DIETARY LIPIDS, LIPID REGULATION,

AND RESISTANCE EXERCISE RESPONSES

A DISSERTATION

by

CHUN-WEN CHEN

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Steven E. Riechman
Committee Members,	James D. Fluckey
	Stephen F. Crouse
	Stephen B. Smith
Head of Department,	Richard B. Kreider

August 2017

Major Subject: Kinesiology

Copyright 2017 Chun-Wen Chen

ABSTRACT

Being the largest tissue that accounts for 40-50% of overall metabolism in non-obese individuals, skeletal muscle is a modifiable target for reducing the risk of chronic diseases. Resistance exercise induces cell signaling pathways that promote muscle protein synthesis via muscle contraction, inflammation, and hormones. Lipids facilitate these mechanisms through the provision of energy and building blocks for cell regeneration, regulation of membrane permeability for hormones, and formation of lipid rafts for signaling molecules. Exercise increases free fatty acids and mobilizes several kinases, the factors that activate the skeletal muscle lipid regulator PPARδ. The purpose of this study was to test the hypotheses that dietary lipids would improve skeletal muscle adaptations to resistance exercise training and that resistance exercise would enhance lipid metabolism as demonstrated by the upregulation of PPARδ.

The first study examined the changes in skeletal muscle mass, strength, peak power, and quality in response to a 12-week whole-body progressive resistance exercise training (8 sets/12 reps, 70% 1RM) with different levels of dietary cholesterol supplementation. Secondary analyses included determination of the association between dietary fatty acids and skeletal muscle adaptation. No effects of dietary

cholesterol on the training-induced muscle adaptation was observed. Exploratory analyses of dietary fatty acids suggested potential effects on adaptations. The second study explored the effects of dietary cholesterol and fatty acids on the muscle protein synthesis and soreness induced by a short-term high-intensity unilateral leg resistance exercise (5 set/reps until failure, 85% 1RM). Muscle protein synthesis rate was not significantly different between the exercised and non-exercised legs during the 22 hours after the exercise. In the high cholesterol intake group, muscle PPARδ protein content was 38.9±24.1% higher in the exercised than the non-exercised legs and soreness levels were 91.6±3.6% lower than the low cholesterol intake group. The third study investigated the effects of a 10-week whole-body progressive resistance exercise training (8 sets/12 reps, 75% 1RM) on muscle PPARδ protein content. Before the training, PPARδ protein content acutely increased by 49.1±0.29% after one bout of exercise and the increase was inversely proportional to body fat percentage. Resting muscle PPARδ protein content increased by 114.7±0.32% after the training.

The findings in these studies provided insights into the potential effects of lipids on skeletal muscle adaptation and the mechanism of lipid regulation induced by resistance exercise.

ACKNOWLEDGEMENTS

I would like to thank my dissertation committee chair, Dr. Steven E. Riechman, for your guidance and support that gave me the confidence to achieve my goals. The freedom you allowed me to explore my path while being supportive was invaluable. You have shown me the way to think rationally and how not to be trapped by the dogma. Thank you for standing by me through some of my tough times. I could not have accomplished my graduate studies without you.

I would like to thank my dissertation committee. Dr. James D. Fluckey, your lectures expired me to explore facts in different perspectives. It is always inspiring to talk with you. When I was struggling with the experimental protocols in the MBL, your advice, generosity, and humor helped me relax and make the proteins move to where I wanted them to be. Dr. Stephen B. Smith and Dr. Stephen F. Crouse, thank you for all the guidance and feedback on my studies. You have set the examples of being professional and successful in academia.

I would like to thank the members of Human Countermeasures Laboratory. Chang Woock Lee, you are the big brother who takes care of everyone in the lab. You have guided me through many things and helped me overcome challenges. Thank you for

being so reliable and supportive. Steve, I could not express how much I appreciated your immediate help whenever I needed you.

I would like to thank my fellow graduated and current graduate students for your friendship and assistance. Heath Gasier, Mats Nilsson, Nicholas Greene, Michael Wiggs, and Brandon Macias, thank you for your generosity and patience to guide me through the experimental protocols. I could not have finished these studies without your support.

