EFFECTS OF POST-WORKOUT SUPPLEMENTS AND RESISTANCE TRAINING ON SERUM METABOLOME, MUSCLE STRENGTH AND MUSCLE HYPERTROPHY

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ABSTRACT

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Introduction. Human metabolism is a complex mixture of different metabolites and metabolic pathways that are interrelated. Compared to standard biomarker assessments, metabolomics gives a broader perspective of the whole ensemble thus deepening the understanding of human physiology and pathophysiology. The aim of this study was to provide a comprehensive overview of the effects of different post-exercise supplementation regimens and different resistance training programs on serum metabolome and resistance training adaptations.

Methods. 60 healthy men volunteers (mean ± SD: age 32.6±6.7 y; height 1.80±0.1 m; weight 82.8±10.4 kg and BMI 25.5±3.0 kg/m^2) were included in the study. All the participants went through a resistance training program, including a 4-week familiarization phase and the actual 12-week intervention phase. Before the intervention phase, the subjects were randomly split into protein (PROT), carbohydrate (CHO) or protein+carbohydrate (PROT+CHO) groups and further into hypertrophic (HYP) or maximal+power (MAX+POW) resistance training groups. Fasting blood samples were collected and metabolites were analyzed by an automated high-throughput serum nuclear magnetic resonance (NMR) spectroscopy, body composition was assessed by dual-energy X-ray absorptiometry (DXA), cross-sectional area (CSA) of *vastus lateralis* was assessed by ultrasound and maximal isometric strength was measured in leg extension dynamometer before and after the 12-week intervention phase.

Results. The serum metabolome profiles did not differ significantly after the different training and supplement regimens in between group comparisons. Thereafter, the study groups were combined to examine the changes in dyslipidemia biomarkers. Although there were no significant changes, the tendency was towards more beneficial metabolite profile: LDL cholesterol (mean ± SE: -1.1 ±2.8 %), HDL cholesterol (+4.2 ±1.9 %), serum cholesterol (-0.4 ±1.6 %), triglycerides (-1.7 ±3.4 %). Conversely, in blood glucose the change was adverse (+0.9 ±1.3 %). Muscle strength (p=0.001) and size increased (p=0.003) in all study groups. MAX+POW group increased more CSA of *vastus lateralis* (9.5 ±16.9 % vs. 6.9 ±16.8, % p=0.04) than HYP group. Although the finding was not statistically significant, HYP group had a greater increase in maximal isometric strength (13.8 ±42.9 %, p=0.001) compared to MAX+POW group (6.7 ±44.8 %, p=0.008). Of the supplement regimens, PROT group (p=0.001) and of the training programs, HYP group (p=0.001) had the most pronounced effects on all the variables of body composition shifting the values towards leaner body composition.

Conclusion. The main finding of this study was that the present resistance training intervention resulted in healthier metabolite profiles among the whole group of participants. Especially, dyslipidemia biomarkers shifted towards better values reducing the risk of having metabolic diseases. Moreover, enhancements were produced also in body composition and maximal muscle strength. The importance of resistance training should be highlighted as it appears to have broad beneficial influence in metabolism and body composition in previously untrained men thus resulting in a better health state.

Keywords: post-workout supplements, resistance training, serum metabolome, metabolomics, muscle strength, muscle hypertrophy, body composition

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ABBREVIATIONS

APO A_1	Apolipoprotein A_1
APO B	Apolipoprotein B
BCAA	Branched-chain amino acids
BMI	Body mass index
CHO	Carbohydrate supplementation group
CSA	Muscle cross-sectional area
CVD	Cardiovascular diseases
HDL	High-density lipoprotein
HYP	Hypertrophy training group
IDL	Intermediate-density lipoprotein
LDL	Low-density lipoprotein
MAX+POW	Combined maximal and power training group
MS	Mass spectrometry
mTOR	Mammalian target of rapamycin
NMR	Nuclear magnetic resonance
PROT	Protein supplementation group
PROT+CHO	Combined protein and carbohydrate supplementation group
RFD	Rate of force development
TG	Triglyceride
VL	Vastus lateralis
VLDL	Very-low-density lipoprotein
VO_2max	Maximal oxygen uptake

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1 INTRODUCTION

Nowadays, there are more and more sedentary people. Such a lifestyle has led drastically to increased prevalence of obesity, metabolic syndrome and cardiovascular diseases in which the metabolic pathways are closely related. These diseases influence many people's health and even threaten their life world-wide. (Krauss et al. 2000; Kujala et al. 2013.)

Human metabolism is a complex mixture of biochemical pathways which are in close relation to one another. Proper functioning of these pathways prevents development of metabolic diseases. (German et al. 2005.) Metabolites, the building blocks of metabolism, are important message transporters and as such they are great measures of physiologic state (Orešič et al. 2007). Traditionally, biochemical assessments have been done, e. g. for total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride, as fat metabolism has been studied. However, with these measurements it may be impossible to find the real factors underlying in impaired metabolism. For understanding the whole metabolism, more comprehensive assessments are needed. (German et al. 2005.)

Several years ago, a methodology of metabolomics has been applied in the field of epidemiology. It has been used to interpret complex effects of environment and lifestyle habits in the field of pathophysiology. Metabolomics enables exploring structures of small metabolites, their function and synergism within human cells, tissues, blood and bodily secretions (Orešič et al. 2007.) A promising method of metabolomics, nuclear magnetic resonance spectroscopy (NMR), has been used in several epidemiological studies (e. g. Mora et al. 2009; Nicholson et al. 2011; Wiklund et al. 2014). On a metabolome level, it provides comprehensive quantitative information on various amino acids, glycolysis intermediates, fatty acid composition and degree of saturation and lipoprotein subclass distributions (Wiklund et al. 2014).

Adequate nutritional inputs and physical activity form the base for regulating normal metabolic processes and further lowering risk of metabolic diseases.

(German et al. 2005; Kujala et al. 2013; Soininen et al. 2015). For example, benefits of physical activity have been seen in serum lipids (Hu et al. 2001), body composition, glucose and insulin (Sillanpää et al. 2009), amino acid metabolism (Yan et al. 2009), lipoprotein profiles and higher levels of polyunsaturated relative to total fatty acids (Kujala et al. 2013) in the active individuals compared with inactive individuals. Metabolomics has not been applied to sports medicine yet. However, it is a likely method as it has the potential to identify the biomarkers associated with performance, fatigue, and even sports-related disorders. (Yan et al. 2009.) With such promising results from studies of metabolomics, in the future physical activity may be more applied as a treatment for metabolic disorders and cardiometabolic diseases (Kujala et al. 2013).

The aim of this study was to examine the effects of different post-exercise supplementation regimens and different resistance training regimens on metabolic factors of human metabolism, the emphasis being on fat metabolism. Furthermore, the effects of nutrition and resistance training on muscle hypertrophy and muscle strength were studied.

2 NUTRITION

2.1 Recommendations of protein and carbohydrate intakes for resistance training

Proper nutrition is a cornerstone for adequate development, both physical and intellectual. Therefore, a diet should consist in proper amounts of proteins, carbohydrates, fats, vitamins and minerals. (Wiktorowska-Owczarek et al. 2015.) Nutrition has an important role in resistance training as it prepares the body for a workout, enhances recovery from the training and promotes training adaptations such as skeletal muscle hypertrophy (Slater & Phillips 2011). An intake of 1.6-1.7 g/kg/d of protein is recommended for resistance training athletes (Phillips 2004). That amount of ingestion is usually easily achieved by resistance training athletes (Phillips 2004; Slater & Phillips 2011). The topic has been much debated over past few years but it seems that intakes greater than above mentioned offer no further benefit and even promote increased amino acid catabolism and protein oxidation (Moore et al. 2009). Considering proper doses of protein per meal, Morton & colleagues (2015) summarize dose of 20 g of protein being the maximally effective protein dose per meal for healthy, young individuals (Morton et al. 2015).

A range of 4-7 g/kg/d of carbohydrate intake are considered a reasonable amount for resistance training athletes to cover demands due to resistance training. Too low carbohydrate intake can result in impaired training or competition performance in any session or event that relies on rapid and repeated glycogen breakdown. (Slater & Phillips 2011).

2.2 Nutritional demands after a resistance training workout

Muscle tissue does not undergo significant cell replacement through life. Therefore, an efficient method is required to avoid apoptosis and maintain skeletal mass. (Schoenfield 2013.) Acute periods of imbalance, created by resistance training, between muscle protein synthesis and muscle protein breakdown are

needed, resulting in a positive net protein balance with enough protein ingestion. Over time, these individual acute periods of positive protein balance provide a net gain in muscle fiber content and furthermore in cross-sectional area resulting in hypertrophy. (Phillips 2004; Tang & Phillips 2009; Tipton et al. 1999.) Due to this elevation in muscle protein synthesis, it is most advantageous to ingest protein and generate hyperaminoacidemia in the post-resistance training period (Morton et al. 2015; Tipton et al. 1999). Also, as infused and ingested amino acids have been compared they seem to be equally effective for producing hyperaminoacidemia and net muscle protein synthesis (Tipton et al. 1999). Furthermore, it may not be necessary to include nonessential amino acids to create an anabolic response from muscle after exercise which highlights importance of the role of essential amino acids (Naclerio & Larumbe-Zabala 2016).

Sport increases the neuromuscular and physical demands. Post-workout nutrition is essential to support metabolic repair and nutrition requirements, especially for activities that require multiple daily workouts or repeated bouts of exertion. The aim of the post-workout nutrition is to prevent muscle glycogen loss and catabolism while augmenting glycogen repletion and muscle protein synthesis, stimulating muscle recovery pathways, and reducing inflammatory and catabolic constituents. (Lynch 2013.) A primary goal of post-workout nutrition is to replenish glycogen stores. Various studies have shown a reduction of glycogen stores ranging from 12 % to 38 % after resistance training regimen. Furthermore, muscle glycogen content mediates intracellular signaling and is therefore crucial part of muscle protein synthesis to occur. However, there exists evidence that the importance of glycogen re-synthesis is diminished for goals that are not specifically focused on the performance of multiple exercise bouts in the same day. Either way, it has been proven that adding protein to a post-workout carbohydrate meal can improve glycogen re-synthesis. (Aragon & Schoenfield 2013.)

Dietary protein is essential to activate the muscle protein synthesis pathway. In particular, mammalian target of rapamycin (mTOR) that signal initiation factors, such as p70S6K and 4EBP, is responsible for activating messenger RNA translation initiation and ribosomal activity. These events are rate-limiting steps for controlling protein synthesis. Replenishing muscle glycogen content post-workout

is important in many ways: it mitigates tissue damage, inflammatory markers, and upregulate the specific signaling pathways for muscle protein synthesis. (Lynch 2013.)

Ingesting protein after resistance exercise is essential to maximize post-exercise anabolism (Tang & Phillips 2009). Also, post-workout nutrition seems to attenuate muscle protein breakdown because spiking insulin levels reduce proteolysis. However, it is not clear how much effect the spiking insulin levels and protein breakdown have on muscle growth. Earlier, the optimal dose of protein and carbohydrate has been discussed but in addition to that the timing of protein intake is of importance. According to different studies done on that field it is suggested, that delaying post-workout nutrient intake may impede muscular gains. (Aragon & Schoenfield 2013.)

Even if nutrition has an essential role in enhancing muscle gain, the role is still small compared to the stimulus of exercise itself (Morton et al. 2015). A meta-analysis of Cermak & colleagues (2012) found only 3 of the 16 studies showing statistically significant gains in lean mass with protein supplementation (Cermak et al. 2012).

2.3 Post-workout supplementation

Due to their absorptive properties, carbohydrate and protein drinks are leading sources for post-exercise refueling. However, there is a disagreement as to which extent one of the two macronutrients is most effective after workout session, specifically as it relates to nutrient timing and supporting recovery. Some experts support use of carbohydrate only recovery supplement, while others favor the 4:1 ratio of carbohydrate to protein, and then some advocate protein only. In theory, the consumption of macronutrients and the timing of such may have their effect on the neuromuscular response to exercise by counteracting the negative physiological state that follows. The study of Lynch (2013) demonstrated that a beverage, primarily comprised of protein led to better post-exercise replenishment for

subsequent physical tests than a drink that comprised mainly of carbohydrates. (Lynch 2013.)

2.3.1 Protein supplementation

Protein supplementation is most commonly used to enhance muscle growth post-workout. A meta-analysis of Cermak and colleagues showed that protein supplementation during resistance-type exercise training (>6 wk) significantly augments the gains in fat-free mass, type I and II muscle fiber cross-sectional area (CSA) and one repetition maximum (1 RM) leg press strength within younger and older subjects compared with resistance-type exercise training without a dietary protein based cointervention. (Cermak et al. 2012.) High-quality proteins such as whey, casein and soy protein can support muscle protein synthesis. (Tang & Phillips 2009.) However, different studies have shown that the consumption of whey protein hydrolysate stimulates muscle protein synthesis more than either casein or soy. Moreover, the leucine content of the protein seems to be closely related to increased degree of muscle protein synthesis and quicker digestion. (Tang et al. 2009; Tang & Phillips 2009)

According to various studies protein supplementation provides a positive ergogenic effect on various exercise training adaptations. As a result to the increasing demand for protein supplements, sports nutrition companies and manufacturers have developed protein supplements in several forms, such as premixed protein beverages, bars, and powder supplements. However, the protein supplements contain traditionally large quantities of added sugar. To overcome this problem, sugar alcohols, or polyols, have been used in the supplements to substitute sugar. Sugar alcohols are a form of low-digestible carbohydrates that are used because of their tendency to maintain steadier blood glucose and insulin levels. On the other hand, these supplements are often high in total fat, saturated fat, and cholesterol which are associated with cardiovascular diseases and obesity. (Dugan et al. 2012.)

2.3.2 Carbohydrate supplementation

Ingestion of protein after resistance training has been studied to great extent. Instead, carbohydrate dosage and timing relative to resistance training is lacking cohesive data. Thus, there are no general uniform recommendations for carbohydrate intake. Of much importance is carbohydrate availability during and after endurance training. However, it is known that for goals being not specifically focused on the performance of multiple exercise bouts in the same day, the need of glycogen resynthesis is greatly diminished. For the goal of maximizing rates of muscle gain it seems more important to meet daily carbohydrate need instead of specific timing. Furthermore, it is well known that carbohydrate availability during and after exercise is of greater concern for endurance as opposed to strength or hypertrophy goals. (Aragon & Schoenfield 2013.)

2.3.3 Combined protein and carbohydrate supplementation

The primary purpose of combined carbohydrate and protein intake is to stimulate insulin release beyond that seen with amino acid ingestion alone. It is supposed that insulin improves net protein balance. (Morton et al. 2015.) Koopman et al. (2007) pointed out greater influence of combined carbohydrate and protein supplementation compared to carbohydrate supplementation alone. In the study, they observed increased S6 phosphorylation in both type I and II fibers in both treatments immediately after exercise. Furthermore, phosphorylation of S6 in type I fibers immediately post-exercise was substantially higher in PROT+CHO than in CHO only treatment and no differences occurred between treatments in type II fibers. The combined ingestion of protein and carbohydrate further elevates the phosphorylation status of signaling factors 4E-BP1, S6K1, and S6 during recovery from a strength training workout. (Koopman et al. 2007.)

Still, there are controversial results as it has been shown that there is no benefit of co-ingestion carbohydrate and protein on stimulating muscle protein synthesis. This finding was proven in circumstances where resistance training was combined with adequate protein intake. Adequate protein dose for optimal muscle protein

synthesis is relatively low, only 2-3 times basal resting level. (Morton et al. 2015.) Combined protein and carbohydrate supplementation has been shown to enhance post-workout glycogen re-synthesis. However, despite of beneficial results in acute studies examining post-exercise nutrition there is a lack of long-term studies examining the co-ingestion of protein and carbohydrate near training. (Aragon & Schoenfield 2013.)

