0.6
Body fat (kg)	18.8 ± 2.0	21.5 ± 1.9	21.2 ± 1.7	20.6 ± 1.1
Lean body mass (kg)	59.1 ± 1.7	59.0 ± 1.0	60.4 ± 1.2	59.6 ± 0.7

7.2 Energy intake

7.2.1 4-day food diaries

The subjects kept food diaries for 4 days at the beginning of the 12-week training period. Dietary intakes are reported in table 3. All three groups reported consumption of approximately 20 E% proteins and 40 E% carbohydrates. Total energy intake was significantly lower in the protein group compared to the carbohydrate group (P = 0.023). The protein group differed significantly from the carbohydrate group also in fat intake (P = 0.019). The dietary intake did not differ significantly between protein and carbohydrate groups with relation to body weight (total energy per kg body weight, P = 0.264) at the time of the dietary recall. The protein + carbohydrates group did not differ between these two groups in total energy or total energy with relation to body weight.

TABLE 3. Dietary intakes (mean ± SE).

	Carbohydrate (n = 21)	Protein (n = 22)	Protein + Carbohydrate (n = 25)	All (n = 68)
Kcal/day	2938.0 ± 141.0	2349.0 ± 141.0[a]	2451.2 ± 135.7	2549.3 ± 87.8
Kcal/kg/day	35.0 ± 2.0	29.6 ± 1.9	29.3 ± 2.1	31.0 ± 1.3
Protein, g	147.6 ± 10.1	122.0 ± 6.9	120.4 ± 5.9[a]	128.2 ± 4.5
Protein/kg, g	1.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
Protein, %	20.0 ± 0.6	21.2 ± 1.1	20.2 ± 1.1	20.5 ± 0.6
Fat, g	114.7 ± 8.1	85.7 ± 6.6[a]	93.8 ± 5.9	96.8 ± 4.2
Fat/kg, g	1.4 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Fat, %	35.2 ± 1.8	32.5 ± 1.2	34.6 ± 1.2	34.1 ± 0.8
Carbohydrate, g	292.9 ± 19.4	236.5 ± 18.1	250.6 ± 19.5	257.5 ± 11.5
Carbohydrate/kg, g	3.5 ± 0.3	3.0 ± 0.3	3.0 ± 0.3	3.2 ± 0.2
Carbohydrate, %	40.1 ± 2.2	40.0 ± 1.2	40.3 ± 1.6	40.2 ± 0.9

[a]Significantly different from carbohydrate group (P < 0.05).

7.2.2 Energy intake for recovery day and exercise day

One of the four days that subjects kept food diaries was usually an exercise day. Table 4 presents dietary intakes on recovery days and exercise days as means. In relation to body weight, the protein and protein plus carbohydrate groups differed significantly from the carbohydrate group only in protein intake (P = 0.034 and P = 0.032 respectively) on recovery days. However, when estimated as absolute values, total energy intake was significantly lower in the protein group when compared to the carbohydrate group (P = 0.025) on recovery days. The protein and protein plus carbohydrate group differed significantly from the carbohydrate group in protein intake (P = 0.010 and P = 0.020 respectively) on recovery days. Moreover, the protein group differed significantly from the carbohydrate group in carbohydrate intake (P = 0.027) on non-exercise days. In contrast, the carbohydrate group differed significantly from the protein group in protein E% (P = 0.040) on exercise days.

TABLE 4. Dietary intakes on recovery days and exercise days (mean ± SE).

	Carbohydrate		Protein		Protein + Carbohydrate	
	Recovery	Exercise	Recovery	Exercise	Recovery	Exercise
Kcal/day	2812.9 ±223.2	2852.4±281.8	2168.7 ± 108.9[a]	2363.3±151.3	2419.3 ± 160.5	2560.3±153.4
Kcal/kg/day	34.0 ± 2.3	34.7 ± 3.6	27.3 ± 2.0	30.2 ± 3.0	29.6 ± 2.6	31.1 ± 2.4
Protein, g	142.2 ± 12.7	136.9 ± 20.5	106.9 ± 5.0[a]	144.3 ± 7.6	109.3 ± 6.0 [a]	136.7 ± 8.5
Protein/kg, g	1.7 ± 0.1	1.6 ± 0.2	1.3 ± 0.1[a]	1.8 ± 0.1	1.3 ± 0.1[a]	1.6 ± 0.1
Protein, %	20.3 ± 0.8	18.6 ± 1.7	20.1 ± 1.2	25.1 ± 1.7 [a]	18.7 ± 1.6	22.0 ± 1.9
Fat, g	100.6 ± 7.0	108.7 ± 11.7	78.5 ± 5.9	86.8 ± 7.5	93.6 ± 8.4	83.0 ± 7.6
Fat/kg, g	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
Fat, %	32.6 ± 1.1	34.0 ± 2.1	32.3 ± 1.3	32.9 ± 2.2	34.6 ± 1.5	28.6 ± 1.3
Carbohydrate, g	299.8 ± 27.2	295.6 ± 22.6	220.2 ± 12.2 [a]	219.7 ± 16.9	251.1 ± 21.9	290.7 ± 22.6
Carbohydrate/kg, g	3.6 ± 0.3	3.6 ± 0.5	2.8 ± 0.3	2.8 ± 0.3	3.0 ± 0.3	3.5 ± 0.3
Carbohydrate, %	42.5 ± 1.9	42.0 ± 2.6	40.6 ± 1.2	37.1 ± 1.4	41.1 ± 1.9	45.2 ± 1.8[b]

[a]Significantly different from the carbohydrate group ($P < 0.05$); [b]significantly different from the protein group ($P < 0.05$).

7.3 Body composition and strength measures

Table 5 presents body composition and body mass for all three groups. Subjects were randomly assigned to either the power-strength or hypertrophic-strength training group. Although hypertrophy-strength and power-strength altered muscle hypertrophy differently, no differences in muscle strength were noticed and the differences in fat mass were also small. Moreover, no major and consistent effect of training type was observed on the supplementation response for lean or fat masses. Therefore, the training groups were pooled.

TABLE 5. Body mass, fat mass, fat free mass, and 1 RM measurements pre- and post intervention (mean ± SE).

