THE EFFECT OF NUTRITION ON PHYSIOLOGICAL RESPONSES OF RESISTANCE TRAINING IN MUSCLE AND BLOOD IN YOUNG MEN

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ABSTRACT

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Background. Exercise results in increased blood flow to the active skeletal muscles, which have potential to enhance delivery of nutrients to target receptors. The combined effect of muscular contraction and the increased availability of nutrients have the potential to enhance training responses. The purpose of this study was to examine, if the nutritional variances between individuals are associated with the responses and adaptations of heavy resistance training (RT) in muscle and blood.

Methods. The test group consisted of 21 young men (18-30 years) who did not have a regular RT background. They went through supervised RT program practicing two times a week for 21 weeks. Their maximum strength, muscle size, muscle fiber types, body composition and hormonal content of the blood were measured in the beginning, in the middle and at the end of the study. The strength test consisted of bilateral dynamic leg press in David 200-eguipment (David Fitness and Medical, Finland). Muscle biopsies were taken before and after 21 week training period from the vastus lateralis muscle using Bergström's 5 mm biopsy needle technique. The muscle fiber types were determined by histochemical ATPase colouring (Brooke & Kaiser 1970). The sizes of the cells were determined by anti-dystrofin antibody. The muscle cross-sectional area (CSA) of the quadriceps femoris (QF) muscle was determined before and after 21 week training period using magnetic resonance system (MRI) (GE Signa Exite HD 1.5 T) and analyzed with OsiriX (version 2.7.5) software. Blood samples were drawn from the antecubital vein before the muscle work, right after exercise, 15 minutes and 30 minutes after exercise. Serum hormones were analyzed with an immunometric chemiluminescence method with an Immulite® 1000 (DPC, Los Angeles, USA). The dietary diaries were registered during four to five consecutive days around the other measurements. The diaries were analyzed using the Micro Nutrica nutrient analysis software version 3.11 (The Social Insurance Institution of Finland). The data was analyzed using IBM SPSS Statistics 19 software. Mean value, standard deviation, Pearson's correlation, student's t-test, repeated measures ANOVA and regression analysis were used.

Results. During 21 weeks of resistance training maximal strength ($p<0.001$), the sizes of type 1 and type 2 muscle cells ($p=0.001$), the surface area of QF ($p<0.001$), the body weight ($p<0.001$) and the lean body mass increased. The consumption of protein (g/kg body mass/d) increased ($p=0.047$) from the beginning to the end of the study. The consumption of monounsaturated fatty acids ($r= -0.534$, $p =0.018$) and fat ($r= -0.473$, $p=0.041$) correlated inversely with absolute 1RM development. The water consumption ($r= -0.557$, $p= 0.025$) correlated inversely with absolute cell type 1 and 2 size changes. Especially the amount of water ($r=0.687$, $p=0.001$) and carbohydrates ($r=0.608$, $p=0.006$) correlated with the increase of percentage fat. Sex hormone binding globulin (SHBG) ($p<0.001$) and growth hormone (GH) ($p=0.049$) levels rose after the single exercise. Cortisol levels right after muscle stress correlated inversely with the amount of cholesterol ($r =- 0.747$, $p=0.008$). Especially water ($r=0.785$, $p<0.001$) correlated with insulin levels after exercise. The basal levels of hormones or their changes induced by training did not correlate with any chronic training adaptations in muscle ($p>0.05$).

Conclusions. During the regular RT the consumption of protein increases, however if dietary recommendations are met, the individual differences in protein or carbohydrate consumption do not affect the chronic adaptations in the muscle. The effect of vitamin D on chronic adaptations is interesting and has to be studied more in future. Acute hormonal elevations of testosterone, insulin and cortisol after resistance exercise can be affected by nutrition. Also the changes in body composition during 21 weeks of RT can be affected by nutrition.

Keywords: hormonal responses, muscle growth, nutrition, resistance training

TIIVISTELMÄ

Katajavuori, Marja. 2014. Ravinnon vaikutus voimaharjoittelun aiheuttamiin lihaksen sekä veren fysiologisiin vasteisiin nuorilla miehillä. Jyväskylän Yliopisto, Liikuntabiologian laitos. Liikuntafysiologian Pro gradu -tutkielma. 95 s.

Taustatieto. Harjoittelu saa aikaan lisääntyneen verenvirtauksen aktiivisissa luurankolihaksissa. Tämä mahdollistaa ravintoaineiden paremman kulkeutumisen kohdereseptoreilleen. Lihassupistuksen ja lisääntyneen ravintoaineiden kulkeutumismahdollisuuden yhteisvaikutus mahdollistaa harjoitusvasteiden paranemisen. Tämän pro gradu -tutkielman tarkoitus oli selvittää, onko yksilöiden ravitsemuksellisilla eroilla ja raskaan voimaharjoittelun aiheuttamilla lihaksen ja veren vasteilla yhteyttä.
Menetelmät. Koehenkilöryhmässä oli 21 nuorta miestä (ikä 18 - 30 vuotta), joilla ei ollut aiempaa kokemusta säännöllisestä kuntosaliharjoittelusta. He harjoittelivat kuntosalilla ohjatusti kahdesti viikossa 21 viikon ajan. Tutkimuksen alussa, puolivälissä ja lopussa heiltä mitattiin maksimivoima, lihaksen pinta-ala, lihassolutyypit, kehonkoostumus ja veren hormonipitoisuudet. Voimatesti koostui bilateraalisesta dynaamisesta jalkaprässistä David 200-laitteessa (David Fitness and Medical, Finland). Lihassolunäytteet otettiin nelipäisestä reisilihaksesta Bergströmin 5 mm:n lihassolunäyteneulatekniikalla ennen ja jälkeen 21 viikon harjoittelun. Lihassolutyypit määritettiin histokemiallisella ATPaasi värjäyksellä (Brooke & Kaiser 1970). Lihassolujen koot määriteltiin anti-dystrofiini vasta-aineella. Ennen ja jälkeen 21 viikon harjoittelun nelipäisen reisilihaksen poikkipinta-ala määritettiin magneettikuvauksella (GE Signa Exite HD 1.5 T) ja analysoitiin OsiriX-ohjelmalla (versio 2.7.5). Verinäytteet otettiin kyynärtaivelaskimosta ennen lihastyötä, heti lihastyön jälkeen, 15 minuuttia ja 30 minuuttia harjoituksen jälkeen. Seerumin hormonit analysoitiin Immulite® 1000-laitteella (DPC, Los Angeles, USA). Ravintopäiväkirjoja täytettiin 4 - 5 päivän aikana muiden mittauspäivien läheisyydessä. Päiväkirjat analysoitiin Micro Nutrica ravintoanalyysi -ohjelman versiolla 3.11 (The Social Insurance Institution of Finland). Materiaali analysoitiin IBM SPSS 19 tilasto-ohjelmalla, jossa käytettiin keskiarvoa, keskihajontaa, Pearsonin korrelaatiota, Studentin t-testiä, toistomittausten ANOVAa ja regressioanalyysia.
Tulokset. Voimaharjoittelu lisäsi maksimivoimaa ($p<0,001$), tyypin 1 ja 2 lihassolujen kokoa ($p<0,001$), nelipäisen reisilihaksen pinta-alaa ($p<0,001$), kehon painoa ($p<0,001$) sekä rasvattoman massan määrää ($p<0,001$). Proteiinin kulutus (g/kg kehon paino/vrk) kasvoi ($p=0,047$) harjoittelujakson aikana. Nautittujen tyydyttyneiden rasvahappojen ($r= -0,534$, $p =0,018$) ja rasvan määrä ($r= -0,473$, $p=0,041$) korreloivat käänteisesti maksimivoiman absoluuttisen kehityksen kanssa. Nautitun veden määrä korreloi käänteisesti lihassolujen absoluuttisen pinta-alan kasvun kanssa ($r= -0,557$, $p= 0,025$). Erityisesti veden ($r=0,687$, $p=0,001$) ja hiilihydraattien ($r=0,608$, $p=0,006$) nauttiminen korreloi kehon rasvaprosentin lisääntymisen kanssa. SHBG -hormonin ($p<0,001$) ja kasvuhormonin ($p=0,049$) tasot nousivat yksittäisen harjoituksen jälkeen. Harjoituksen jälkeiset kortisolitasot korreloivat käänteisesti kolesterolin määrän kanssa ($r = - 0,747$, $p=0,008$). Erityisesti vesi ($r=0,785$, $p=0,000$) korreloi harjoituksen jälkeisen insuliinitason kanssa. Lepotilan hormonitasot tai kuormituksen jälkeiset hormonitasojen nousut eivät korreloineet lihasten kroonisten harjoitteluvasteiden kanssa ($p>0,05$).
Johtopäätökset. Säännöllisen voimaharjoittelun aikana proteiinin käyttö lisääntyy. Kun syödään ravintosuositusten mukaisesti, ei kuitenkaan yksilöiden välisillä nautittujen proteiinien tai hiilihydraattien määrällä tässä tutkimuksessa ole vaikutusta lihaksen kroonisiin harjoitteluvasteisiin. D-vitamiinin osuus kroonisissa harjoitteluvasteissa on mielenkiintoinen ja jatkossa sitä tulee tutkia enemmän. Ravinnolla voidaan vaikuttaa testosteroni-, insuliini- ja kortisolipitoisuuksien akuuttiin nousuun voimaharjoituksen jälkeen. Myös 21 viikon voimaharjoittelujakson aikana tapahtuviin kehon antropometrisiin muuttujiin voidaan vaikuttaa ravinnolla.

Avainsanat: hormonivaste, lihaskasvu, ravinto, voimaharjoittelu

CONTENT

ABBREVIATIONS

ACTH	adrenocorticotropin hormone
CRH	corticotropin-releasing hormone
CSA	cross-sectional area
GH	growth hormone
HDL	high-density lipoprotein
LBM	lean body mass
LDL	low-density lipoprotein
MPB	muscle protein breakdown
MPS	muscle protein synthesis
MRI	magnetic resonance imaging
NPB	net protein balance
1 RM	one repetition maximum
RE	resistance exercise
RT	resistance training
SHBG	sex hormone-binding globulin
VDR	vitamin D receptor

1 INTRODUCTION

Acute responses and chronic adaptations of resistance training are linked together (figure 1). Every single exercise session causes transient changes in physiological function. The hormonal and metabolic environment created by an acute exercise bout modulates the magnitude and direction of adaptations. Resistance exercise (RE) invokes a sequential casade consisting of muscle activation, signaling events, protein synthesis and muscle fiber hypertrophy. Muscle activation means that α-motoneurons activate muscle fibers to produce force. The neuromuscular interaction determines which muscle fibers are activated and the amount of force exerted. Only those motor units recruited will respond and adapt to RE. Signaling events mean contraction and stretching of muscle fibers that stimulate various muscle signaling pathways independently of changes in hormones and growth factors, anabolic hormonal responses and immune/inflammatory responses. During eccentric actions, the myofibrils of the muscle fiber stretch while producing force. Repetitive overstretching leads to sarcomere disruption and membrane damage. It leads to muscle soreness, but also provides an important stimulus for muscle growth: it promotes neutrophil mobilization and invasion into the muscle tissue. Neutrophils degrade damaged muscle tissue. Also protein synthesis increases after RE as an acute response. The nutrition consumed and the intensity, volume and other parameters of the RE effect on the magnitude of these acute responses. (Bird 2010; Spiering et al. 2008b)

If a single RE is heavy enough, it causes tiredness that temporarily lowers the nervous capacity to activate muscles to their maximum. The acute effects of training are related to the type of exercise (isometric, concentric or eccentric). Skeletal muscle can generate about 30 % more tension during eccentric than concentric actions, which explains why maximal eccentric actions evoke a greater signaling response in humans (Eliasson et al. 2006). The acute effects of RE are also related to the amount of load, intensity, duration, exercise order, recovery time and the individual performance level. The elevated hormonal levels get back to their normal levels quite soon after RE. Several weeks of training does not necessarily affect to these normal levels. (Erola 2000, 1; Prasartwuth et al. 2005; Spiering et al. 2008; Thalacker-Mercer 2009.)

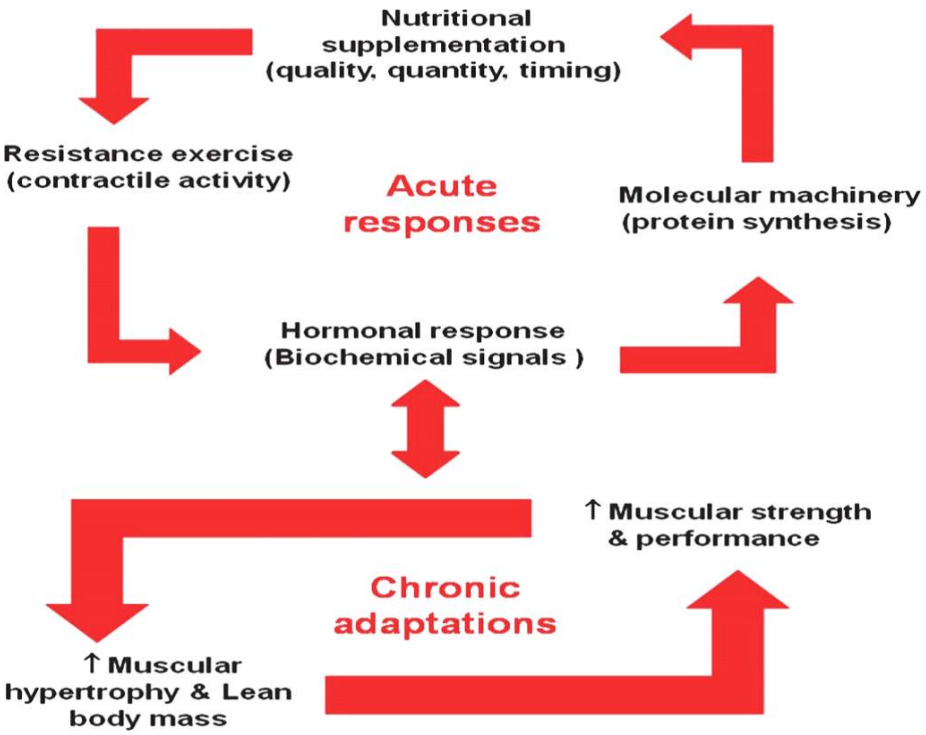

FIGURE 1. The pathway of adaptation model (Bird 2010, 82).

In addition to the nervous system, muscles also get tired when energy stores finish or when the chemical status of the muscle changes through the accumulation of the metabolic waste. RE creates endocrine response that with nutrition enhances protein synthesis and affects muscle hypertrophy. An exercise results in increased blood flow to the active skeletal muscles, which has the potential to enhance the delivery of nutrients to target receptors. The combined effect of muscular contraction and the increased availability of nutrients have the potential to enhance the adaptations to RT. Nutritional variances between individuals influence changes in muscle protein synthesis. (McArdle 2007, 541–543; Phillips 2004; Phillips et al. 2009; Volek 2004).

The purpose of this study was to examine, if nutrition has acute responses or chronic adaptations in muscle and in blood following the 21 weeks RT in young non-trained men.

2 SPORT NUTRITION

2.1 Proteins

The body of an average-sized adult contains between 10 and 12 kg of protein, with the largest quantity located within the skeletal muscle mass (McArdle et al. 2007, 32). Protein is one of the most important nutrients. During the digestion, protein hydrolyzes to its amino acid constituents. There are 20 different amino acids and their derivatives, which are working in the human body. Amino acids can be divided in two groups: necessary ones and unnecessary ones. The body can synthesize unnecessary amino acids; however necessary amino acids, the group of 9 different acids, need to be obtained regularly from the nutrition. (Nelson & Cox 2000, 115–129.) Amino acids join together. Two joined amino acids produce a dipeptide, three joined amino acids produce tripeptide and so on. A combination of more than 50 amino acids is named polypeptide, and that form is called protein. (McArdle et al. 2007, 32.)

Daily need of protein is recommended to be 10-15% of daily energy consumption. It is more meaningful to count protein requirement relative to the body mass. In Finland that recommendation is 0.8g/kg per day for all men and women, aged 19 years and above, independent of physical status. It is argued for years how much exercising increases the need of protein. Athletes might need to consume 50-100% more protein for exercise-related energy production, post-exercise muscle damage repair, and muscle hypertrophy. However, it is more useful to talk about the optimum of protein instead of need of protein. The need only guarantees that the nitrogen balance does not go negative. The optimum makes the improvement possible. An optimum needs to be evaluated individually depending on the target. (Campbell & Leidy 2007; Hulmi 2013a; Lemon 2000.)

There is no protein store in the human body except the structural proteins that are in use. If energy used is higher than energy obtained from the food or the protein amount of the food is too small, body needs to use muscles' own protein. Also if glycogen stores (form of custody of carbohydrates in the body) are empty, body sacrifices proteins from the muscles to build carbohydrates. (Niemi 2006, 30.) Proteins are obtained for example

from eggs, milk, meat, fish and poultry (McArdle et al. 2007, 33).

2.2 Carbohydrates

Carbohydrates can be classified into monosaccharides, oligosaccharides and polysaccharides. Monosaccharides are basic units of carbohydrates. Monosaccharides consist of glucose, fructose and galactose. Glucose forms in the body through digestion of more complex carbohydrates. Glucose is also synthesized in the liver from other compounds. Fructose is the sweetest simple sugar obtained from fruits and honey. Galactose does not exist freely. The body converts galactose to glucose. Oligosaccharides form when 2 to 10 monosaccharides bond chemically. Sucrose, lactose and maltose are examples of oligosaccharides. Sucrose means ordinary sugar. Lactose is found from milk products as milk sugar. Maltose occurs in beer and cereals. Polysaccharides describe the linkage of more than 10 monosaccharides. Polysaccharides are divided in plant and animal polysaccharides. Starch and fiber are the common forms of plant polysaccharides. Glycogen is the form of animal polysaccharides. Glycogen is the storage carbohydrate. It is stored in the liver and in the muscles. During exercise intramuscular glycogen provides the major carbohydrate energy source for active muscles. Glycogen in the liver rapidly reconverts to glucose for release into the blood as an extramuscular glucose supply for exercise. (McArdle et al. 2007, 7-9,13.)

It is suggested that the optimum for carbohydrate consumption would be about 5-6 g/kg/day, depending on the level of the exercise. The percentage amount of the carbohydrates would be then about 55% from the total daily consumption. (Haff et al. 2003.) Like the optimum of proteins there are also individual differences in carbohydrate consumption. Some top athletes for example consume much more carbohydrates than previous recommendations say, some athletes avoid carbohydrate consumption. In both ways it is possible to gain good results. Different practicing methods work in different individuals. (Hulmi 2013b) Excessive carbohydrate consumption, like every other excessive consumption as well, makes extra carbohydrate change to body fat (Niemi 2006, 23).

2.3 Fats

Lipids belong to one of main groups: simple lipids, compound lipids or derived lipids. The simple lipids are named “neutral fats” and they consist of triglycerides. Triglycerides consist of glycerol molecule and three fatty acid chain. All lipid-containing foods consist of a mixture of different proportions of saturated and unsaturated fatty acid chains. Saturated fatty acids are obtained from products of animal origin. Unsaturated fatty acids are called soft fat and they are liquid in room temperature. Triglycerides constitute the major storage form of fat in fat cells. (McArdle et al. 2007, 18-19; Niemi 2006, 33, 35.)

Compound lipids are triglycerides combined with other chemicals. They represent about 10% of the total fat in the body. One compound lipid is lipoprotein. Lipoproteins provide the possibility of transporting lipids in the blood. The liver and small intestine produces high-density lipoproteins (HDLs), which contain the highest percentage of proteins (50%) of the lipoproteins and the least total lipid (20%) and cholesterol (20%) of the lipoproteins. Very-low-density lipoproteins (VLDHs) and low-density lipoproteins (LDLs) contain highest percentage of lipid and cholesterol instead. (McArdle et al. 2007, 24.) HDLs are good form of cholesterol. HDLs transport cholesterol away from the walls of the veins to the liver. HDLs keep veins open and decrease the risk of coronary artery disease. LDLs are bad cholesterol. LDLs transport cholesterol to tissues and vein walls forming nests that are the beginning for the atherosclerosis. (Niemi 2006, 36-37.)