Finally, I would like to give special thanks to my parents Jeng-Long Chen and Shu-Yin Lu, and my little brothers Chun-Yu and Chun-Hao Chen, for supporting me in any way you can. You let me know there is a place where I am always welcome and a group of people who always love me.

On the journey through my graduate study, I have met the best advisor and professors who guided me, great colleagues who supported each other, and amazing friends to have fun together. I am thankful to everyone who makes my ten years in Texas A&M University so amazing.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Dr. Steven E. Riechman (advisor), Dr. Stephen Crouse, Dr. James D. Fluckey of the Department of Health and Kinesiology, and Dr. Stephen B. Smith of the Department of Nutrition and Food Science.

All work for the dissertation was completed by Vincent Chun-Wen Chen with assistance from Dr. Steven Riechman, Dr. James Fluckey, Dr. Chang Woock Lee, Dr. Steve Bui, Dr. Heath Gasier, Dr. Mats Nilsson, Dr. Brandon Macias, Dr. Nicholas Greene, Dr. Michael Wiggs, Dr. Kevin Shimkus, Dr. Teak Lee, and Sara Safdari.

Funding Sources

This work was made possible in part by American Egg Association, Sydney and J.L. Huffines Institute for Sport Medicine and Human Performance, Texas Chapter of the American College of Sports Medicine, Department of Health and Kinesiology at Texas

A&M University, College of Education and Human Development at Texas A&M
University, and Office of Graduate and Professional Studies at Texas A&M University.

TABLE OF CONTENTS

Page

ABSTRACT ii

ACKNOWLEDGEMENTS iv

CONTRIBUTORS AND FUNDING SOURCES vi

TABLE OF CONTENTS viii

LIST OF FIGURES x

LIST OF TABLES xiii

CHAPTER I INTRODUCTION AND REVIEW OF LITERATURE 1

- Resistance Exercise and Fatty Acid Oxidation 6
- Resistance Exercise and Skeletal Muscle 9
- Lipid 16
- Resistance Exercise and Lipid Metabolism 31
- Peroxisome Proliferator-Activated Receptors 35
- Summary 49

CHAPTER II THE EFFECTS OF DIETARY CHOLESTEROL AND FATTY ACIDS ON SKELETAL MUSCLE RESPONSE TO RESISTANCE EXERCISE TRAINING 50

- Overview 50
- Introduction 51
- Methods 55
- Results 65
- Discussion 84
- Conclusion 87

CHAPTER III THE EFFECTS OF DIETARY CHOLESTEROL AND FATTY ACIDS ON SHORT-TERM HIGH-INTENSITY ECCENTRIC RESISTANCE EXERCISE 88

Overview........ 88
Introduction........ 89
Methods 93
Results 104
Discussion........ 109
Conclusion 112

CHAPTER IV THE EFFECT OF RESISTANCE EXERCISE ON SKELETAL MUSCLE PPARδ PROTEIN CONTENT 113

Overview........ 113
Introduction........ 114
Methods 117
Results 124
Discussion........ 133
Conclusion 136

CHAPTER V CONCLUSION 137

Dietary Lipids and Resistance Exercise........ 138
Resistance Exercise and PPARδ........ 139
Final Conclusion........ 140

REFERENCE 141

LIST OF FIGURES

Figure 1. The Proposed Relationship among Dietary Lipids, Resistance Exercise-induced Skeletal Muscle Adaptation, and the Regulation of Endogenous Lipids 4

Figure 2. Resistance Exercise-induced Cell Signaling 11

Figure 3. Regulation of Cholesterol in Skeletal Muscle 19

Figure 4. Cholesterol Synthesis 20

Figure 5. General Structure of Fatty Acids 23

Figure 6. Lipid Transport and Metabolism 26

Figure 7. Exercise-induced Lipid Metabolism 33

Figure 8. The Structure of PPARs 36

Figure 9. The Heterodimer of PPAR and RXR 39

Figure 10. The Effects of Exercise on Lipid Regulation via PPARδ 47

Figure 11. Timeline of Study 1 55

Figure 12. Example of DEXA Scan Image of Right Thigh 63

Figure 13. Correlation of Change in Thigh Lean Mass Gain to Dietary (A) Omega 6 Fatty Acids, (B) PUFA, (C) MUFA, (D) Omega 6 to Omega 3 Fatty Acids Ratio, and (E) GLA, per KG Lean Mass, in All Participants 74