	Carbohydrate			Protein			Protein + Carbohydrate				
	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ	Time, P	Group x Time, P
Body mass, kg	82.0±2.6	82.7±2.6	0.7±0.4	83.9±2.3	83.6±2.3	-0.3±0.4	85.0±2.2	85.3±2.2	0.3±0.3		
TFM, kg	18.4±2.1	18.4±2.1	-0.0±0.4	20.8±1.9	19.3±1.8[a]	-1.5±0.4[b]	20.3±2.1	19.7±1.6[a]	-0.6±0.3	0.001	0.032
TFM/kg, g	218.4±19.0	216.7±19.5	-1.7±4.2	239.6±17.2	223.5±16.2[a]	-16.1±3.6[b]	233.5±15.0	225.0±14.5[a]	-8.4±2.5	0.091	0.019
TrFM, kg	8.8±1.3	9.1±1.3	0.3±0.2	9.5±1.1	8.7±1.1[a]	-0.8±0.2[b]	10.2±1.0	10.0±1.0	-0.2±0.1	0.065	<0.001
TrFM/kg, g	103.8±12.3	106.4±12.3	2.6±1.9	114.3±10.6	104.8±9.9[a]	-9.6±2.2[b]	117.9±10.0	114.6±9.9[ab]	-3.3±1.5	0.355	<0.001
AnFM, kg	1.9±0.3	1.9±0.3	-0.0±0.0	2.2±0.2	2.0±0.2	-0.2±0.0	2.3±0.2	2.2±0.0	-0.1±0.0	<0.001	0.014
AnFM/kg, g	22.6±2.4	22.3±2.4	-0.3±0.5	25.4±2.2	23.0±2.0	-2.4±0.5	26.0±2-0	24.9±1.9	-1.1±0.4	0.019	0.010
TLM, kg	60.0±1.7	60.9±1.7[a]	0.9±0.3	59.8±1.0	61.0±1.1[a]	1.2±0.3	61.2±1.2	62.1±1.2[a]	1.0±0.2	<0.001	0.738
TLM/kg, g	748.6±17.1	751.7±17.1	3.1±4.2	720.5±16.5	736.5±15.5[a]	16.0±3.5[b]	725.4±14.4	734.0±13.9[a]	8.6±2.5	0.039	0.040
LLM, kg	24.5±0.8	25.0±0.7	0.5±0.2	24.1±0.5	24.6±0.6	0.5±0.1	24.9±0.5	25.3±0.5	0.4±0.1	0.003	0.945
LLM/kg, g	300.3±8.5	303.6±8.6	3.3±1.9	290.3±7.2	297.0±7.1[a]	6.6±1.6	295.0±6.3	298.9±6.4[a]	3.9±1.5	0.115	0.334
1 RM	229.9±8.2	252.1±8.5	22.2±2.6	221.9±7.8	245.1±7.6	23.2±2.2	221.9±8.1	249.9±8.4	28.0±2.4	<0.001	0.421

Δ, absolute change; TFM, total fat mass; TFM/kg, total fat mass per kg; TrFM, trunk fat mass; TrFM/kg, trunk fat mass per kg; AnFM, android fat mass; AnFM/kg, android fat mass per kg; TLM, total lean mass; TLM/kg, total lean mass per kg; LLM, leg lean mass; LLM/kg, leg lean mass per kg; [a]Significant different from baseline pre intervention measurement (P < 0.05); [b]significantly different from the carbohydrate group.

7.3.1 Training habituation period

4 weeks of training habituation was conducted before the supplements were provided and during this period, the training was similar for all subjects. The habituation period increased total and leg fat free mass (P < 0.001) (figures 7 and 8), and there were no differences in the groups later randomized into different supplement groups (P > 0.7). Total body fat mass, trunk fat mass, and android (abdominal) fat mass decreased significantly after habituation training without differences in the groups later randomized into different supplement groups (P > 0.15) (figures 9, 10, and 11). Muscle strength (1 RM and isometric) increased after the habituation period (P < 0.001) (figures 12 and 13) and there were no differences in the groups later randomized into different supplement groups (P > 0.08).

7.3.2 Total lean mass and leg lean mass

Significant increases following the resistance training for all three groups were seen in total fat free mass (P < 0.001) and leg fat free mass (P = 0.003), and there was no effect of training type on total fat free mass (P = 0.064) and leg fat free mass (P = 0.212) (figures 7 and 8). Therefore, in the following results the two different training types were pooled and used as a covariate. Relative fat free mass changes were larger in the protein group compared with the carbohydrate group (P = 0.03). As a reminder, the first 4 weeks were habituation and after that subjects were randomized to different groups in which they consumed appropriate post-exercise supplementations during 0–12 weeks. Thus pre to 0 week was habituation period, during which subjects did strength-endurance training without consuming supplementations.

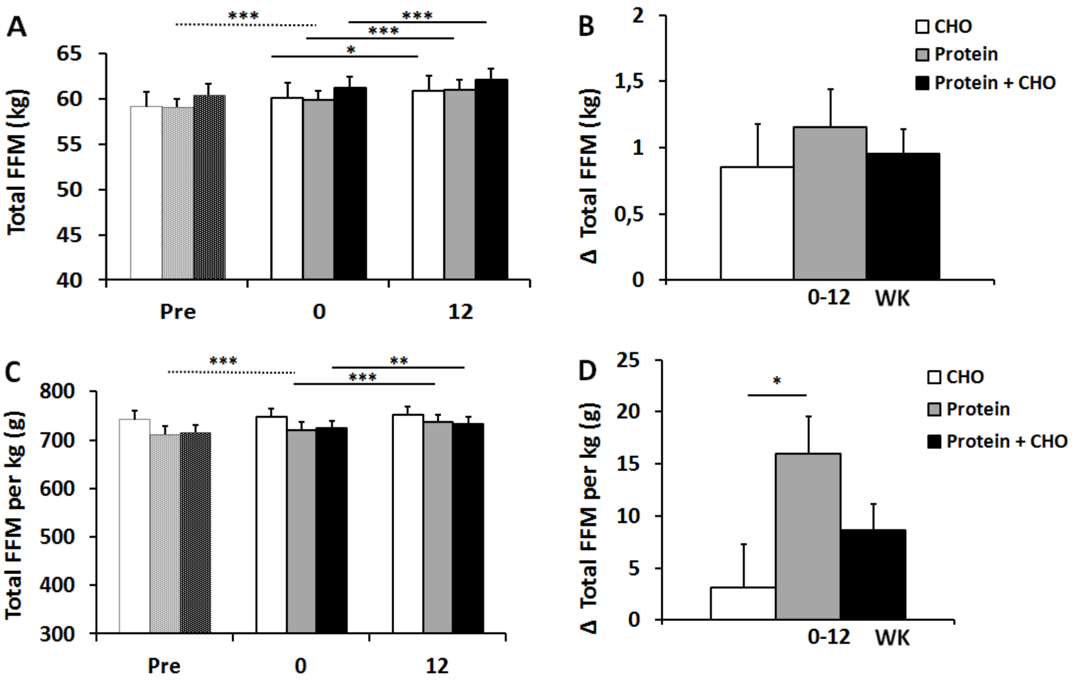

FIGURE 7. Total fat free mass (A), total fat free mass changes (B), relative fat free mass (total fat free mass divided by the body weight) (C), and relative fat free mass changes (D) from the beginning of supplementation to the end of the training period in carbohydrate, protein and protein plus carbohydrate groups. * A and C-figures depict statistical changes between weeks 0 and 12. * B and D-figures depict statistical difference in the change between different supplementations. * P < 0.05, ** P < 0.01, *** P < 0.001, significant differences between weeks 0 and 12 and between different supplementations. Dashed lines are at pre and the difference to the week 0 is depicted just as a single line as no supplementation was provided before week 0.