Simple and compound lipids form derived lipids. Cholesterol is the most known compound lipid. It exists only in animal tissue. (McArdle et al. 2007, 26.) Cholesterol is required for the body to function sufficiently. It is not only bad thing like believed. Normally we just get too much cholesterol. (Niemi 2006, 36-37.) Cholesterol is needed for hormonal production and to build cell membranes, bile acids and vitamin D. Cholesterol is an important component of biological membranes. It increases membrane viscosity, which increases the exposure of membrane proteins to extracellular fluids. It is also essential for the formation of lipid rafts, which function as platforms for the assembly of components of signaling pathways through protein sorting and construction of signaling complexes. That is why depletion of cholesterol can induce protein

missorting and reduced signal transduction. (Freeman & Solomon 2004; Lucero & Robbins 2004; Simons & Toomre 2000.) Cholesterol is built in the liver, however it is also obtained from the food. The food consumed is the most important factor when controlling cholesterol amount. Especially hard fat raises cholesterol levels. Genetic factors have also some effect on levels. The recommendation of daily consumption of cholesterol is 250-330 mg. (Niemi 2006, 36-37.)

Essential fatty acids need to be obtained from the food, because body cannot form them from any other fatty acid. Most of these are obtained from fish. The normal limits for the usage are 1-2g/kg/day, 25% from the daily consumption. RT does not increase the need of fat like it increases the need of proteins and carbohydrates. Small fat consumption should not be so harmful for improvement, but thinking from the health point of view consuming fat is important. (Laatikainen 2011; Niemi 2006, 33,35.)

Many people believe that fat is not good for health. Especially saturated fatty acids are said not to be good for the health. Most of the hidden fats are specifically these saturated fatty acids. Unsaturated fatty acids have believed to have positive effects on health. However, the newest studies say that there is no difference between low- and high-fat diets (studied until diets consisting 45% fat) when it is considering heart and vascular deceases. Sufficient and versatile fat intake is actually only good for health. When fat is consumed, the need of linoleic acids and α-linoleic acids of essential amino acids are easier to satisfy. When CHO are replaced by fat in the diet, HDL-cholesterol increases and triglycerides decrease. If this replacement is done using fat products versatile, LDLs will not increase at all. When fat is used in a meal at the same time with CHO, insulin and glucose responses decrease. Additionally, when using vegetable oil and margarines the supply of vitamin E and D may increase. (Laatikainen 2011.)

2.4 Water

Water makes up 40-70% of body weight. Nutrients travel in solution. Waste products leave the body through the water in urine and feces. Water has heat-stabilizing qualities as it absorbs considerable heat with changes in temperature. This quality maintains a relatively stable body temperature during exercise. 2.5 l water is needed daily. Exercising raises the need of consumed water. (McArdle 2007, 74-75.)

2.5 Alcohol

Alcohol is energy-producing, not essential nutrient. The energy amount it contains is not nutritionally valuable, as it does not contain any important nutrients like vitamins or minerals. Alcohol can be equated to sugar. Alcohol use effects on dehydration, slows down recovery and lowers alertness that easily leads to injuries. (Niemi 2006, 43)

2.6 Protective nutrients

Protective nutrients mean minerals and vitamins that cannot be produced by the body, at least not the amounts required. They need to be obtained from the food. Normally we get enough all the other ones except iron, phosphate and vitamin D. (Niemi 2006, 45-46.)

Minerals are important for regulating enzymes working with energy metabolism, muscles or nerves. Minerals can be divided in two sub groups: macro minerals and trace minerals. Macro minerals consist of calcium, chloride, magnesium, phosphorus, potassium, sodium and sulphur. The lack of these minerals leads to a disorder in body function. These disorders can be explained by biochemical dysfunctions. Boron, cobalt, chromium, copper, fluoride, iodine, iron, manganese, molybdenum, selenium and zinc are trace minerals. The need of daily consumption of trace minerals is smaller than the need of macro nutrients. Physical stress changes the levels of minerals in blood. All minerals except iron are obtained when following normal Finnish diet. (Rehunen & Fogelholm 1993, 110.)

Vitamin A, D, E and K are fat-soluble. Vitamin C and B are water-soluble. Fat-soluble vitamins are stored in the body and that is why overconsumption is unhealthy. Water-soluble vitamins' stores are small and extra vitamins are excreted away from the body with urea. Body cannot form vitamins, so they need to be obtained from food. Vitamin K and vitamin B are the only ones built also in the colon but, however, they are absorbed poorly anyway. Three vitamins have precursors, which body can change as a vitamin; vitamin A is formed from carotenoids obtained from vegetables, vitamin D is formed on the skin with the effect of ultraviolet radiation and B-vitamin, niacin, is formed from tryptophan- amino acid. The first symptoms of the lack of vitamins are

tiredness and touchiness. The lack of fat-soluble vitamins is seen before water-soluble ones. The overuse is not happening if eating normally. Only vitamin supplements can raise the amount too high. (Niemi 2006, 45-46.)

Especially about the effects of vitamin D on exercising has been studied much during the previous years. D-vitamin can be gotten from the sun and food. Recommended levels for vitamin D are 75nmol/l. (Visser et al. 2006.) Approximately 1700IU, 42,5µg, daily is needed for 95% of the population to reach 75nmol/L (Vieth et al. 2007). Despite these doses, the human body appears to be able to metabolize more than these levels and the body tends to stop solar synthesis of vitamin D at the level equivalent to 10000IU, 250 µg that is the safe upper limit (Vieth 1999). A deficiency of vitamin D is associated with an increase level of fat in skeletal muscle tissue and therefore it has been hypothesized that vitamin D insufficiency is a possible contributor to obesity. (Foss 2009; Gilsanz et al. 2010.)

There may not be any detectable vitamin D receptors (VDR) on skeletal muscle tissue, despite a series of studies suggest this. Despite the lack of vitamin D receptor expression directly on skeletal muscle cells, there appear to be impairments to physical function and reduced skeletal muscle hypertrophy associated with VDR knockout mice. (Minasyan et al. 2009; Van Leeuwen et al 2001; Wang & DeLuca 2011.) Vitamin D has been showed to be positively associated with androgen status (higher testosterone and lower sex-hormone-binding-globulin) (Wehr et al. 2010). Supplementation of vitamin D to correct a deficiency may improve athletic performance in athletes (Cannell et al. 2009). However, daily supplementation with 25 or 10 µg of vitamin D for 16 weeks did not improve muscle strength or power measured by the jump test, handgrip test or chair-rising test in the population with low baseline vitamin D status (Knutsen et al. 2014).

3 SKELETAL MUSCLE

3.1 Skeletal muscle structure and function

Skeletal muscle comprises nearly 40% of body weight and contributes 50-75% of all proteins. In the muscle tissue there are about 22% proteins and the rest is water (Westcott & La Rosa Loud 2013). Muscles consist of muscle fibers, which contain smaller functional units that are called myofibrils. Myofibrils contain even smaller units called myofilaments. Myofilaments consist mostly (85%) of two proteins myosin and actin (figure 2). There are also 12-15 other proteins that have structural function or affect protein filament interaction during muscle action. (McArdle et al. 2007, 362)

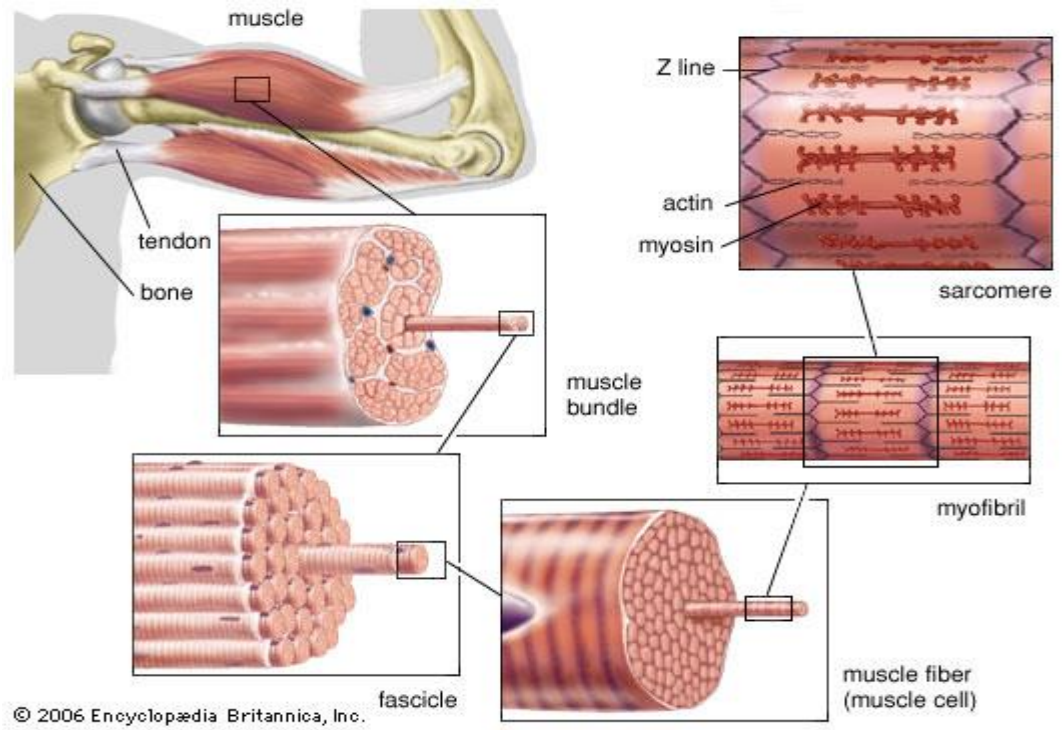

FIGURE 2. The structure of the striated muscle (Kids Britannia 2013).

There are three different types of muscles in the human body; striated muscle, smooth muscle and cardiac muscle (McArdle et al 2007, 380). Skeletal muscle tissue is very adjustable. It can change its functionality, metabolism and shape as a response to the external stimulus such as contractile activity and nutrient availability. (Hawley et al. 2011; Roy et al. 1991.)

Skeletal muscles are important as an amino acid reservoir, for energy consumption and for fuels for other tissues (brain, immune cells). Nevertheless, there are not many free amino acids inside the muscle cell, as most of the amino acids will be synthesized as proteins when they enter inside the cell (Guyton & Hall 2000, 792–795.) The number of proteins and free amino acid changes continuously, due to protein synthesis and protein break down happening all the time. 1-2% of proteins are synthesized and broken down daily. (Wagenmakers 2001.)

Muscle cells function by the ability of actin and myosin filaments to overlap and slide back with the use of ATP-energy. Calcium works as stimulator, magnesium as regulator, sodium makes the process to go faster and potassium slows down the process. (Marjanen & Soini 2007,35)

3.2 Muscle fiber types

Skeletal muscles consist of two different muscle fiber types that differ from each other by metabolism and contracting abilities. Type 1 muscle cells, named slow muscle cells, can use fat as energy source. Slow muscle cells are activated in activities long in duration. Fast muscle cells, type 2 cells, are unable to do that and they have to store extra fat inside the cell. Type 2 cells are activated in heavy loads, explosive exercises or when exercise is significant fatiguing. Fast muscle cells can still be divided sub groups depending on their fatiguing qualities - the bigger force production the faster fatigue. (McArdle et al 2007, 380-383; Spiering et al. 2008). Relative amounts of muscle cells vary a lot among individuals. Studies show that relative amounts of different fiber types are affected by genotype (45%), muscle biopsy mistakes (15%) and environment (40%). Physical activity is part of environmental factors and it explains the differences between limbs or muscle groups. Type 2 muscle fibers have a greater capacity for hypertrophy following RE training than type 1 fibers. (Karjalainen et al. 2006; Simoneau & Bouchard 1995; Spiering et al. 2008.)

Muscle glycogen is shown to be depleted by 30-40% after resistance exercise, especially in type 2 muscle fibers (Roy & Tarnopolsky 1998; Tesch et al. 1998). Specific type 2 muscle fiber glycogen depletion may limit performance during high volume workouts. Glycogen resynthesis is slow during the exercise, whereas

carbohydrate supplementation during exercise can attenuate the rate of muscle glycogen depletion. Carbohydrate, provided after exercise, can speed the rate of glycogen resynthesis after exercise, which may enhance performance. (Volek 2004.)

Previous studies show that nutrition, especially protein and glucose, increases muscle fiber area. Cribb and Hayes (2006) reported greater increases in type 2 muscle fiber area after 10 weeks of RT when subjects consumed a protein and glucose supplement immediately before and after exercise. Another study says that type 2 muscle fiber area of the vastus lateralis muscle increased with RT; however the increase tended to be greater when consuming mixed-food instead of specified diet. Type 1 fiber area stayed unchanged with RT in both all groups. (Campbell et al. 1999.)

4 PHYSIOLOGICAL CHANGES IN MUSCLE

4.1 Protein synthesis and muscle size

Protein synthesis is a biological process, in which a cell forms proteins from amino acids. Progressive heavy RT provides the stimulus for muscle size development. Training causes tissue microtraumas, which require sufficient protein to sustain the muscle-building processes. The process leads to increased strength and size. This growth of the muscle cell is called hypertrophy. (Hulmi et al. 2007; Häkkinen et al. 2001; Westcott & La Rosa Loud 2013.)

Feeding has been shown to be a simple and effective method to alter rates of protein synthesis (Svanberg et al. 2000). Some amino acids also regulate protein breakdown, however their effect is less important in magnitude than the effect of amino acids controlling protein synthesis. (Bohe et al. 2003; Kadowaki & Kanawaza 2003).

4.1.1 Net protein balance

Adding to forming of proteins in protein synthesis, proteins are also constantly being broken down by external cues. These processes include feeding and physical activity. Net protein balance (NPB) is defined as muscle protein synthesis (MPS) minus muscle protein breakdown (MPB). When net protein balance remains positive increased skeletal muscle mass accretion can happen (Hulmi et al. 2010). Measurements of the rates of protein synthesis and breakdown during acute experiments have established the following knowledge:

1) At rest the rate of protein synthesis is slower, than the rate of protein breakdown, which results in a net catabolic state, in other words the NPB is negative. (Campbell et al. 2009; Drummond et al. 2009).

2) Protein ingestion improves protein synthesis to the positive NPB (Borsheim et al. 2002; Drummond et al. 2009; Miller et al. 2003).

3) After an acute session of resistance exercise body accelerates the building of new muscle tissue proteins, though it also increases the rate of protein degradation. NPB is improved, however if proteins are not ingested, NPB does not improve to the point of becoming positive. Muscle cells namely take the building blocks they need from the muscle protein break down earlier. Exercising without proper food does not give positive results. (Ilander & Mursu 2008, 384; Phillips 2004; Phillips et al. 2005.)

4) When eating only carbohydrate after RT, NPB may slightly improve, however the improvement is minor and delayed compared with the ingestion of amino acids. (Bird 2006; Borsheim et al. 2004.)

5) The combination of feeding proteins or mixtures of amino acids and resistance exercise result in the greatest net anabolic state, in other words positive NPB exists (figure 3) (Phillips et. 2005). Protein, whey and milk proteins in particular, stimulate the greatest rise in muscle protein synthesis, result in greater muscle cross-sectional area and enhance exercise recovery. (Hulmi et al. 2010).

The events 1) and 2) typically balance each other over time and skeletal muscle and fat-free mass are unchanged. Muscle hypertrophy theoretically is achieved from the accumulated periods of positive protein balance by feeding and exercise. (Burd et al. 2009; Phillips 2004; Phillips et al. 2009.)

Improved cellular signaling might also be one possible explanation for greater skeletal muscle hypertrophy in persons with higher dietary and serum cholesterol. Cholesterol may also play a role as an essential building block to repair microtears that occur in the skeletal muscle membrane with RT. This may not be the major mechanism of cholesterol-induced muscle hypertrophy. Immediate reductions in serum cholesterol following exercise causing muscle injury have been suggested to be the part of the process to repair the membrane damage. (Riechman et al. 2007; Shahbazpour et al. 2004.)

Summarized, proteins affect directly being used for protein synthesis. Carbohydrates affect indirectly being used as a fuel for the muscle to make exercising as heavy as

possible. Carbohydrates also decrease the breakdown of the muscle proteins. Also fat works indirectly through the changes in hormone stages of the body. (Ilander & Mursu 2008, 379.)

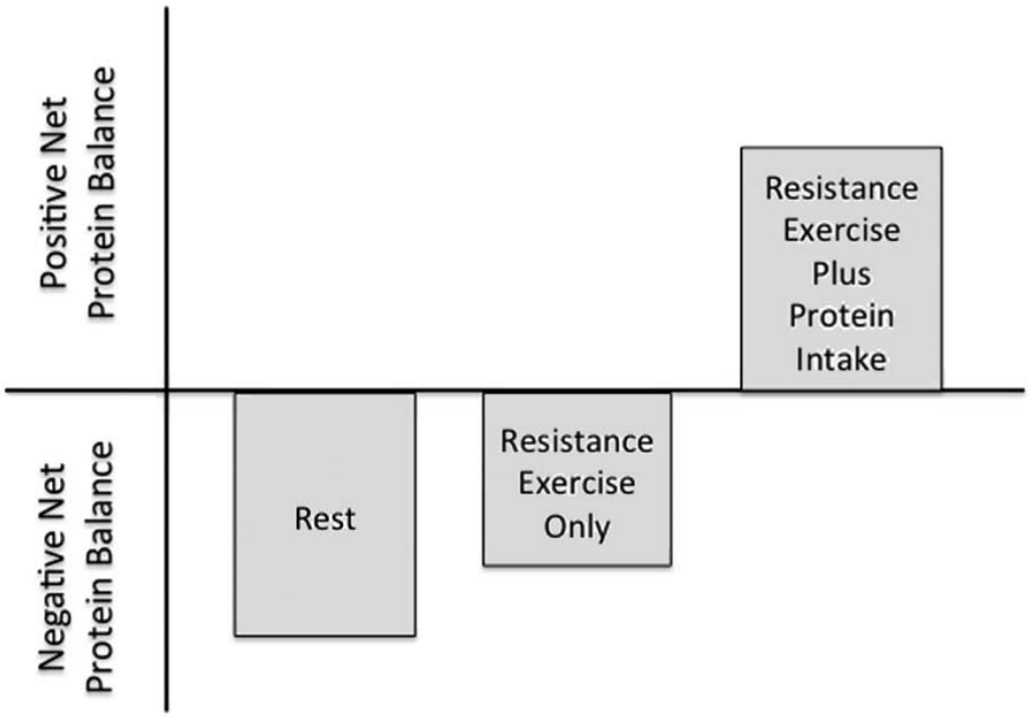

FIGURE 3. Net protein balance (Campbell et al. 2012, 6).

Findings regarding the impact of dietary intake on long-term RT- mediated skeletal muscle growth are equivocal. Some studies suggest that higher daily intakes of dietary protein and EAAs enhance the magnitude of skeletal muscle hypertrophy during RT. (Campbell and Leidy 2007.) Studies have reported that when amino acid availability increases, improves the activity of anabolic reactions of skeletal muscles too. (Biolo et al.1997). Carbohydrate (1g glucose/body mass kg) taken immediately and 1 hour after a session of resistance exercise resulted in higher plasma glucose and insulin, decreased myofibrillar protein breakdown and urea nitrogen excretion, and slightly increased fractional muscle protein synthetic rate (Roy et al. 1997). Rasmussen et al. (2000) reported that consumption of both protein and carbohydrate results in even greater effects on protein balance. Protein synthesis was stimulated 400% above pre-exercise values when a protein and carbohydrate supplement (6 g EAAs and 35 g sucrose) was

consumed 1 or 3 h after a session of resistance exercise. (Rasmussen et al. 2000.) Panneman's et al data showed a lower net protein synthesis for the diet high in vegetable protein than for an equivalent amount of protein provided in a diet high in animal protein (Campbell 1999). By contrast to the studies supporting proteins, others report that muscle mass during long-term RT is not enhanced by higher quantities and source of dietary protein. Thalacker-Mercer et al (2009) reported that intrinsic or extrinsic factors other than nutrient ingestion apparently impaired the anabolic response in non-responders. There were no associations between the magnitude of myofiber hypertrophy and any dietary intake. (Thalacker-Mercer et al 2009.)

4.1.2 Nitrogen

Protein molecules contain about 16% nitrogen. During catabolism, protein first degrades into its component amino acid. The amino acid molecule then loses its nitrogen in the liver to form urea. The remaining amino acid is either converted to a new amino acid, converted to carbohydrate or fat, or catabolized directly for energy. Urea leaves the body in solution as urine. Excessive protein catabolism promotes fluid loss because urea must be dissolved in water for excretion. (McArdle 2001, 32, 36.)