3 RESISTANCE TRAINING

Resistance training develops muscle endurance, power, speed and agility, increases muscle hypertrophy, sport performance, balance and coordination (Kraemer et al. 2003). Traditionally resistance training is split into subclasses of endurance, maximal and power resistance training. Maximal resistance training has two subclasses: neural and hypertrophic resistance training. (Ratamess et al. 2009.) The pennation angle of muscle cells, muscle length, joint angle and muscle contractile speed effect on the force production of skeletal muscle (Gulch 1994). Used mode of muscle work, intensity, volume, exercises and exercise order, recovery time between series and training frequency influence the progression of force production (Kraemer & Ratamess 2004). Heavy loading (85-100 % 1 RM) develops absolute force production whereas moderate loading (30-60 % 1 RM) should be used in developing explosive force production (Peterson et al. 2004). Despite great amount of resistance training studies, it remains unknown what kind of resistance training protocol is the most effective in the light of the most anabolic or sensitizing effects (Morton et al. 2015).

3.1 Hypertrophic resistance training

Influence of resistance training on muscle growth is a complex phenomenon that is dependent of numerous physiological systems and signaling pathways. Muscle growth occurs in a sequential cascade in a following manner:

1) Muscle activation,
2) Signaling events arising from mechanical deformation of muscle fibers, hormones and inflammatory responses,
3) Protein synthesis due to increased transcription and translation
4) Muscle fiber hypertrophy. (Spiering et al. 2008.)

Hypertrophy is important in increasing maximal force production. On a cellular level hypertrophy results because of increases in muscle cross-sectional area

creating new contractile units. That again increases force production. (Folland & Williams 2007.) Increase of contractile units can occur either by adding sarcomeres in series or in parallel (Schoenfield 2013). As a result to long-term resistance training, hypertrophy occurs in type I and II muscle fibers. In human bodies hyperplasia does not occur after resistance training. Muscular growth is a multifaceted process. It starts with recruitment of motor units which cause a transformation in muscle fiber units. Thereafter, different hormones (insulin-like growth factor, testosterone, and growth hormone), immunological responses and inflammation responses get activated. The activation has influence on various signaling factors in a muscle fiber. Particularly, Akt/mTOR signaling pathway and activation of satellite cells are important regarding muscle fiber growth. (Spiering et al. 2008.) Other anabolic signaling pathways are mitogen-activated protein kinase (MAPK) and calcium-(Ca2+) dependent pathways (Schoenfield 2013).

Maximum gains in muscle hypertrophy are achieved by training regimens that produce significant metabolic stress while maintaining a moderate degree of muscle tension (Schoenfield 2013). In a resistance training regimen aiming to hypertrophy, subsequent features should be followed:

- Load of 70-100 % 1 RM.
- Number of repetitions being 1-12 (Favoring repetition number from 6 to 12). (Kraemer et al. 2000.)
- Recovery time of 2-3 minutes in between the series when main exercises are practiced and 1-2 minutes in between the series of complementary exercises.
- The number of series is high and several exercises have been done for the same muscle group. (Ratamess et al. 2009.)
- Following features should be modified occasionally:
    - Joint angle and planar
    - Exercise speed (Schoenfield 2013).

All three types of muscle actions (concentric, eccentric, concentric-eccentric) can cause significant hypertrophy at impressive rates when sufficient frequency,

intensity and duration of work are given. In designing a resistance training program, progression and individualization should be emphasized. (Wernbom et al. 2007.) Regarding progression, low volumes are recommended in the initial phase of training. Low volume has been shown to be sufficient in the early stages of training. Furthermore, it may improve exercise adherence and avoid unnecessary damage allowing hypertrophy to take place earlier. After initiation phase an individual starts to adapt to the training stimulus. Thereafter, the overall volume or intensity possibly need to be gradually increased to result in continued physiological adaptations and other strategies (e.g. periodization) can be introduced to progress still further. (Wernbom et al. 2007.)

3.2 Neural resistance training

Sports, work and daily living require maximal force production. To clarify, more power is produced when the same amount of work is completed in a shorter period or when a greater amount of work is performed during the given period. (Cormie et al. 2011; Ratamess et al. 2009.) Heavy resistance training with slow velocities improves maximal force production whereas power training (light to moderate loads at high velocities used) increases force output at higher velocities and rate of force development (RFD) (Häkkinen & Komi 1985). Power is the product of force and velocity. As the force output of muscle increases, the velocity of shortening decreases. (Cronin & Sleivert 2005; Kawamori & Haff 2004) This relationship is demonstrated as the highest power output attainable during a given movement or repetition, and has been viewed as an exceedingly important testing variable and training objective. Improving power performance requires increase of maximal RFD, force production at slow and fast contraction velocities, enhancing stretch-shortening cycle performance, and improved coordination of movement pattern and skill. (Ratamess et al. 2009).

Besides morphological adaptations, appropriate activation of the muscles involved is needed to generate maximal power during a movement (Cormie et al. 2011). Neural adaptations take place after individual resistance training session that again results in increase of force production (Gabriel et al. 2006). The primary changes

to take place are motor unit recruitment, firing frequency, synchronization and inter-muscular coordination. Considering motor unit recruitment, increased ability to rapidly recruit high-threshold motor units enhances generation of maximal muscular power (Cormie et al. 2011.) Presumably, these adaptations occur by three different ways which are increased motor unit recruitment, preferential recruitment of high-threshold motor units and lowering of the thresholds of motor unit recruitment (Sale 1988). Resistance training increases firing frequency which allows greater magnitude of force generated during contraction and impacts the RFD (Cormie et al. 2011). Theoretically, motor unit synchronization occurs due to resistance training and it is a nervous system adaptation that enhances with the coactivation of numerous different muscles to improve RFD (Semmler 2002). Inter-muscular coordination refers to the appropriate activation of agonist, synergist and antagonist muscles while performing a movement (Cormie et al. 2011). Regarding factors on a cellular level, muscle cross-sectional area and muscle fiber type are muscular factors that could contribute to high-power output. Having a higher percentage of fast-twitch muscle fibers may be beneficial in high-power outputs. (Kawamori & Haff 2004.)

Exercise order should be based on complexity of exercises, meaning that the most complex exercises should be performed early in a workout. Recovery period for power training is similar to resistance training. Taking the needed rest ensures the quality of each repetition being performed in a set. The recommended training frequency for novice is 2-3 days a week stressing the total body. (Ratamess et al. 2009.) Traditionally, the number of repetitions in neural resistance training is 1-6 repetitions (Spiering et al. 2008), and the load should be at least 80 % 1 RM to initiate neural adaptations and to achieve maximal gains in strength (Häkkinen et al. 1985; Peterson et al. 2004). Moreover, one study showed that maximal gains were elicited at by a mean intensity of 60 % of 1 RM for untrained individuals (Rhea et al. 2003). However, progression is needed in prolonged training because of gradual neural adaptations (Peterson et al. 2004). In regard of exercise intensity, it has been highlighted to train until momentary muscular failure assuring that all the available motor units and muscle fibers are actively recruited (Fisher et al. 2011).

There exist inconsistent results of the load that produces the highest power output. (Kawamori & Haff 2004). It seems that maximal power output differs, if it is expressed relative to a dynamic strength measure (% 1 RM). However, the load that maximizes power output is supposed to be between 30-60 %. In optimal case, every individual's maximal power output (P_{max}) should be determined and they should train at that load. (Cronin & Sleivert 2005.) It is suggested to use a mixed training strategy using both heavy and light loads. This is because most sports involve a mixture of activities that span the force-velocity capability of muscle. Mixed training strategy is also the best and safest course of action for those interested in improving the power output of muscle. (Cronin & Sleivert 2005.) For athletes, it is suggested to train with loads that they usually encounter in their athletic events (Kawamori & Haff 2004).

4 METABOLOMICS AND METABOLIC HEALTH

Metabolic health refers to health state of an individual related to metabolism and metabolic function (German et al. 2005; Orešič et al. 2007.) whereas metabolomics is a methodology which focuses on comprehensive metabolic profiling of multiple molecular pathways (Soininen et al. 2015). Traditionally, total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride concentrations have been assessed in assessments studying fat metabolism. However, completeness of plasma lipids reflecting metabolic state, is much more complex and biochemical pathways are not isolated systems in human body. (German et al. 2005; Orešič et al. 2007.) In that sense, traditional assessments do not give comprehensive overview of the health state of an individual. Proper functioning of metabolism prevents development of metabolic diseases (German et al. 2005). Genes and environmental factors affect fat metabolism. With traditional measurements, it may be impossible to track possible changes in fat metabolism. Metabolomics is a new method targeted to explore structures of small metabolites, their function and synergism within human cells, tissues, blood and bodily secretions. (Orešič et al. 2007.)

4.1 Applying metabolomics

Development of analytical approaches has been in huge progress in last two decades. Their target is to analyze different cell products, such as those from gene expression, proteins, and metabolites. These approaches are so-called ‘omics approaches which include genomics, transcriptomics, proteomics, and metabolomics. These important tools have been applied to understand the biology of an organism and its response to environmental stimuli or genetic perturbation. (Roessner & Bowne 2009.) Metabolomics has been used to understand better and wider different diseases. It helps to interpret complex effects of environment and lifestyle habits in the field of pathophysiology (Orešič et al. 2007).

4.1.1 General methods

Blood remains the reservoir of metabolic assessment for most applications of routine health because it is a central reservoir integrating across the entire organism. Blood is in the central role in transporting metabolites. Therefore, metabolic profiling is capable of simultaneously recognizing metabolic status and suggesting optimal strategies for intervention in metabolic diseases. With metabolite profiling it is possible to distinguish whether a disorder is due to substrate imbalances or catalytic activities. (German et al. 2005.)

The most usual methods of metabolomics are mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR). (Orešič et al. 2007.) Also, chromatographic systems have been used in metabolomics. These technologies provide first look at integrated metabolism revealing how it affects human health. Earlier, mass spectrometry has been more popular and because of its sensitivity, it is useful in studying and comparing metabolites of different sizes (German et al. 2005). However, there are some drawbacks of MS. The analysis of the quantitative MS is robust, the per-sample costs are high and it cannot analyze lipoproteins. NMR instead is currently the only methodology which enables reproducible high-throughput metabolite quantifications in a cost-effective manner. The only disadvantage of NMR is that it is not as sensitive for metabolite detection as MS. However, a few minutes' measurement time is enough to capture a comprehensive molecular signature from a serum sample. (Soininen et al. 2015.)

4.1.2 NMR spectroscopy

NMR spectrometers and techniques have been routinely used in many kinds of biomolecular and clinical research (Ala-Korpela 1995). The method provides absolute quantitative information on about 140 metabolic measures. (Inouye et al. 2010; Soininen et al. 2009) NMR quantifies the most abundant metabolites in biofluid, typically those above 10 micromoles in concentration (Nicholson et al. 2011). Considerable progress has been achieved in basic research and in clinically oriented applications of specific type of NMR, high-throughput proton nuclear

magnetic resonance spectroscopy in which hydrogen protons are normally assessed (Ala-Korpela 1995). The benefit of NMR metabolomics is its ability to measure the concentrations of standard biomarkers, such as various cholesterol measures, triglycerides, and creatinine, but in the same experiment it provides also quantitative molecular data on lipoprotein subclasses. These subclasses are lipids, fatty acids and apolipoproteins as well as various low-molecular-weight metabolites, such as amino acids, glycolysis-related metabolites, and ketone bodies. (Soininen et al. 2009.)

This methodology is still in the middle of development and only small amount of information of human metabolome can be tracked. Traditionally, for example basic glucose and cholesterol measurements have been done but the developers of the NMR method aim tracking a metabolic fingerprint which would show wide biochemical unities in different diseases and their treatments. Specifically, discovering human metabolomes helps to see how genetic variation affects metabolic phenotype of complex diseases. (Orešič et al. 2007.) However, at the current moment there is no single technology available to analyze the entire human metabolome.

Sample processing and data analysis are hard but once optimized, these methods produce highly quantitative data on many metabolites simultaneously. (German et al. 2005). Because the NMR method produces great number of data, data handling needs to be systematic and well developed with metabolomics. (Orešič et al. 2007.) The current way of thinking needs to be challenged in a way that it is possible to deal with large data sets and distinguish between noise and real sample-related information. Still, scientists are optimistic and they believe that in the future, metabolomics may enable development of new approaches in medicine that will be predictive, preventative, and personalized. (Roessner & Bowne 2009.) Under optimal circumstances, metabolomics is cost- and time-effective as an individual's metabolome can be tracked with one single measurement (Soininen et al. 2015).

4.2 Metabolic health

The background of most diseases is found in abnormal enzyme activity, improper substrate balance or abnormal metabolic regulation. These influences are acting to disturb normal metabolism. (German et al. 2005.) Nowadays, growing state of sedentary lifestyle leads to an increased tendency for poor metabolic profiles, obesity, and cardiovascular diseases resulting in a severe health burden (Krauss et al. 2000; Kujala et al. 2013). Long-term abnormalities in metabolism cause chronic and metabolic diseases. For a proper functioning of metabolism, all the metabolic pathways must act appropriately and metabolic needs must be balanced by nutritional inputs. New applications of metabolomics help to integrate single metabolites to create a comprehensive strategy facing the health challenges. The developers of the technology assessing human metabolism aim to develop a method that is personalized and focuses on prevention rather than diagnosis. With metabolomics measurements, treatments can be tailored to the molecular basis for the processes of diseases. (German et al. 2005.)

4.2.1 Epidemiological studies

Atherogenic dyslipidemia, elevated blood pressure and elevated plasma glucose are the most widely recognized metabolic risk factors. Atherogenic dyslipidemia is a state in which lipoprotein abnormalities occur, such as elevated serum triglyceride and apolipoprotein B concentrations, increased small LDL particles and reduced level of HDL cholesterol. These factors alone or combined speed up progression of atherosclerotic disease. Also, other metabolic risk factors appear individually to be atherogenic, such as a prothrombotic state and a proinflammatory state. (Grundy et al. 2005.)

The metabolic syndrome and type II diabetes are increasing in prevalence in both developed and developing countries (Gill & Malkova 2006). The metabolic syndrome is a constellation of endogenous risk factors that increase the risk of developing both atherosclerotic cardiovascular disease (CVD) and type 2 diabetes. The syndrome is strongly associated with the presence of abdominal obesity.

However, the syndrome can occur among people without abdominal obesity indicating impaired functioning of metabolism. (Grundy et al. 2005.) Moreover, hyperglycemia seems to be closely related to lipid and lipoprotein metabolism, meaning that hyperglycemia and dyslipidemia are likely to share similar pathophysiological mechanisms. (Stancáková et al. 2011.) Hyperglycemia can be associated with detrimental lipid profiles among non-diabetic individuals also (Zhang et al. 2008). Factors or mechanisms explaining the development of the metabolic syndrome remain poorly understood. Therefore, they are intensely investigated as their understanding could help designing novel therapeutic strategies. (Wiklund et al. 2014.)

With traditional assessments, it is possible to measure several single biomarkers such as serum cholesterol. Measuring cholesterol as a biomarker gives a quantitative estimate of disease risk of an individual within a population. However, it does not provide sufficient information to conclude why cholesterol is accumulated or which would be appropriate intervention to cut out the problem. With metabolite profiling it is possible to find answers to these questions. There are several mechanisms causing accumulation of cholesterol, such as abnormal absorption of cholesterol through the intestine, excessive production of cholesterol through endogenous biosynthesis and too slow conversion of cholesterol into bile acids. With metabolite profiling the mechanism causing accumulation of cholesterol can be tracked. (German et al. 2005.)

Lately, NMR has been used in several epidemiological studies to explore large number of subjects. Basic idea of the method is explained in the paragraph of methods. NMR gives a deep understanding of health status on serum metabolome level (Kujala et al. 2013). In the field of cardiometabolic diseases, NMR method has been applied to study influence of metabolism in overweight and obese premenopausal women with and without metabolic syndrome (Wiklund et al. 2014), risk and presence of cardiovascular diseases (Mora et al. 2009), and associations of serum metabolome and differing glucose tolerance levels (Wang et al. 2014). It has been used to study many other conditions as well, such as the use of hormonal contraception (Wang et al. 2016), the reasons underlying mortality (Fischer et al. 2014) and the influences of genetic and long-term environmental

background on human metabolic profile (Nicholson et al. 2011). With NMR metabolomics, there has been a finding of four biomarkers which predict the risk of short-term death. These biomarkers are albumin, glycoprotein, VLDL lipoprotein particle size, and citrate. These biomarkers are implicated in various pathophysiological mechanisms, including fluid imbalance, inflammation, lipoprotein metabolism and metabolic homeostasis. (Soininen et al. 2015.)