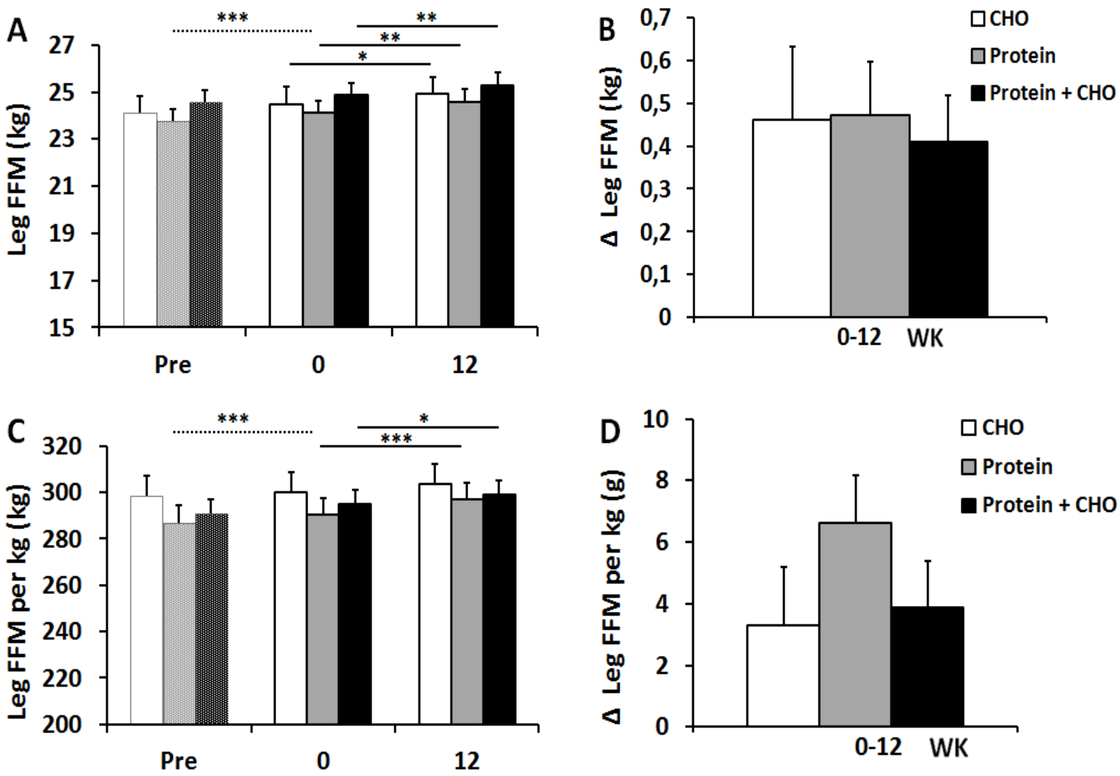

FIGURE 8. Leg fat free mass (A), leg fat free mass changes (B), relative leg fat free mass (leg fat free mass divided by body weight) (C), and relative leg fat free mass changes (D) from the beginning of supplementation to the end of the training period in carbohydrate, protein and protein plus carbohydrate groups. * the A and C figures depict statistical changes between weeks 0 and 12. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significant differences between weeks 0 and 12. Dashed lines are at pre and the difference to the week 0 is depicted just as a single line as no supplementation was provided before week 0.

7.3.3 Change for total fat mass

Total fat mass ($P = 0.001$) was decreased due to resistance training, and there was no effect of training type on total fat ($P = 0.064$). Therefore in the following results the two different training types were pooled, but used as a covariate. A nutrition x time interaction was detected for total fat mass ($P = 0.032$). However, total fat mass ($P = 0.001$) decreased following the resistance training in the protein group only (figure 9). This change was larger in the protein group compared with the carbohydrate group ($P = 0.03$), with training group as a covariate.

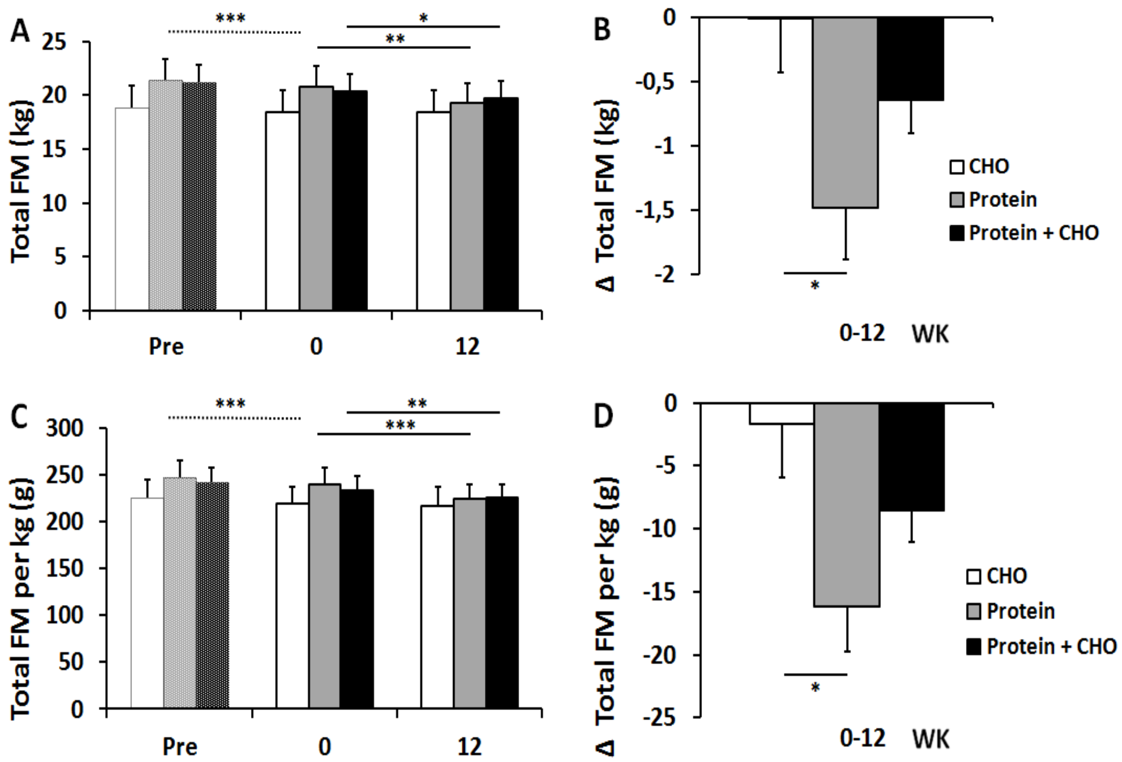

FIGURE 9. Total fat mass (A), total fat mass changes (B), relative fat mass (total fat mass divided by the body weight) (C), and relative fat mass changes (D) from the beginning of supplementation to the end of the training period in carbohydrate, protein and protein plus carbohydrate groups. * in A and C-figures depict statistical changes between weeks 0 and 12. *in B and D-figures depict statistical difference in the change between different supplementations. * P < 0.05, ** P < 0.01, *** P < 0.001, significant differences between weeks 0 and 12 and between different supplementations. Dashed lines are at pre and the difference to the week 0 is depicted just as a single line as no supplementation was provided before week 0.