Nitrogen balance exists when nitrogen intake (protein) equals nitrogen excretion. The opinions about the amount of protein required for the most positive nitrogen balance are between this previously mentioned 0.8g/kg/day and 2.8g/kg/day. (Lemon 2000). Campbell et al 2002 evaluated influence of RT on nitrogen balance and amino acid utilization. During the first six weeks of intervention, urinary nitrogen excretion decreased and nitrogen balance increased both among trained and non-trained men, when they consumed diets that contained the recommend daily amount for protein and sufficient energy. Campbell et al. (1995) demonstrated that the subjects of high-protein group had greater nitrogen balance, rates of leucine turnover and uptake for protein synthesis. The subjects of low-protein group had greater efficiencies of nitrogen retention and utilization for protein synthesis. These findings support metabolic adaptation to the constant protein intake and the achievement of increased efficiency of nitrogen retention and amino acid utilization. The aim is to achieve and maintain physiological homeostasis. (Campbell & Leidy 2007.) According to that aim resistance exercise does not increase the need of dietary protein in fact, it improves utilization of

protein, which may actually lower the protein requirement during training (Thalacker-Mercer 2009).

The positive nitrogen stage of the body is useful when trying to gain more muscles. Positive nitrogen stage is not necessary; however when combining the large consumption of protein with RT it is possible to gain and maintain positive energy stage also when dietary energy consumption is small. (Demling & DeSanti 2000.)

4.2 Muscle strength

Muscle strength is determined by the ability of the nervous system to recruit motor units in concert with the number of muscle contractile units in cross-section (Shirreffs 2005). Muscle mass is the major determinant of physical strength, thus the loss of lean mass is thought to be a major contributor to functional decline and disability (Janssen et al. 2004a; Janssen et al. 2004b). A large proportion of maximal strength decline is due to a reduction in the size and number of fast twitch, type 2, muscle fibers (Nilwik et al. 2013).

Dietary and serum lipids and lipid-lowering drugs (statins) are associated with many skeletal muscle pathologies including muscle weakness. Therefore it is hypothesized that lower dietary cholesterol intake, serum cholesterol and statin use would be associated with reduced skeletal muscle responses. Riechmans et al. (2007) showed in their study that when protein consumption was standardized, there was a strong direct association of average dietary cholesterol consumption ($r= 0.448$, $p= 0.001$) to the magnitude of lean mass gains. The highest mean dietary cholesterol was also associated with greater strength gains and appendicular muscle hypertrophy as compared to the lowest dietary cholesterol. Statin users also had greater lean mass gains, independent of dietary and serum cholesterol. The study of Riechmans et al. (2007) suggests that the effect of blood cholesterol is greater in men than in women. The direct association between dietary cholesterol and changes in strength supports the potential anabolic role of cholesterol. (Riechman 2007.) Protein supplementation is superior to carbohydrate supplementation alone in terms of muscular strength (Cermak et al. 2012).

4.3 Body composition

Correlation analysis support that the changes in protein metabolism, especially in skeletal muscle are related to changes over time in body composition (Campbell & Leidy 2007). Reduced muscle mass is largely responsible for reduced resting metabolism, which is typically accompanied by increased fat accumulation (Phillips 2007; Wolfe 2006).

Protein or EAA supplementation in combination with RT has been shown to significantly increase fat-free body mass, myofiber cross-sectional area and whole muscle cross-sectional area greater than a non-energetic or carbohydrate placebo. (Bird et al. 2006; Cribb et. al 2007; Hartman et al. 2007; Josse et al. 2010.) In addition to that thermogenesis and satiety increase (Halton & Hu 2004). Riechman et al. (2007) showed that cholesterol intake had an effect on the change in lean mass over 12 weeks of RT (Riechmal et al. 2007).

5 PHYSIOLOGICAL RESPONSES IN BLOOD

Exercise results in increased blood flow to the active skeletal muscles, which has the potential to enhance hormone interactions. Muscular contraction together with increased availability of hormones promotes an anabolic environment. Resistance exercise-induced hormones such as insulin, growth hormone, insulin-like growth factor-1, testosterone, sex-hormone binding globulin and cortisol have major regulatory roles in protein, carbohydrate and lipid metabolism (Volek 2004). Nutrition has also effects on hormones (table 1) (Ilander & Mursu 2008, 380).

The amount of muscle mass recruited directly affects the metabolic and hormonal responses to RE. RT that utilizes large muscle masses, moderate loads (10RM), short rest periods (1 min) and high total work, maximize the hormonal response to an exercise. Hansen et al. (2001) demonstrated the importance of exercise-induced hormonal responses by examining two groups of subjects who performed identical upper body RE programs for 9 weeks. One group performed additional lower-body RE to stimulate large increases in circulating hormones. Subjects training the upper and lower body and thus experiencing greater circulating hormonal concentrations increased arm strength by 37% compared to 9% of the other group. These results indicate that RE-induced hormonal responses potentiate strength gains following long-term training. Gains in strength and hypertrophy can occur with little to no change in circulating hormones; indicating that hormonal responses potentiate, but are not responsible for adaptations to RT. (Hansen et al. 2001.)

TABLE 1. Effects of nutrients on hormones. Edited from the table of Ilander and Mursu 2008. "+" with nutrient means huge consumption of that nutrient, "-" with nutrient means a small consumption of that nutrient. Below GH, insulin and testosterone "+" means, that the amount of hormone increases when consuming that nutrient," –" means that the amount of hormone decreases, empty box means that there is not effect known. (Ilander & Mursu 2008, 380.)

Nutrient	Growth hormone	Insulin	Testosterone
Fat +	+		+
Fat -	-		-
CHO +	-	+	
Animal protein		+	+
Plant protein		+	-
Protein +		+	-

The most important anabolic hormones to stimulate protein synthesis have been suggested to be testosterone, insulin and growth hormone (Ilander & Mursu 2008, 379). The changes in hormonal levels are associated with potential for muscle size and strength. It is reported that individuals, who exhibit large growth hormone (GH), testosterone and insulin like growth factor-1 (IGF-1) responses after resistance exercise would have greater training-induced adaptations. (Beaven et al. 2008a; Beaven et al. 2008b; Hansen et al. 2001; Migiano et al. 2009; Ronnestad et al. 2011.) High levels of hormones increase the interaction with receptors, which have hypertrophic importance in the post-workout period when muscles are primed for anabolism. Hormonal spikes enhance intracellular signaling so that post-exercise protein breakdown is attenuated and anabolic processes are heightened leading to a greater super compensatory response. (Schoenfeld 2013). This simplistic theory has been, however, criticized (West et al. 2010; West & Phillips 2012).

5.1 Insulin

Insulin plays a key role in regulating liver and muscle glycogen stores by controlling circulating blood sugar levels. Elevated blood sugar causes secretion of additional insulin, which facilitates cellular glucose uptake and glycogen formation and inhibits

further insulin secretion. (McArdle et al. 2007, 13.) Different glycemic indexes of the food result differentially elevated insulin levels. (Van Loon et al. 2000a; Van Loon 2000b.) Insulin also activates protein synthesis by activating components. In long term, insulin also increases the cellular content of ribosomes to augment the capacity of protein synthesis. Because of the big size of amino acids the delivery inside the muscle cells needs to be done by active mechanisms. (Ilander & Mursu 2008, 380; Proud 2006.)

The ability of insulin to suppress protein breakdown in human skeletal muscle is thought to be greater after resistance exercise than at rest (Biolo et al. 1999). Current knowledge proposes that exercising is not the reason but the explanation might be amino acid differences. Positive effect of insulin on protein synthesis existed, when amino acid delivery to the muscles increased. No change or a decrease in protein synthesis existed, when amino acid concentration decreased and amino acid delivery stayed same or decreased. Although insulin can directly stimulate initiation of translation, its stimulatory effect on human skeletal muscle protein synthesis is modulated by increases in muscle perfusion and amino acid delivery and availability for the muscle tissue. When muscle perfusion increases as a consequence of hyperinsulinemia, more tissue is exposed to the nutrients contained in the blood. (Fujita et al. 2006.)

Because amino acids increase insulin, there has been some interest in combining protein with carbohydrate to maximize insulin secretion. Enhanced insulin levels resulting from carbohydrate combined with protein have a favorable effect on net protein balance because insulin has only a modest effect on protein synthesis in the absence of amino acids. (Drummond et al. 2009; Kimball et al. 2002; Williams et al. 2002.) Some studies say that carbohydrate co-ingestion does not affect muscle protein synthesis rate during recovery from resistance-type exercise under conditions where ample protein is being ingested, so the amount of protein shows to be the main factor in insulin secretion, like mentioned earlier. (Koopman 2007).

5.2 Testosterone

Testosterone is a steroid hormone synthesized from cholesterol (Buresh et al. 2009). It is an androgen hormone that has anabolic effects on muscle tissue (Volek 2004).

Testosterone is known to have potent effects on contractile tissue accretion when administered pharmalogically (Crewther et al. 2011; Hayes et al. 2010). Very low-fat diets reduces testosterone levels. Very high consumption of dietary protein decreases testosterone levels. Considering that fact, protein overconsumption is not needed. (Ilander & Mursu 2008, 379; Sallinen ym. 2004.) Testosterone may also contribute indirectly to muscle protein accretion by potentiating the release of other anabolic factors such as GH and IGF-1 (Veldhuis, et al. 2005; Sculthorpe et al. 2012).

5.3 Sex hormone binding globulin

Sex hormone binding globulin (SHBG) is a protein transferring other proteins for sex steroids, regulating circulating concentrations of unbound hormones and their transport to target tissues. SHBG is primarily synthesized in the liver. (Avvakumov et al. 2010.) SHBG is influenced by metabolic and hormonal factors. Genetic differences also contribute to inter-individual variations in plasma SHBG levels. Body mass and the relative amount of adipose tissue versus lean muscle in particular, is one of the most important determinants of plasma SHBG levels. (Pugeat et al. 2010; Stone et al. 2009.)

5.4 Growth hormone

Growth hormone (GH) is secreted by the anterior pituitary gland and released in pulsatile fashion mostly during sleep. The GH mediates both anabolic and catabolic processes. (Velloso 2008.) GH induces mobilization of triglycerides and incorporates amino acids into various proteins, including those in skeletal muscle (Vierck et al. 2000). GH increases muscle and skeletal growth, protein synthesis, lipolysis and glucose conservation (Nakagawa et al. 2002; Van Loon et al. 2003). In the study of West and Phillips' (2012) it was shown GH to correlate positively both with change in type 1 and type 2 fibers CSA ($r= 0.36$, $p<0.01$; $r= 0.28$, $p<0,05$). Growth hormone also has a function to increase the use of fat as energy and to have a positive impact to body composition. (Ilander & Mursu 2008, 379.)

There is a linear relationship between the magnitude of the acute increase in GH release and exercise intensity. Because the GH response to acute resistance exercise is dependent on the work-rest interval and the load and the frequency of the resistance

exercise used, the ability to equate intensity across different exercise protocols is desirable. A high volume training regimen typically used by bodybuilders to promote maximal muscle hypertrophy, resulted in a greater GH response compared to a high intensity training regimen typically used by competitive weight lifters to promote maximal muscle strength or power. (Williams et al. 2002.)

Each nutrient has independent effect on regulation of GH secretion; Glucose or carbohydrate-rich meals decrease GH levels that may be followed by a rebound hypoglycemia-induced rise in GH (Nakagawa et al. 2002; Van Loon et al. 2003.) Amino acids can increase GH levels; however physical training and high-protein diets reduce the effect (Chromiak & Antonio 2002). The amount of fat in energy increases the amount of GH, when the lack of fat decreases it. (Ilander & Mursu 2008, 379; Volek et al. 2001).

5.5 Cortisol

Cortisol is an adrenal steroid hormone that is regulated by pituitary adrenocorticotropin (ACTH). ACTH is under the influence of hypothalamic corticotropin-releasing hormone (CRH). Cortisol increases hepatic lipolysis and proteolysis to fuel hepatic glucogeonesis. This protects blood glucose and glycogen levels (Bloomer et al. 2000; Koch et al. 2001; Williams et al. 2002).

Cortisol is frequently elevated after resistance exercise protocols designed to elicit hypertrophy and is considered to be catabolic and counteractive to hypertrophy (Kraemer and Ratamess 2005; Spiering et al. 2008; Tarpenning et al. 2001). However, this relation is probably not that simple, because in the study of West and Phillips (2012) cortisol was positively correlated with change in whole-body LBM ($r = 0.29$, $P<0.05$). It also correlated with increases in type 2 fiber area positively ($r=0.35$, $p<0.01$). (West & Phillips 2012.)

Most of the studies show that carbohydrate or carbohydrate-protein before or after exercise does not alter the cortisol response. (Bloomer et al. 2000; Koch et al. 2001; Williams et al. 2002.) In contrast some studies demonstrate cortisol to be sensitive to feeding. Tarpenning et al. (2001) showed that carbohydrate intake during an acute

session of resistance exercise significantly decreased cortisol response. The reduction in post-resistance exercise cortisol was significantly related to increases in muscle fiber hypertrophy (Tarpenning, et al. 2001; Volek 2004.) Independent of external thermal stress, hypohydration potently amplifies the exercise-induced responses of cortisol (Judelson et al. 2007; Maresh et al. 2006).

6 PURPOSE, PROBLEMS AND HYPOTHESES

The gym program was the same for all subjects, nutritional habits were not controlled. The aim of the study was to find an explanatory factor from nutritional habits for individual differences in muscle strength gain, muscle fiber size changes, body composition changes and in acute hormonal changes following a heavy resistance exercise.

The research problems and the hypotheses of the present study were:

1. Do nutritional habits cause differences in the subjects' chronic adaptations in muscle?

   Hypothesis: The increased availability of nutrients has the potential to enhance the adaptations to RT (Mc Ardle 2007, 541-543; Phillips et al. 2009). Carbohydrate consumption is important to maximize exercise volume and results (Volek 2004). Especially type 2 muscle fiber has in some studies correlated with maximal power, grows greater when consuming carbohydrates and proteins (Cribb & Hayes 2006).

2. Do nutritional habits have impact on subjects' hormonal levels?

   Hypothesis: The nutrition effects on the magnitude of acute hormonal responses (Bird 2010; Hansen et al. 2001). Different glycemic indexes of the food result differentially elevated insulin levels (Van Loon et al. 2000a). Feeding, particularly fat, decreases circulating testosterone; however it increases growth hormone (Volek et al. 2001).

3. Does RT period change nutritional habits?

   Hypothesis: RT increases the consumption of proteins and carbohydrates (Niemi 2006, 33, 35).

4. Does nutrition make differences between anthropology results of subjects?

Hypothesis: Proteins have been shown to increase fat-free body mass greater than carbohydrates (Josse et al. 2010). Cholesterol intake has the effect on the change in lean mass (Riechmal et al. 2007).

7 METHODS

7.1 Subjects

The study involved 21 young male subjects. The age of the subjects varied between 19 and 30 years. The voluntary subjects were recruited for the study by advertising in newspapers and through email lists. They needed to be able to commit to the study for six months that included 21 weeks of supervised RT (gym training with weights) with two practices per week and measurements in the beginning, in the middle and in the end of the study. The subjects were informed about the design of the study, risks and discomfort. They also had the right to discontinue the study at any time. After this information they signed written informed consent form to participate in the study, which had been approved by the ethics committee of the University of Jyväskylä. The study was conducted according to the Declaration of Helsinki.

All the subjects went through a medical examination and none of them had any obstacles to perform heavy RT. They were moderately active; however none of them had previous regular experience about RT. They all had normal body shape. The limit for the subjects' height was 165-185 cm, for the weight 60-90 kg and body mass index (BMI) had to be less than 30. None of the subjects had regular need for nutritional supplements or pharmacological substances that might affect the measured variables. None of them were vegetarian what would have been an obstacle for participation. Physical characteristics of the subjects' are presented in the table (table 2).

TABLE 2. Physical characteristics of the subjects.

N	Age (yr.)	Height (cm)	Body mass (kg)	LBM(kg)	Fat (%)
21.0	26±4	182±5.9	75.5±7.8	62.6±5.5	16.9±3.9

Subjects were randomly assigned to three groups; whey protein, placebo and control groups. The mean age, the body weight or the 1RM-result of the leg press did not vary among the groups. Whey protein and placebo groups practiced the same amount and both had pre- and post-training drink to consume. They did not know whether their drinks contained protein or not. The amount of protein content was so small that it did

not matter in this report. In this report only whey protein and placebo groups are examined and they are examined as one group (n=21).

7.2 Experimental design

The study consisted of four measurements; the start measurement, the second start measurement, the middle measurement and the end measurement (figure 4). The aim of the first start measurement was to be a practicing possibility for the subjects - When accomplishing the official start measurement and the two later measurements, learning would not be affecting to the results anymore. All the measurements were carried out at the same time of the day to exclude the effects of daily variations (Vissing et al. 2005; Sedliak et al. 2007).

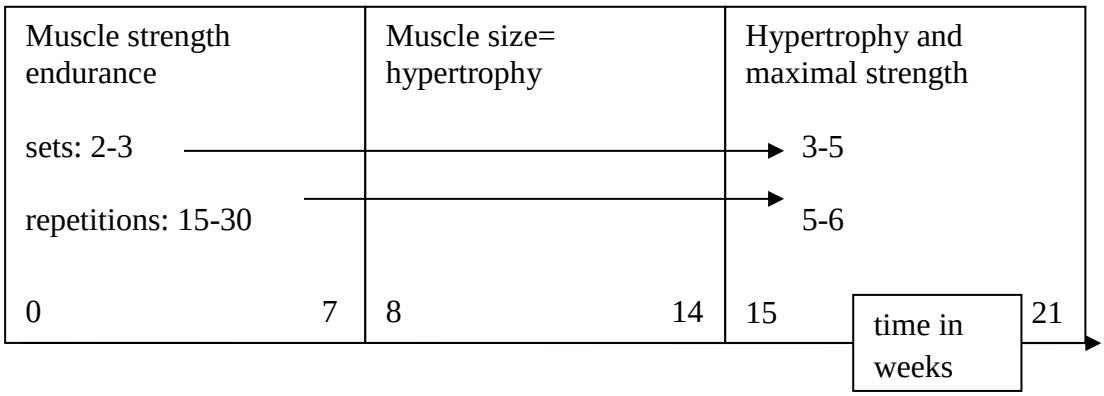

FIGURE 4. Gym program protocol.

The subjects trained with supervision in the gym two times a week for 21 weeks. The instructor controlled techniques and motivated for the best possible result. RT was done progressively starting with 40% and ending to 85% from the maximum loads, that were determined individually from the measurements for every exercise throughout the study. The aim of the first seven weeks was to make RT familiar and improve muscle strength endurance. Next seven weeks concentrated on increasing muscle size. Adding to muscular hypertrophy last seven weeks was used to optimize gains in maximal strength as well. The amount of sets increased from 2-3 sets to 3-5 sets. 15-30 repetitions in the beginning decreased to 5-6 repetitions in the end. There was 2-3 minutes break between the sets. Between the gym days minimum two days break was required. In every practice thigh muscles were activated by using knee extensors with two different exercises, bilateral leg press and bilateral knee extension and one exercise, bilateral

knee flexion, for the knee flexors. The training program included also exercises for the other big muscle groups of the body (chest and shoulders, upper back, upper arms, trunk extensors and flexors, hip abductors and adductors and ankle extensors), but the main focus of the practice was in knee extensors. They were chosen to be the focus due to its shape and function has been studied most and the results of this study can be compared with the previous studies. In each workout knee extensors were the first or the second exercise on the program. The first exercise was alternated between bench press movement of the other large muscle group, chest and shoulders.

The measurements were done in Viveca in Jyväskylä, in the laboratory of the Department of Biology of Physical Activity and in the magnet center of middle Finland in Jyväskylä. The exercising was done in the gym in Jyväskylä.

7.3 Measurements

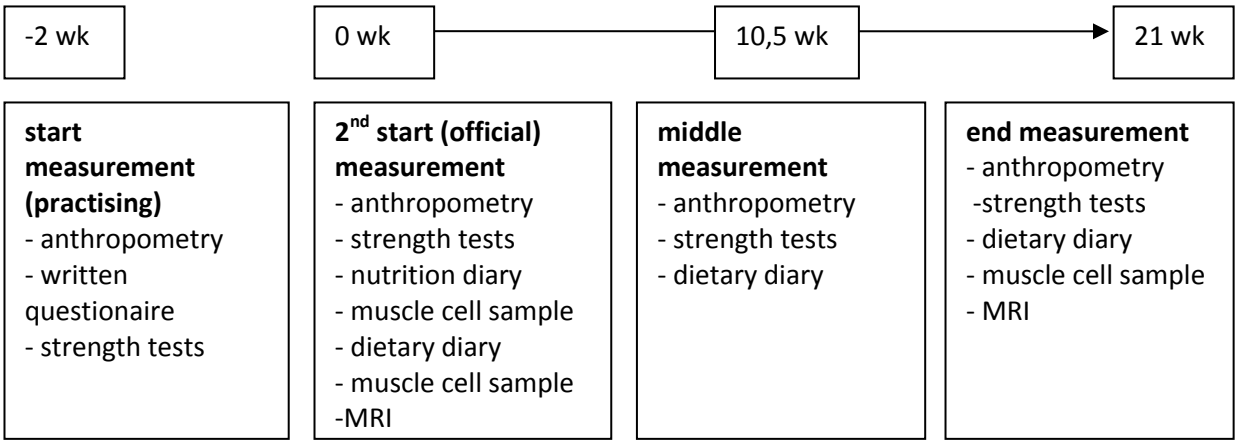

FIGURE 5. The measurement protocol.