Metabolites are important message transporters and therefore they are great measures of physiologic state (Orešič et al. 2007). It is important to highlight that certain amounts of metabolites do not tell much. Researchers and doctors need to understand how pathways and their respective reactions function before making conclusions and giving practical applications considering diet, drugs and lifestyle for example. (German et al. 2005.) Also, some details concerning subject need to be considered when interpreting the data, such as individual variation and individuals' different adaptations to external and internal stimuli. (Orešič et al. 2007.)

4.2.2 Physical activity studies

Physical activity, and especially long-term physical activity, maintains good metabolic profile and further lowers risk of metabolic diseases. (Kujala et al. 2013; Soininen et al. 2015). Moreover, physical activity offers protection against CVD (Gill & Malkova 2006). Benefits of physical activity have been seen in serum lipids and lipoproteins (Hu et al. 2001, Kujala et al. 2013), body composition, blood pressure, glucose and insulin (Sillanpää et al. 2009). Anyhow, the magnitude of benefits in lowering risk of metabolic diseases and CVD is heterogenous: some individuals experience greater reductions than others (Gill & Malkova 2006).

Earlier, assessments of biochemical markers have been used among athletes but there is no information concerning one's metabolism as an ensemble. There are various biochemical assessments used for evaluating athletes' physical status but no available universal method for the diagnosis and monitoring. In the future, metabolomics could provide a novel analytical platform to monitor athletes'

physiological state and diagnose the disorders induced by exercise. Even if metabolomics has been used in biomedical sciences, it has not been applied to sports medicine. Metabolic studies have the potential to identify the biomarkers associated with performance, fatigue, and even sports-related disorders. The level of endogenous metabolites will change accordingly as physical exercise will deplete the nutrition, energy, elevate the metabolism, and generate more metabolic products. (Yan et al. 2009.) The serum NMR metabolomics platform enables concurrent examinations of various metabolic pathways (Soininen et al. 2015), which could presumably add our knowledge in sports-related changes in metabolism.

Yan et al. (2009) have shown remarkable differences on metabolome between rowers and control subjects. Strength-endurance type of sport affected glucose metabolism, lipid metabolism, oxidative stress and amino acid metabolism. More accurately, professional rowers exhibited significant elevation of alanine, lactate, cysteine, glutamic acid, valine, glutamine, and some unidentified compounds, notably declined level of B-D-methylglucopyranoside, citric acid, palmitic acid, linoleic acid, and oleic acid. However, no biochemical parameters (e.g. hemoglobin, testosterone, and creatine kinase) had a significant difference in long-term trained rowers compared to control subjects which highlights the need of measurements which assess the whole human metabolome. (Yan et al. 2009.)

Recently, promising NMR method has been used in a couple of studies to reveal differences in the serum metabolome between persistently active and inactive individuals (Kujala et al. 2013; Mukherjee et al. 2014) Figure 3 shows the results of different metabolic factors between active and inactive twins. In that study twin pairs, discordant for physical activity for >30 years and individuals from three population-based cohorts who were persistently (± 5 years) active or inactive, were studied in comparison with one another to consider differences in their metabolic health. Serum NMR metabolomics were applied to create a comprehensive coverage of systemic metabolism. The results of the study illustrate persistent physical activity being associated with a characteristic multivariate metabolic profile both in twins and in pairs identified from the population cohorts. The main findings were better lipoprotein profiles and higher levels of polyunsaturated

relative to total fatty acids in the active individuals compared with inactive individuals. Furthermore, lower isoleucine and lower glycoprotein concentrations relate to persistent physical activity. The results support the efforts for increased physical activity as a treatment for metabolic disorders and cardiometabolic diseases. (Kujala et al. 2013.)

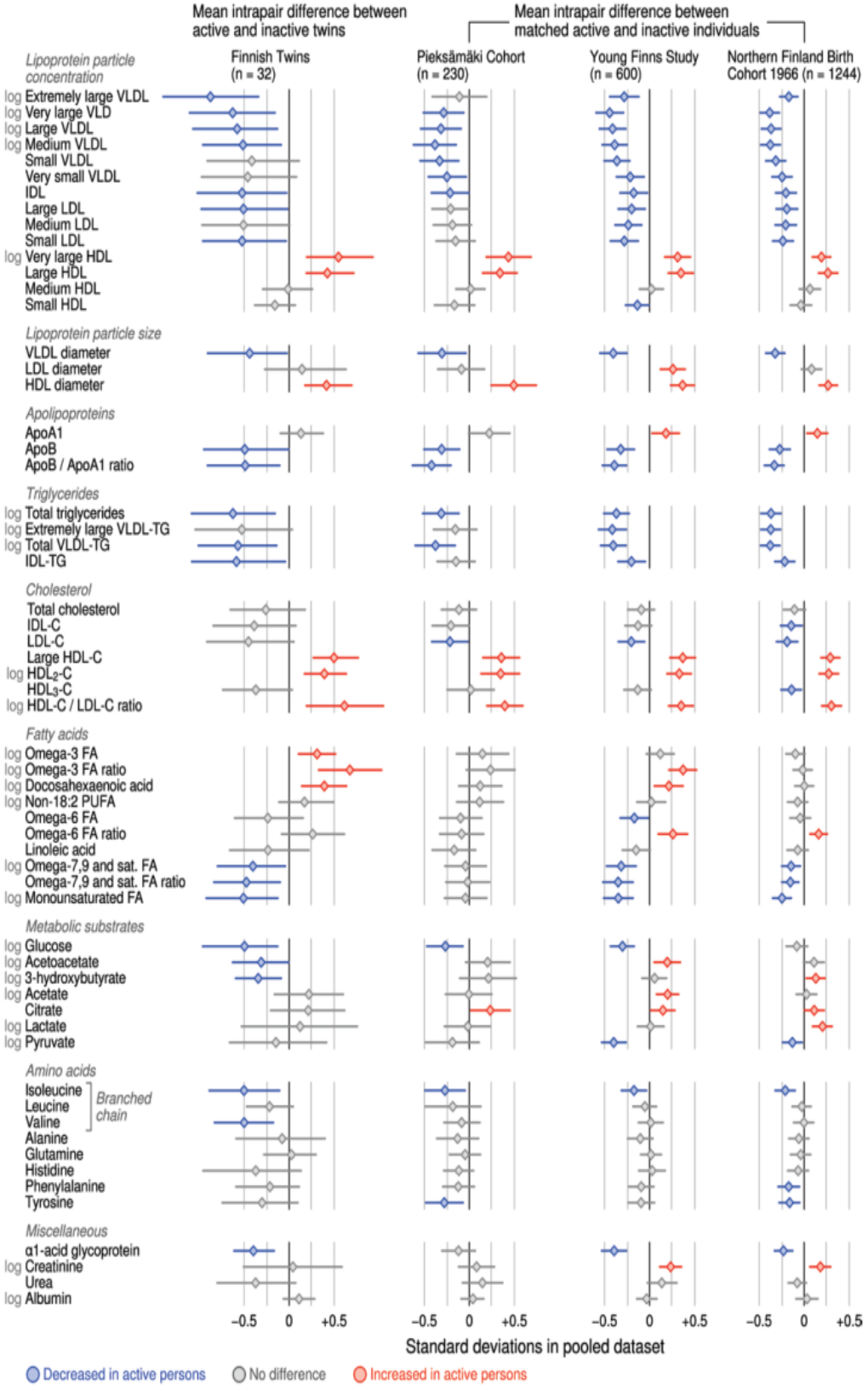

FIGURE 1. Results of the study of Kujala and colleagues (2013).

5 SERUM METABOLOME

The metabolome consists of all small molecules which can be found in a specific cell, organ or organism. The metabolome, the transcriptome, the genome and the proteome form the building block of systems biology. Nowadays, most of the human genome, transcriptome and proteome are known and the data are electronically available. Unfortunately, this is not the case with the human metabolome. There is a database created for human metabolome called *Human metabolome database* (*HMDB*). It brings together quantitative, chemical, physical, clinical and biological data of thousands of endogenous human metabolites. *HMDB* could be even wider but to make it both relevant and reasonable, the database includes metabolites with following criteria: the compound must weigh <1500 Da, it should be found at concentrations greater than 1 micromole in one or more biofluids or tissues and it should be of biological origin. (Wishart et al. 2007.)

In the following section, main metabolic groups and their metabolites are described. In this thesis, the main interest is in lipoproteins and apolipoproteins, and in specific lipids. Therefore, some subgroups are excluded.

5.1 Lipoproteins and apolipoproteins

Lipoprotein particles consist of an insoluble lipid core surrounded by a coat of phospholipid, free cholesterol and apolipoproteins. Each class of lipoprotein particle has its specific apolipoproteins. Apolipoproteins stabilize lipoprotein structure and play an essential role in regulating metabolism. (Walldius & Jungner 2004.) The classification of lipoproteins is based on the density at which they float by ultracentrifugation. (Mahley et al. 1984.) In recent years, the value of lipoprotein subclass data in understanding the complex pathways in lipoprotein metabolism, has raised. For example, total cholesterol sums up all the cholesterol molecules in circulation but it does not distinct the lipoprotein particle it carries with. The lipoprotein metabolism is complex and it involves various particle subclasses that have different and even opposite biological roles. LDL and HDL particles are good

represents of such case and most commonly known lipoprotein subclasses. (Soininen et al. 2015.)

5.1.1 Lipoproteins

The plasma lipoproteins are commonly split into six major classes which are chylomicrons, chylomicron remnants, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). (Mahley et al. 1984.) Our specific interest - regarding this study - is in the lipoproteins and not in chylomicrons or chylomicron remnants. It is commonly known that high concentration of HDL cholesterol is associated with lower risk of CVD (Krauss et al. 2000), whereas high concentration of LDL cholesterol in the circulation has an additive effect on CVD risk (Davidson et al. 2011).

VLDLs transport triglycerides and cholesterol from the liver for redistribution to various tissues. Thereafter, the triglycerides of VLDL are hydrolyzed to free fatty acids by lipoprotein lipase generating a series of smaller, cholesterol-enriched lipoproteins including IDLs and LDLs. The LDLs are formed as end-products of VLDL catabolism and they are the major cholesterol-transporting lipoproteins in the plasma. HDLs seem to arise from several sources such as the liver and intestine. (Mahley et al. 1984.)

Different lipoproteins have their specific diameter of particle. The size of a lipoprotein is from the biggest to the smallest in a subsequent manner: VLDL, IDL, LDL and HDL. By contrast, the density of lipoproteins from the greatest to the lowest is reversed. The densities for VLDLs, IDLs, LDLs and HDLs are < 1.006 g/ml, 1.006-1.019 g/ml, 1.019-1.063 g/ml and 1.019-1.063 g/ml, respectively. (Mahley et al. 1984.) In figure 2 specific particle sizes of lipoproteins and their direction in circulation are illustrated.

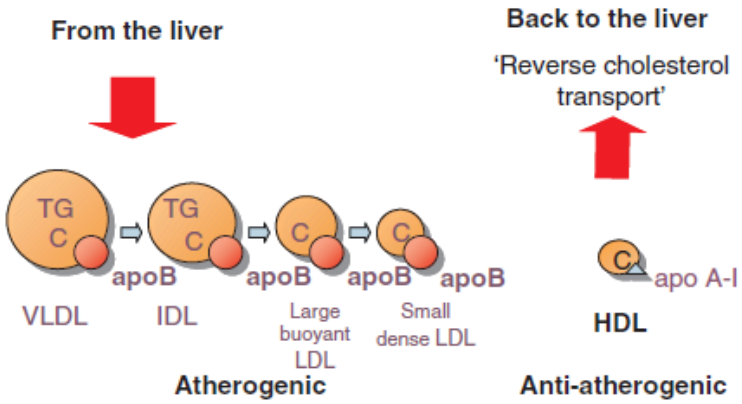

FIGURE 2. Atherogenic and anti-atherogenic lipoproteins (Walldius & Jungner 2004).

Recent understanding of lipoproteins highlights the importance of lipoprotein size and subclass composition in addition to traditionally screened lipid concentrations. For example, patients with type 2 diabetes typically present abnormalities in lipid concentrations, lipoprotein size and subclass composition. At least LDL size seems to be inversely associated with incident of diabetes. Furthermore, VLDL particle size and small HDL particles were shown as significant contributors to incident of diabetes. (Festa et al. 2005.) Also, it has been proposed that total LDL particle concentration predicts better risk of cardiovascular diseases than does LDL cholesterol. Concerning HDL, it seems that the size of the lipoprotein is the one that matters: in the study of Mora et al. (2009) only large HDL particles were associated with lower CVD risk. (Mora et al. 2009.)

5.1.2 Apolipoproteins

Specific apolipoproteins have several major functions, for example transport and redistribution of lipids among various tissues, role as a cofactor for enzymes of lipid metabolism and maintenance of the structure of the lipoproteins. The co-operation of apolipoproteins and their specific cell surface receptors is essential in delivery of lipids to specific cells. (Mahley et al. 1984.)

Apolipoprotein A_1 (apo A_1) is the major component associated with HDL cholesterol. (Walldius & Jungner 2004.) It has been found also in chylomicrons but

it is rarely present in significant amounts on chylomicron remnants, VLDL, their remnants, or LDL. The two major synthesis sites of apo A_1 synthesis are the intestine and the liver. (Mahley et al. 1984.) Low levels of apo A_1 have consistently been associated with an elevated risk of cardiovascular events (Walldius & Jungner 2004). Apolipoprotein B (apo B) is a primary apolipoprotein of chylomicrons, VLDL, IDL and LDL lipoproteins (Mahley et al. 1984). Apo B is synthesized in the intestine. With dietary triglycerides and free cholesterol absorbed from the gut lumen it forms chylomicron particles. Apo B is essential for the binding of LDL particles to the LDL receptor which causes influx of LDL into the cell and absorption of cholesterol. Therefore, an excess of apo B-containing particles is a crucial part in the atherogenic process. It has been concluded that baseline apo B level is even stronger predictor of cardiovascular risk than LDL cholesterol. (Walldius & Jungner 2004.)

Apo B summarizes the number of atherogenic lipoproteins whereas apo A_1 represents the atheroprotective capacity. Therefore, the ratio of apo B to apo A_1 reveals individuals' lipoprotein balance and thus serves as a good predictor of CVD risk. Other apolipoproteins are apolipoprotein C and E. Apo C is associated with chylomicrons, VLDL- and HDL lipoproteins. (Walldius & Jungner 2004.) Apolipoprotein E is a component of chylomicrons, chylomicron remnants, VLDL, IDL and HDL (Mahley et al. 1984; Walldius & Jungner 2004).

5.2 Lipids

Lipids, more commonly known as fats, are essential elements of the diet. They are used as highly energetic material in human metabolism. (Wiktorowska-Owczarek et al. 2015.) Fat is absorbed by the cells of the small intestine in the form of fatty acids and cholesterol. Thereafter, esterification of fatty acids to triglycerides and of the cholesterol to cholesterol esters takes place. Subsequently, lipoprotein particles are formed containing an outer core of phospholipids, cholesterol and apolipoproteins, with an inner core of neutral lipids, meaning primarily triglycerides with some cholesterol esters. (Cianflone & Paglialunga 2006.) Lipids consist of a carbon chain and carboxyl group and they are non-water soluble. Most

of the lipids are hydrophobic and amphipathic. Amphipathic lipids have a polar head which is hydrophilic as well as a non-polar head which is hydrophobic. (Ranallo & Rhodes 1998.) Lipids exist as simple and complex compounds. Esters of fatty acids and various alcohols are included in the simple fats. (Wiktorowska-Owczarek et al. 2015.) Lipids have important tasks as components of cell membrane, energy reserve and message molecules (German et al 2005).