7.3.4 Changes for trunk and android fat mass

Trunk fat mass (P = 0.065) was unchanged following the resistance training, and there was no effect of training type on trunk fat (P = 0.455). On the contrary, significant decreases following the resistance training for all three groups were seen for android fat mass (P < 0.001) as measured by DXA. There was small effect of training type on android fat (P = 0.044). Therefore, in the following results the two different training types were pooled, but used as a covariate. A nutrition x time interaction was detected for trunk fat mass (P < 0.001) and android fat mass (P = 0.014). Both, trunk fat mass (P

= 0.001) and android fat mass (P = 0.010) decreased following the resistance training in the protein group (figures 10 and 11). These changes were larger in the protein group compared with the carbohydrate group (P < 0.001 and P = 0.01, respectively), with training group as a covariate.

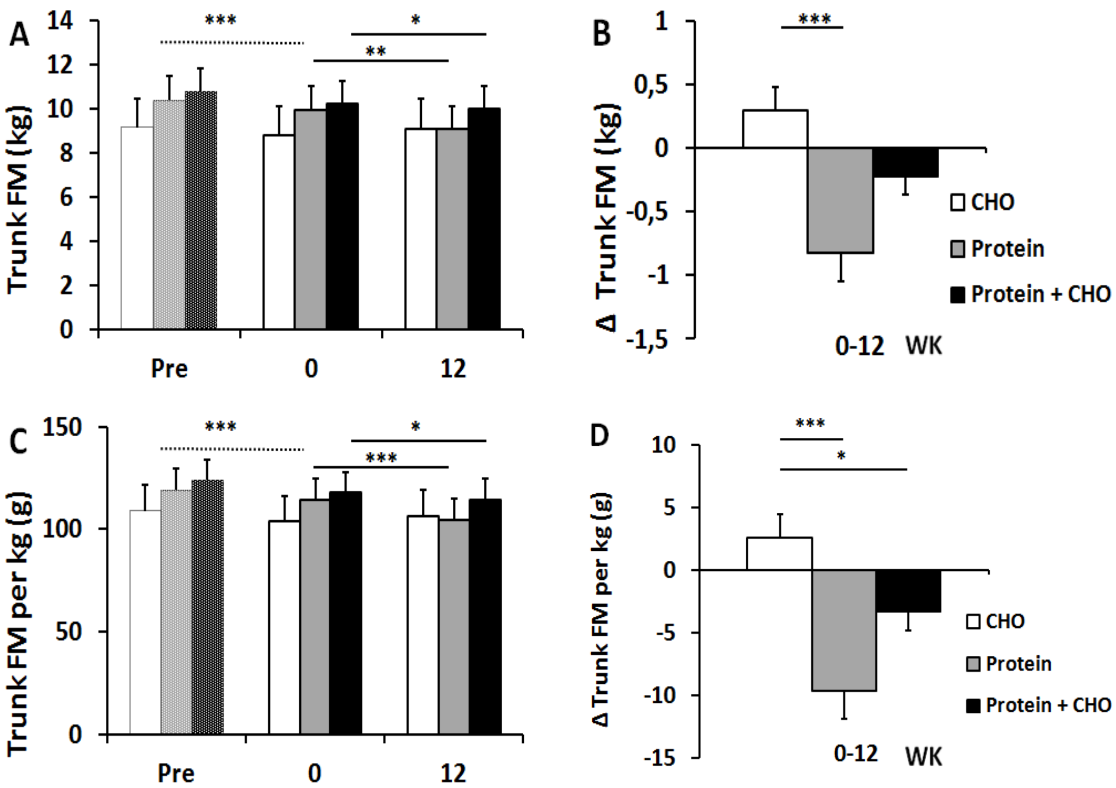

FIGURE 10. Trunk fat (A), trunk fat mass changes (B), relative trunk fat mass (trunk fat mass divided by the body weight) (C), and relative trunk fat mass changes (D) from the beginning of supplementation to the end of the training period in carbohydrate, protein and protein plus carbohydrate groups. * in A and C-figures depict statistical changes between weeks 0 and 12. *in B and D-figures depict statistical difference in the change between different supplementations. * P < 0.05, ** P < 0.01, *** P < 0.001, significant differences between weeks 0 and 12 and between different supplementations. Dashed lines are at pre and the difference to the week 0 is depicted just as a single line as no supplementation was provided before week 0.

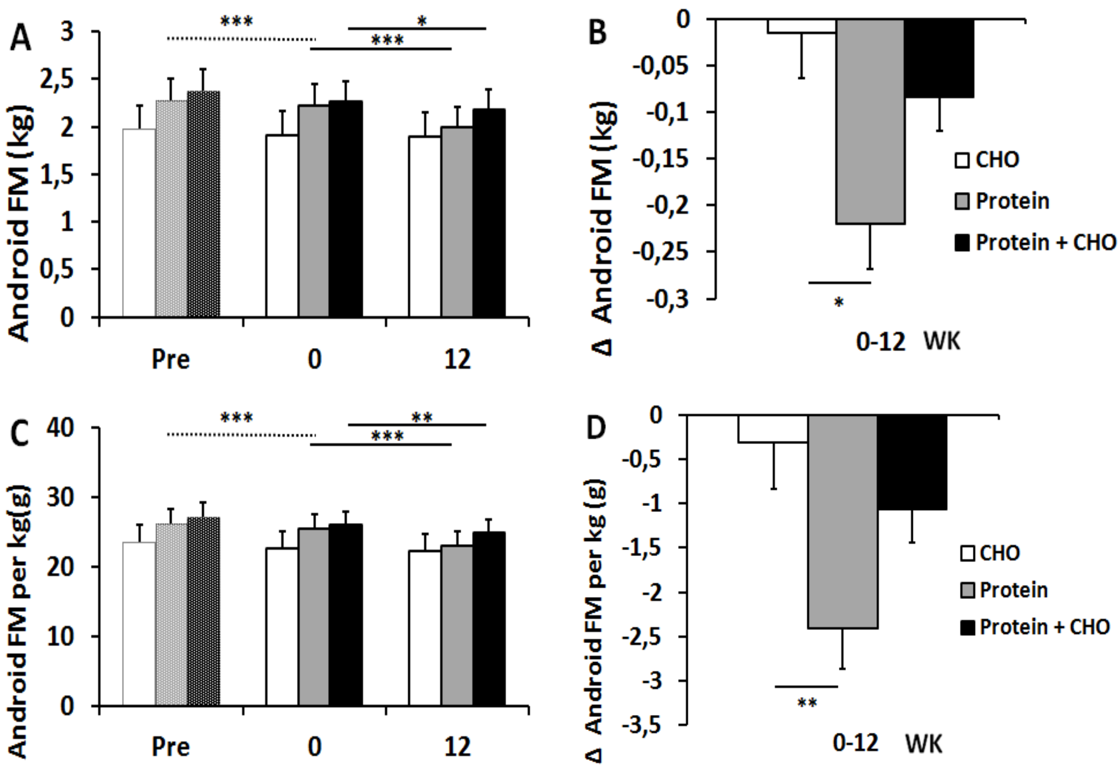

FIGURE11. Android fat mass (A), android fat mass changes (B), relative android fat mass (android fat mass divided by the body weight) (C), and relative android fat mass changes (D) from the beginning of supplementation to the end of the training period in carbohydrate, protein and protein plus carbohydrate groups. * in A and C-figures depict statistical changes between weeks 0 and 12. * in B and D-figures depict statistical difference in the change between different supplementations. * P < 0.05, ** P < 0.01, *** P < 0.001, significant differences between weeks 0 and 12 and between different supplementations. Dashed lines are at pre and the difference to the week 0 is depicted just as a single line as no supplementation was provided before week 0.