The first start measurement included only strength tests, anthropometry, blood sample and a written questionnaire. In the second start measurement (official), in the middle measurement and in the end measurement also nutrition dairy was used. Muscle cell sample and magnetig resonance imaging (MRI) were taken in the second start measurement and in the end measurement (figure 5). 2-3 hours before measurements coffee and smoking were not allowed. Alcohol consumption in the measurement day and previous days were forbidden. Adding to these measurements subjects took part in acute heavy resistance exercise. The aim was to determine acute blood variables

(appendix 1).

7.3.1 Morning measurement

Morning measurement is a collective term for several measurements done during the same measurement appointment. It always included anthropometry measurements and in the first morning measurement also written questionnaire belonged to that protocol. Morning measurement was done without eating or smoking. The day before measurements had to be without physical stress. If the power test was made before morning measurement, minimum two days of rest had to follow the power test.

7.3.2 Anthropometric measurements

Anthropometry measurements were done after overnight fasting. The height was measured with 0.1 cm accuracy by the measuring tape placed to the wall. The weight was measured with 0.1 kg accuracy by the calibrated scale. The percentage of fat was determined from biceps, triceps brachii, subscapular and iliac crest skinfolds with Durnin & Womersley's (1974) formula. Body fat percentage and amount of total body muscle mass (kg) was measured by bioelectrical impedance using an Inbody720 machine (Seoul, Korea). The subjects were advised to come to test in normal hydration status. Too less or much drinking, sauna and alcohol must have been avoided.

7.3.3 Strength tests

Before the test subjects warmed up for 5 minutes with a bicycle ergometer. The strength tests consisted of two lower limb and one upper limb movements. The first lower limb movement was maximal isometric leg press with 107º knee ankle in David 200-eguipment (David Fitness and Medical, Finland). The aim was to produce as fast as possible as much power as possible. The time given to produce power was three seconds. Three trials were given. The second lower limb movement was bilateral dynamic leg press also with David 200-equipment with the knee angle less than 70º. The maximum power was measured from 3-5 attempts. After each attempt, the load was increased until the subject was unable to extend his legs to full-extended 180º knee angle position. The highest successful load was determined as 1 RM. The only upper

body movement in the test was maximal bench press by sitting in David 210-equipment (David Fitness and Medical, Finland). Elbows were in 90 º angle compared to upper arms. The aim again was to produce as much power as possible quickly.

There was always a few minutes break between the trials of the same movement. Between the different movements the break length was the time needed for explanation of the next technique. Every subject was cheered in every single trial to motivate in order to make the best possible result. The best result from the trials was used in the statistical analysis. The forces produced in isometric measurements were converted to digital format on the computer. Signal 2.15 software (Cambridge Electronic Design Ltd., Cambridge, UK) with a sampling frequency of 2000Hz was used to analyze maximal power. Before the power test two exercise-free days was required. In the first power test a small tattoo mark was drawn to subject's leg in order to make the coming ultratonic, MRI and biopsy measurements always from the same area.

7.3.4 Dietary diaries

In order to observe the eating habits subjects filled dietary diaries in the beginning, in the middle (after 10.5 weeks) and in the end of the training period. The diaries were registered on four to five consecutive days. One of these days had to be Saturday or Sunday. It was important to write down everything very meticulously; the amount of the food, the place where it was consumed, the time and the brand. If the food was something rare, also content of nutrients should have been mentioned. The subjects were given an example about registering. During this diary writing period eating habits had to be normal.

7.3.5 Muscle biopsy

Muscle biopsies were taken before (pre) and after (post) 21 week training period. The biopsies were taken from the vastus lateralis muscle midway between the patella and greater trochanter about 2.5 cm depth. The sample taker was an experienced doctor. In the first measurement ultrasonic testing was made for the sample taking place to be sure the needle would not touch blood veins or nerves. Circumstances were sterilized. The sample taking area was anaesthetized with Lidocain- local subcutaneous anaesthetic and

cleaned by amisept-disinfectant. The doctor made 1 cm cut to the skin with a surgeon knife and took the muscle sample using Bergström's 5 mm biopsy needle technique. (Bergström and Hultman 1966.) The sample inside the needle was checked by size and quality and then it was set to a cork. The muscle sample was cleaned of any visible connective or adipose tissue and blood. The piece of muscle taken for muscle fiber size analyze was frozen in isopentane cooled to (-160º) in liquid nitrogen and thereafter stored in the freezer (-80º).

7.3.6 Muscle cross-sectional area

The muscle cross-sectional area (CSA) of the quadriceps femoris muscle was determined before and after 21 week training period using MRI (GE Signa Exite HD 1.5 T). During the measurement the legs of the subject were kept parallel and strapped with a belt and a special cast designed to standardize the measurement as well as possible. Four axial-plane MRI scans were taken; the first image was 4 cm above the midway between the patella and greater trochanter. The next three scans were taken at 2, 4, and 6 cm towards the patella. The MRI- images were analyzed with OsiriX (version 2.7.5) software.

7.3.7 Blood sample

Blood samples were drawn from the antecubital vein using disposable needles. Blood was obtained during acute heavy resistance exercise. The samples analyzed in this study were obtained before the muscle work, right after exercise, 15 minutes and 30 minutes after exercise. Blood was centrifuged for 10 minutes in 4 ºC 3500RPM and then stored in -80ºC for the later hormone analysis.

7.4 Analyzes

7.4.1 Dietary diary analyze

The diaries were analyzed using the Micro Nutrica nutrient-analysis software version 3.11 (The Social Insurance Institution of Finland). The energy intake was mentioned with total energy (1000kJ), total energy per weight (kJ/kg) and protein, carbohydrate

and fat intake related to the weight (g/kg). In the beginning of the study there were more data decreasing towards the end of the study. Only eight subjects completed the diary until the end. That is why mean value of one, two or three diaries - the beginning, midway and end - are used. In these cases when comparing the changes in eating to the development of some other factor, only these eight subjects are analyzed.

7.4.2 Muscle cell sample analyze

Muscle fiber types. Muscle cell samples were cut by cryostate –(20ºC) to 10 μm thick sections cross-sectionally. Four different muscle fiber types (I, IIa, IIb ja IIc) were determined by histochemical ATPase colouring (Brooke & Kaiser 1970). They were separated from each other using four different preincubate-solution; pH 4.2, pH 4.6, pH 9.4 ja pH 10.3. Because there were only a small amount of each subtypes, all fast muscle fiber types, IIa, IIb ja IIc were counted together when doing statistical analyzes.

Muscle fiber sizes. The same 10 μm thick pieces as in the muscle cell type determination were used in the muscle fiber size determination. The borders of the muscle cells were determined by anti-dystrophin antibody. Pictures from the muscle cells with borders were saved and analyzed by Tema Image-Analysis System – equipment (Scan Beam). An Olympys BX-50F light microscope (Olympus Optical, Tokyo, Japan) with Olympus colour CCD camera (Colorview III, Olympus Optical, Tokyo, Japan) and Analysis® Software (Version 5.0, Soft Imaging System GmbH, Munster, Germany) were used for imaging and analyzing. The change of average sizes of muscle fiber types during 21 weeks, were compared.

7.4.3 Blood sample

Serum insulin, testosterone and SHBG were possible to be analyzed with an immunometric chemiluminescence method with an Immulite® 1000 (DPC, Los Angeles, USA). Serum insulin was also analyzed by the ADVIA Centaur insulin assay (Bayer, LTD, USA). Serum testosterone could be analyzed also with ELISA (IBL, Hamburg, Germany). All samples of each subject were assayed in the same assay run.

7.4.4 Statistics

The data was analyzed using IBM SPSS Statistics 19 software. All data was checked to be normally distributed. In every category there is shown a mean value and standard deviation for all subjects. Pearson's correlation was used to find dependencies between the variables. Student's t-test and repeated measures ANOVA were used to determine if the results of the measurements done in the different phases of the study significantly differed from each other. Regression analysis was used to estimate the relationships among variables.

In all statistical tests differences were significant when $p \leq 0.05$. In this study "*" means significant ($p=\leq 0.05$), "**" means highly significant ($p=\leq 0.01$).

8 RESULTS

8.1 Nutritional status

Average energy intake among subjects was about 10000-11000kJ a day. That means 140-150kJ per kg of body mass. Protein levels were 1.4-1.6 g/kg, carbohydrate levels were 3.7-4.0 g/kg, fat levels were 1.3-1.4g/kg (table 3). There were no statistical differences ($p<0.05$) in total energy, energy per weight, carbohydrates and fat consumption between the week 0, 10.5 and 21. The consumption of protein increased significantly ($p=0.047$) from the beginning to the end of the study, however the difference between weeks 0 and 10.5 or 10.5 and 21 did not exist (table 4). High correlation between weeks 0, 10.5 and 21 in the protein amounts of the subjects shows that the increase in protein levels occurred in every subject's habits (table 5).

TABLE 3. Mean (±SD) nutritional intake in the beginning, in the middle and in the end of the study.

Variable	**0 wk**	**10,5 wk**	**21 wk**
Energy (1000 kJ)	10.01±1.9	10.82±2.9	11.3±2.8
Energy (kJ/kg)	139.58±20.0	145.12±35.2	153.34±31.6
Protein (g/kg)	1.43±0.3	1.6±0.5	1.59±0.4
CHO (g/kg)	3.74±0.7	3.98±0.8	4.03±1.1
Fat (g/kg)	1.36±0.2	1.28±0.4	1.39±0.4

TABLE 4. P-value of variables between weeks 0, 10.5 and 21. E= total energy, Prot=proteins and CHO=carbohydrates.

	E (1000 kJ)	**E (kJ/kg)**	**Prot (g/kg)**	**CHO (g/kg)**	**Fat (g/kg)**
0 vs. 10.5	0.32	0.57	0.21	0.21	0.62
0 vs. 21	0.13	0.23	0.05 *	0.52	0.82
10.5 vs. 21	0.64	0.53	0.93	0.92	0.4

TABLE 5. Relationships between protein consumptions in the weeks 0, 10.5 and 21.

	Prot (g/kg)	**p-value**
0 vs. 10.5	0.82	0.014*
0 vs. 21	0.92	0.001*
10.5 vs. 21	0.91	0.002*

The percentage amounts of protein, CHO and fat stayed about the same (table 6). The sum of these three variables may not come up to 100% due to alcohol being counted in even if it is not shown in the table.

TABLE 6. Percentage of energy sources in different phases of the study.

Variable	**0 wk (%)**	**10.5 wk (%)**	**21 wk (%)**
Protein (g/kg)	17.3	18.6	18.2
CHO (g/kg)	45.5	47.33	44.33
Fat (g/kg)	37.2	33.47	34.56

Subjects' eating habits mostly followed recommendations (table 7a, table 7b). The consumption of vitamin D was low compared to the newest recommendations. The amount of niacin and selenium was doubled, the amount of vitamin C a bit less than doubled, the amount of vitamin B12 was tripled and the amount of sodium was even 15 times higher than required. (Ravitsemusneuvottelukunta 2012.)

TABLE 7a. The sources, functions and a need of vitamins compared to the average consumptions in the study. Vitamins that are consumed in amounts greater than recommended, are bold. Vitamins that are consumed in amounts less than recommended are underlined. (modified from the table of Niemi 2006, 55; Ravitsemusneuvottelukunta 2012.)

Vitamin	**Source**	**Function**	**Need/day**	**Average in the study**
Vitamin A	meat, vegetables, margarin	night vision, cell growth and specialization	0.9 mg	1.27 mg
Vitamin D	fish, vegetable fat, sun	bones, teeth (kalsium absorption)	10µg	6,8 µg
Vitamin E	vegetable oils, full corn products	immune system	10 mg	11.7 mg
Thiamine (vitamin B1)	full corn, meat	neuromuscular function, carbohydrate metabolism	1.4 mg	1.5 mg
Riboflavin (vitamin B2)	milk, meat, corn	energy-metabolism	1.6 mg	2.4 mg
Niacin	corn, milk	energy metabolism, skin, mucous membranes	19 mg	**40.5 mg**
Folic acid	full corn, vegetables, fruits, berries, meat	blood cell synthesis, protein metabolism	0.30 mg	0.32 mg
Kobalamin (vitamin B12)	milk, meat, fish, kidney	red and white cell synthesis, nervous system	0.0020 mg	**0.0071 mg**
Vitamin C	fruits, berries, vegetables	immune system, collagen, minimizing muscle soreness	75 mg	**120.7 mg**

TABLE 7b. The sources, functions and a need of minerals compared to the average consumptions in the study. Minerals that are consumed more than recommended are bold. (modified from the table of Niemi 2006, 64; Ravitsemusneuvottelukunta 2012.)

Mineral	**Source**	**Function**	**Need/day**	**Average in the study**
Calsium	milk products	bone and teeth tissue, muscle contraction	800-900 mg	1398 mg
Sodium	salt (sodium chloride)	fluid balance, nerve and muscle irritability	230 mg	**3051 mg**
Potassium	corn, milk, meat	acid-base balance, neuromuscular system	3500 mg	4667 mg
Magnesium	vegetables, full corn, coffee	enzyme activator, neuromuscular system	350 mg	464 mg
Iron	blood food, vegetables	oxygen delivery	9 mg	13,8 mg
Zinc	almost in all food	energy metabolism, antioxidant	9 mg	15,3 mg
Selenium	meat, cheese, corn products	function of liver, improves immune system function, prevent inflammation diseases	0.06 mg	**0.1 mg**

8.2. Chronic reactions of resistance training

8.2.1 Anthropometry

TABLE 8. Changes in body composition during 21 weeks of RT. LBM= lean body mass.

			0 vs 21 (%)	p-value
Weight (kg)	0 wk	75.5±7.76	4.0±2.63	<0.001
	21 wk	78.6±8.65		
LBM (kg)	0 wk	62.6±5.45	3.9±2.19	<0.001
	21 wk	65.0±5.85		
Fat (%)	0 wk	16.9±3.94	1.0±6.92	0.64
	21 wk	17.0±4.00		

The body weight and the lean body mass had statistical changes during this 21 week training period. The body mass increased average 4.0 kg, that being lean body mass (average 3.9 kg). The percentage fat did not change. Compared to changes in other variables fat varied quite a lot among the subjects. About half gained more fat, when other half lost fat. The biggest gain in fat was 15.4%, biggest lost was -8.9%. For example the weight gain did not vary so much. Only one subject lost 700g during the study, others gained even 7.9kg. The same with lean body mass – none of the subjects lost any of it. The gains varied from 0.9 kg to 9.3 kg (table 8). The subjects were not instructed to change their diet habits anyhow nor were they suggested to decrease their fat mass.

Changes in the weight or the lean body mass did not have any significant correlation with nutrients, whereas the percentage fat did (table 9). The more subject's diet consisted water, carbohydrates, vitamin C, potassium and magnesium the higher was the increase of relative fat. Especially the amount of water (p=0.001) and carbohydrates (0.006) affected. Water explained 52.3% (figure 6) and carbohydrates explained 29.9% (figure 7) from the percentage fat change.

TABLE 9. Correlations between nutrients and the fat% change during the 21 weeks of RT. The fat% change is a relative change from the 0 wk value. The more these nutrients were consumed the more percentage fat increased.

fat % change	r	p-value	
Water	0.687	0.001	**
CHO	0.608	0.006	**
Vitamin C	0.467	0.044	*
Kalium	0.521	0.022	*
Magnesium	0.549	0.015	*

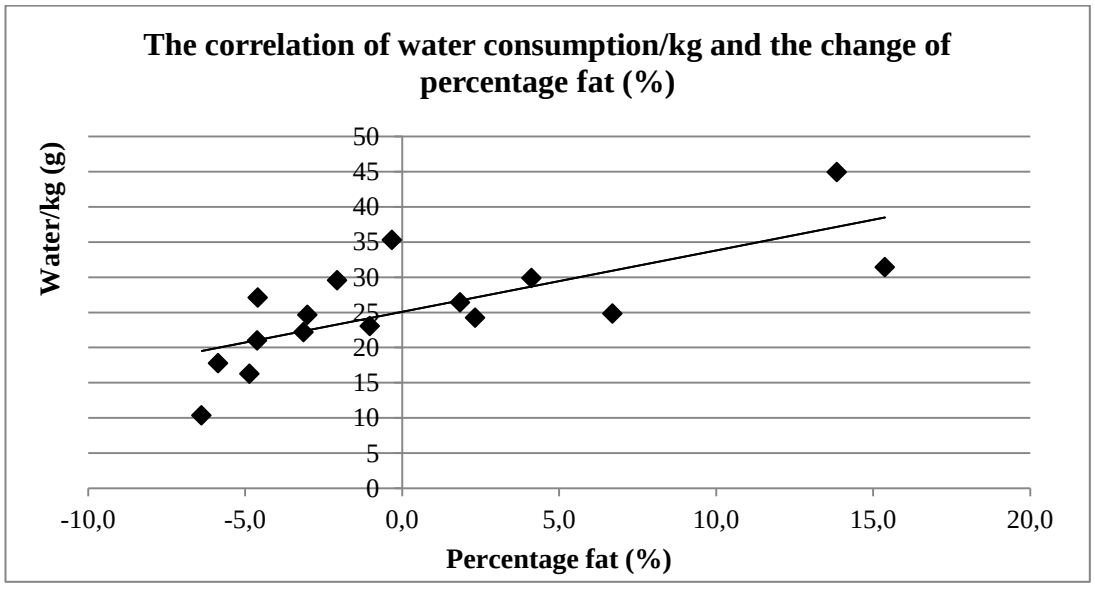

FIGURE 6. The correlation of water consumption/kg and the percentage fat change. ($Y=0.8722x+25.091$. $r^2=0.5226$). The water consumption explained 52.3% from the percentage fat change. The more water was consumed the more fat% increased.

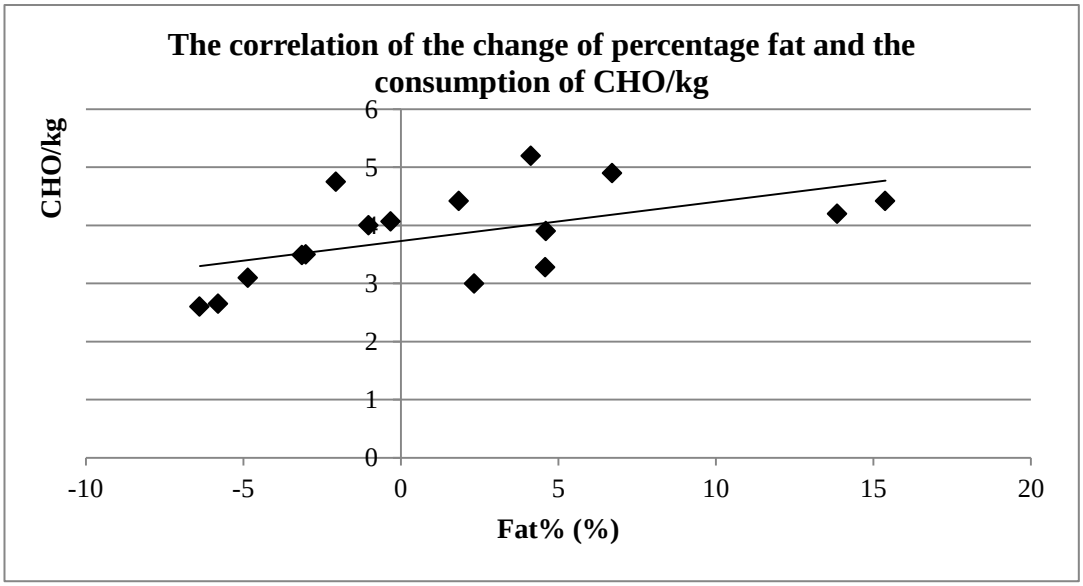

FIGURE 7. The correlation of the change of percentage fat (%) and the consumption of CHO/kg ($Y=0.0676x+3.729$. $r^2 = 0.2991$). The CHO consumption explained 29.9% from the fat% change. The more CHO was consumed the more fat% increased.