Figure 14. Correlation of Change in Lean Mass Gain to Dietary (A) Short-chain Saturated Fatty Acids, (B) Saturated Fatty Acids, and (C) GLA per KG Lean Mass in Men 75

Figure 15. Correlation of Change in Thigh Strength (TS) to Dietary LA in Men 76

Figure 16. Correlation of Change in Thigh Muscle Quality- Strength (TMQ-S) to Dietary LA in Men 76

Figure 17. Correlation of Change in Lean Mass Gain to Dietary DHA per KG Lean Mass in Women 77

Figure 18. Correlation of Change in Thigh Lean Mass to Dietary Omega-6 to Omega-3 Fatty Acids Intake Ratio per KG Lean Mass in Women. 77

Figure 19. Correlation of Change in Thigh Lean Mass to Dietary GLA per Lean Mass in LC 78

Figure 20. Correlation of Change in Composite Peak Power (CP) to Dietary (A) Omega-3 Fatty Acids, (B) PUFA, (C) MUFA, (D) LA, and (E) GLA per KG Lean Mass in LC 78

Figure 21. Correlation of Change in Thigh Peak Power (TP) to Dietary (A) PUFA, (B) MUFA, (C) LA, and (D)GLA per KG Lean Mass in LC 80

Figure 22. Correlation of Change in Whole Body Muscle Quality-Peak Power (WBMQ-P) to Dietary (A) Omega-3 Fatty Acids, (B) Short-chain Saturated Fatty Acids, (C) PUFA, (D) MUFA, (E) LA, and (F) GLA per KG Lean Mass in LC 81

Figure 23. Correlation of Change in Thigh Muscle Quality-Peak Power (TMQ-P) to Dietary (A) PUFA, (B) LA, and (C) GLA per KG Lean Mass in LC 82

Figure 24. Correlation of Change in Thigh Lean Mass to Dietary MUFA per Lean Mass in MC 83

Figure 25. Correlation of Change in Thigh Lean Mass to Dietary (A) Omega-6 Fatty Acids and (B) Omega-6 to Omega-3 Fatty Acids Ratio per Lean Mass in HC 83

Figure 26. Timeline of Study 2 93

Figure 27. Soreness Visual Analogue Scale 99

Figure 28. Dietary Cholesterol and FSR 105

Figure 29. Cholesterol and Soreness 106

Figure 30. Correlation of Overall Soreness to Dietary (A) EPA, (B) DPA, and (C) DHA 107

Figure 31. PPARδ Protein Content in CON and EX Legs in LC and HC Groups 108

Figure 32. Timeline of Study 3 117

Figure 33. Acute Effect of Resistance Exercise on PPARδ Protein Content 127

Figure 34. The Relationship between Change in PPARδ Protein Content and Body Fat Percentage 127

Figure 35. Changes in PPARδ Protein Content between Men and Women 129

Figure 36. Effect of Resistance Exercise Training on PPARδ Protein Content 130

Figure 37. Correlation of PPARδ Protein Content to (A) Total Cholesterol and (B) LDL after Resistance Exercise Training 131

LIST OF TABLES

Table 1. Baseline Measurement by Cholesterol Intake Groups 66

Table 2. Baseline Measurement by Gender.. 67

Table 3. Daily Proportion of Nutrients at Two Weeks before, at the beginning of, and throughout the Training... 68

Table 4. Changes before and after Training.. 70

Table 5. Correlation of Dietary Fatty Acids to Skeletal Muscle Adaptation to Resistance Exercise Training... 72

Table 6. Baseline Measurements in LC and HC Groups...................................... 104

Table 7. Subject Baseline and the Changes in Body Composition....................... 125