7.3.5 Changes for 1 RM, isometric strength, and cross sectional area of quadriceps femoris

Significant increases following resistance training for all three groups were also seen for 1 RM (P < 0.001) as measured by dynamic leg press, and for isometric strength (P < 0.001) as measured using an isometric leg extension dynamometer (figure 12). However, there were not significant differences between groups. Also cross sectional area of quadriceps femoris increased following the resistance training for all three groups (P < 0.001) as measured by ultrasound (figure 13).

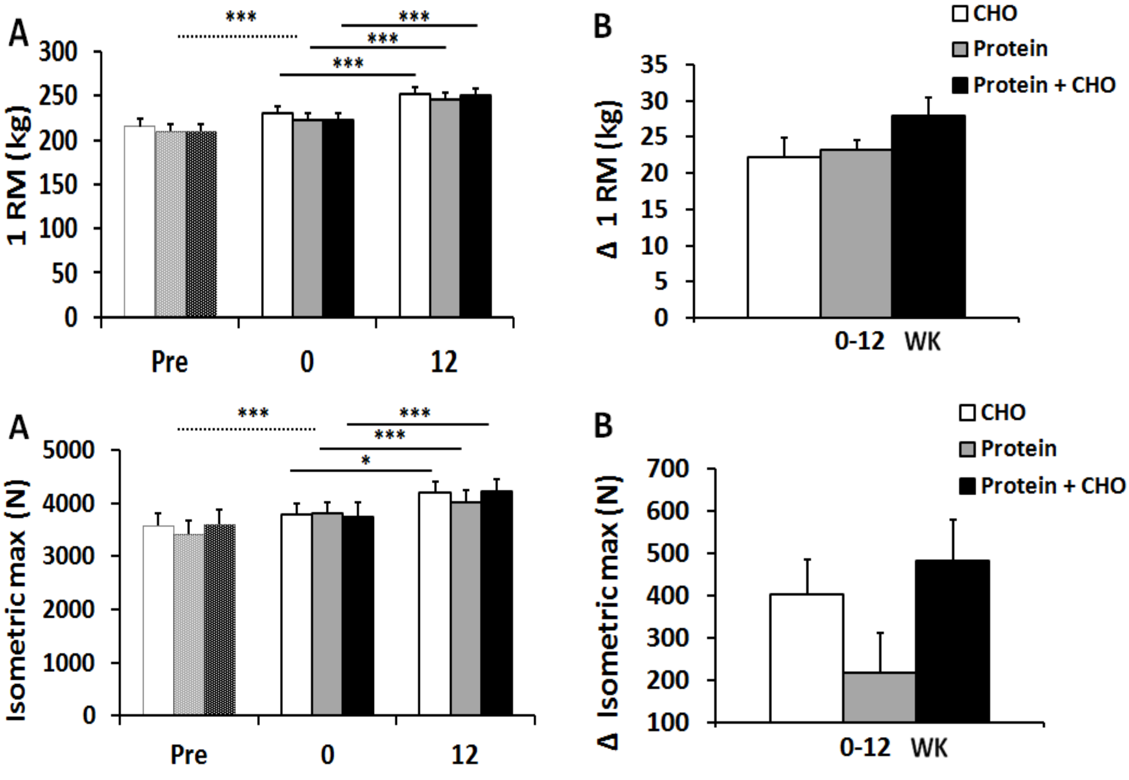

FIGURE 12. Maximal dynamic strength 1RM (A) and absolute changes in gains in 1RM (B), isometric strength (C) and changes in isometric strength (D) from the beginning of supplementation to the end of the training period in carbohydrate, protein and protein plus carbohydrate groups. * in A-figure depicts statistical changes between weeks 0 and 12. * P < 0.05, ** P < 0.01, *** P < 0.001. Dashed lines are at pre and the difference to the week 0 is depicted just as a single line as no supplementation was provided before week 0.

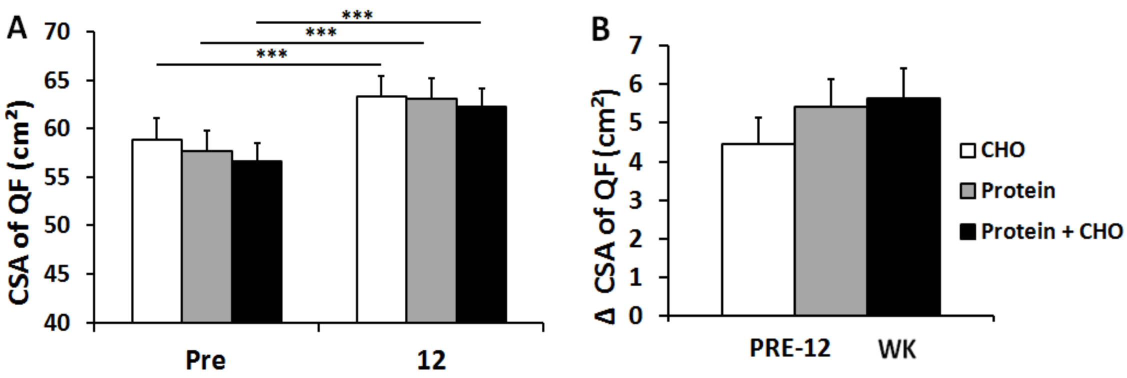

FIGURE 13. Cross sectional area (CSA) of quadriceps femoris (QF) (A) and absolute changes in CSA of QF (B) from the beginning of supplementation to the end of the training period in carbohydrate, protein, and protein plus carbohydrate groups. * in A-figure depicts statistical changes between weeks 0 and 12. * P < 0.05, ** P < 0.01, *** P < 0.001, significant differences between weeks pre and 12. Dashed lines are at pre and the difference to the week 0 is depicted just as a single line as no supplementation was provided before week 0.

7.3.6 Correlations between energy intake plus macronutrients intakes and fat mass

Table 6 and figure 14 represent correlations between total energy intake and relative trunk fat mass per kg on recovery days. Also correlations between macronutrients, fat and carbohydrate, intake and relative trunk fat mass are presented on table 5 and figure 14. Correlations between total energy, fat plus carbohydrate intake and relative android fat mass on recovery days are presented on table 7 and figure 15.

TABLE 6. Correlations between total energy, fat plus carbohydrate intake, and the relative trunk fat mass change during the 12 weeks of resistance training on recovery days. The more energy or macronutrients were consumed the less trunk fat mass decreased.

Trunk fat mass per kg change	**r**	**p-value**	
Energy per kg	0.436	0.013	*
Fat per kg	0.542	0.001	**
CHO per kg	0.404	0.022	*

TABLE 7. Correlations between total energy, fat plus carbohydrate intake, and the relative android fat mass change during the 12 weeks of resistance training on recovery days. The more energy or macronutrients were consumed the less android fat mass decreased.