8.2.2 One repetition maximum

Subjects' maximal strength, one repetition maximum (1RM) in leg press, enhanced 19.6 % during the 21 week training period (table 10). This means 31.5 kilograms on average. The relative strength gain, 1RM/kg, was 14.6 % during these 21 weeks. This means 310g/kg. The absolute strength development, 1 RM, correlated with the 1RM/kg development significantly ($r=0.813$, $p<0.001$). This means that the same individuals who reached the best absolute results also were in the top in the list even if compared the result to their size (figure 8).

TABLE 10. The 1RM shown together with the 1RM/kg development in the different phases of the study.

	0 wk	**21 wk**	**0 vs 21 wk (%)**
1RM (kg)	166.4±28.5	198±29.1	19.6±7.2
1RM/kg (kg)	2.21±0.3	2.52±0.3	14.6±6.7

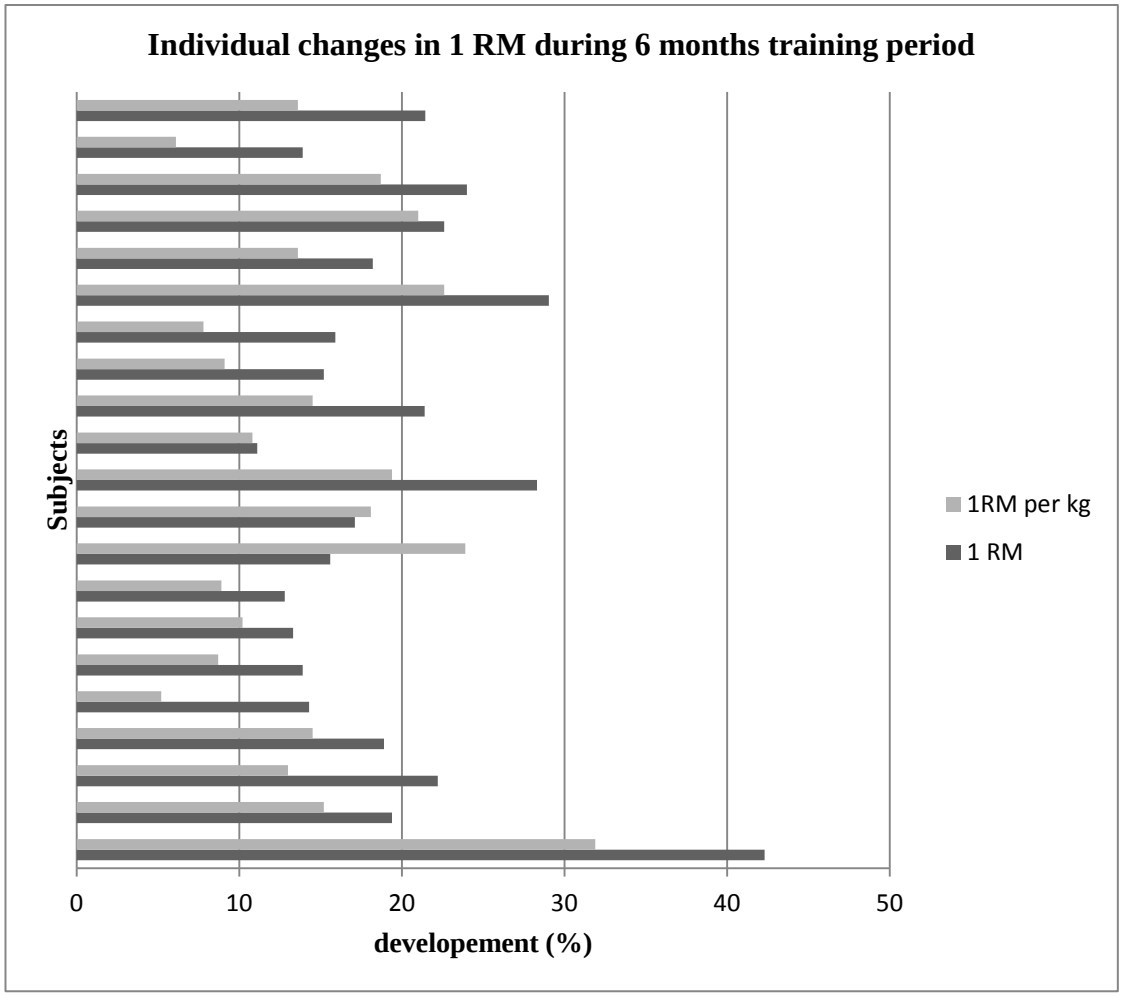

FIGURE 8. Individual gains in both absolute and relative RM.

The development was fastest in the beginning (figure 9). In the halfway of the training period the gain in muscle strength was 13.3% (21.4 kg). Compared to that point the latter half of the training period still enhanced individuals 1RM 5.7% (10.1 kg). Both changes between 0 wk and 10.5 wk and 10.5 wk and 21 wk were statistically very significant ($p < 0.001$).

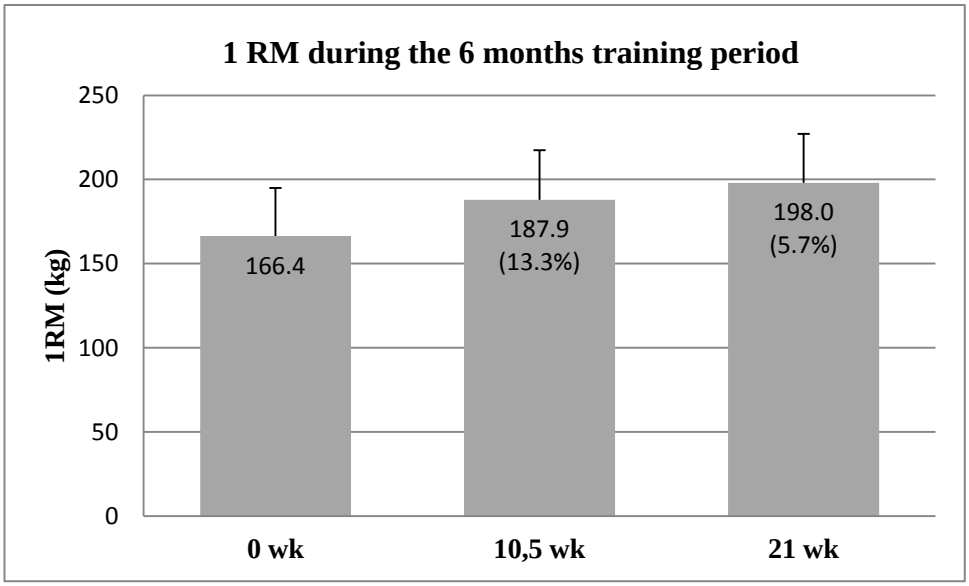

FIGURE 9. 1RM development during the 21 weeks of RT.

Closer inspection of the high and low responders.

3 high- and 3 low-responders are checked more carefully. 15% (3 from 21 individuals in this study) is a normal amount of low- and high-responders in a study and that is set as a limit for a special attention in this study. (Hubal et al. 2005.) The biggest personal development was 42.3% with the 55 kg difference, starting from 130 kg and ending to 185 kg. The smallest gain was 11.1% with 30 kg difference, starting from 225 kg and ending to 250 kg. In many cases the most developed subjects were the ones who started with the lowest results and the smallest gains were among the best 0 wk subjects (figure 10). The development is also viewed as absolute kilograms despite the relation to the starting result. The amount of kilograms is changed relative to the body size (figure 11). The same three individuals got the best results comparing the development to the starting result. The results of the weakest individual are slightly different.

Subject's eating habits followed the trend. These nutrients that contrariwise correlated with results were exactly used like that: less by the individuals with biggest gains, most by the ones with smallest gains (table 11a and table 11b). A few significant correlations are vizualized more specifically (figure 12a and 12b). Both high- and low- responders consumed vitamin D, selenium, water, lactose, dietary fibre, calcium, vitamin B2 and vitamin C more than average values. High-responders consumed all the other nutrients less than average. The low-responders ate over averages except vitamin A, vitamin B12, folid acid and iron. The amounts of nutrients correlated with 1RM results are considered

relative to the size of the subject, g/kg. The absolute amount of a bigger subject does not have an effect on results.

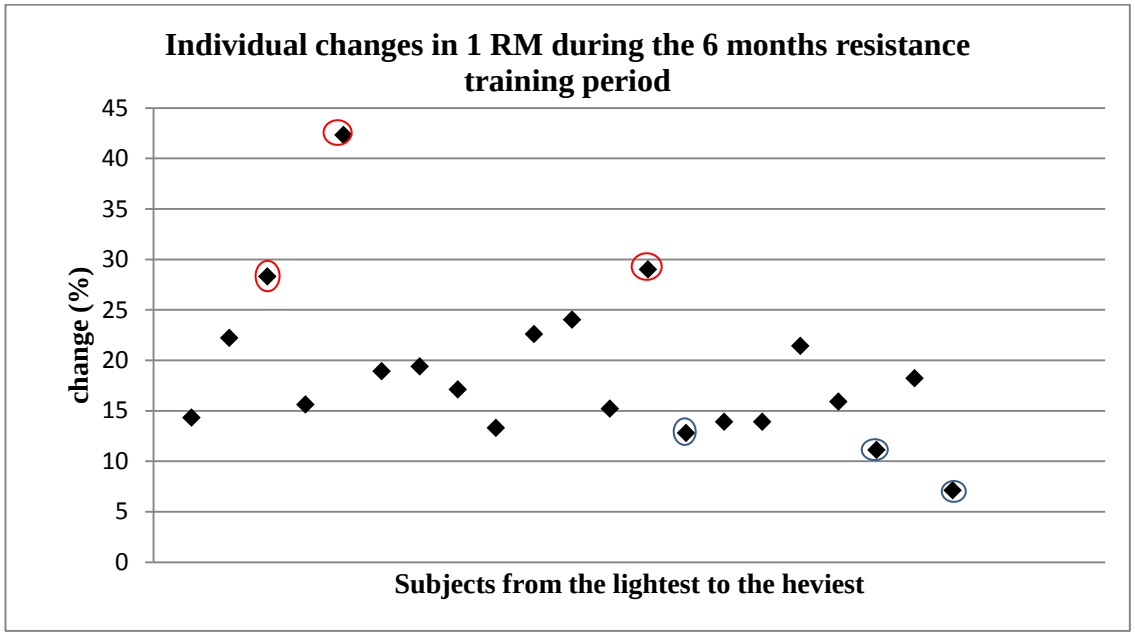

FIGURE 10. Individual relative changes to the starting result in the 1 RM during the 21 week training period. The size of a person is not taken in to consideration. Three relatively biggest gains are marked with red color and three relatively smallest gains are marked with blue color. Nutritional habits of these individuals are examined more closely.

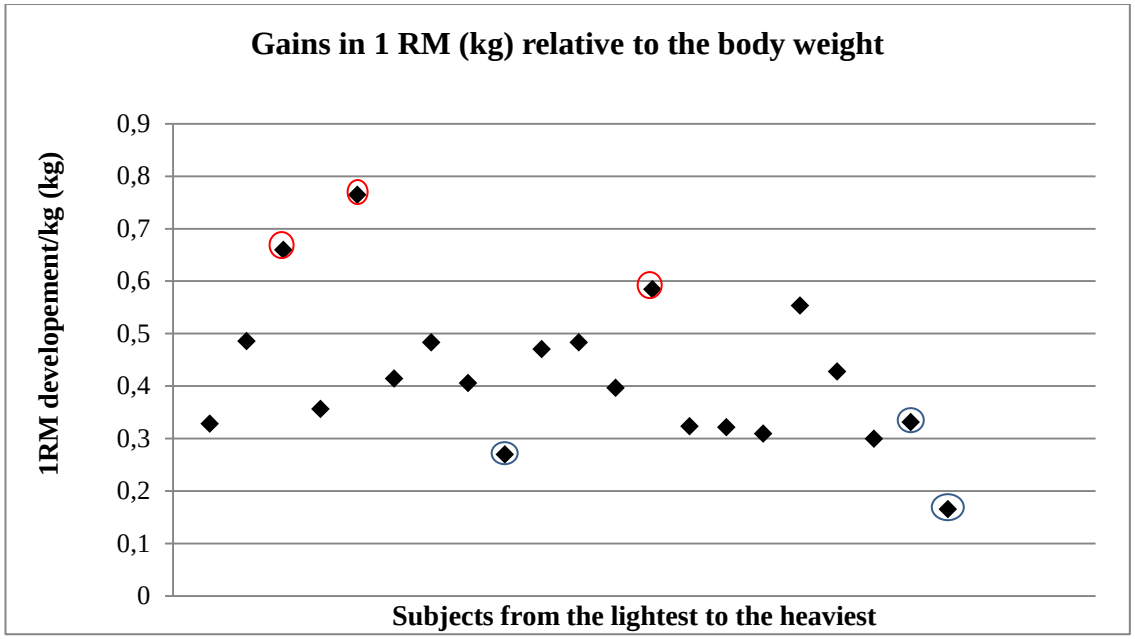

FIGURE 11. Individual absolute gains in 1RM compared to the body weight. Three relatively biggest gains are marked with red color and three relatively smallest gains are marked with blue color. Nutritional habits of these individuals are examined more closely.

TABLE 11a). Nutrients that correlated with absolute RM change.

RM change		
	**	p-value
Monounsaturated fatty acid	-0.534	0.018
	*	
Fat	-0.473	0.041

TABLE 11b). Nutrients that correlated with relative RM change.

RM/kg change		
	**	p-value
Fat	-0.611	0.005
Monounsaturated fatty acid	-0.627	0.004
Vitamin E	-0.593	0.007
Vitamin B12	-0.667	0.002
Folic acid	-0.590	0.008
Calsium	-0.590	0.008
	*	
Saturated fatty acid	-0.499	0.030
Multisaturated faty acid	-0.463	0.046
CHO	-0.518	0.023
Starch	-0.533	0.019
Vitamin A	-0.524	0.021
Vitamin B1	-0.538	0.018
Vitamin B2	-0.464	0.046
Magnesium	-0.559	0.013
Iron	-0.574	0.010

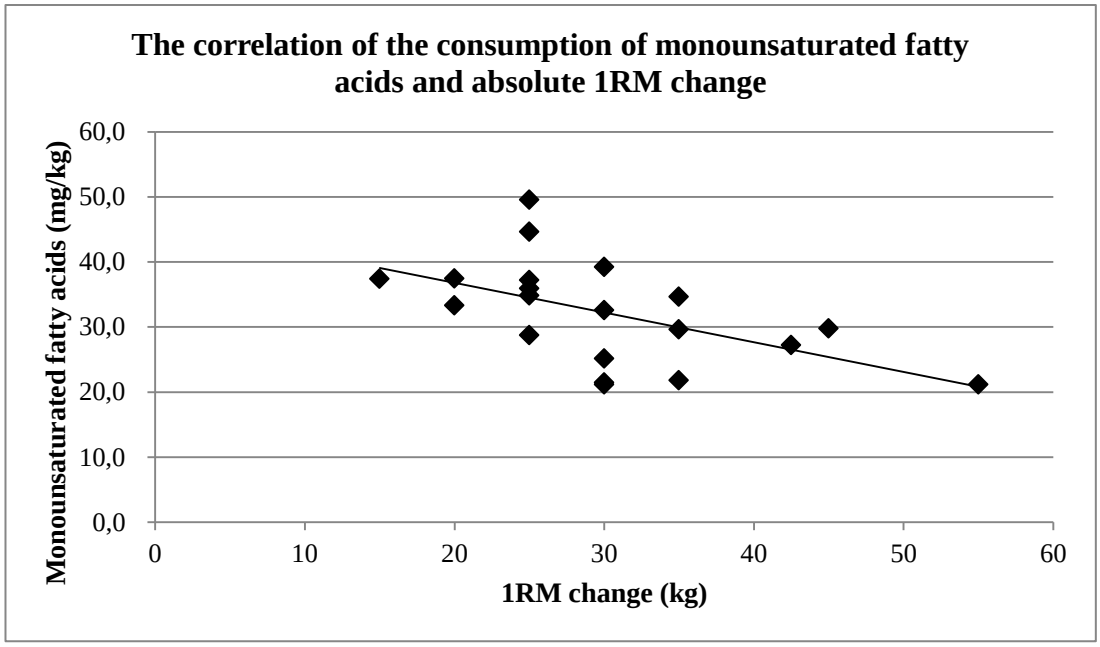

FIGURE 12a. The relationship of the consumption of monounsaturated fatty acids and absolute 1RM in kg.

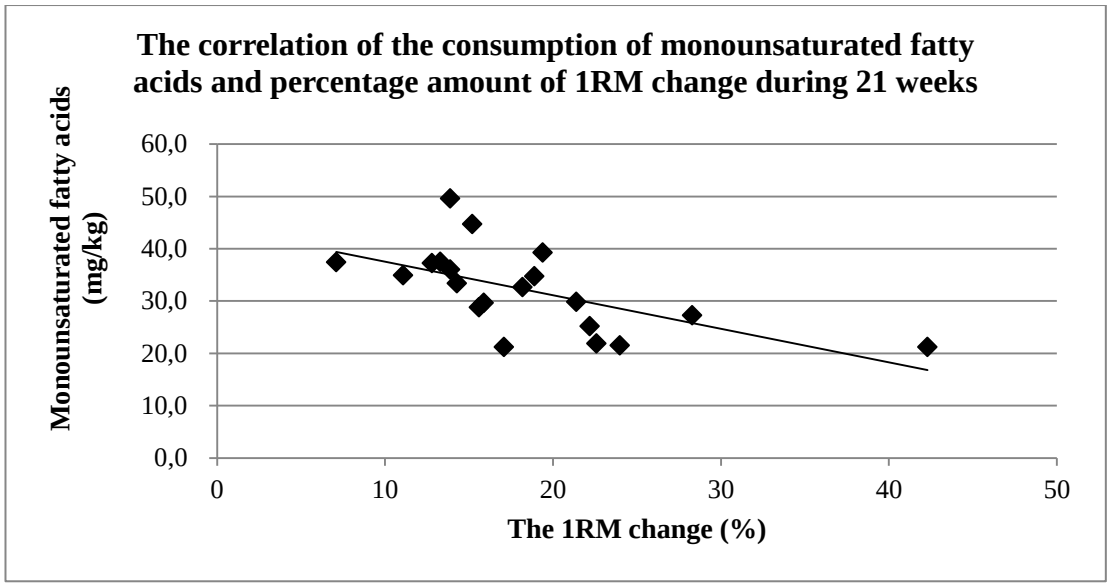

FIGURE 12b. The relationship of the consumption of monounsaturated fatty acids and percentage amount of 1 RM change during 21 weeks.

8.2.3 Muscle fiber size

When counting all muscle fiber types together the average growth in size during the 21 week training period was 49.3%. The cell type 1, slow cell type, grew 42.8%. The cell type 2, fast cell type, grew 55.0%. In most cases cell type 2 grew more than cell type 1. (figure 13).

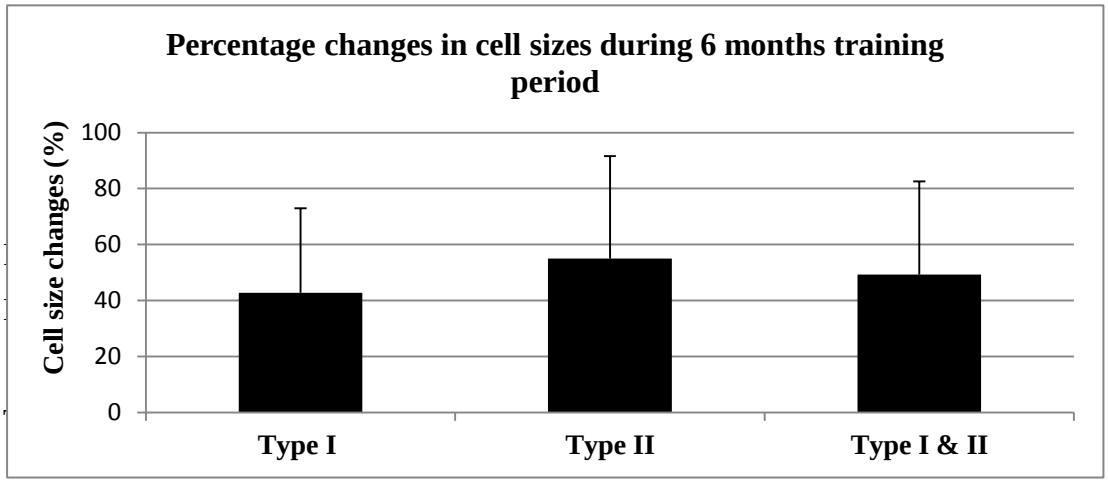

FIGURE 13. Average growths of cell type 1 and 2 separately and together during the 21 week RT period.