5.2.1 Fatty acids and triglycerides

Triglyceride (TG) molecule is made of three fatty acid chains which have been esterified into a glycerol molecule. TGs are hydrophobic and therefore they are stored in lipid droplets into subcutaneous and deep visceral adipose tissue. The major part of adipose tissue is in abdominal cavity. These depots represent approximately 95 % of the total energy stores in human body. Fat storage functions as energy storage and serves for heath production. Lipid droplets are located adjacent to mitochondria in cells. TGs are stored also in skeletal muscle, lipoproteins, plasma ketones and ketoacids. They may function as readily available fuel for oxidative metabolism, especially during exercise. (Ranallo & Rhodes 1998; Bonen et al. 2006.)

Hydrolysis of TG is initiated by physiological signals, such as catecholamines, cortisol and reduced insulin concentrations. In the hydrolysis of TG, fatty acids are released into the circulation and to several tissues, including skeletal muscle. These circulating fatty acids are a primary source for skeletal muscle fatty acid oxidation, and precursors for the formation of intramuscular fatty acyl coenzyme A, diacylglycerols, ceramides and TGs. The total quantity of intramuscular TG is only 1-2 % of the TG depot. (Bonen et al. 2006.)

It is well known that high concentration of TGs is in association with higher risk of cardiovascular diseases. Energy intake that exceeds energy consumptions leads to a higher concentration of TGs. (Krauss et al. 2000.) An imbalance between fatty acid uptake and fatty acid disposal cause an accumulation of intracellular TG. Rather than an increased delivery of free fatty acids to muscle, it seems more likely

that impaired disposal via oxidation is the principal basis for accumulation of TG deposition in muscle and other potentially active products of fatty acids. (Wolfe 2006.)

Omega-3 and omega-6 fatty acids are polyunsaturated fatty acids and essentials for the human body as they cannot be synthesized by humans in sufficient amounts. Therefore, they need to be provided with food. Omega-3 fatty acids have a significant role in the process of blood coagulation, inflammation, regulation of blood vessel contractility and proper brain and eye retina functioning (Wiktorowska-Owczarek et al. 2015). Reduction of saturated and trans-fatty acids as well as increased consumption of mono- and polyunsaturated fatty acids is important in the treatment of dyslipidemia and prevention of CVD risk (Ooi et al. 2013). Also, Jelenkovic et al. (2014) showed that higher concentrations of serum omega-6 fatty acids and lower concentrations of monounsaturated fatty acids are strongly associated with lower triglyceride and VLDL particle concentrations. (Jelenkovic et al. 2014).

5.2.2 Cholesterol

The basic structure of cholesterol is a sterol nucleus, which is synthesized from multiple molecules of acetyl coenzyme A. The sterol nucleus can be modified to whether cholesterol, cholic acid or a steroid hormone by adding various side chains. Cholesterol is obtained by the diet but the liver, as well as some cells in small amounts, synthesize cholesterol. In the diet, most cholesterol is in the form of cholesterol esters. (Guyton & Hall 2011, 793, 826-827.) Plasma lipoproteins transport cholesterol in the circulation and cholesterol is stored in the liver as cholesterol esters (Hoving 1995).

Cholesterol esters consist of free cholesterol and one molecule of fatty acid. About 70 % of the cholesterol in the lipoproteins of the plasma is in the form of cholesterol esters. Factors that affect plasma cholesterol concentration are the amount and type of fat ingested every day as well as some hormones, such as insulin and thyroid hormone. (Guyton & Hall 2011, 826-827.) Inadequate transport of cholesterol

leads to accumulation of cholesterol esters in blood vessel walls, uptake by macrophages, formation of foam cells and subsequent plaque formation. This state is known as a disease called atherosclerosis. (Hoving 1995.)

The primary function of cholesterol is forming specialized structures, mainly membranes with phospholipids, in all cells of the body. Other functions of cholesterol are its conversion to cholic acids and of the cholic acids into bile salts; conversion into adrenocortical hormones; progesterone, estrogen, and testosterone. (Hoving 1995; Guyton & Hall 2011, 826-827.)

6 PURPOSE OF THE STUDY AND RESEARCH QUESTIONS

The aim of the study was to provide a comprehensive overview of the associations between different post-exercise supplementation regimens and different resistance training regimens with serum metabolome. The empirical part of the thesis is based on 16-week resistance training study in which the subjects (n=60) were recreationally active men with normal BMI. More in detail, the purpose of the study was to clarify the effects of both protein and carbohydrate supplementation and their combination as well as resistance training on different metabolic factors of human metabolism, muscle hypertrophy and muscle strength. Because research evidence is scarce regarding metabolomics and physical activity and diet, the research questions are set to be rather wide.

Research questions and hypotheses are as follows:

Do different post-workout supplements have effect on serum metabolome?

Hypothesis: Protein supplementation results in gains in muscle cross-sectional area (Cermak et al. 2012, Cribb et al. 2007, Hulmi et al. 2009, Morton et al. 2015), increases in lean body mass/fat-free mass (Cribb et al. 2006, Ha & Zemel 2003, Naclerio & Larumbe-Zabala 2015, Volek et al. 2013), decreases in different fat variables (Ha & Zemel 2003; Mekary et al. 2015; Westcott 2012), and counteracts better the post-workout catabolic state that occurs than carbohydrate supplementation (Lynch 2013). Therefore, it seems probable that there would be greater adaptations on a serum metabolome level as well after consuming protein supplement.

Do different resistance training regimens affect serum metabolome?

Hypothesis: Hypertrophic resistance training induces greater adaptations in serum metabolome than neural resistance training since hypertrophic resistance training produces greater metabolic stress post-exercise in human body (Schoenfield 2013).

7 METHODS

7.1 Subjects

A total of 86 healthy, recreationally active men participated in the study. Recruiting was done by newspaper, email and university web page advertisements. The inclusion criteria required subjects being between the ages of 18 and 45 years old. Smokers and subjects with chronic diseases or those having abnormal resting ECG or using prescribed medications were excluded from the study. Concerning training subjects were expected not to have earlier background of more than one year of systematic resistance training by the time of enrolling in the study. The study was conducted in Jyväskylä at spring term 2014. Total duration of the study was 16 weeks. During the study, the subjects were not allowed to ingest any other nutritional supplements than those provided excluding basic vitamins and mineral supplements. Prior attending the study, subjects went through medical screening. Following comprehensive verbal and written explanations of the study, all subjects signed the written informed consent to the study. The study was conducted according to the Declaration of Helsinki and approved by the Ethical Committee at the University of Jyväskylä and by the Ethical Committee of the Central Hospital in Jyväskylä.

7.2 Study protocol

7.2.1 Study design

Subjects went through baseline measurements in the week 0 and after that a familiarization period of muscle-endurance training was followed for four weeks. Under that time subjects conducted whole-body workouts two times a week. Of the exercises, bilateral leg press, bilateral knee extension and bilateral knee flexion were performed in each workout. The other main muscle groups (chest and shoulders, upper back, trunk extensors and flexors, and upper arms) were exercised once a week in a rotating manner. On average nine exercises were included in a

workout. The number of sets was 2-3 for every exercise and the number of repetitions varied from 10 to 15 per set. Recovery time was two minutes between the sets. Training loads were 50-80 % of 1 RM progressing throughout the familiarization period.

Prior to randomization of the subjects, there was a drop-out of eight subjects during the familiarization period resulting in 78 subjects (age 34.4 ± 1.3 yr, height 179.9 ± 0.8 cm, weight 83.6 ± 1.4 kg) starting the resistance training study with different supplementary nutrition and differing resistance training programs. This group of subjects went through pre-measurements in the week 4 and consequently, was randomized into three supplementary groups: whey protein (PROT, n=25), carbohydrates (CHO, n=25) or whey protein + carbohydrates (PROT + CHO, n=28). The study groups were created in a way that the subjects of one group were equal as height, weight and body mass were taken into consideration. In this case a double-blind protocol was used. The exact nutrient amounts of every post-workout drink are declared below in the paragraph of nutritional supplementation.

Thereafter, the subjects were further split into two different resistance training regimens being either a group aiming especially for muscle hypertrophy and strength (HYP, n=37) or a group aiming especially for muscle strength and power (MAX+POW, n=23). The training phase lasted for 12 weeks and post-measurements took place right after that in the week 16. In the pre- and post-measurements, the following measurements were performed: blood sample collection, cross-sectional area (CSA) of *vastus lateralis* taken by ultrasound imaging and measurements of maximum isometric force exertion in leg extension dynamometer. Figure 3 illustrates the study design and the different phases of the study.

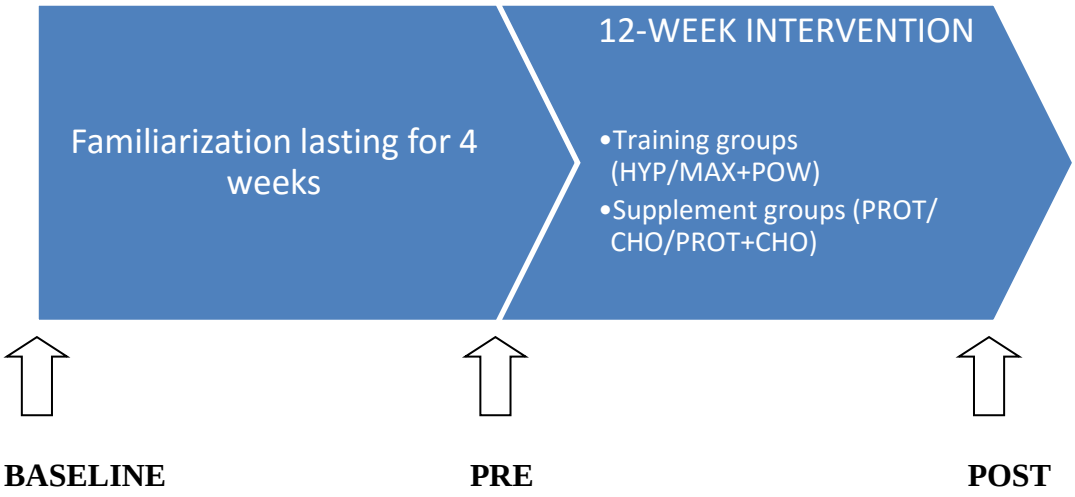

FIGURE 3. Study design. Arrows indicate the timepoints of the measurements.

7.2.2 Training protocol

In the program of MAX+POW group, the emphasis was set on developing power strength features and in the program HYP group the emphasis was set on developing maximal force and increasing muscle mass. During the training phase, the subjects trained 2-3 times a week, depending on the phase of the training program. Each training session was supervised to control correct training technique. The individual loads were determined by the strength tests for all main exercises. The intensity of training increased progressively through the training period. Emphasis of the training program was on muscles of the lower body. The exercises used in the workouts were the same as in the familiarization period with similar division.

7.2.3 Training program

The 12-week training period was split further into three different blocks. Every block consisted of four weeks of resistance training, with five to seven exercises in each training session. For the MAX+POW group, the magnitudes were 25 %, 75 % and 87.5 % for power resistance training sessions and 75 %, 25 % and 12.5 % for the maximal resistance training sessions, respectively. Instead, the magnitudes

of training sessions were split as follows for the HYP group: 100 %, 75 % and 25 % for the hypertrophic resistance training sessions and 0 %, 25 % and 75 % for the maximal strength training sessions, respectively. Subjects in the MAX+POW group performed 9 exercises, 2-5 sets and 5-15 repetitions in each set whereas subjects of HYP group performed 9 exercises, 2-4 sets, and 6-15 repetitions in each set per session. Recovery time was 2-3 minutes for the MAX+POW group, and 1-2 minutes for the HYP group depending on repetitions and machines used. For the MAX+POW group training loads were 40-60 % 1 RM and 80-95 % 1 RM for the HYP group. Training protocol for MAX+POW group included both power and maximal strength training whereas training protocol for HYP group included hypertrophic and maximal resistance training for 12 weeks, resulting in 32 sessions in total.

7.2.4 Nutritional supplementation

Depending on the group division, protein, carbohydrate or combined post-workout supplement was taken immediately after each resistance training session in a double-blind fashion. Protein and carbohydrate supplements were provided by Northforce (Kuusamon Juusto Oy, Kuusamo, Finland). The protein supplement consisted of whey concentrate (37.5 g) of which 30 g was protein, 5 g lactose and < 1g fat. The carbohydrate supplement consisted of maltodextrin (34.5 g) of which 30 g was carbohydrate. These two post-workout supplements were isocaloric whereas the third supplement was a combination of protein and carbohydrate supplements together, resulting in 37.5 g of whey protein concentrate (30 g of whey protein) and 34.5 g of maltodextrin. The supplements were mixed with 0.5 L of non-caloric sugar-free drinks (FUN Light provided by Orkla Foods Finland, Turku, Finland), hence all the three post-workout supplements were equal in taste, mixture and appearance. The flavors of the non-caloric sugar-free drinks were selected depending on the week and subject’s preference (either strawberry, forest fruit, pomegranate-strawberry, apple-pear or raspberry-lemon). The subjects were advised to eat regular mixed meal based on the Finnish Nutrition Recommendations 2014 within 1-2 hours of the finishing of the resistance training bout.

7.3 Data collection and analysis

7.3.1 Blood sample collection

Venous blood samples were drawn from the radial artery into serum tubes (Venosafe; Terumo Medical Co., Leuven, Hanau, Belgium) after overnight fasting in the mornings of baseline, pre- and post-measurements. Subjects were instructed to rest for 8 h minimum the night before and were instructed to refrain from strenuous physical activity for at least 48 h. Blood samples were taken by an experienced laboratory nurse. The blood samples were stored in room temperature for 10 min and thereafter centrifuged for 10 minutes at 3 500 rpm. Following the centrifugation, the serum samples were stored in a freezer in the temperature of -80 °C and then transported to Kuopio where the actual analysis with NMR spectroscopy platform took place.

7.3.2 Metabolite measurement

Blood samples were analyzed with a high-throughput serum nuclear magnetic resonance (NMR) metabolomics platform in Kuopio, Finland with an optimized measurement and analysis protocol. For the precise sample preparation and NMR protocol see the article of Soininen et al. (2009). The basic idea of NMR involves nuclear absorption and emission of radiofrequency radiation to reveal information about magnetic nuclei (Bothwell & Griffin 2011). The method provides a richly informative functional datum, a spectrum, in which the concentration of each detectable hydrogen-containing metabolite (in case of ^{1H} NMR spectroscopy) is represented quantitatively by the area under its specific profile. The spectrum consists of the sum of the intensities of the spectra, of individual metabolites. Each chemically distinct hydrogen atom in the molecule creates a peak in the spectrum. (Nicholson et al. 2011.) The information from an NMR experiment clarifies four things about a sample: 1) how much of it is present (quantification of resonance peaks) 2) what it is (identification of the resonances), 3) metabolite environment of the metabolite and 4) kinetics or behavior of the metabolite (Bothwell & Griffin 2011.) The method and the procedure of the method are described more in detail

elsewhere (e.g. Ala-Korpela 1995; Bothwell & Griffin 2011; Inouye et al. 2010; Soininen et al. 2009).

The NMR method is based on three molecular windows: LIPO (lipoproteins), LMWM (low-molecular weight metabolites) and LIPID windows. LIPO and LMWM windows are applied to native serum and LIPID window is applied for serum lipid extracts. The LIPO window includes the information, e.g. on the lipoprotein subclass distribution and lipoprotein particle concentrations for 14 lipoprotein subclasses, whereas the LMWM includes the information on various low-molecular-weight metabolites. (Inouye et al. 2010.) In addition, the LIPID window includes detailed molecular information on various serum lipid constituents, e.g., free and esterified cholesterol, sphingomyelin and omega-3 fatty acids (Tukiainen et al. 2008). Figure 4 depicts the spectral characteristics of LIPO and LMWM windows.