Android fat mass per kg change	**r**	**p-value**	
Energy per kg	0.449	0.01	**
Fat per kg	0.569	0.001	**
CHO per kg	0.454	0.009	**

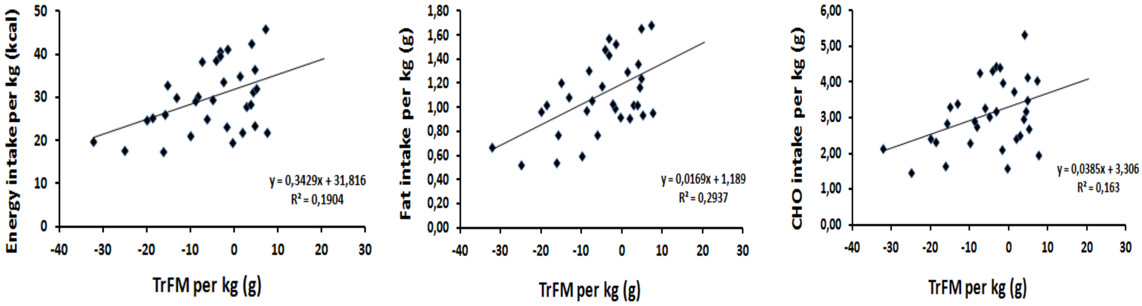

FIGURE 14. The correlation of relative energy intake (A), relative fat intake (B), relative carbohydrates intake (C) and the relative trunk fat change. The energy intake explained 19.04 %, the fat intake explained 29.37 %, and carbohydrates intake explained 16.3 % from the trunk fat change. The more energy, fat, and carbohydrates were consumed the less trunk fat decreased.

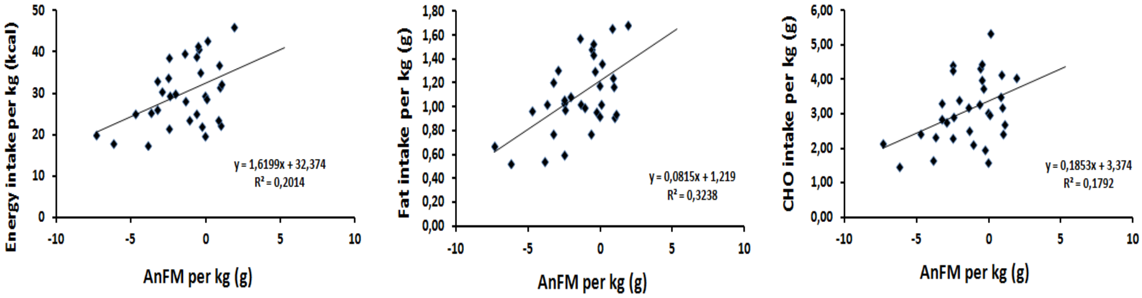

FIGURE 15. The correlation of relative energy intake (A), relative fat intake (B), relative carbohydrates intake (C) and the relative android fat change. The energy intake explained 20.14 %, the fat intake explained 32.38 %, and carbohydrates intake explained 17.92 % from the android fat change. The more energy, fat, and carbohydrates were consumed the less android fat decreased.

8 DISCUSSION

The purpose of this study was to examine the effects of different post-exercise supplementation regimens on resistance training adaptations. More specifically, the purpose of the study was to find out the effects of protein and carbohydrate supplementation on body composition and strength. The main finding of the study was that whey protein supplementation after resistance training sessions reduces total fat, trunk fat, and android fat mass when compared to the group supplemented with carbohydrates as a form of maltodextrin (glucose). Additionally, significant increases following the resistance training for all three groups were seen in 1 RM as measured by dynamic leg press. Moreover, cross sectional area of the quadriceps femoris increases following the resistance training for all three groups as measured by ultrasound.

Changes in body composition. In the present study, subjects were randomly divided into three different post-exercise supplementation groups; protein, protein plus carbohydrate or carbohydrate. After resistance training a significant increase of total fat free mass ($P < 0.001$) and leg fat free mass ($P = 0.003$) were identified in all three groups. The protein group lost significantly more fat than the carbohydrate group. As a result, additional calories in the form of whey protein probably did not add up fat. In the present study, the protein group consumed 37.5 g of whey (30 g protein) after each exercise.

It has been well established that protein intake in the context of resistance training increases post-exercise muscle protein synthesis rates and inhibits muscle protein breakdown in both younger and older populations. This allows net muscle protein accretion during the acute post-exercise recovery period. However, during prolonged resistance training, the results are not consistent. Some studies report greater gains in fat-free mass, muscle fiber size, and/or muscle strength after protein supplementation (Josse et al. 2010; Vieillevoye et al. 2012; Walker et al. 2010), while others (Bemben et al. 2010; Hoffman et al. 2009) failed to demonstrate such benefits (see meta-analysis by Cermak et al. 2012). It has been showed that carbohydrates co-ingestion with protein does not further augment protein-stimulated muscle protein synthesis or protein-mediated inhibition of muscle protein breakdown after resistance exercise (Gorissen et al. 2014; Koopman et al. 2007; Staples et al. 2011). However, research examining long-term effects of supplementing protein and carbohydrates versus protein within the context of exercise is limited. Nonetheless, according to Bird et al. (2006a), consuming the combined carbohydrate and essential amino acids supplement tends to promote larger gains in muscle mass and in muscle fiber size and muscle strength than non-energic placebo, carbohydrate or amino acids only, but this was significant between the groups only in the measures of fiber size.

In the present study, the long term effects of protein plus carbohydrate in the context of resistance training were examined. Subjects were randomly assigned to either the power-strength or hypertrophic-strength training group. However, although hypertrophy-strength and power-strength altered muscle hypertrophy differentially, no differences in muscle strength were noticed. In addition, no major effects of training type were seen on the supplementation response and therefore the training types were pooled for the current thesis. In present study, all three groups increased their cross sectional area of quadriceps femoris muscle as measured by ultrasound. These increases tended to be greater as a mean level in the protein and protein plus carbohydrate group when compared to the carbohydrate group, but were not, however, significantly greater. Also, as measured by DXA, total fat free mass increased in all three groups. This finding is in contrast with Andersen et al. (2005) who reported that only the protein group experienced muscle hypertrophy. It is, however, plausible that resistance training will increase cross sectional area of skeletal muscle provided that total energy intake is adequate. For instance, Westcott et al. (2009) demonstrated that 10 week resistance training has typically increased lean mass on average by 1.35 kg.

No major effect of protein on muscle adaptations were noticed when the results were expressed in absolute terms. However, relative fat free mass (total fat free mass divided by the body weight) increased significantly more in the protein group when compared to the carbohydrate group. Previously Cribb et al. (2007) also observed an increase in fat free mass in resistance-trained males supplementing with whey protein. Also a recent meta-analysis demonstrated that resistance training combined with protein supplementation leads to greater hypertrophy compared to carbohydrate or no energy supplementation (Cermak et al. 2012). This may be due, at least in part, to different protein distribution throughout the day between groups. It is unlikely that adding additional protein to meals that were already protein rich has the same effect as achieving a higher daily protein intake by adding protein to meals that were previously protein poor. (Layman. 2009.) In the present study, the protein and protein plus carbohydrate groups consumed protein right after each exercise but in contrast, the carbohydrate group did not.