These two individuals whose type 2 muscle cells grew most consumed less sucrose and vitamin C than the subjects in average. Selenium, starch and vitamin D were consumed more than in average. Two individuals who developed least consumed less vitamin D and multi-saturated fatty acids than average. There were no nutrients explaining the difference of smaller and bigger growths of cell type one and two in one person (figure 14).

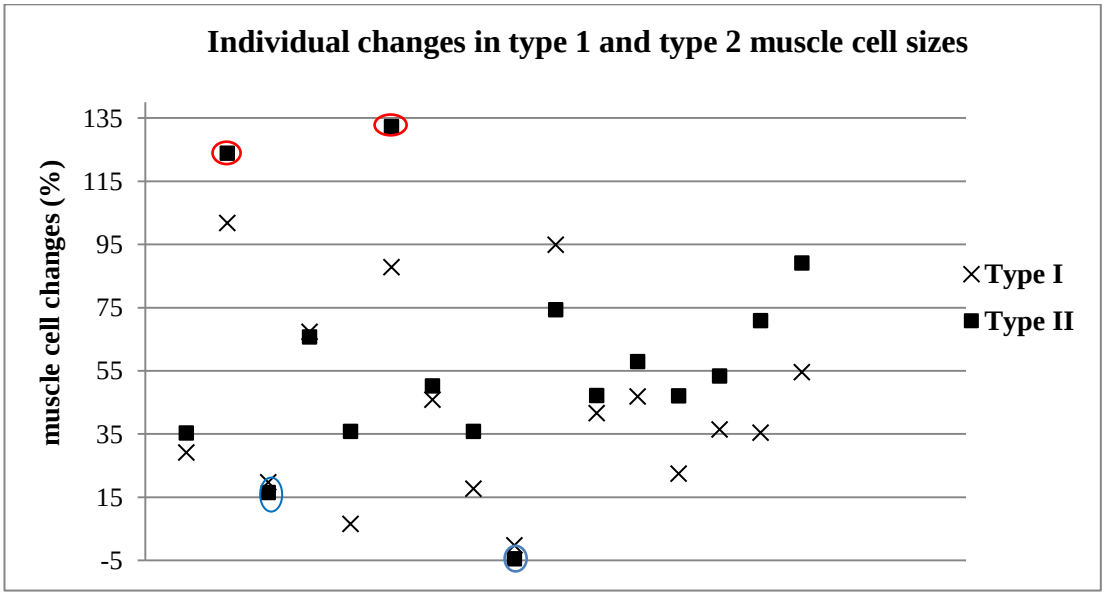

FIGURE 14. Individual changes in type 1 and type 2 muscle cell sizes. The best percentage gains in type 2 cells are marked with red circle. The weakest percentage gains are marked with blue circle.

Type 1 cells, slow muscle cells, did not correlate with the maximal strength, the weight or the lean body mass. Type 2 cells correlated (r= 0.639, p= 0.006) with the maximum strength. The bigger the fiber sizes the bigger was the 1RM result. Also the growth in type 2 cells correlated with starting 1RM, but inversely (r= -0.515, p= 0.035). The bigger starting 1RM, the less the size of type 2 cells changed. (table 12, figure 15.)

TABLE 12. The correlation coefficients of muscle fiber types, strength and body composition. RM=repetition maximum, LBM= lean body mass.

Variable	**RM 0 wk**	**RM 0-21 wk**	**weight 0 wk**	**weight 0-21 wk**	**LBM 0 wk**	**LBM 0-21 wk**
Type 1, 0 wk	0.053	0.24	0.697	0.195	0.052	0.814
Type 2, 0 wk	0.006 **	0.18	0.757	0.826	0.084	0.162
Type 1 change	0.052	0.066	0.947	0.471	0.007**	0.032*
Type 2 change	0.035*	0.052	0.589	0.807	0.003**	0.152

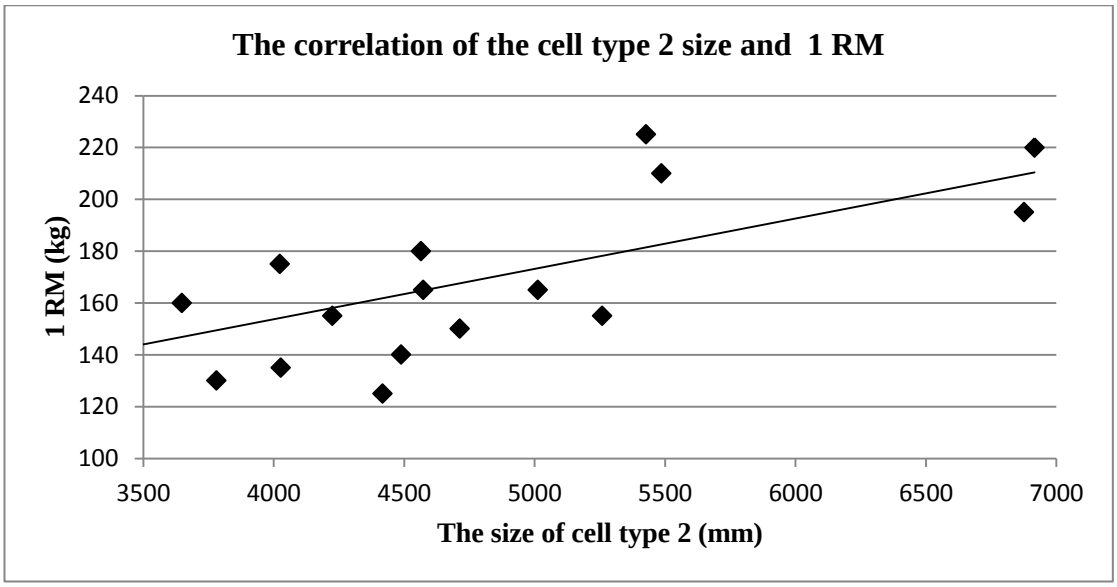

FIGURE 15. The size of cell type 2, fast cell type, explains 40.9 % about the maximal strength. 1RM and cell type 2 size correlated with the formula y= 0.0194x + 75.947.

Average type 1 and type 2 muscle fiber size changes correlated inversely with the starting lean body mass (type 1: r= -0.624, p= 0.007, type 2: r= -0.674, p= 0.003). The bigger the lean body mass, the smaller the average growth of the muscle fiber sizes. Furthermore, the 21 weeks growth in cell type 1 correlated with lean body mass change

(r= 0.521, p= 0.032). The more lean body mass grew the bigger were the growth of the type 1 muscle fiber sizes. (table 12, figure 16, figure 17.)

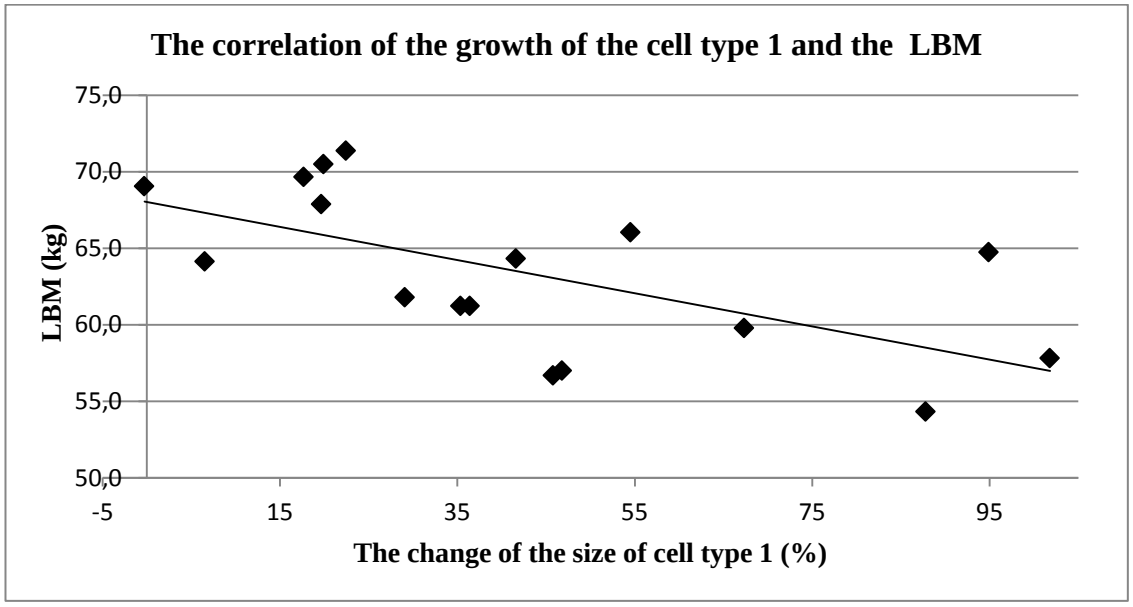

FIGURE 16. LBM (lean body mass) explains 38.8 % about the muscle fiber size growth of cell type 1. LBM and Cell type 1 size changes correlated with the formula y=-0.183x+68.012.

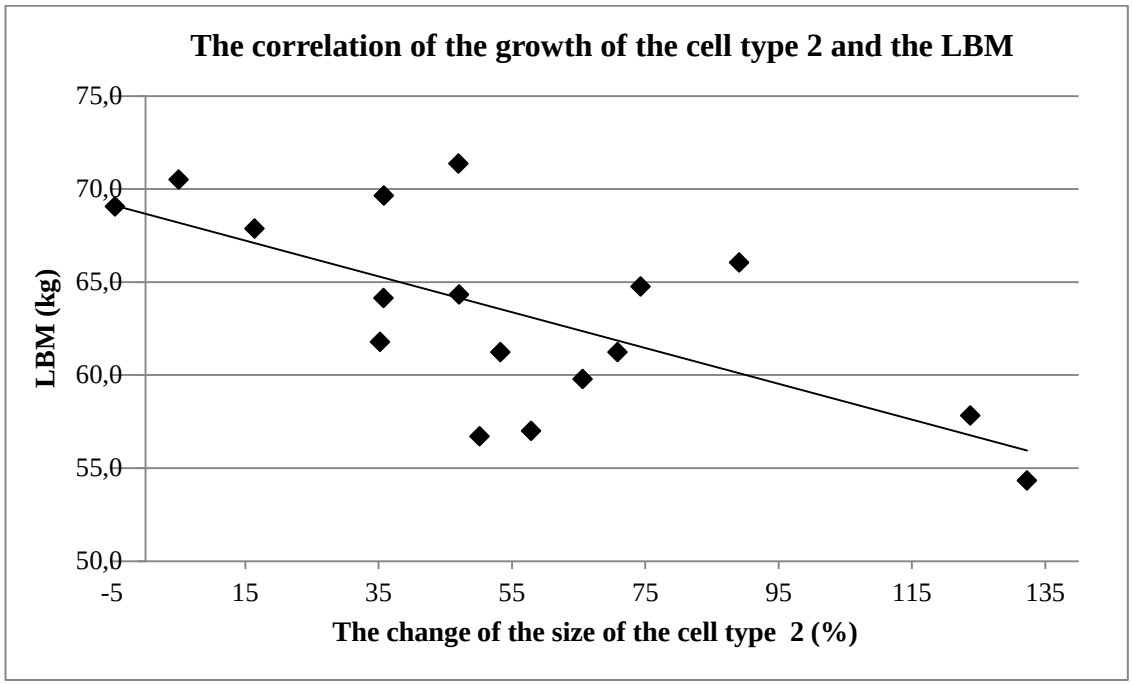

FIGURE 17. LBM explains 45.1 % about the muscle fiber size growth of cell type 2. LBM and Cell type 2 size changes correlated with the formula y=-0.0961x+68.67.

The muscle cell growth did not correlate with any other nutrients than water. The less water was consumed the more muscle cells grow (table 13).

TABLE 13. Correlations of muscle fiber sizes and water.

	*	r.	p-value
Abs type 1 change	water	-0.590	0.016
Type 1 change/kg	water	-0.565	0.023
Abs type 2 change	water	-0.577	0.019
Type 2 change/kg	water	-0.513	0.042
Abs type 1&2 change	water	-0.557	0.025
Type 1&2 change/kg	water	-0.578	0.024

8.2.4 Muscle surface area

During the 21 weeks training period the surface area of quadriceps femoris developed 7.6 % on average, which means 7.7 cm² growth (figure 18). The muscle size in the beginning did not affect relative development (r= -0.199, p=0.459). In the group of three best results there were subjects both with 70cm² and 96cm² areas in the beginning. The size development of quadriceps femoris and absolute 1RM development correlated together (r= 0.694, p=0.003), even if only one of three subjects with best 1RM was in the group of subjects with biggest percentage growth in muscle size (figure 19).

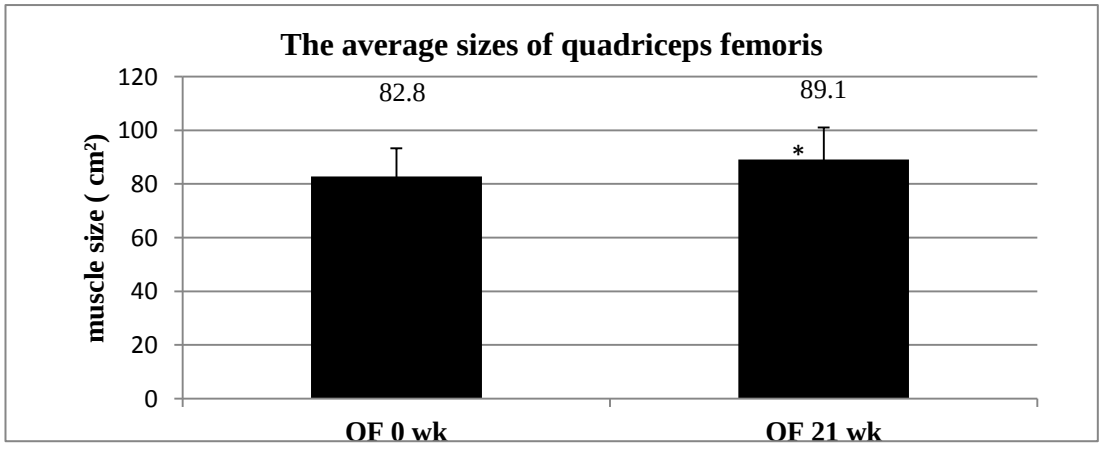

FIGURE 18. The average area of subjects' quadriceps femoris muscle before and after 21 wk long RT period. The difference between areas was very significant (p< 0.001).

There were no nutrient, which would had been consumed more to get bigger surface area change. In common, individuals who gained more ate less vitamin A and multisaturated fatty acids than average, however also the subjects with weakest gains ate vitamin A less than in average. Neither among best nor weakest gains, there was a nutrient that was consumed more than average among subjects (figure 19).

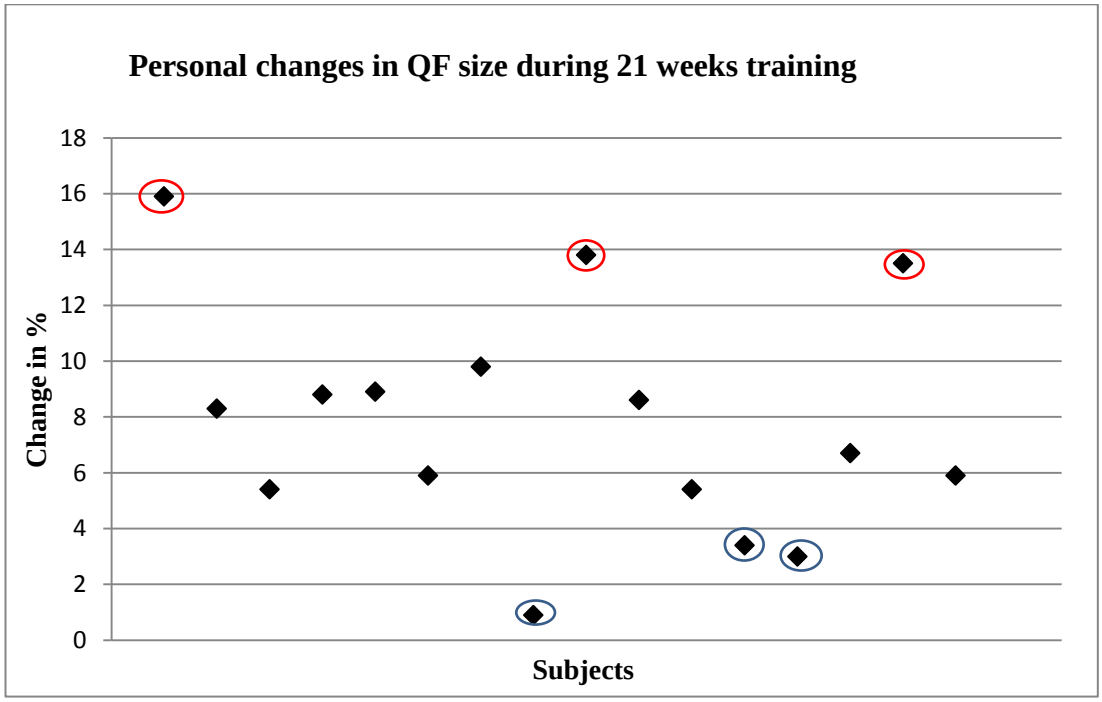

FIGURE 19. Personal changes in the size of quadriceps femoris (QF) during 21 weeks RT. The best relative changes are marked with red circles. The weakest results are marked with blue circles.

The growth of muscle surfaces did not correlate with any nutrient. When considering separately the muscle size in the beginning and in the end of the study, it was shown that the amount of consumed vitamin C, water and potassium correlated with muscle size in both phases. The more these three nutrients were consumed the bigger was the muscle area (table 14).

TABLE 14. The correlation of nutrients and the quadriceps femoris surface area in the beginning and in the end of the study.

		0wk			21 wk	
		r	p-value		r	p-value
**	Vitamin C	0.608	p= 0.010		-	
*	Water	0.517	p= 0.034	Water	0.587	p=0.013
	Potassium	0.532	p= 0.028	Potassium	0.597	p=0.011
				Vitamin C	0.552	p=0.022

8.3 Acute reactions of resistance training

The normal circumstance levels or rises of hormones measured in this study did not correlate with 1 RM results or muscle size.

8.3.1 Testosterone

There were no significant rise in testosterone levels after acute exercise bout (pre-post, p=0.329). Instead the decrease of testosterone levels was significant (post 15-post 30, p= 0.022) (figure 20). The higher the testosterone level was before muscle test the higher the acute reactions were after exercise. The levels before and after measurement correlated very significantly (pre-post r= 0.812, p<0.001). Immediate recovery of testosterone levels did not follow the same trend; however levels after 15 and 30 minutes correlated again significantly (post15'-post30' r= 0.751, p<0.001, n=22).

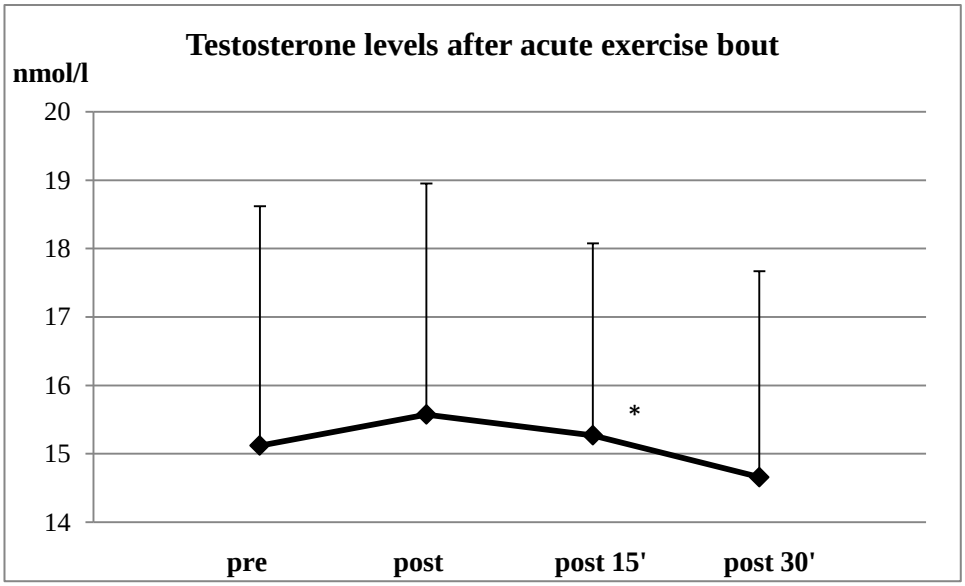

FIGURE 20. Acute testosterone levels before the exercise, right after the acute exercise bout, after 15 minutes of rest and after 30 minutes of rest. ”*” means statistically significant change between marked result and previous one. n= 22

Testosterone levels did not correlate with any other nutrient than vitamin D. The higher the consumption of vitamin D was, the smaller the testosterone levels were in normal circumstances before acute RT (r=-0.475, p= 0.026). Vitamin D did not have an effect on testosterone levels after exercise.