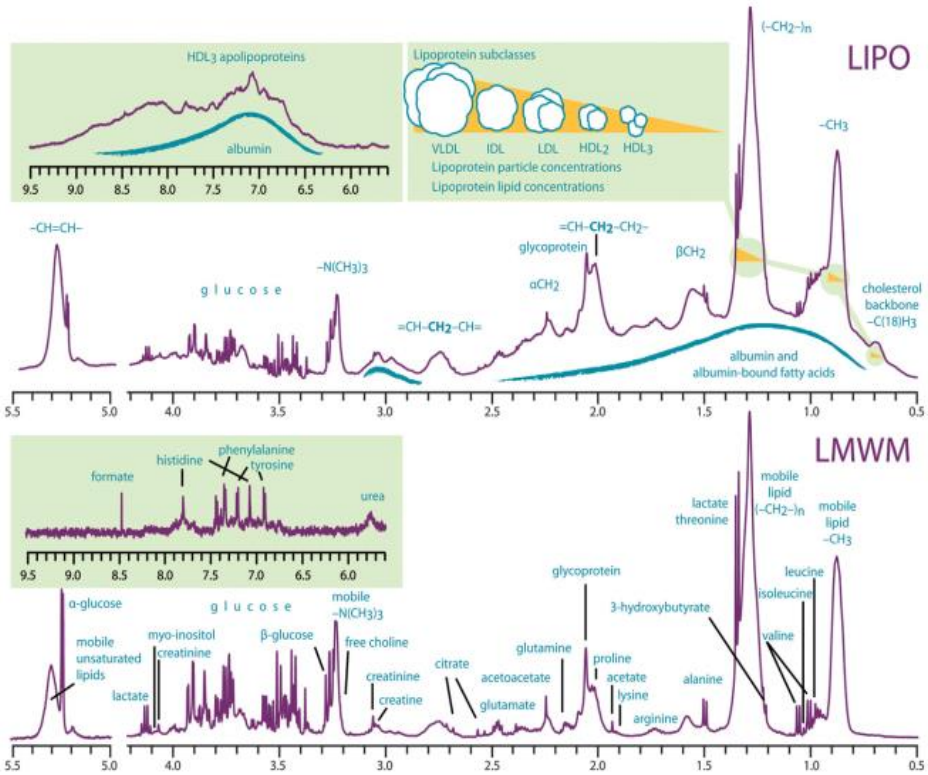

FIGURE 4. The NMR spectral characteristics and the metabolic contents of the two molecular windows- LIPO and LMWM windows (Soininen et al. 2009).

7.3.3 Body composition measurements

Body composition was assessed by Dual-energy X-ray absorptiometry (DXA, Lunar Prodigy Advance, GE Medical Systems – Lunar, Madison WI, USA) in the baseline, pre- and post-measurements. The DXA measurements were conducted following a 12-h overnight fast and 24-h absence of alcohol and strenuous exercise. The subjects were positioned lying on their back in a supine position on the DXA platform with light clothing. Arms were aligned on the sides of the trunk with palms facing the thighs. Legs were together secured with non-elastic straps at the level of knees and ankles. All metal objects were removed before the scan. Analyses (using enCORE 2005, version 9.30 and Advance 12.30) provided total, lean and fat mass. All the analyses were done by the same technician. Automatically generated regions of the legs were manually adjusted by the technician in a manner that hamstrings and gluteal muscles were included. More information of the regional analyses conducted is provided in the study of Hulmi et al. (2015).

7.3.4 Ultrasound imaging

CSA of the knee extensor muscles *vastus lateralis* was measured by ultrasound imaging (model SSD-2000, Aloka, Tokyo). The measurements were conducted in the pre- and post-measurements. Panoramic cross-sectional images were obtained at 50 % of the femur length. Three images without noise were saved for analyses phase. The CSA was analyzed manually with ImageJ software (version 1.44p; National Institutes of Health, Bethesda, MD, USA). The two closest values of these measurements were averaged. The method has been validated earlier in the laboratory of the Department of Biology of Physical Activity with MRI measurement as a reference (Ahtiainen et al. 2010). Ultrasound image is created by the echo of sound-wave pulse. At first, the probe placed on the skin of the patient emits the sound-wave pulse and records its echo. An electronic current is applied across the component material of the transducer to cause a slight change in conformation that induces a sound-wave pulse. Also, a tiny electric current is generated when a rebounding sound-wave pulse strikes it in return. (Walker et al. 2004.)

7.3.5 Strength measurements

The determination of maximal isometric bilateral leg press force (maximal voluntary contraction, MVC) was done by a horizontal leg extension dynamometer (Biology of Physical Activity, University of Jyväskylä, Finland) and the analyses were done by Signal 2.15 software (Cambridge Electronic Design Ltd. 1997-2004). Before the baseline measurements subjects were familiarized once to learn the correct techniques in the strength test devices. Isometric strength was already then performed maximally to assess the reliability of the testing between the preliminary session and the actual pre-test session in the subjects.

Subjects were seated with a hip and knee angle of 110 degree and 107 degree, respectively. They were instructed to push as fast and hard as possible with the whole surface of the foot, and to exert the maximal force for 3-4 seconds following verbal command. Concerning the right position while performing the maximal force, subjects were instructed to keep their back and hips in contact with the bench of the dynamometer and tight grip should be taken of the handles of the dynamometer lasting throughout the movement. Subjects performed 2-3 warm-up trials prior to the actual measurement in which they needed to exert a sub-maximal effort. After the warm-up, at least three (max. 5) maximal trials with one minute rest in between the trials were performed. The maximal trial with the highest maximal force measured was used for statistical analysis.

7.4 Statistical analysis

All data was analyzed using IBM SPSS Statistics 22 software (SPSS, Chicago, IL, USA). In the beginning, data was checked for normality. If there were variables which were not normally distributed, the variables were log transformed. Individual samples T-test was done to compare if there existed differences in the variables between two different training regimens. The differences in the variables between different nutrition supplements were analyzed using one-way ANOVA. Bonferroni post hoc tests were performed to localize differences between supplement groups. Paired samples T-test was run to analyze within group

differences at two different time points (PRE, POST). Baseline values were checked as well in order to see if some abnormalities occurred but because of no abnormalities detected they were not included in the final analysis since the main interest was in the time points of pre- and post-measurements. Correlation analyses were run using Pearson's Product Moment coefficient. For the data that was not normally distributed even after log transformation, the corresponding non-parametric tests (Mann-Whitney U test, Kruskal-Wallis one-way analysis of variance, Wilcoxon signed rank test and Spearman correlation) were run to check for the differences between training groups, supplement groups, time-points and for correlation, respectively. The level of significance was set at $P \leq 0.05$. Microsoft Office Excel 2007 software was used to calculate means, standard errors and delta percentages. Also, the same software was used for graphical illustration of the results.

8 RESULTS

8.1 Participants

Of the 78 subjects, 18 participants were excluded from the data analysis, due to noncompliance with the exercise training sessions, inaccurate DXA measurements or incomplete NMR data, leaving 60 subjects in the main analysis. The mean values and standard deviations (SD) of the general characteristics are as follows: age 32.6±6.7 yr, height 1.80±0.1 m, weight 82.8±10.4 kg and body mass index (BMI) 25.5±3.0 kg/m^2. There were no differences between the supplementation groups in the rate of noncompliance and drop-outs (PROT n=4; CHO n=8; PROT+CHO n=6) but between the training groups, MAX+POW group had relatively higher noncompliance rate (HYP n=5; MAX+POW n=13). Finally, the division of the subjects for the supplement groups was PROT n=21, CHO n=17 and PROT+CHO n=22 and for the training groups HYP n=37 and MAX+POW n=23.

8.2 Serum metabolome

Because of the vast number of serum metabolome variables which were statistically analyzed (n=47), only the most remarkable group and time differences or changes and the most important variables are reported here. See the whole serum metabolome variable list and abbreviations in appendix 1. In the first section, all the means and standard errors of the variables which created statistical significances are reported in the tables 1 and 2. Thereafter, the statistically significant results of the different subgroups are reported.

Most of the changes were within group differences and different supplement regimens seemed to produce more changes than different training regimens. Statistical between groups changes were found only in serum cholesterol (p=0.033) as training groups were compared and in omega-3 fatty acids as supplement groups were compared. With Bonferroni Post hoc test the statistical difference was found to be between PROT and PROT+CHO groups (p=0.022).

TABLE 1. Serum metabolome characteristics in pre- and post-measurements for hypertrophy resistance (HYP) and combined maximal and power resistance (MAX+POW) groups (mean ± SE). *Significant difference from pre-measurements (**p ≤0.01). [a]Significant difference from the hypertrophy group (p≤0.05).

	HYP		**MAX+POW**	
	Pre	Post	Pre	Post
Serum-C [mmol/l]	4.23±0.14	4.28±0.13	4.45±0.17	4.25±0.18[a]
ApoB/ApoA$_1$	0.69±0.02	0.65±0.02**	0.72±0.02	0.71±0.02
Glycoprotein [mmol/l]	1.35±0.03	1.40±0.02**	1.36±0.04	1.46±0.05**

TABLE 2. Serum metabolome characteristics in pre- and post-measurements for protein (PROT), carbohydrate (CHO) and combined (PROT+CHO) groups (mean ± SE). *Significant difference from pre-measurements (*p ≤0.05, **p ≤0.01). [a]Significant difference from the protein group (p≤0.05).

	PROT		**CHO**		**PROT+CHO**	
	Pre	Post	Pre	Post	Pre	Post
L-HDL-P [nmol/l]	0.70±0.07	0.73±0.07	0.73±0.08	0.83±0.07*	0.63±0.07	0.69±0.07
M-HDL-P [nmol/l]	1.55±0.06	1.59±0.06	1.52±0.06	1.62±0.05*	1.55±0.04	1.63±0.05*
HDL-C [mmol/l]	1.08±0.05	1.10±0.05	1.10±0.05	1.18±0.04*	1.08±0.04	1.10±0.04
HDL$_2$-C [mmol/l]	0.62±0.04	0.64±0.05	0.63±0.04	0.70±0.04*	0.60±0.03	0.63±0.04
Serum-C [mmol/l]	4.37±0.17	4.30±0.19	3.98±0.21	4.16±0.20	4.52±0.17	4.33±0.17**
ApoB/ApoA$_1$	0.68±0.03	0.65±0.03	0.71±0.03	0.66±0.02*	0.72±0.03	0.70±0.03
FAw3 [mmol/l]	0.36±0.02	0.33±0.02	0.39±0.02	0.38±0.02	0.39±0.02	0.41±0.02[a]
Gp [mmol/l]	1.35±0.03	1.41±0.04*	1.32±0.04	1.40±0.04**	1.39±0.04	1.45±0.04*

8.2.1 Changes in standard lipid test biomarkers

No significant differences were found neither in the dyslipidemia biomarkers between or within the training groups (Table 3) nor between or within supplement groups (Table 4). Even though the differences were small, some beneficial changes were seen: the concentrations of cholesterol in LDL particles decreased in every study group, the concentrations of cholesterol in HDL particles increased in every study group and the concentrations of serum TGs remained stable or decreased in every study group. Also, beneficial decreases of serum cholesterol were produced in all the other study groups except HYP and CHO groups. The overall changes for all the subjects were the followings: LDL cholesterol -1.1 ±2.8 %; HDL cholesterol +4.2 ±1.9 %; serum cholesterol -0.4 ±1.6 %; serum TG -1.7 ±3.4 %; glucose +0.9 ±1.3 %.

TABLE 3. Standard blood biomarker characteristics in pre- and post-measurements for hypertrophy resistance (HYP) and combined maximal and power resistance (MAX+POW) groups (mean ± SE). [a]Significant difference from the hypertrophy group ($p \leq 0.05$).

	HYP		**MAX+POW**	
	Pre	Post	Pre	Post
LDL-C [mmol/l]	1.69±0.08	1.61±0.08	1.70±0.10	1.68±0.08
HDL-C [mmol/l]	1.10±0.03	1.14±0.03	1.06±0.03	1.09±0.04
Serum-C [mmol/l]	4.23±0.14	4.28±0.13	4.45±0.17	4.25±0.18 [a]
Serum-TG [mmol/l]	1.21±0.08	1.21±0.08	1.11±0.06	1.08±0.06
Glucose [mmol]	4.73±0.06	4.75±0.07	4.84±0.08	4.87±0.09

TABLE 4. Standard blood biomarker characteristics in pre- and post-measurements for protein (PROT), carbohydrate (CHO) and combined (PROT+CHO) groups (mean ± SE).

	PROT		**CHO**		**PROT+CHO**	
	Pre	Post	Pre	Post	Pre	Post
LDL-C [mmol/l]	1.57±0.10	1.49±0.08	1.80±0.11	1.74±0.10	1.72±0.11	1.71±0.11
HDL-C [mmol/l]	1.08±0.05	1.10±0.05	1.10±0.05	1.18±0.04	1.08±0.04	1.10±0.04
Serum-C [mmol/l]	4.37±0.17	4.30±0.19	3.98±0.21	4.16±0.20	4.52±0.17	4.33±0.17
Serum-TG [mmol/l]	1.17±0.07	1.17±0.08	1.23±0.15	1.22±0.15	1.12±0.06	1.10±0.05
Glucose [mmol/l]	4.68±0.08	4.71±0.10	4.82±0.08	4.81±0.06	4.82±0.09	4.87±0.11

8.2.2 Changes in lipoprotein particles and apolipoproteins

Lipoprotein particles. In the subgroup of lipoprotein particles, the only statistically significant differences were seen in large-sized HDL particle concentration within CHO group (p=0.023) and in medium-sized HDL particle concentration within CHO (p=0.039) and PROT+CHO groups (p=0.040) in which the particle concentrations increased.

Lipoprotein diameter. No significant differences were produced as the diameter of different sized lipoprotein particles were examined neither within nor between groups. Figure 5 depicts the differences of large- (A) and medium-sized (B) HDL particle concentrations from pre- to post-measurements for the supplement groups.

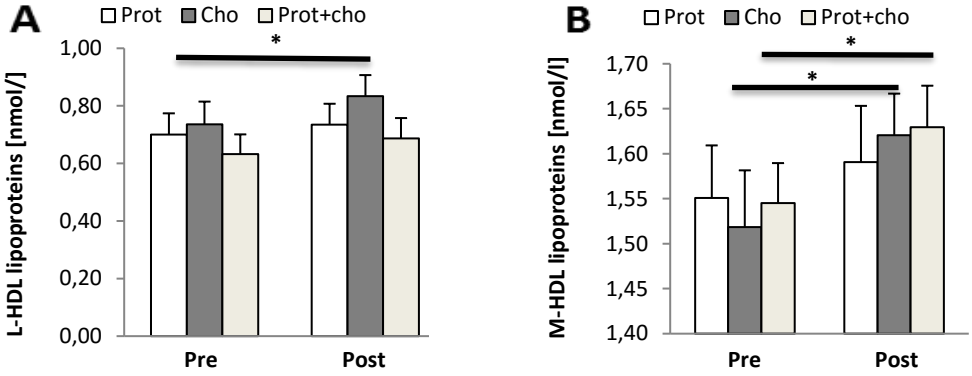

FIGURE 5. Differences in large sized HDL-lipoprotein particle concentrations (A) and medium sized HDL-lipoprotein particle concentrations (B) from pre- to post-measurements for protein (PROT), carbohydrate (CHO) and combined (PROT+CHO) groups (mean ± SE). *Significant difference from pre-measurements (*p ≤0.05).

Apolipoproteins. Statistically significant differences in the ratio of apolipoprotein B to apolipoprotein A_1 were seen in both training and supplement groups as post-measurements were compared to pre-measurements. In the training group, the decrease of the ratio was statistically significant for HYP group (p=0.007) and in the supplement group, the decrease was statistically significant for CHO group (p=0.023) (Figure 6).

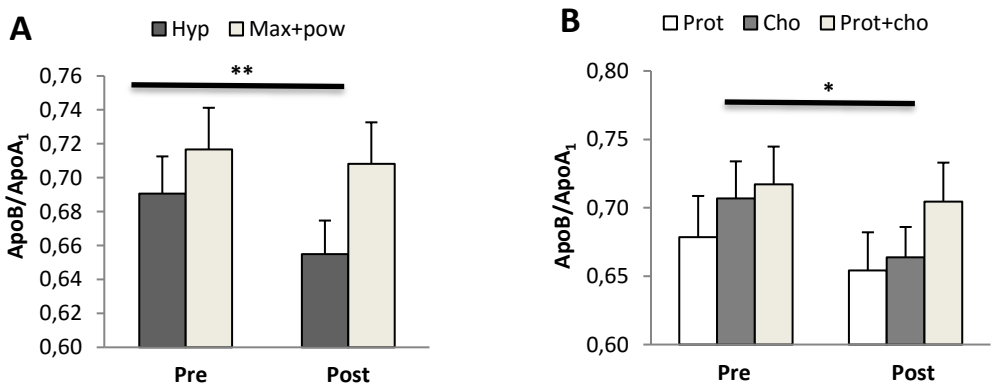

FIGURE 6. Differences in ratio of apolipoprotein B to apolipoprotein A_1 from pre- to post-measurements for hypertrophy resistance (HYP) and combined maximal and power resistance (MAX+POW) groups (A) and for protein (PROT), carbohydrate (CHO) and combined (PROT+CHO) groups (B) (mean ± SE). *Significant difference from pre-measurements (*p ≤0.05, **p ≤0.01).