In the post-absorptive state, protein breakdown increases more than protein synthesis due to resistance training. Amino acid ingestion in the context of resistance training increases protein synthesis and attenuates protein breakdown. The increase in amino acid availability from protein supplementation could potentially increase translational efficiency. In this process messenger RNA directs the amino acid sequence of a growing polypeptide during protein synthesis. Increased translational efficiency may be one explanation for the greater increase in fat free mass during resistance training when protein is ingested. (Candow 2004.) This indicates that to maximize skeletal muscle hypertrophy, it is important to include protein in post-exercise supplements or foods.

A higher loss of fat mass was observed in the protein group (~1.5 kg change from 0 to 12 weeks) when compared to the carbohydrate group (~0.0 kg), the combination group being in between (~0.6 kg). Recent increases in the number of obese individuals and individuals suffering from lifestyle-related diseases, such as type 2 diabetes, which accompany obesity, have become a serious social problem. Therefore, strategies for prevention and mitigation of obesity and lifestyle-related diseases are thought to be an extremely important public health issue. (Sakurai et al. 2012.) However, already small (3–5 kg) weight loss may reduce the overall incidences of diabetes by 58 percent in subjects with impaired glucose tolerance (Tuomilehto et al. 2001). It is suggested that a protein-rich diet promotes reduction of body fat. However, besides the amount of protein intake, also the quality of protein seems to be an important factor especially when the goal is to reduce fat mass. Especially whey protein appears to be effective in reducing fat mass. A recent meta-analysis of protein confirms that whey protein promotes weight loss and reduction of body fat (Sousa et al. 2012). For instance, Frestedt et al. (2008) demonstrated that the supplementation with whey protein led to greater reductions of body fat (6.1 %) and a higher maintenance of lean mass compared to supplementation with carbohydrate. In addition, Belobrajdic et al. (2004) investigated how proteins from different sources affect body weight and fat mass of rats. They concluded that a high-protein diet with whey protein decreased body weight and reduced visceral and subcutaneous fat deposition more compared to meat-based protein diet. Moreover, Loenneke et al. (2012) found moderate to strong correlations between variables indicating that the quality of protein may play an important role in regulating abdominal fat.

In the present study, total energy intake was higher in the carbohydrate group compared to the protein group, but not, however, significantly higher when presented as relative to body mass. To analyze the importance of the basal diet of the subjects on the fat mass changes, two subjects from the protein group, who consumed the least energy, and two subjects from the carbohydrate group, who consumed the most energy were removed. However, the results did not change much. For instance, even after this change, trunk fat mass of the protein group decreased significantly more, for instance, compared with the carbohydrate group. Due to this, at least in this case, supplementations combined with resistance training are thought to be the main reason for the results obtained. Thus, it is suggested that protein supplementation in the context of resistance training either A) induces reduction in fat mass due to resistance training or B), protein intake does not block the fat mass loss effects of resistance training while carbohydrates do.

There is evidence that whey suppress appetite (Westerterp-Plantenga et al. 2009). Additionally, a high protein intake also seems to be able suppress the following day's energy intake more than an isoenergetic amount of carbohydrate (Stubbs et al. 1995). The mechanisms responsible for the high satiating effect of protein are not known. Consequently, the inhibition of energy intake caused by the high-protein diet may be due to, for instance, difference of energy density and mechanisms like release of gut peptides, liver metabolism and a direct central effect of certain amino acids (Peters & Harper 1987). In the present study, the protein group consumed only approximately 2350 kcal per day while the carbohydrate group consumed approximately 2900 kcal. During resistance training consuming less than 2400 kcal per day can be considered relatively little. Two recent meta-analyses suggest that during energy restriction consuming a high-protein diet results in enhanced fat loss (Abargouei et al. 2012; Chen et al. 2012). This may be one explanation for why the fat mass of the protein group, especially trunk and android fat, decreased so much in our study.

A higher loss of android fat mass was observed in the protein group (~0.2 kg change from 0 to 12 weeks) when compared to the carbohydrate group (~0.0 kg), the combination group being in between (~0.1 kg). There is a substantial amount of literature referring to obesity as a major risk factor in the development of diabetes and other lifestyle-related diseases (Sakurai et al. 2012). In addition, it is increasingly recognized that for a given BMI, central rather than lower body fat distribution confers greater risk of lifestyle-related diseases of obesity (Bigaard et al. 2005). Moreover, Freemantle et al. (2008) showed that there is a strong association between measures reflecting abdominal obesity and the development of type 2 diabetes. Although DXA measures the total fat of android region, both visceral and subcutaneous, it has been observed that the correlation between visceral fat and android fat is relatively strong (R = 0.78) (Hill et al. 2007). Due to this, android fat as determined by DXA is quite comparable with more-established measures of abdominal or visceral fat.

In addition to the effect on energy intake, a high-protein diet may increase resting energy expenditure (REE) more than a high-carbohydrate diet. This may be due to higher post-prandial thermogenesis of protein compared with carbohydrate. (Skov et al. 1999.) REE represents the minimum amount of energy required to maintain cellular processes at rest, and it has been regarded as a gauge for metabolic homeostasis. Moreover, Hackney et al. (2009) investigated how protein supplementation combined with resistance training would influence post-exercise REE. They found that at 24 h after resistance exercise, REE was greater in the protein group compared with the carbohydrate group. They suggested that this elevation was mediated by preferentially increasing amino acid availability in skeletal muscles that were damaged during the acute resistance exercise session. (Hackney et al. 2009.)

Unfortunately, the mechanisms by which timing the intake of protein facilitates increases REE are unclear. It is postulated that this strategy increases amino acid delivery and uptake to the working muscles, leading to the activation of multiple cell signaling transduction pathways, e.g. the mammalian target of rapamycin (mTOR). Moreover, activation of the mTOR pathway may lead to acute and long term up-regulation of muscle protein synthesis. In addition, a combination of feeding and exercise may interact and influence hormone concentrations during the exercise and in the post-exercise period. For example, the hormone cortisol is released after resistance exercise. Cortisol increases protein degradation and decreases protein synthesis in skeletal muscle cells. However, it has been suggested that protein supplementation consumed after exercise may reduce cortisol concentrations 24 h after the acute resistance exercise. Due to this, it is possible that a post-exercise reduction in cortisol could increase muscle protein synthesis and elevate metabolic activity thereby leading to the elevation in REE. (Hackney et al. 2009.) However, on daily basis, a higher satiating effect of protein when compared with carbohydrate is probably more important factor as reducing fat mass than differences in resting energy expenditure (Skov et al. 1999).