8.3.2 SHBG

Sex hormone binding globulin (SHBG), levels rose very significantly after the acute exercise bout ($p<0.001$). Still after 15 minutes the level of SHBG was statistically high compared to the normal circumstances (p= 0.042). After 30 minutes no statistical differences existed any more. The decrease of levels was constant all the way until 30 minutes. The SHBG levels decreased significantly during first 15 minutes ($p<0.001$) and the decrease continued during the next 15 minutes being significant again (p=0.001) compared to previous measurement point (figure 21).

Sex hormone binding globulin levels correlated very strongly before and after acute exercise bout and also during recovery phase (pre-post r= 0.988, p= 0.000; post-post15’ r= 0.992, p= 0.000; post15’-post 30’ r= 0.995, p= 0.000, n=22). None of the nutrients interrelated with SHBG levels before or after exercise.

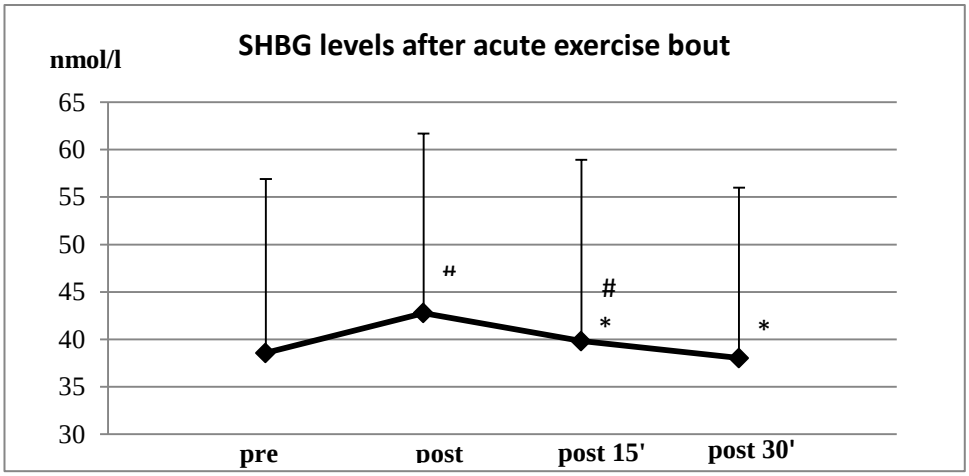

FIGURE 21. Acute SHBG levels before the exercise, right after acute exercise bout, after 15 minutes rest and after 30 minutes rest.” #” means statistically significant change between marked result and normal circumstances (pre). ”*” means statistically significant change between marked result and previous one. n= 22

8.3.3 Cortisol

Cortisol levels did not have a significant difference before and after acute exercise bout (p=0.944). The levels varied a lot between the subjects. Almost all the subjects had smaller cortisol levels after than before the exercise: Contrariwise few subjects reacted to exercise with a very high rose in levels. 15 minutes after the exercise followed a significant rose in cortisol levels (p= 0.047) (figure 22).

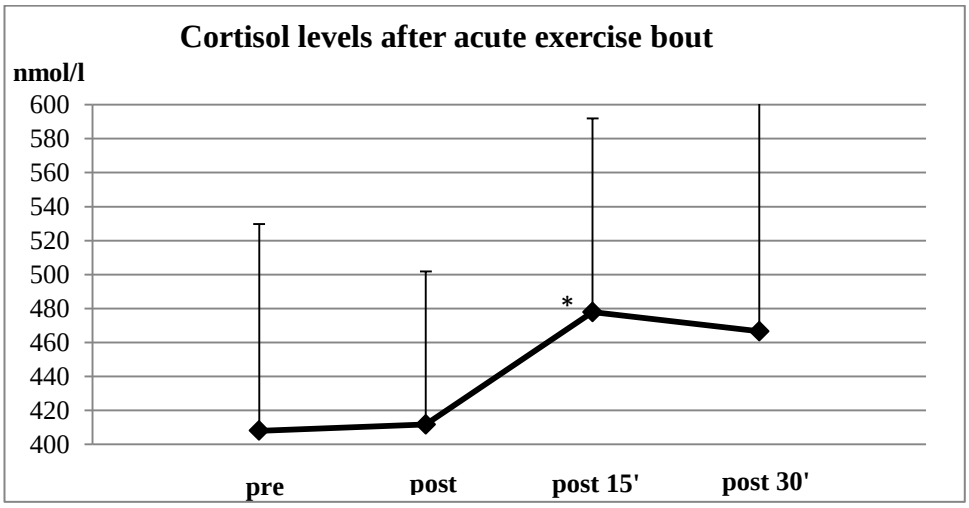

FIGURE 22. Acute cortisol levels before the exercise, right after acute exercise bout, after 15 minutes rest and after 30 minutes rest. ”*” means statistically significant change between marked result and previous one. n= 12.

Cortisol levels of normal state did not correlate with the cortisol levels after heavy acute exercise bout. But the rises of cortisol levels during recovery phase correlated significantly (post-post15' r= 0.605 p= 0.049; post15'-post30' r=0.840 p=0.005). Cortisol levels right after exercise bout correlated inversely with cholesterol levels. The higher the consumption of cholesterol the smaller the increase of cortisol level (r =-0.747, p=0.008). Also starch and monounsaturated fat correlated with cortisol levels, and again contrariwise (r=-0.614, p= 0.045; r= -0.612, p= 0.045).

8.3.4. Growth hormone

Growth hormone levels rose significantly continuously since 30 minutes after the acute exercise bout (figure 23). The difference between the level before and after the exercise just exceeded the limit of significance (p=0.049). The next two phases were already more significant (pre-post15, p= 0.005; pre-post30, p=0.01). Growth hormone levels did not correlate with each other in any phase of measurement. None of the nutrients had any effects on hormone levels either.

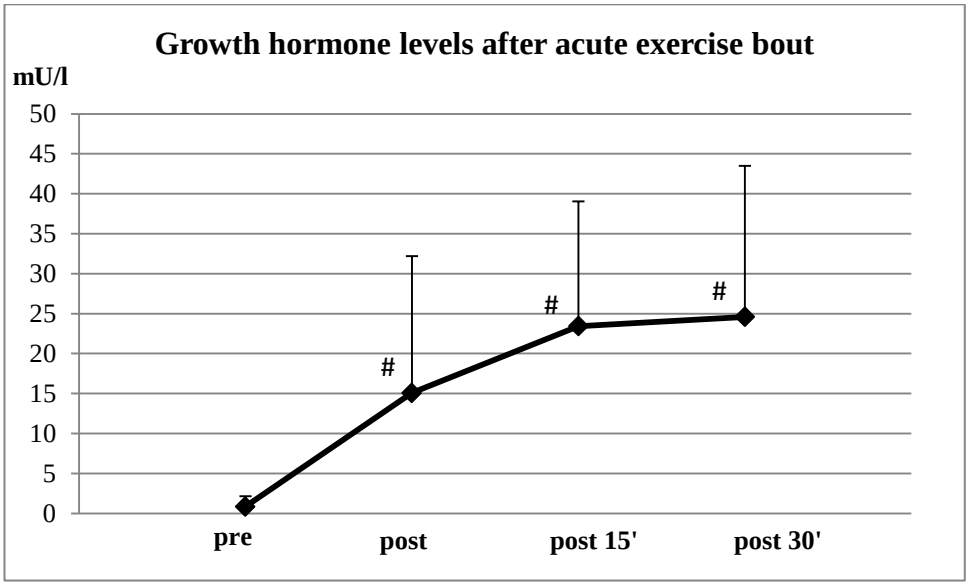

FIGURE 23. Acute growth hormone levels before the exercise, right after acute exercise bout, after 15 minutes rest and after 30 minutes rest. ." #" means statistically significant change between marked result and normal circumstances (pre). n= 8

GH was the only hormone that correlated with the hormone level rises of the other hormones. The rises are calculated as percentage amount to the normal circumstances.

GH correlated strongly both with testosterone levels (r=0.81) and cortisol levels (r=0.89) right after acute exercise bout. After 15 minutes correlations still stayed high (testosterone r= 0.87; cortisol r=0.81).

8.3.5 Insulin

Insulin levels did not rise significantly after the acute exercise bout (figure 24). The highest point in insulin levels was after 15 minutes recovery from the exercise. The rise in the point of 15 minutes was significant when comparing to the level right after exercise (p= 0.004), but it did not correlate with the normal circumstance levels.

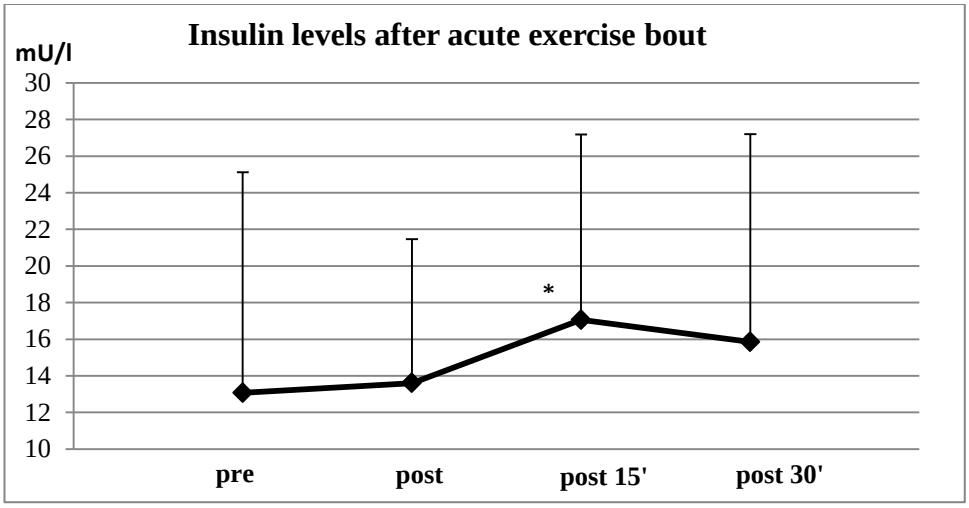

FIGURE 24. Acute insulin levels before the exercise, right after acute exercise bout, after 15 minutes rest and after 30 minutes rest. .” *” means statistically significant change between marked result and previous one. n= 19

Insulin levels correlated with many nutrients. The amount of consumed water had a very significant effect in every measurement phase. The more water was consumed the more insulin levels rose. Also calcium and magnesium consumption correlated with every phase of the measurement. Proteins, carbohydrates, niacin and natrium correlated with values taken just after acute exercise bout however correlations were not very strong. Potassium as well as zinc correlated both right after acute exercise bout and also during the early recovery. In addition to this zinc also correlated with insulin in normal circumstances. Alcohol consumption had an effect on recovery. The more alcohol was consumed the longer insulin levels stayed higher (table 15).

TABLE 15. The correlation of insulin and nutrients before and after an acute RT. Only nutrients with significant correlation are mentioned in that table.

	pre		**post**		**post15'**		**post30'**	
Nutrient	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**	**r**	**p-value**
Water	0.579	0.009	0.785	0.000	0.753	0.000	0.741	0.000
Protein	-	-	0.492	0.032	-	-	-	-
CHO	-	-	0.457	0.049	-	-	-	-
Alcohol	-	-	-	-	0.486	0.035	0.54	0.017
Niacin	-	-	0.459	0.048	-	-	-	-
Natrium	-	-	0.458	0.049	-	-	-	-
Potassium		-	0.603	0.006	0.504	0.028	-	-
Calsium	0.508	0.026	0.558	0.013	0.499	0.3	0.463	0.046
Magnesium	0.475	0.4	0.702	0.001	0.689	0.001	0.594	0.007
Zinc	0.534	0.019	0.626	0.004	0.598	0.007	-	-

9 DISCUSSION

The strength and muscle size increased as expected during the 21 weeks training period. The eating habits of the subjects mostly followed the general nutrition recommendations in Finland. The consumption of protein (g/kg/d) increased significantly from the start to the end of the study. In many categories nutrients did not have any effects on the responses to RT. Vitamin D, water and selenium were the nutrients that most often correlated with the RT responses. D-vitamin and selenium were the nutrients that individuals with the best gains in one repetition maximum and muscle fiber size growth consumed more than an average subject. The individuals with the smallest gains consumed less vitamin D. Vitamin D also correlated positively with the testosterone levels. The more water was consumed the more percentage fat and insulin levels increased.

The meaning of nutritional habits in gaining results

There were no statistical changes in nutrition diaries. Therefore diaries written in any phase of the study could be used for comparison. Protein was the only nutrient, the amount of which (g/kg) changed during the study. Because protein consumption rose evenly in every subject's eating habits, it was not an obstacle to use the mean value of one to three nutrition diaries.

The rise in protein levels might be due to awareness in healthy nutritional habits. The subjects might have consciously changed their eating habits. They might have been more interested in searching information about nutrients than earlier when knowing that the aim of the 21 weeks practicing was to gain strength. About the importance of proteins is written in media more than about the importance of other nutrients, so adding the amount of protein is a natural choice for a beginner. Some persons have a habit to think that eating only most important nutrients will gain the best results. With nutrition "much is better"- phrase does not work. These wrong thoughts lead to nutrient deficiencies: There are almost 50 essential nutrients that can be classified to protective nutrients and energy nutrients. Protective nutrients are nutrients, lack of which causes deficiency disease typical for them. These are proteins, vitamins and minerals. Energy nutrients are carbohydrates, fats and proteins. Even if nutrients can partly change to

other nutrients or replace and supplement each other, every nutrient has its own specialties and that is why it is important to get enough all nutrients and in good balance. (Peltosaari et al. 2002, 9.) Protein consumption of the subjects (mean: 1.4-1.6g/kg/day) was in normal limits, clearly more than the minimum general recommendation 0.8g/kg/day. On the contrary, the use of carbohydrates was a bit small vs. general recommendations (3.7-4.0g/kg/day vs. 5-6g/kg/day). Carbohydrate fuels the body with the right kind of energy for heavy workouts and saves amino acids for muscle building and recovery. Specific type 2 muscle fiber glycogen depletion may limit performance during high volume workouts. The consumption of carbohydrates increases when the duration of exercise increases. A little amount of carbohydrates causes small muscle glycogen stores, which deteriorates strength abilities and makes high-intensity practicing difficult to continue. A partial deficit of glycogen stores already causes the use of amino acids for rebuilding of glucose and the use of amino acids as energy. And even if some studies say that the meaning of carbohydrates for strength athletes is not the biggest thing, there is no reason to limit the use, in order that the actions mentioned before don't suffer (Haff et al. 2003). Every individual just has to find the most suitable way to eat. (Hulmi 2013a.)

When adding the amount of proteins, should also be careful if water needs to be consumed more. A huge consumption of protein may cause water balance problems. For example metabolism of 400 kJ (95 kcal) of protein requires 350g water. The same amount of fat or carbohydrates consumes only 50 grams. If the amount of protein is more than 15 % from energy, the amount of water consumed especially needs to be taken care. (Peltosaari et al. 2002, 83, 88.)

The smaller the amount of energy is, the higher the relative amount of protein needs to be. The breakdown of muscle proteins and muscle damages are the higher the less energy and carbohydrates are consumed combined in exercising. (Niemi 2006, 30.) The breakdown of muscle protein and muscle damages are the highest when the training program is changed harder, when new exercising methods are used, when is practiced again after the break or when practicing is started for the first time. In these cases the need of protein to maintain the positive nitrogen balance increases. Although, beginners work with lower power and amounts of repetitions are quite small, so the need of protein may not crucially differ from experienced athletes. Protein appears to have the

greatest effect on promoting satiety, whereas the relative satiety value of fats and carbohydrates is less clear. The energy density of a meal or food, defined as an energy content of a given weight of the food (kcal/g), has a strong influence on both satiation and satiety. The lower the energy density of the meal or food, the greater the satiety that it produces. (Gallaher 2008.)

The consumed protein supplements are in form of liquids, powders and pills of purified protein. These supplements often contain proteins "predigested" to simple amino acids through chemical action in laboratory. Advocates believe that simple amino acid molecules are absorbed more readily. This does not occur. The healthy small intestine readily absorbs amino acids when they exist in more complex di- and tripeptide forms rather than in simple amino acid form. A concentrated amino acid solution draws water into the intestine. This often precipitates intestinal irritation, cramping, and diarrhea. (McArdle 2007, 35.)

In many cases also micronutrients work together. One example is that when the ratio of micronutrients relative to each other is not in balance, it might lead for example to cramps. In that case it would mean there is too much potassium in nutrition compared with lime and magnesium. Also the lack of vitamins B1 and B2 can affect cramps. (Marjanen & Soini 2007, 35). Subjects did not suffer from the lack of any of the nutrients. In contrast the amount of niacin and selenium was double, the amount of vitamin C a bit less than double, the amount of vitamin B12 was triple and the amount of sodium was even 15 times higher than needed. It is said that Finnish people use salt a lot and this research supports that claim. Sodium leads to increase in blood pressure and further the risk of arterial disease based on some, but not all studies (Taylor et al. 2013). Vitamin B12 is obtained from the same food that includes a lot of protein (milk, meat, fish) so it is very natural that also the amount of vitamin B12 was risen. (Niemi 2006, 64.)

Power lifters tend to have "overeating days", because it is thought to be a useful method for gaining more muscle mass. The theory is to destabilize the hormone balance by huge amount of dietary fat. It has not been possible to give statistically significant results for that thought. (Ilander & Mursu 2008, 379; Volek et al. 2001.) Like mentioned before, fat consumption increases HDL amount. The limited use of saturated fatty acids

decreases LDL. However, the limited use of saturated fatty acids at the same time with increased amount of CHO decreases also HDL. From the point of view if the total cholesterol/HDL- ratio, increasing the amount of CHO causes the most unfavorable effect, even worse than saturated fat. (Laatikainen 2011.)

Anthropometry

Like Thalacker-Mercer (2009) reported that the average body weight did not change during the 16 week of RT and it also did not change in this study. (Thalacker-Mercer 2009). Percentage fat varied a lot instead. That might be the result of eating habits. This study reported that the more water, carbohydrates, vitamin C, potassium and magnesium were consumed the bigger was the increase of percentage fat. Especially the amount of water and carbohydrates affected. Protein did not affect on the percentage fat, however it was the only nutrient, amount of which changed statistically during the training period. This supports the theory, that when adding the percentage amount of protein to the diet, it does not automatically mean, that it is healthier and good-caloric when the subjects already consume relatively large amount of protein. (Peltosaari et al. 2002, 88.)

In this study it was not separated, where in the body lean mass or fat increased or decreased most. The development was just dealt with as whole. Thalacker-Mercer reported that in his study most of the lean mass increases were in the thigh compartment, because the training regimen focused on knee and hip extensor muscle groups (Thalacker-Mercer 2009). It is natural that only these muscles develop that are exercised, but about fat accumulation there are conflicting reports. It should be studied more why people lose fat from totally different body parts compared to each other despite the movements they do. In this study there were no correlation between any nutrients and lean body mass change. In Riechman's study there was a greater increase in lean mass with higher dietary cholesterol intake. (Riechman et al 2007.) Maybe protein products include cholesterol and cholesterol correlates with lean body mass through protein or the effect of cholesterol as a precursor of testosterone, but those are only speculations. Vitamin D that has many positive effects on health is showed to decrease when body mass increases. For every $1kg/m^2$ increase in BMI, it appears that serum vitamin D is reduced 1.15% (Vimaleswaran et al. 2013).

In this study other daily calorie expenditure was not asked. Activities of daily living (everything we do that is not sleeping, eating or exercising) may help explain why some people can maintain their body weight, while others gain weight (Monorem & Thopson 2008). Also times of protein ingestion were not reported. It is studied that approximately 10g of EAAs during a meal stimulates muscle protein synthesis. It has been demonstrated that a plateauing of muscle contractile protein synthesis follows approximately these 10 grams of EAAs, meaning that intake of EAA above this threshold does not significantly contribute to the accretion of skeletal muscle (Hulmi et al. 2010). Instead of that the amount of times the 10 g EAA threshold is reached is meaningful. It is inversely related to the percent central abdominal fat. (Cuthbertson et al. 2005.) However, not all studies support the frequent eating, even in contrast (Areta et al. 2013).