8.2.3 Changes in cholesterol and fatty acids

Cholesterol. In the subgroup of cholesterol, the study intervention resulted in statistically significant between group and within group differences. In the training group, a statistically significant between group difference was produced in serum cholesterol concentration at the post-measurement time point (p=0.033) (Figure 7). Within group differences were seen in the concentrations of serum cholesterol for CHO group (p=0.044), HDL cholesterol concentrations for CHO group (p=0.046), HDL_2 cholesterol concentrations for CHO group (p=0.044) and in serum cholesterol concentrations for PROT+CHO group (p=0.003) (Figure 8). There were both increases and decreases in the concentrations of different cholesterol variables.

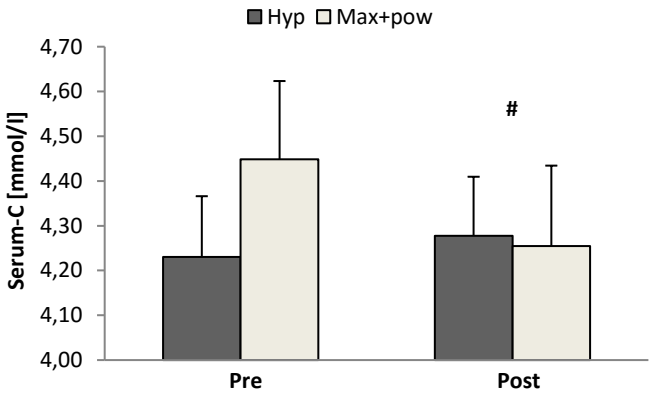

FIGURE 7. Differences in serum cholesterol concentrations from pre- to post-measurements for hypertrophy resistance (HYP) and combined maximal and power resistance (MAX+POW) groups (mean ± SE). #Significant difference from hypertrophy group in post-measurements (#p ≤0.05)

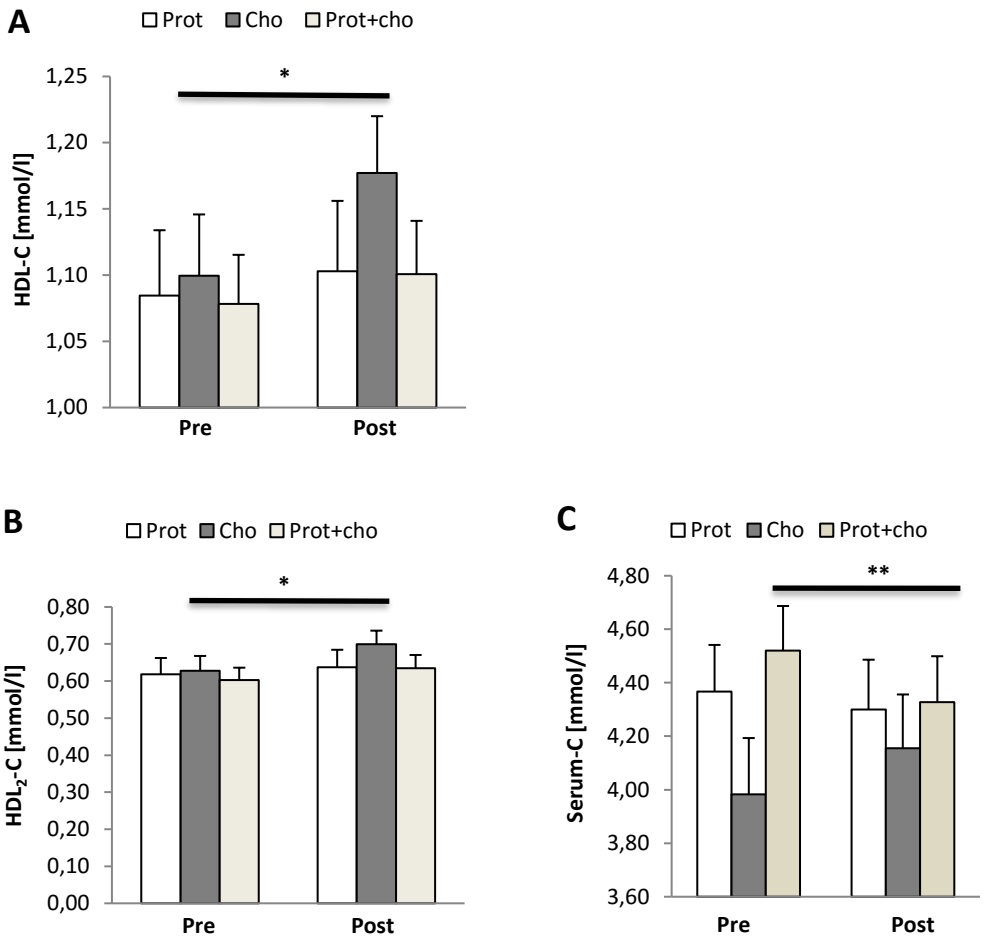

FIGURE 8. Differences in concentrations of HDL cholesterol (A), HDL_2 cholesterol (B) and serum cholesterol (C) from pre- to post-measurements for protein (PROT), carbohydrate (CHO) and combined (PROT+CHO) groups (mean ± SE). *Significant difference from pre-measurements (*p ≤0.05, **p ≤0.01).

Fatty acids. The study intervention created a statistically significant difference in omega-3 fatty acid (FAw3) concentration between PROT and PROT+CHO groups (p=0.022) (Figure 9). Statistical significance remained even though the data analysis was controlled with pre-measurement values (p=0.049). No statistically significant within group differences were found.

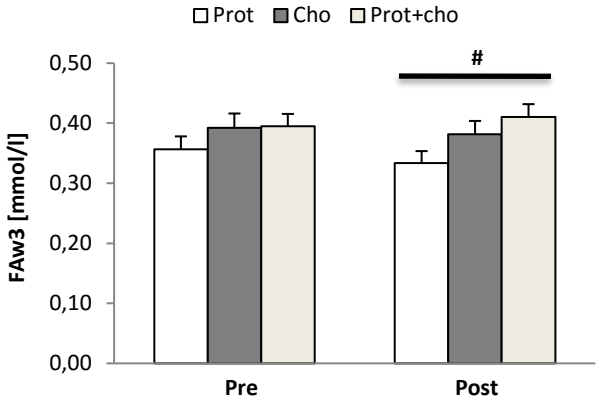

FIGURE 9. Differences in omega-3 fatty acid concentrations from pre- to post-measurements for protein (PROT), carbohydrate (CHO) and combined (PROT+CHO) groups (mean ± SE). #Significant difference from protein group in post-measurements (#p ≤0.05).

8.2.4 Changes in glycoproteins

The most pronounced differences were found in glycoprotein concentrations as significant increases were seen in both training (HYP p=0.004; MAX+POW p=0.004) and all the supplement groups (PROT p=0.043; CHO p=0.003; PROT+CHO p=0.025) (Figure 10). No between group differences were found.

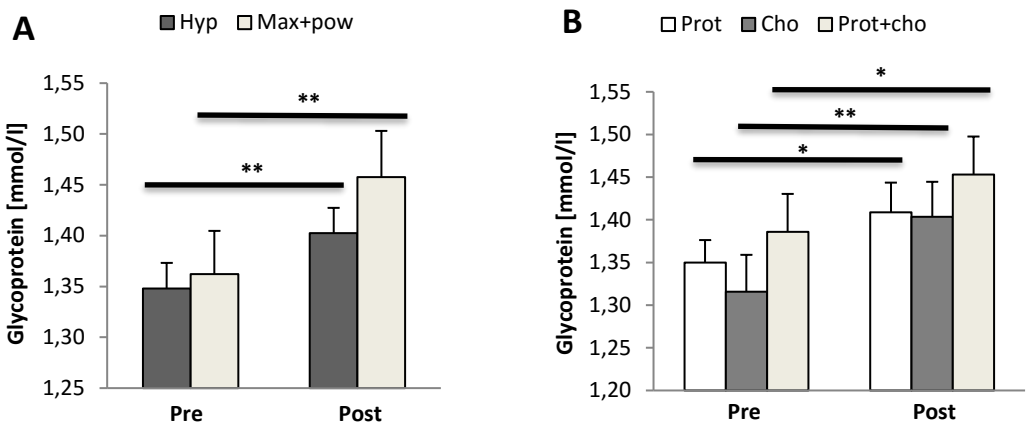

FIGURE 10. Differences in glycoprotein concentrations from pre- to post-measurements for hypertrophy resistance (HYP) and combined maximal and power resistance (MAX+POW) groups (A) and for protein (PROT), carbohydrate (CHO) and combined (PROT+CHO) groups (B) (mean ± SE). *Significant difference from pre-measurements (*p ≤0.05, **p ≤0.01).

8.2.5 Correlations for serum metabolome variables and body composition characteristics

The associations of the changes in body composition (BMI, lean mass, fat mass, fat percentage) were examined with the changes of those serum metabolome variables, which created the most pronounced time or group differences. The associations were explored by using Pearson correlation coefficient. The accurate correlation coefficients and significances can be seen in the table 5 as all the subjects were analyzed in one group. Interestingly, there seems to be somewhat linear relationship for concentration of HDL cholesterol (HDL-C) and lean mass (Figure 11). However, the relationship did not last anymore as the subjects were split into the training groups and the supplement groups.

TABLE 5. Correlation coefficients for changes in body composition (Δ%) and changes in serum metabolome (Δ%) for all the subjects. r=Pearson's correlation coefficient. *Significant correlation (*p ≤0.05, **p ≤0.01, ***p ≤0.001).

	BMI (Δ%)	**Fat mass (Δ%)**	**Lean mass (Δ%)**	**Fat percentage (Δ%)**
	r	**r**	**r**	**r**
L-HDL-P (Δ%)	0.222	0.018	0.418**	-0.059
M-HDL-P (Δ%)	0.308*	0.091	0.428**	0.013
HDL-C (Δ%)	0.325*	0.111	0.362*	0.025
HDL_2-C (Δ%)	0.340**	0.122	0.408**	0.030
$ApoB/ApoA_1$ (Δ%)	0.349***	0.250	-0.222	0.271*
FAw3 (Δ%)	0.439***	0.251	0.199	0.178

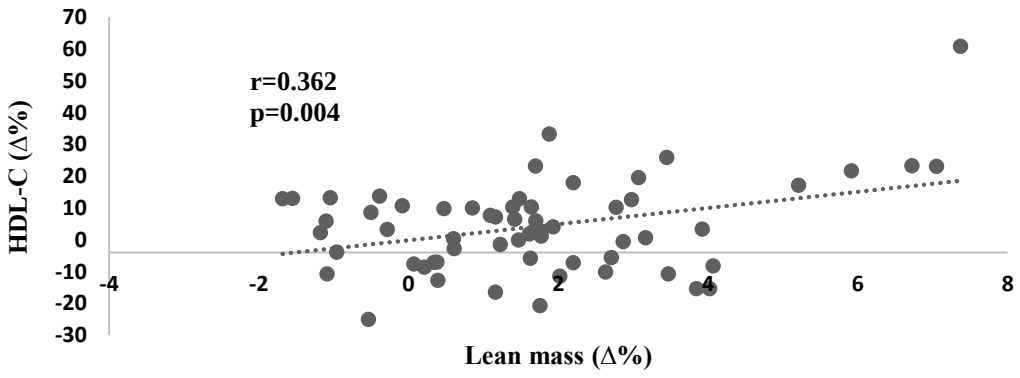

FIGURE 11. Correlation for change of HDL-C with change of lean mass. r=Pearson's correlation coefficient, p=significance (2-tailed). Linear regression line illustrates the relationship of HDL-C and lean mass.

8.3 Muscle hypertrophy and strength

All the subjects increased their maximal isometric strength in leg press (+13.0 ±2.4 %) as well as the muscle hypertrophy in *vastus lateralis* (VL +10.9 ±2.7 %) As the results were explored after splitting into the training and the supplement groups, following results were found: in the training groups both groups increased maximal isometric strength and the changes due to the intervention were statistically very

significant (HYP p=0.001; MAX+POW p=0.008). However, the intervention did not create statistically significant differences between the groups. Regarding muscle hypertrophy, both training groups showed gains in VL muscle mass. The increase in CSA of VL for the MAX+POW group was statistically significant (p=0.033). However, the statistical power in the analysis of CSA was lower (HYP n=6; MAX+POW n=8) due to lacking data and inaccurate ultrasound images. Therefore, the obtained results should be examined with caution. Also, it should be mentioned here that HYP group had greater CSA of VL (26.3 cm^2 vs. 21.6 cm^2) already in the pre-measurements and the difference was statistically significant (p=0.046).

Also, as the strength and hypertrophy results of the supplement groups were explored, it can be concluded that in those groups maximal isometric strength increased statistically significantly in every group (PROT p=0.001; CHO=0.001; PROT+CHO p=0.005). The study intervention resulted in hypertrophy of VL in all the supplement groups. However, no significant differences were created as post-measurement results were compared to pre-measurement results. Tables 6 and 7 present means and standard errors of maximal isometric strength and CSA of VL in different training and supplement groups, respectively.

TABLE 6. Muscle hypertrophy and strength characteristics in pre- and post-measurements for hypertrophy resistance (HYP) and combined maximal and power resistance (MAX+POW) groups (mean ± SE). *Significant difference from pre-measurements (**p ≤0.01, ***p ≤0.001).[a]Significant difference from hypertrophy group in pre-measurements (p≤0.05). [b]Significant difference from hypertrophy group in post-measurements (p≤0.05).

	HYP		**MAX+POW**	
	Pre	Post	Pre	Post
Max isometric strength [N]	3694.2±137.4	4202.4±196.5***	4137.6±205.1	4416.4±297.0**
CSA of VL [cm^2]	26.3±1.4	28.1±1.1	21.6±1.5[a]	23.7±1.3**[b]

TABLE 7. Muscle hypertrophy and strength characteristics in pre- and post-measurements for protein (PROT), carbohydrate (CHO) and combined (PROT+CHO) groups (mean ± SE). *Significant difference from pre-measurements (**p ≤0.01, ***p ≤0.001).

	PROT		**CHO**		**PROT+CHO**	
	Pre	Post	Pre	Post	Pre	Post
Max isometric strength [N]	3823.2±201.8	4154.1±283.0***	3808.7±164.5	4269.2±222.7***	3946.1±230.4	4426.9±336.5**
CSA of VL [cm^2]	23.9±1.9	25.5±2.1	22.7±2.8	23.3±2.4	23.5±0.8	26.2±0.9

8.4 Body composition

Overall, as all the subjects were examined as one group, BMI remained almost the same (+0.17 ±0.3 %) whereas fat mass and fat percentage decreased (-4.1 ±1.2 %; -4.3 ±1.0 %, respectively) and lean mass increased (+1.7 ±0.3 %). For the tests of significance, the data was split into the training groups (HYP; MAX+POW) and the supplement groups (PROT; CHO; PROT+CHO). There were no statistically significant differences in BMI as these two timepoints (pre vs. post) were compared. However significant differences in fat mass, fat percentage, and lean mass were detected in both training groups and in PROT and PROT+CHO groups as differences within groups were examined (Tables 8 and 9). Only in the CHO group the differences of fat mass and fat percentage from pre- to post-measurements did not produce statistical significance.

Moreover, in every training and supplement group the changes tended to be towards more muscular body and smaller bodily fat storages. As between group changes were examined, no significant differences were found. Tables 8 and 9 present means and standard errors of body composition characteristics for different training groups and different supplement groups, respectively.

TABLE 8. Body composition characteristics in pre- and post-measurements for hypertrophy resistance (HYP) and combined maximal and power resistance (MAX+POW) groups (mean ± SE). *Significant difference from pre-measurements (*p ≤0.05, **p ≤0.01, ***p ≤0.001).