The results from previous studies on the effect of whey protein supplementation during resistance training on fat mass are conflicting. Volek et al. (2013) demonstrated that whey protein supplementation did not promote fat loss more that carbohydrate supplementation. Arciero et al. (2014) demonstrated that whey protein combined with resistance training is an effective way to reduce total fat mass and abdominal fat mass. This is consistent with the results of present study, where total fat, trunk fat, and android fat mass of the protein group reduced significantly more when compared to the carbohydrate group. Studies examining effects of resistance training combined with protein plus carbohydrate supplementation on fat mass reduction is very limited. In the present study, fat mass of the protein plus carbohydrate group decreased more than the fat mass of carbohydrate group but the difference was not significant. However, resistance training alone, without supplementation, also reduces fat mass. For instance, Mekary et al. (2015) investigated 10 500 healthy men during a 12-year follow-up and concluded that resistance training is an effective way to reduce or prevent gain in waist circumference and, therefore, abdominal obesity. In addition, Westcott et al. (2009) demonstrated that 10 week resistance training reduced fat mass approximately 1.7 kg.

Whey is considered a high-quality protein because of its abundance of essential amino acids, especially BCAA (Ha & Zemel 2003), which are necessary for stimulating protein synthesis and supporting muscle growth (Borsheim et al. 2002). BCAA, i.e. leucine, isoleucine, and valine, play important role in protein metabolism. It has been demonstrated that leucine promotes protein synthesis but also regulates fat and glucose metabolism (Kimball et al. 2006; Matthews 2005). Moreover, Crozier et al. (2005)

demonstrated that during energy restriction, leucine supplementation results enhanced fat loss and muscle protein synthesis. Additionally, Nishimura et al. (2010) demonstrated that mice that received more isoleucine in their diet compared to placebo group, gained less weight than the placebo group. Their results further indicate that in isoleucine group, the adiposity of liver and skeletal muscles were less than in the control group. Due to this, isoleucine may enhance to reduce the triglycerides levels of liver and skeletal muscles. This may be due to the fact that isoleucine affects fatty acid oxidation. (Nishimura et al. 2010.)

In addition to leucine and isoleucine, calcium may be another reason why whey helps to promote reduction of body fat (Ha. & Zemel. 2003). Some studies suggest that dairy products' content of calcium decreases accumulation of body fat and also accelerates weight and fat loss during energy restriction (Zemel 2003; Zemel et al. 2000; Zemel et al. 2002) Due to this, whey supplementation might increase lean body mass and decrease fat mass especially when energy is restricted. Zemel et al. (2000) demonstrated that the calcitrophic hormones, for instance parathyroid hormone, which respond to low calcium diets, promote adipose tissue storage. Additionally, high calcium diets suppress these hormones and thus, inhibit adiposity. (Shi et al. 2001; Zemel. 2003; Zemel et al. 2000.) This may be, at least partially, one possible explanation why fat mass of protein group in present study decreased and at the same time lean body mass increased.

Changes in strength. In present study, cross sectional area of the quadriceps femoris and total fat free mass of all three groups increased. The resistance training period also increased 1 RM in all three groups, which is consistent with previous meta-analyses (Cermak et al. 2012; Pasiakos et al. 2014). However, the protein plus carbohydrate group increased the most in terms of relative 1 RM, measured by dynamic leg press, but this increase was not statistically significant. Also Bird et al. (2006a) demonstrated that a protein plus carbohydrate group increased 1 RM more than other groups. Moreover, this increased were significantly greater when compared with a placebo group. However, in Bird et al. (2006a) study, the placebo group received a non-energetic supplementation.

There are a couple of explanations for why all three groups increased their 1 RM without any significant differences between the groups. Dynamic leg press is a quite complex movement involving movement at a multiple number of joints. This may lead to the fact that early gains in strength are not due to only muscle hypertrophy but also to neural adaptations. (Chilibeck et al. 1998.) Especially in case of individuals without earlier experience of regular strength training, increases in strength are usually caused by both neural factors and hypertrophy, at least during a short strength training period. Resistance training also leads to increased protein turnover and further to increased protein need (Biolo et al. 1995). However, as mentioned earlier, the carbohydrate group consumed a relatively high amount of protein per day. This amount of protein may be sufficient to meet the increased protein requirement. Moreover, this may account for the relatively small differences in 1 RM between all the three groups.

Limitations of the study. Methodological limitations of the present study include the lack of food recording more than once during the training period. Theoretically, some of the subjects could have changed their dietary habits during the training period. However, it would be uncommon that one group but not the others would make a systematic change in habitual diet. In addition, due to collecting only one dietary record, is not seen how energy intake and macronutrients intake changed during the time subjects consumed supplements compared to time that subjects did not consume supplements. All of the subjects returned dietary records during the training period with supplements. However, some of the subjects returned food diaries during the first weeks while other returned their diaries during the eighth week of training. Additionally, only 40 of all subjects returned the diaries. It should also be noted that 4-day dietary records are not able to pick up relatively small differences in total energy intake. This is due to limited accuracy of food diaries.

All of the subjects were given a supplement after each exercise and all of them consumed it immediately. Subjects mixed the powder with the juice themselves. However, it is possible that a small amount of the powder was not mixed and consequently that small amount of the powder remained on the bottom of the shaker and was thus not consumed by the subject. In addition, subjects were instructed to avoid other kinds of sports besides the resistance training program given by the present study. They were allowed to do only one other strenuous physical activity per week in addition to this resistance training. However, subjects did not fill out a training diary during the resistance training period. Due to this, it is possible that subjects did more strenuous physical activity that went undetected.

Practical applications. In the present study, whey protein supplementation combined with resistance training promoted more total fat, trunk fat, and android fat mass loss compared to carbohydrate supplementation. In addition, whey protein promoted a greater relative fat-free mass increase as compared to consuming a carbohydrate supplement. The accumulation of abdominal fat mass correlates with an increased risk of cardiovascular disease, diabetes, hypertension, frailty, and certain cancers. Due to this, supplementation with whey protein combined with resistance training may be an effective way to decrease total fat and abdominal fat mass.

Conclusions. The present study examined the effects of supplementation with protein, protein plus carbohydrate, or carbohydrate during a 12-week resistance training program and included 68 healthy, recreational active men. The main finding of the study was that the intake of protein supplementation after each resistance training session during the 12-week period was shown to reduce total fat, trunk fat, and android fat mass significantly more compared to the intake of carbohydrate. A group that consumed protein plus carbohydrate supplementation showed results that were in between those of the protein and carbohydrate groups. These results are in accordance the previous findings on supplementation combined with resistance training. In addition, significant increases following the resistance training for all three groups were seen for 1 RM. Moreover, cross sectional area of quadriceps femoris increased following the resistance training for all three groups. Additionally, protein supplementation seemed to decrease energy intake when compared to carbohydrate group. This may, at least in part explain the results in fat mass. These results highlight the importance of diet and resistance training to reduce fat mass and increase lean mass, and due to this, underline the possible positive influences that protein supplementation with resistance training can have on overall health. A limitation of the present study, however, is that only one dietary analysis was conducted in order to determine the energy intake and the macronutrients intake during the training period. Due to this, is not seen how energy intake and macronutrients intake may have changed during the time that subjects consumed supplements compared to the time that subjects did not consume supplements. Thus, in the future, more investigations will be needed to further explore the potential combination of resistance exercise and whey protein supplementation for reducing the total fat and the visceral fat mass.

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