1 RM and muscle fiber size

The development in strength abilities was researched calculating the new record relative to previous record measurement. In that calculating method it is not possible to keep development in the same level for a long time, because the previous "zero level" is every time higher making the relative development weaker time after time (13.3% vs. 5.7%), even if the absolute amount of gained strength were the same. Anyway in this study also the development in absolute kilograms did change less towards to the end of the study (21.4kg vs. 10.1 kg.) Development is faster in the beginning because even if muscle mass does not develop any faster than in the end nervous system learns how to recruit all the muscle cells needed for one attempt (Campbell et al. 1999).

Different starting levels in force production make it difficult to choose, how to really compare development to get the real results. In this study the absolute 1RM development is compared relative to starting measurement result. The problem in this method is that if one person starts from lower level than the other one, the first person's relative development is automatically better than the one's starting from the higher level. This problem can be compared to the example mentioned above about faster development in the beginning compared to the end; If one person starts from 130 kg ending up to 185 kg and the other one starting from 225 kg ending up to 250 kg, the relative development difference is 42.3% vs. 11.1%. It indicates that the first person would have been almost four times better by developing, even if the real kilogram

difference is just 30 kg (55kg vs. 25kg), what means about two times better instead of four. One could say that the best way to compare would be just the absolute kilograms between starting and final 1 RM measurement, ignoring the starting level. This would be possible, if the persons were the same size. 1RM goes together with the muscle size and the smaller person has not the same possibility to gain the same size of muscles than the bigger person, because the area for the muscles in the body is smaller. (Janssen et al. 2004a; Janssen et al. 2004b.) That was a reason why I chose to compare absolute kilograms compared to the body weight in kilograms. In fact two best and two worst results were the same persons in both methods anyway.

When a person eats a lot, improvement in muscle strength relative to the body weight may stay lower. In this study strength gain was measured both with and without body weight relation. Persons eating less, that means in most cases also lightest persons of the group, got better results in both measurements. This was actually not a surprise, when comparing 1RM relative to previous result without body weight relation. It is normal that lightest persons are the ones who start with smallest results. Like reported before, it is for these persons easier to get higher percentage development than for the bigger persons with heavier starting results. When 1RM gain in kilograms was divided by the body weight, the subjects were more on the same line. In that case the same persons got the best gains, what means that also other things than size has the effect of their good results. Anyhow, it is difficult to find a way to evaluate strength gain results among people with different body sizes and starting strengths. Maybe comparing the 1RM gains to muscle mass results got from inbody-measurement would have been one effective way for measuring.

The person who had the best gain ate more vitamin D and selenium compared to average values. The second best consumed more lactose, dietary fiber, calcium, vitamin B2 and vitamin C. The persons with the smallest gains ate all nutrients over averages. Studies have both shown and not shown a correlation between D-vitamin and muscle strength. For example Grimaldi et al. 2013 showed that vitamin D was significantly associated with arm and leg muscle strength when controlling age and gender (Grimaldi et al. 2013). Knutsen et al. 2014 did not find any correlation between D-vitamin and strength gain measured by the jump test, handgrip test or chair-rising test (Knutsen et al. 2014). Protein was not in the list of most consumed nutrients. Some individuals seeking

hypertrophy may benefit from higher protein intake during RT whereas others may not. Maybe each individual's propensity for myofiber hypertrophy in the first months of training is determined by factors independent of macronutrient intake. (Bamman et al. 2007; Kim et al. 2007).

This study gave contradictory results about water: the less water was consumed the more muscle cells grew. The study also showed that the more water was consumed the better was the 1RM result. The size of muscle cells and 1RM result should correlate, so this result was against expectations.

In this study type 1 cells, slow muscle cells did not correlate with maximal strength, type 2 cells did: The bigger the type 2 muscle fiber sizes the bigger was the 1RM result. But the bigger starting 1RM and lean body mass, the less muscle fiber sizes changed. This supports the theory that the less gym practicing is done, the easier it is to gain results faster. Muscle mass growth happens fast among beginners, because of technique learning and better innervation and partly because of muscle cell growth. The growth can be noted both in fast and slow muscle cells, though the growth is faster in fast muscle cells. Especially exercising affects type 2x cells. In this study any other sport activities than supervised gym practices were not taken into consideration. Aerobic training is said to weaken muscle development. This happens because of the change of 2a cells to type 1 cells by aerobic training instead of new born type 1 cells. That means that fast power producing muscle cells change to aerobic endurance muscle cells. (Ilander & Mursu 2008, 383; Prasartwuth ym. 2005.)

The more person has type 1 muscle cells the likely the person has less fat than the person with plenty of type 2 muscle cells. It is also easier to lose weight for the person with many type 1 cells. (Tanner et al. 2001). It could have been studied the correlation of cell type 1 and percentage fat. This study already reported that the more lean body mass grew the bigger were the growth of the type 1 muscle fiber sizes but because this was not a relative number to body fat we cannot say if the difference in body shape happened. Nutrients did not explain the difference in muscle cell growths between subjects. Some other factors are affecting.

The size development of quadriceps femoris and absolute 1RM development correlated together (r= 0.694, p=0.003), even if only one of three subjects with best 1RM was in the group of subjects with biggest percentage growth in muscle size. There was no nutrient that would have been affecting to the results. The muscle size in the beginning did not affect relative development (r= -0.199, p=0.459). When considering separately the muscle size in the beginning and in the end of the study, it was shown that the amount of consumed vitamin C, water and potassium correlated with muscle size in both phases. The more these three nutrients were consumed the bigger was the muscle area.

Hormones

Subjects had different hormonal levels in normal circumstances and these levels correlated to the levels after RT, except GH and insulin. The higher was the hormone level before RT the higher were the acute reactions after muscle stress. It is important to note that hormone levels measured after an acute bout of exercise do not always occur in parallel with chronic adaptations. That is why acute hormone levels are not usually predictive of long-term hypertrophic responses to regular RT. (Coffey et al. 2006; Schroeder et al. 2013; West & Phillips 2012.) Gender, age and training status influence to the release of the hormones (Kraemer & Ratamess 2005). It has been estimated that genetic differences can account for approximately half of the variation in athletic performance (Crewther et al. 2011). In this study hormone levels did not correlate with each other as much as could have thought according to previous studies. Only rises in GH correlated with testosterone and cortisol. Also none of the hormone levels in the normal circumstances or the levels after RT correlated with 1RM or muscle size in this study.

Testosterone. Previous studies said that testosterone correlates negatively with fat and protein consumption but these claims could not be proved in this study. Only the consumption of D-vitamin correlated with testosterone levels. It correlated negatively and only in normal circumstances. D-vitamin also correlated with 1RM and muscle size, but positively. This is a bit contradictory, because 1RM correlates with muscle mass and muscle mass should correlate with testosterone levels, what means D-vitamin should have correlated with all of these. Testosterone correlated with growth hormone release like

mentioned it literature. IGF-1 was not measured in this study, so correlation with that hormone is not known.

Insulin. Insulin is an anabolic hormone that responded in this study to many nutrients. The anabolic state is easy to keep high when consuming carbohydrates and proteins regularly. On the other side that might prevent the use of fat as energy, what means that fat is stored in the body. If the aim is to lose percentage fat of the body, the limitation of insulin excretion might help. (Ilander & Mursu 2008, 380.) In this study insulin did not correlate with percentage fat. Alcohol consumption had an effect on recovery levels of insulin. The more alcohol was consumed the longer insulin levels stayed higher. The subjects did not consume alcohol one day before measurements. The amount of consumed water had a very significant effect in every measurement phase, what is strange, because water has no calories to affect with insulin. Water has to have a secondary influence through other food containing water. Insulin levels did not rise right after exercising like other hormone levels did.

GH. Studies said that both carbohydrate-rich and fat-rich meals decrease GH levels. In this study there were no connection between any nutrient and GH. Murine studies indicate that the effects of GH on muscle function and mass are dependent on insulin like growth factor 1 (IGF-1) (Kim et al. 2005). In this study IGF-1 was not measured. As the regulation of GH becomes clearer, it is possible that a shared mechanism such as neural drive, muscle activation or metabolic stress that could affect both GH and muscle adaptation may explain the association of GH with hypertrophy. GH is known to have gluconeogenic action as well as liberate substrates such as fatty acids and amino acids. It is unknown whether exercise-induced changes in GH could also be modulating these energy-releasing and tissue-remodelling processes leading to an improved phenotype with training. (Inagaki et al 2011; Sakharova et al 2008; West & Phillips 2012).GH was the only hormone, which amount in blood continued rising until 30 minutes after exercising.

Cortisol. Even if cortisol is catabolic hormone, there is no need to be afraid of it. It has important tasks to do during the muscle work. Furthermore, muscle work deletes negative influences of cortisol. Hormonal environment can be manipulated toward a profile favorable for anabolism by using post-exercise carbohydrate-protein

supplementation. In that way hormones have a greater effect on net protein synthesis immediately after RT. (Biolo et al 1999.) These probably are the reasons for the effectiveness of RT rather than an attenuation of cortisol release (Williams et al. 2002). In rest quantity of cortisol is worth keeping an eye on by diet.

Cortisol levels varied a lot between subjects. That might be due to personal psychological reactions and nutritional habits. Excitement and stress increase the amount of cortisol and adrenalin as well as increasing protein breakdown. (Peltosaari, Raukola & Partanen 2002, 85.) The higher the consumption of cholesterol the smaller the increase of cortisol level ($r=-0.747$, $p=0.008$). Also starch and monounsaturated fatty acids correlated inverse with cortisol levels ($r=-0.614$, $p=0.045$; $r=-0.612$, $p=0.045$). Like previous studies said nutrition affect to the rise of cortisol.

Future studies

Food timing. In this study the timing of nutrition was not paid attention to. The subjects mentioned the point of time in their diaries, but these markings were not analyzed. Anyway the timing might be important to maximize the anabolic response (Tipton, Rasmussen, Miller, Wolf, Owens-Stovall, Petrini & Wolfe 2001), although some have suggested that the timing effect has been overrated (Schoenfield et al. 2013).

Numerous studies say that the best time to ingest extra protein is just before or just after a strength training session (Cribb & Heyes 2006; Elliot et al. 2006; Josse et al. 2010; Poole et al. 2010). One reason for the timing effect may be increased skeletal muscle circulation. It makes maximal nutrient transport possible during and for a short period following exercise. (Levenhagen et al. 2001; Tipton et al. 2001.) In the study of Anderson et al. (2005) after 10 weeks of training, the study participants who took the supplement pre- and post-workout gained significantly more lean weight than those who ingested the supplement morning and evening (+2.8 kg vs. 1.5 kg). Also bench press and squat strength results were better (+12.2 kg vs. 9.0 kg ; + 20.4 kg vs. 16.1 kg). Also increases in type 2a muscle fiber cross sectional area and contractile protein content were higher. (Anderson et al. 2005.)

The best effect is when consuming protein and carbohydrates both before and after the training (Andersen ym. 2005). Ingesting carbohydrates before or during a RT session will increase the total amount of work that is performed during the workout (Kulik et al. 2008). Skeletal muscle glycogen is depleted during RT exercise. When it is depleted, the intensity and the total work volume are compromised. (Campbell et al.2012). Also Volek (2004) reported that the consumption of protein and carbohydrate supplement immediately before exercise resulted in increased amino acid delivery to muscle and greater net muscle protein synthesis compared with consumption of the supplement at various times after exercise. This may provide the best anabolic situation for muscle growth (Volek 2004). About protein it should be noted, that it is recommended to consume it through the day rather than consuming a single protein-rich meal (Westcott & La Rosa Loud 2013).

Pre- or post-exercise ingestion of protein or EAA can increase muscle protein synthesis and result in a positive net protein balance (Tipton et al. 2007; Tang et al. 2009; Koopman et al. 2005). Optimal recovery includes reducing muscle soreness and muscle damage after workout. In this context protein ingestion has been reported to have favorably impact. (Flakoll et al. 2004.) Research also has shown that ingesting protein before a training session elicits a greater increase in resting energy expenditure 24 hours after the workout compared to carbohydrate (Hackney et al. 2010.)

It should be noted, that only small percentage of amino acids are oxidized for energy during exercise. That is far below carbohydrates and fats. (Jeukendurp & Gleeson 2006, 62) Because proteins limited role in oxidation and their ability to improve acute resistance exercise performance, amino acids should not be ingested before a resistance exercise bout, with the belief that they will improve the performance of the following workout. (Campbell et al. 2012). In the future the type of protein could be paid attention to. The quality of protein consumed after resistance exercise can determine the acute amplitude of MPS and lean mass gains (Cribb et al. 2006; Hartman et al. 2007; Tang et al. 2009).

CHO ingestion before exercise may reduce muscle and liver glycogen loss associated with an acute bout of resistance exercise, and this may be of importance for athletes involved in multiple training bouts per day (Bird 2010). A single RT session can reduce

skeletal muscle glycogen stores from 24% to 40%, depending on the duration and intensity (Slater & Phillips 2011). If skeletal muscle glycogen is depleted and not purposefully replenished a subsequent resistance exercise workout will be compromised (Campbell et al. 2012). Enhanced recovery enables greater training volume and increased training volume increases muscle hypertrophy (Volek, 2004; Hulmi et al. 2010). CHO ingestion during the exercise bout also may shift the exercise-induced hormonal milieu toward a profile more favorable for anabolism (Roy et. 1998; Tarpenning et al. 2001).

Only few studies have investigated the effects of the timing of dietary fat intake. The reason for this is that RT is an anaerobic activity, relying on the phosphagen system and carbohydrate oxidation for ATP production. Dietary fat is not the energy to fuel a resistance exercise bout. (Campbell et al. 2012.)

Preparing meal. The meaning of preparing meal is to grow amino acid, glucose and insulin levels of blood and keep the level high during the whole exercising time. The meal should be liquid to be absorbed fast, and to contain fast absorbing proteins and mainly EAAs. Whey protein and maltodextrin are the best choices. Also carbohydrates that are absorbed and drained well are recommended. The meal should be eaten not earlier than 10 minutes before the training. In that way the insulin level does not raise too high before the training and effect negatively. To maximize muscle gain both enhancing the building and minimizing the breakdown of muscle protein should be thought. (Ilander & Mursu 2008, 379-380.)

Recovery meal. After practicing only carbohydrates are not enough, because carbohydrates do not enhance new proteins to be built effectively and no anabolic effect exists (Borsheim et al. 2004). Consuming protein, especially EAAs after RT, changes the muscle protein balance positive (Bird et al. 2006; Nosaka et al. 2006; Power et al. 2009). Added to muscle gain protein recovery drink in long term decreases overtraining accidents, maintains resistance and decreases muscle soreness. (Flakoll et al. 2004). EAA concentration alone cannot entirely explain the improved rate of recovery. In the case of whey protein one explanation may be that whey provides a post-exercise insulin response such that NPB can be slightly smaller and glycogen resynthesis occurs more rapidly. (Nosaka et al. 2006; Bird et al. 2006; Power et al. 2009.) 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). Enhanced exercise recovery may be partially also explained by the transcript levels. For example gene –expression of cyclin-dependent kinase 2 (cdk2), a factor that positively affects cell proliferation and animal size has been shown to increase in humans after acute and chronic bouts of resistance exercise, but only when whey protein is ingested around training times. Compared to that one week of low protein diet decreased several transcript levels in muscle that relate positively to cell proliferation. At the same time negatively affecting transcript levels were increased. (Hulmi et al. 2008; Hulmi et al. 2009.) If eaten the preparing meal before the training there is not so hurry to get the recovery meal after practicing (Ilander & Mursu 2008, 380).

IGH-1. In this study IGF-1, insulin-like growth factor, was not paid attention to. It is said that other hormones' function is connected to IGF-1 and that is why it could be measured in the future studies. IGF-1 is an anabolic hormone that stimulates growth in almost all tissues. It is responsible for many anabolic effects of GH. IGF-1 is produced under regulation by GH in the liver. During intense physical training exercised muscles are the primary producers and majority of circulating IGF-1 is taken up by working musculature. (Frystyck et al. 2003; Goldspink 2005; Van Loon et al. 2003.) IGF-1 is similar to insulin. It acts to stimulate protein synthesis, suppress proteolysis and increases the mean myotube diameter and the number of nuclei per myotube. (Harrige 2007.) The combination of testosterone and GH has been shown to confer a synergistic effect on muscle IGF-production (Vingren et al. 2010).

The nutrition does not affect IGF-1 levels, but for binding protein 1 (IGFBP-1) it is meaningful. IGFBP-1 is the most rapid regulator in plasma after meals and may contribute to glucose regulation by countering the insulin-like, hypoglycemic effects of free IGF-1. The IGF-I system does not take part in meal-related glucose regulation but does contribute to glucose disposal during the fasted state. (Volek 2004.)

Decline of circulating IGF-1 levels has been found to correlate with losses of muscle mass and strength (Hand et al. 2007). This may indicate that there is a threshold for systemically produced IGF-1. Below that threshold muscle development is compromised. This need to be researched more, because blood levels of IGF-1 do not

always correlate with post-exercise increases in muscle protein synthesis. (Zou et al. 2011.) The research of Spangenburg et al. (2008) indicated that a functional IGF-1 is not obligatory for muscle growth.

There are some studies supporting that there is a significant association between IGF-1 and those individuals, who respond favorably to hypertrophy-type training (West & Phillips 2012). It needs to be studied further weather training status influences the morphological response to acute exercise-induced hormonal elevations. Some researchers have proposed that post-exercise hormonal fluctuations may be permissive for untrained individuals but follow a dose-response relationship in those with considerable training experience. (Tremblay et al. 2004.)

Validity and reliability

First starting measurement was done to minimize variability caused by learning. Always when possible there were the same measurers making the measurements in order to avoid differences in the way of measuring. There was more than one attempt to find the best result in strength measurements. In the leg there was a small tattoo as a mark to keep the measurement point as same through the study in biopsy measurements.

The blood analysis of this study was collected in the beginning of the training period and thus does not address how the acute hormone response may have changed during the training period. West et al. (2009) have anyway reported acute hormone responses and resting hormone concentrations to be similar in the beginning and at the end of 15 weeks of training. The measurements were done always at the same time of the day to minimize error factors caused by timing.

In the nutrition diary analyses it is uncertain whether subjects answered honestly about the amount of food consumed. In many correlations parameters between food and some other characteristics there were many factors that could not be controlled during the study. (West & Phillips 2012.) The main thing is that the training program was the same for every subject, because different exercise programs can result in differential responses of muscle protein synthesis after resistance exercise. (Burd et al. 2010; Moore et al. 2005.)

10 CONCLUSIONS

The 21 weeks of RT increased 1RM, both type 1 and type 2 muscle fiber size, muscle surface area, body weight and LBM. The regular RT increased the consumption of protein. Chronic adaptations of resistance training correlated with some of the nutritional habits. Many nutrients surprisingly correlated inversely with gains in 1RM. also the water consumption correlated inversely with muscle fiber size growths. When subjects were examined individually, the meaning of vitamin D emerged, even if it did not meet statistical significance. Especially the amount of water and CHO correlated with percentage fat change. The normal circumstance levels or rises of hormones did not correlate with chronic adaptations in muscle or with anthropology variables. SHBG and GH levels rose after the exercise. GH correlated strongly both with testosterone and cortisol levels right after exercise. Cortisol levels after muscle stress correlated inversely with cholesterol levels. Many nutrients, especially water, correlated with insulin levels after exercise. Vitamin D consumption correlated inversely with testosterone levels in normal circumstances.

In conclusion:

1) If dietary recommendations are met, the individual differences in protein or carbohydrate consumption do not have major effect on the chronic adaptations in the muscle. The effect of vitamin D on choric adaptations has to be studied more.

2) Hormonal elevations of insulin, cortisol and testosterone after RE can be affected by nutrition. Only SHGB and GH levels grow right after acute RE, but they are not affected by any nutrients. Neither the levels of hormones in normal circumstances or after exercise do not correlate with chronic adaptations in muscle, so with nutritional habits cannot be affected to chronic adaptations through hormonal changes.

3) The regular resistance training increases the consumption of protein however the protein was not associated for bigger chronic developments.

4) Nutritional habits have effects on anthropometry. The more relative carbohydrate or water is consumed the higher was the increase of relative fat.

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12 APPENDIXES

APPENDIX 1

Acute resistance exercise protocol

Protocol

- 2 min warm up with a bicycle ergometer
- Isometric leg dynamometer 3 maximum attempts/ recovery 1min
- Dynamic leg press 2x10 repetitions 30% from 1RM/ recovery less than 1min
    - leg press 10 RM, 75% from 1RM/ recovery 2min
    - leg press 10 RM/ recovery 2min
    - leg press 10 RM/ recovery 2min
    - leg press 10 RM/ recovery 2min
    - leg press 10 RM/ recovery 2min

    The amount of resistance was changed in every series according to the ability to accomplish the previous series.

Blood samples were taken

- pre
- post 0
- post 15 min
- post 30 min
- post 1h
- post 48h