	HYP		**MAX+POW**	
	Pre	Post	Pre	Post
BMI [kg/m^2]	25.4±0.4	25.4±0.6	25.6±0.7	25.6±0.7
Lean mass [kg]	61.2±1.0	62.5±1.0***	59.1±1.2	59.8±1.2**
Fat mass [kg]	19.6±1.3	18.3±1.2***	18.4±2.1	17.9±2.0*
Fat percentage [%]	22.8±1.1	21.4±1.1***	21.8±2.0	21.3±1.8**

TABLE 9. Body composition characteristics in pre- and post-measurements for protein (PROT), carbohydrate (CHO) and combined (PROT+CHO) groups (mean ± SE). *Significant difference from pre-measurements (*p ≤0.05, **p ≤0.01, ***p ≤0.001).

	PROT		**CHO**		**PROT+CHO**	
	Pre	Post	Pre	Post	Pre	Post
BMI [kg/m^2]	25.7±0.7	25.6±0.7	24.5±0.6	24.6±0.6	26.0±0.6	26.1±0.7
Lean mass [kg]	59.9±1.1	61.1±1.1***	60.3±1.8	61.3±1.8**	61.0±1.2	61.9±1.3***
Fat mass [kg]	21.3±2.1	19.8±2.0**	15.7±1.5	15.2±1.4	19.7±1.8	18.9±1.7*
Fat percentage [%]	24.2±1.9	22.6±1.8***	19.6±1.7	19.0±1.6	22.9±1.6	22.0±1.6**

8.5 Correlations for body composition variables and muscle hypertrophy and strength

The associations of the changes in body composition (BMI, lean mass, fat mass, fat percentage) were examined with the changes of muscle hypertrophy and strength measures by using Pearson correlation coefficient. No significant correlations occurred neither when all the subjects were put in a group nor after splitting in HYP group. In the MAX+POW group, significant correlations were found between the change of VL and the change of fat mass (r=-0.881**; p=0.004) as well as between the change of VL and the change of fat percentage (r=-0.952**; p=0.001). These analyses were run by using Spearman's rho because the variables were not normally distributed.

9 DISCUSSION

The purpose of this study was to provide a comprehensive overview of the effects of different post-exercise supplementation regimens and different resistance training regimens on serum metabolome and resistance training adaptations. The primary interest of the study was to explore the effects of both protein and carbohydrate supplementation and their combination as well as differing resistance training regimens on different metabolic factors of human metabolism, muscle hypertrophy and muscle strength. The intriguing findings of the current study are presented here as follows:

1) Post-workout supplements had influence on serum metabolome. However, as the supplement regimens were compared, only one statistically significant between group change was found between PROT and PROT+CHO groups (omega-3 FAs; -6.4±7.8 % vs. +3.9±4.9, p=0.022). Therefore, it cannot be clearly said that one of the three supplement regimens resulted in greater adaptations in serum metabolome than any other.

2) Resistance training had effects on serum metabolome. However, it is rather difficult to distinct the effects which have been resulted only because of the resistance training. With the study results showing only one group difference between HYP and MAX+POW groups (Serum-C; +1.1±2.4 % vs. -4.4±2.6 %, p=0.033), it cannot be said that hypertrophic resistance training resulted in greater adaptations in serum metabolome than did combined maximal and power resistance training regimen.

Other findings that were under interest:

1) Maximal isometric strength and muscle size increased in both training groups and in all supplement groups. HYP group had slightly greater increase in the improvement of maximal strength than MAX+POW group (13.8±42.9 %, p=0.001 vs. 6.7±44.8 %, p=0.008). However, the difference between the groups was not significant. Instead, rather surprisingly MAX+POW group increased more CSA of VL than HYP group (9.5±16.9 % vs. 6.9±16.8 %, p=0.04).

2) Both training groups ameliorated their body composition towards more lean and less fat-containing body as did all supplement groups. Additionally, of the supplement regimens PROT group (p=0.001) and of the training programs, HYP group (p=0.001) had the most pronounced effects on all the variables of body composition shifting the values towards leaner body composition.

9.1 Adaptations in serum metabolome

The study intervention led to multiple changes in serum metabolome. Both, different training and supplement regimens provoked metabolism of the subjects. Nevertheless, different supplement regimens seemed to produce more changes in serum metabolome than did different training regimens. In both regimens, produced statistical differences were merely within group changes than between group changes. Therefore, it can be said that hypertrophic resistance training regimen was not superior to maximal and power resistance training. Additionally, protein supplementation did not result in more beneficial serum metabolome than did either carbohydrate alone or combined protein and carbohydrate supplementation. To the best of our knowledge, the present study is the first study examining influence of resistance training on serum metabolome using NMR method. Besides, there exist only few studies examining effects of physical activity on serum metabolome.

The only remarkable between group differences were seen in serum cholesterol and omega-3 fatty acids concentrations. The concentration of serum cholesterol in MAX+POW group lowered 4.4 ±2.6 % whereas in HYP group it increased 1.1 ±2.4 %. The concentration of omega-3 fatty acids lowered 6.4 ±7.8 % and 2.8 ±6.7 % in PROT and CHO group, respectively, and increased 3.9 ±4.9 % in PROT+CHO group. Even though only some remarkable differences were detected in the present study, earlier Chorell et al. (2009) have found low-carbohydrate-protein beverage (LCHO-P) to be superior compared to water, low-carbohydrate or high-carbohydrate beverages. More specifically, LCHO-P beverage tended to improve the metabolic status of less fit subjects in the recovery phase. (Chorell et al. 2009.) In this study, the composition of macronutrients was ingested immediately after exercise bout as was the case in the present study also.

As the study intervention created significant differences only with two variables in between group comparisons, the most pronounced changes are discussed here rather in the light of the effects of nutrition and exercise on serum metabolome in general. Therefore, the subsequent changes are presented here for all the subjects without group division. Increases were seen in concentrations of large- (+8.7 ±0.5 %) and medium-sized (+4.7 ±3.0 %) HDL-lipoproteins, HDL- (+3.4 ±5.4 %) and HDL_2-cholesterol (+6.3 ±4.8 %), glycoprotein acetyls (+5.2 ±4.1 %) and glucose (+0.5 ±13.8 %). Conversely, decreases were seen in concentrations of serum cholesterol (-1.1 ±1.3 %), ratio of apolipoprotein B to apolipoprotein A_1 (-3.6 ±4.7 %), omega-3 fatty acids (-1.4 ±1.3 %), LDL-cholesterol (-3.2 ±4.6 %) and serum triglycerides (-0.6 ±5.0 %). Even though the changes are relatively small they are mainly in accordance with previous findings.

In the study of Kujala et al. (2013) the same NMR platform was used to study metabolic differences of twin pairs of which one had been active and the other inactive for a long period of lifetime (>30 y). As in the present study, Kujala et al. (2013) found concentrations of TG and LDL cholesterol being lower in active than in inactive individuals. Besides, within active individuals they found decreased concentrations of VLDL particles and IDL cholesterol, which are known to have atherogenic effects as well. Conversely, higher concentrations of large HDL particles, larger HDL particle size and higher HDL_2 cholesterol were found among

active individuals. (Kujala et al. 2013.) The findings from the present study gives support for the finding of higher concentration of large HDL particles and higher concentration of HDL_2 cholesterol. In addition, in the present study it was found out that also the concentration of medium-sized HDL particles and of HDL cholesterol augmented. In the present study, no significant changes in HDL particle diameter were found.

Inconsistent with earlier studies, the present study produced an increase in glucose concentration. Previously, decreases of fasting glucose resulting after resistance training period have been detected among healthy individuals with normal glucose tolerance (Sillanpää et al. 2009) and among individuals with type 2 diabetes (Fenicchia et al. 2004). It is hard to postulate the reason underlying because also protein group increased its fasting glucose levels. Otherwise, it could have been hypothesized that with carbohydrate supplements, great increases in sugar intakes would have affected the fasting glucose levels. With more controlled diet or multiple diet record analysis the cause of these increases could have been found.

Beyond changes in lipoproteins, Kujala et al. (2013) reported that the ratio of apolipoprotein B to A_1 was lower, serum fatty acid composition had been shifted towards more polyunsaturated profile and α_1-acid glycoprotein levels were lower among active individuals (Kujala et al. 2013). In the present study, lowered ratio of apolipoprotein B to A_1 was demonstrated. However, in the present study no support can be given for the finding of Kujala et al. (2013) considering the shift of serum fatty acid composition towards more polyunsaturated profile (Kujala et al. 2013). In fact, in the present study the concentration of omega-3 fatty acids lowered during the intervention.

Furthermore, in the present study increases in the concentration of glycoprotein acetyls (consisting mainly of α_1-acid glycoproteins) were found. However, in the earlier studies, the increases of the concentrations of α_1-acid glycoproteins have been linked with increases in adiposity (Würtz et al. 2014) as α_1-acid glycoprotein is an inflammatory marker (Cheng 2013). Conversely, in our study fat mass contents decreased. Therefore, in the present study increases in α_1-acid glycoproteins may reflect provoking some other pathways than those linked with

lipid metabolism. The increased concentration may be rather due to strains in muscle tissue because of the resistance training than due to perturbations in lipid metabolism. It is important to mention that the study of Kujala et al. (2014) differed from the present in the amount and type of physical activity. Therefore, that study showed improvements in cardiometabolic health among individuals committed to long-term physical activity (Kujala et al. 2014).

It seems possible that long-term physical activity affects human metabolism more than physical activity lasting for a rather short period. Also, weight reduction studies have provoked metabolism more. Floegel et al. (2014) clarified more strong linkage between serum metabolome and obesity or cardiorespiratory fitness. Instead, diet and physical activity showed weaker association with serum metabolome. Cardiorespiratory fitness seems to reflect long-term exposure and is therefore more strongly linked to serum metabolome than physical activity. (Floegel et al. 2014.)

More pronounced changes and differences have been found in the studies examining overweight, obese and metabolically unhealthy inactive individuals. Wiklund et al. (2014) found out branched-chain amino acids (BCAA), aromatic amino acids, orosomucoid, several species of fatty acids and phospholipids being associated with metabolic syndrome independent of BMI, fat mass, waist circumference and physical activity. Interestingly, no associations with VO_2max and metabolite factors were found even though VO_2max was inversely associated with TG, insulin resistance, BMI and waist circumference. (Wiklund et al. 2014.) Also, Huffman et al. (2011) reported lowered concentrations of circulating free fatty acids and fatty acid by-products in an overweight study including a training intervention (Huffman et al. 2011). It has been proposed that changes in blood lipids would be linked more strongly to changes in body composition rather than improvements in physical fitness (Sillanpää et al. 2009).

Giving support for the finding in the study of Wiklund et al. (2014), other studies have found increased concentrations of BCAA levels as well (Huffman et al. 2011; Kujala et al. 2013; Xiao et al. 2016). Amino acids were excluded from this study as the data concerning amino acids had been already analyzed in another master’s

thesis study. However, no clear changes were found though. The main finding was that the pre-values of leucine, isoleucine and phenylalanine correlated positively with body weight. (Myrberg 2015).

Nevertheless, it has been demonstrated that the weight change is not the only reason in improving serum metabolome. Kraus et al. (2002) showed in their study that regular exercise with minimal weight change had broad beneficial effects on lipoprotein profile. In that study, the amount of exercise was in a key role as greater amounts of exercise had more beneficial effects on lipids and lipoproteins than had the intensity of exercise. More precisely, exercising decreased the concentrations of small LDL and LDL particles and increased the average size of LDL particles. However, the plasma LDL cholesterol concentration remained unchanged. Furthermore, the exercise increased the total HDL concentration, the concentration of large HDL particles, and the average size of HDL particles and decreased the concentrations of TGs and total VLDL TGs with decreases in the IDL concentration, the concentration of large VLDL particles, and the average size of VLDL particles. (Kraus et al. 2002.) That study also supports many of the findings of the present study.

Consistent with previous association, Xiao et al. (2016) found higher overall volume of habitual physical activity being associated with human metabolome and therefore leading to better cardiometabolic health. In that study, physical activity was measured with accelerometers meaning that the whole daily activity could be recorded. Results showed that higher physical activity was associated with lower blood levels of carbohydrates in glucose metabolism. Two monosaccharides, mannose and glucose, were inversely associated with physical activity. Interestingly, overall volume of physical activity and light activity seemed to be more strongly linked with the metabolomic profile than the amount of moderate-to-vigorous physical activity or average intensity of physical activity. Furthermore, duration and intensity of physical activity seemed to have different metabolite association patterns. Also, total physical activity and sedentary time were associated with contrary metabolomics patterns. (Xiao et al. 2016.) Conversely to these study results, in the present study glucose concentration increased in the study group as it was pointed out earlier.

9.2 Strength performance and morphological adaptations

All the subjects increased their maximal isometric strength in leg press. Statistically significant increases lasted also after the division into different training and supplement groups. No group differences were detected. HYP group had slightly greater increase in the improvement of maximal strength compared to combined MAX+POW group (13.8±42.9 % vs. 6.7±44.8 %, respectively). Nevertheless, it may be possible that lower levels of maximal strength in the pre-measurements for HYP group (3694.2±137.4 vs. 4137.6±205.1) have enabled greater gains in maximal strength in the post-measurements. Altogether, the training program resulted in increases of muscle strength. Earlier, protein ingestion has seen to have additive effect on isometric strength compared to placebo group (Hulmi et al. 2009). In this study between the supplement groups, there were no significant differences in the increases of isometric muscle strength.

The results from the ultrasound measures support the fact that the increases of muscle strength may be merely due to neural changes rather than morphological changes. Increases in muscle hypertrophy were seen in all the study groups but no between group differences were produced. The only significant increase was seen in the CSA of *vastus lateralis* of the MAX+POW group in the within group comparison. The finding was rather surprising. Conversely, it was hypothesized that HYP group would increase muscle mass of VL more than MAX+POW group. However, there was a lot of missing data from pre- and post-measurements. Thus, problems in the analysis of ultrasound images could have produced bias. As it was already mentioned, because of the missing data the statistical power in the analysis of CSAs was lower (HYP n=6, MAX+POW n=8). Therefore, the received results cannot be fully trusted. It should be mentioned here as well that HYP group had already in the beginning significantly greater values of the CSA of VL (26.3 cm^2 vs. 21.6 cm^2). Thus, lower pre-values of MAX+POW group may have resulted in relatively greater increment of VL muscle mass during the intervention.

There exists a strong evidence for accretion of muscle CSA with protein ingestion combined with resistance training (Cermak et al. 2012; Cribb et al. 2007; Hulmi et al. 2009; Morton et al. 2015). Also, increases in muscle strength have been detected with protein ingestion (Cermak et al. 2012; Morton et al. 2015; Naclerio & Larumbe-Zabala 2015). Furthermore, it seems that as duration and frequency of the resistance training is increased for untrained and trained individuals, ingestion of protein will result in greater gains in lean mass and muscle strength (Pasiakos et al. 2015). More debatable have been protein timing that lead to the best outcomes considering muscle hypertrophy and strength gains. Even if the research evidence is inconsistent, it seems that there is not so much difference if the protein supplements are consumed before or immediately after a resistance training bout, regarding long-term gains in muscle mass or strength (Pasiakos et al. 2015).

Earlier, co-ingestion of carbohydrate and protein has been thought to stimulate insulin release more than with protein only ingestion. Thus, resulting in an improved net protein balance. In the past, controversial results have been reported of the effect of co-ingestion on muscle protein synthesis and breakdown. The recent studies have shown no additional effect of adding carbohydrate into a post-workout supplementation. Therefore, in the meta-analysis of Morton et al. (2015) it has been concluded that probably there is no need to recommend carbohydrate usage after resistance training exercise. (Morton et al. 2015.) Also, in the recent review article it was concluded that addition of carbohydrate to protein supplements would not enhance increases in lean mass and muscle strength during resistance training program (Pasiakos et al. 2015).

9.3 Body composition

Even though large group differences were not seen in the post-measurements, hypertrophic resistance training regimen resulted in greater adaptations in body composition than did maximal and power resistance training regimen. Both training regimens affected all body composition variables though. When considering different supplement groups, protein supplements had the most pronounced effects on all the variables of body composition shifting the values

towards better body composition. Also, combined supplementation regimen had positive effects on lean mass and fat mass variables whereas carbohydrate alone supplementation had positive effects only on lean mass. Because one underlying aim of the study was increasing muscle mass, it can be deduced here that CHO supplement alone is not sufficient to produce lean mass gaining. Even though the resistance training regimens were targeted to lower extremities, and it was hypothesized that the training of HYP group would result in greater hypertrophy than the training of MAX+POW group, muscle CSA assessment of VL did not produce large group differences. Nonetheless, the differences were seen in the gains of overall lean mass. Therefore, it seems that including only VL muscle in the CSA analysis did not provide wide picture of the overall situation, Also, as it was mentioned earlier, because of the lacking data in the CSA analysis, it is evident that the true results have been obscured.

Giving support to our current findings, protein ingestion was superior to placebo regarding gains of fat-free mass (Cermak et al. 2012). Additionally, gains in lean mass have been produced as well only with resistance training (Westcott 2012). Furthermore, resistance training seems to be efficient in decreasing intra-abdominal fat (Westcott 2012) and waist circumference (Mekary et al. 2015) even without post-workout supplementation. In the meta-analysis of Ha & Zemel (2003) it has been concluded that protein ingestion decreases accumulation of body fat and accelerates weight and fat loss during energy restriction (Ha & Zemel 2003). With the results of the present study, it can be deduced, that measuring only BMI is not sufficient as BMI values remained quite stable but the actual body composition parameters changed.

Resistance training coupled with whey protein ingestion have been detected to result in greater increases in lean mass or fat-free mass compared to other proteins, such as soy protein, casein or carbohydrate (Cribb et al. 2006, Ha & Zemel 2003, Naclerio & Larumbe-Zabala 2015, Volek et al. 2013). Whey protein, which was used as protein supplementation in the current study as well, is high in leucine content. The benefits of leucine are that it is quickly absorbed and results thus in a more pronounced increase in muscle protein synthesis (Ha & Zemel 2003; Tang et al. 2009; Tang & Phillips 2009; Volek et al. 2013). In the meta-analysis of Miller

et al. (2014) whey protein provided enhancements in body composition either combined with resistance training or without exercise coupled with weight loss or weight maintenance diet (Miller et al. 2014). Furthermore, it has been demonstrated that whey protein ingestion combined with resistance training resulted in significant decreases in visceral fat mass among overweight and obese subjects (Arciero et al. 2014)

9.4 Associations between changes in serum metabolome and body composition

A weak association of the concentration of HDL cholesterol with lean mass was detected in the present study. However, the relationship did not remain anymore as the subjects were split into training groups and supplement groups. This may be due to large standard errors. However, in earlier studies HDL cholesterol has seen to be bound with other performance characteristic. In the study of Kujala et al. (2014) the positive correlation between VO_2max and HDL_2 cholesterol and the inverse correlation between VO_2max and glucose and α_1-acid glycoprotein was tracked. Significant correlations remained still after adjustment for age, sex and visceral fat (Kujala et al. 2014). Also, Sillanpää et al. (2009) detected HDL cholesterol to be negatively associated with body weight, BMI, waist girth and body fat percentage (Sillanpää et al. 2009).

Presumably, examining the insulin hormone levels would have given deeper prospect of the lipoprotein and lipid metabolism state. Insulin is an anabolic hormone that is crucial in the regulation of lipid metabolism. More specifically, it promotes lipid synthesis and inhibits lipolysis. (Meshkani & Adeli 2009.) As in the present study, higher increases of the concentrations of glucose and glycoprotein acetyls were produced, insulin levels could have given more understanding of the possible inflammatory state within subjects. Insulin is also linked with VLDL because it inhibits VLDL production from the liver either by decreasing fatty free acid flux from adipose tissue to the liver or by inhibiting the rate of apolipoprotein B synthesis and degradation in hepatocytes (Avramoglu et al. 2003). Also, investigating other lipid metabolism related hormones - such as sex-hormone

binding globulin, testosterone, leptin and adiponectin (Würtz et al. 2014) - could have given more understanding of the changes of lipids and lipoproteins in the present study.

There exist also many other interesting factors that could have been assessed as different metabolites and different pathways are closely interrelated in human metabolism. To highlight some of those, four of the most interesting ones are notified here. One of the confounding factors is diet. Bogl et al. (2013) had an interesting novel study where they investigated the associations between habitual dietary intake and lipoprotein subclass profile in young healthy adults. The main findings were that junk food had adverse effects on lipid and lipoprotein subclass profiles, resulting in increased concentrations of triglycerides, smaller-sized HDL and LDL and large-sized VLDL. These findings were independent of adiposity and other lifestyle factors. Conversely, higher consumption of fish was related to a reduced VLDL particle diameter and VLDL subclass distribution whereas HDL particle diameter increased. These biomarkers reflect a better lipid and lipoprotein profile. (Bogl et al. 2013.)

Sadeghi et al. (2013) have shown visceral fat accumulation to be negatively correlated with apolipoprotein A_1 and positively correlated with the ratio of apolipoprotein B to apolipoprotein A_1 (Sadeghi et al. 2013). As we concluded with the decrease of apolipoprotein B to apolipoprotein A_1, this relation would have been interesting to examine. Another interesting factor is blood pressure, which was not measured in the present study. High blood pressure values have been detected with nonfavorable lipid profiles (Würtz et al. 2014).

Lewis et al. (2010) found even about 10 minutes of exercise inducing an enhance in lipolysis, glycolysis and glucogenolysis lasting for 60 min after completion of exercise. In the study, more fit individuals activate lipolysis, facilitate entry of fatty acids into the tricarboxylic cycle and expand the tricarboxylic acid cycle intermediate pool more than less fit individuals. For examining the state of lipolysis, glycerol concentrations were assessed. (Lewis et al. 2010.) Glycerol was not included in the metabolites in the present study. Presumably, individuals who can activate lipolysis because of exercise, remain lean more likely (Lewis et al.

2010). Therefore, measuring glycerol levels in the present study could have been interesting to assess the subjects' states of lipolysis.

9.5 Strengths and limitations of the study

The present study has both strengths and limitations. Considering the strengths, first it should be said, this was a longitudinal study of 20 weeks of which 16-week training programs were separated. Therefore, some temporality and causality can be seen in the metabolism with the support from earlier research. However, in the light of previous studies to create more beneficial serum metabolite profiles, long term participation in physical activity seems to be needed and more strain of cardiovascular system may be required (e. g. Kujala et al. 2013; Floegel et al. 2014).

Second, because the participants had not been committed to any resistance training program before enrolling the study, they were considered as beginners of resistance training. To minimize the influence of learning effect in maximal efforts throughout the study, the subjects had a 4-week familiarization in the initiation phase. Thus, same resistance training regimen was served for all the subjects in the beginning. After the familiarization phase, the actual study intervention took place with different training regimens and different supplement regimens.

Third, we had a relatively large sample size of 60 participants in the end of the study. Further after splitting participants into training and supplement subgroups, the number of the subgroups was still rather high. It would have been interesting to explore the combinations of different training and supplement groups but it seemed too probable to get unreliable results because of the very small number of those subgroups or at least the statistical power would have been low.

Regarding the limitations of the study, some important remarks should be taken into consideration. First, there was no control group included in this study. As we have seen before (e. g. Kujala et al. 2013; Yan et al. 2009), there really is a great difference in serum metabolome profile in between active and inactive individuals so it would have been interesting to see if the trend was similar in this study. Therefore, in the present study, the possible differences between active and inactive individuals remain unknown. Second, to broaden our understanding of metabolism,

it could have been interesting to collect blood samples also right after the resistance training sessions so that we could have analyzed acute effects of resistance training on serum metabolome as well.

Third, dietary intake assessment was done only once in the beginning of the study. The subjects were informed how to eat healthy with Nordic nutritional recommendations but dietary habits were not controlled throughout the study. Analysis from the dietary intake assessments showed that total energy intake was higher in the CHO group compared to PROT group. However, when the results were presented as relative values to body mass, the significance of differences did not remain anymore. These results are presented in another master's thesis work (Laakso 2015).

Fourth, the findings may be confounded by other factors. To understand more specifically the changes of metabolic markers on a tissue level, muscle biopsies would have been needed to give more insight into this area. There is rather great variety of other genotype and phenotype factors that could affect the concentrations of the metabolites, such as genes and lifestyle factors like diet, the amount of sleep, the level of sedentariness, the amount of other sport activities, and the use of alcohol, cigarettes and drugs.

Study results should be interpreted in the light of the study limitations. Although there exist many confounding factors that were not controlled, we can still see tendencies in metabolism resulting after these specific exercise and supplement regimens. Furthermore, it is unlikely that the confounding factors would have differed among groups. The complexity and great magnitude of metabolic pathways challenges the way of exploring the underlying mechanisms. As the present study was made as a master's thesis, everything could not be considered. Enlarging this study to cover all the factors described above would have resulted in a research work that would have be equivalent to a PhD study.

9.6 Practical applications

It is well established, that exercise affects different pathways of metabolism, such as insulin and glucose metabolism, lipid metabolism and inflammation (Cheng 2013). Human metabolism consists of hundreds of metabolites that form several different pathways that are interrelated. So far, much of work have been done to explore that network but still continuation of research is needed. With the findings from recent studies, it is now well understood that the plasma lipoprotein cholesterol concentrations alone give a limited view on the overall lipoprotein metabolism. Also, measuring only common biochemical parameters gives a narrow perspective of metabolism and the real changes may remain not detected (Yan et al. 2009). Therefore, more metabolites need to be examined, e. g. lipoprotein particle size and concentration. Particularly, for the lipoprotein physiology and pathophysiology deeper insight into compositional variations in the lipoprotein particles should be highlighted. There exist continuation of transfer and exchange of various lipid molecules between the lipoprotein particles and tissues. All in all, traditionally the concentration of lipoproteins has been examined but now the emphasis should be on the quality of lipoprotein particles and the form of transportation. (Kumpula et al. 2010.)

Obesity is a growing, world-wide problem that threatens the life of many people. It has been shown to be associated with adverse serum lipid and lipoprotein profiles but at least as worrying is the finding that even among young adults within non-obese weight range, causative effects of adiposity across multiple cardiometabolic risk factors have been found. More in detail, causal metabolic effects of overweight have been shown as an unfavorable lipoprotein subclass profile and increased concentrations of BCAAs and inflammatory markers, perturbed hormone levels and elevated blood pressure. Promisingly, the metabolite profile seems to be modifiable in young adults through lifestyle changes (Würtz et al. 2014). Therefore, the meaning of health services and health counseling should not be underestimated. Guidance of health experts, e. g. exercise physiologists should be highlighted in the future to mitigate this problem and support individuals to include physical activity and healthy nutrition in their everyday life.

We cannot generalize too much the results received from this study, because the participants were only Finnish men. Opposite gender and other ethnicity could have produced results with different trend. In the light of earlier research, it seems probable, that resistance training two to three times a week did not induce as great changes in metabolism as long-term and great amount of physical activity would have done (e. g. Kujala et al. 2013; Xiao et al. 2016). Moreover, aerobic physical activity may have had resulted in more pronounced changes as it stresses more cardiorespiratory system. At least, it would have caused more changes in the circulatory metabolism which was assessed in the current study. To understand more specifically the changes of metabolic markers on a tissue level, muscle biopsies would have been needed to give more insight into this area.

9.7 Conclusions

The present study examined the effects of three different post-workout supplements (PROT/CHO/PROT+CHO) and two different resistance training programs (HYP/MAX+POW) on serum metabolome, muscle hypertrophy and muscle strength. The actual study intervention including resistance training two to three times a week lasted for 12 weeks and the participants were recreationally active men (n=60). The main finding of the study was that the study intervention resulted in slightly better metabolite profiles among all the participants as compared to pre-measurements. Especially, all the concentrations of dyslipidemia markers, except glucose (HDL cholesterol, LDL cholesterol, serum triglycerides and serum cholesterol) shifted towards more non-atherogenic values. These findings, except for glucose, are in accordance with earlier research work (Kujala et al. 2013). Therefore, it can be concluded that resistance training and post-workout supplements affected serum metabolome. However, it is hard to distinct which differences were due to training and which ones due to supplements. Inclusion of control group could have eased to see the impact of physical activity compared to inactivity on serum metabolome.

Moreover, study intervention led to gains in both isometric muscle strength and in the CSA of *vastus lateralis*. HYP group gained slightly more strength than did MAX+POW group but the increases in the CSA of VL were rather equal and cannot be fully trusted because of a great number of lacking data. Both training groups and all the supplement groups ameliorated their body composition towards more lean and less fat-containing body. However, in comparison between the supplement groups, whey protein group ameliorated its body composition the most. Also, HYP group showed greater adaptations in body composition compared to MAX+POW group.

This study suggests that in order to enhance body composition, whey protein leads to better outcomes than either carbohydrate or carbohydrate plus protein supplements. Thus, it does not seem necessary to add carbohydrate to post-workout supplements. Furthermore, PROT and CHO supplements were isocaloric but PROT+CHO supplement doubled the calorie intake. Hence, the use of it seems unnecessary to avoid excess calories. Considering muscle strength and hypertrophy, resistance training is an efficient way to achieve gains in both. In the light of these study results, resistance training aiming to hypertrophy can be recommended to achieve greater gains, even though the differences between the groups were somewhat small. Giving support to the results of the present study, physical activity creates healthier metabolite profiles (e.g. Floegel et al. 2014; Kujala et al. 2013; Krauss et al. 2002). However, because of the constantly alterable trend of metabolism there still exists a lot to be known. Therefore, multifaceted research work is needed in the future. Hopefully in the future with more research done, we will see physical exercise prescriptions more commonly used as a solution to prevent or alleviate the symptoms of different cardiometabolic diseases.

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APPENDIXES

APPENDIX 1. Serum metabolome variables analyzed in this study.

XXL-VLDL-P	**Concentration of chylomicrons and extremely large VLDL particles**
XL-VLDL-P	Concentration of very large VLDL particles
L-VLDL-P	Concentration of large VLDL particles
M-VLDL-P	Concentration of medium VLDL particles
S-VLDL-P	Concentration of small VLDL particles
XS-VLDL-P	Concentration of very small VLDL particles
IDL-P	Concentration of IDL particles
L-LDL-P	Concentration of large LDL particles
M-LDL-P	Concentration of medium LDL particles
S-LDL-P	Concentration of small LDL particles
XL-HDL-P	Concentration of very large HDL particles
L-HDL-P	Concentration of large HDL particles
M-HDL-P	Concentration of medium HDL particles
S-HDL-P	Concentration of small HDL particles
VLDL-D	Mean diameter for VLDL particles
LDL-D	Mean diameter for LDL particles
HDL-D	Mean diameter for HDL particles
Serum-C	Serum total cholesterol
VLDL-C	Total cholesterol in VLDL
IDL-C	Total cholesterol in IDL
LDL-C	Total cholesterol in LDL
HDL-C	Total cholesterol in HDL
HDL_2-C	Total cholesterol in HDL_2
HDL_3-C	Total cholesterol in HDL_3
FreeC	Free cholesterol
Serum-TG	Serum total triglycerides
VLDL-TG	Triglycerides in VLDL
IDL-TG	Triglycerides in IDL

LDL-TG	Triglycerides in LDL
HDL-TG	Triglycerides in HDL
$ApoA_1$	Apolipoprotein A_1
ApoB	Apolipoprotein B
ApoB/$ApoA_1$	Ratio of apolipoprotein B to apolipoprotein A_1
TotFA	Total fatty acids
UnSat	Estimated degree of unsaturation
DHA	22:6, docosahexaenoic acid
LA	18:2, linoleic acid
CLA	Conjugated linoleic acid
FAw3	Omega-3 fatty acids
FAw6	Omega-6 fatty acids
PUFA	Polyunsaturated fatty acids
MUFA	Monounsaturated fatty acids; 16:1, 18:1
SFA	Saturated fatty acids
Glc	Glucose
AcAce	Acetoacetate
bOHBut	3-hydroxybutyrate
Alb	Albumin
Gp	Glycoprotein acetyls, mainly α_1-acid glycoprotein