Table 8. Coefficient of Determination of Skeletal Muscle PPARδ Protein Content to Serum Lipid Profile... 131

Table 9. Lipid Profile before and after Resistance Exercise Training................... 132

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Physical inactivity and poor nutrition are primary contributors to preventable death in the United States (97). Central to this link among mortality, nutrition, and activity is the role of skeletal muscle function. Skeletal muscle accounts for 40-50% of total body mass in non-obese individuals. It is responsible for more than 50% of overall resting metabolism and increases metabolism by 10-fold during exercise. Skeletal muscle plays a major role in the regulation of electrolytes, acidity, and glucose and lipid metabolism, and therefore, the impact of calorie, fat, glucose, and cholesterol intake on disease is associated with skeletal muscle activity (121).

The metabolism in skeletal muscle is highly regulated by hormones and activity levels, making it one of the potential therapeutic target for metabolic diseases (37). For instance, skeletal muscle takes up and utilizes up to 75% of glucose when stimulated by insulin, making it the primary site of insulin resistance which contributes to metabolic diseases such as type 2 diabetes. Muscle contraction also affects skeletal muscle metabolism independent of insulin and other hormones. For example, muscle contraction increases glucose uptake without the influence of insulin (87). Therefore,

skeletal muscle health and activity have been identified as modifiable targets for reducing the risk of chronic diseases.

Adaptation of skeletal muscle is strongly affected by the workload. High workload, such as resistance exercise, leads to the muscle hypertrophy and strength gain while disuse of muscle may result in the muscle atrophy (144). These adaptations are localized to the working muscle, indicating the regulation by the local signals within the muscle (37). Nutrition and hormones also contribute to the adaptation of skeletal muscle (12). For example, protein and carbohydrate whose uptake are regulated by insulin and growth factors have been identified to be essential for muscle hypertrophy following resistance exercise training (12, 59). While the effects of dietary protein and carbohydrate on the resistance exercise-induced muscle adaptation have been well studied, the effects of dietary lipids on this adaptation are unclear. Most studies investigating lipid metabolism and resistance exercise training focus on the changes of endogenous lipid profile such as triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL) but not the effects of dietary lipids on muscle adaptation. Furthermore, the effects of dietary nutrients have not been monitored or controlled in many exercise training studies.

Exercise training increases the oxidative capacity of muscle by upregulating the expression of proteins involved in the uptake, transport, and oxidation of fatty acids

(FAs) (11, 14, 135). Being the largest tissue and source of energy expenditure, the adaptation of skeletal muscle to exercise may affect general health on the whole-body level thorough energy regulation. Exercise induces expression of metabolic regulators such as PPARδ, which has been identified as a major regulator of lipid metabolism in skeletal muscle and the potential therapeutic target for metabolic syndrome (8, 88, 119, 141). Studies have shown that endurance exercise increases PPARδ expression and that the exercise-induced increase of PPARδ may be beneficial as a clinical treatment for the type 2 diabetes (43, 156). Furthermore, a significantly higher PPARδ mRNA expression has been found in the high fat-fed exercised rats than the exercised-only and high fat-fed-only rats, implying a combined effects of fat intake and exercise on PPARδ expression (76). However, not only does the effects of resistance exercise on PPARδ protein content in humans remain to be elucidated but the studies regarding PPARδ and exercise have been focusing on PPARδ mRNA content, while the mRNA levels do not necessarily predict its protein content. The protein abundance in humans may be affected by transcription, mRNA decay, translation, and protein degradation (151).

The purpose of this dissertation was to investigate the relationship between dietary lipids (FAs and cholesterol) and the skeletal muscle adaptation to resistance exercise training as well as the effects of resistance exercise training on PPARδ, the lipid metabolism regulator in skeletal muscle (Figure 1).

It was hypothesized that dietary lipids would be associated with enhanced skeletal muscle mass, strength, and peak power following resistance exercise training and that resistance exercise training would improve lipid profile and increases the PPARδ protein content as a biomarker of enhanced lipid metabolism in skeletal muscle.

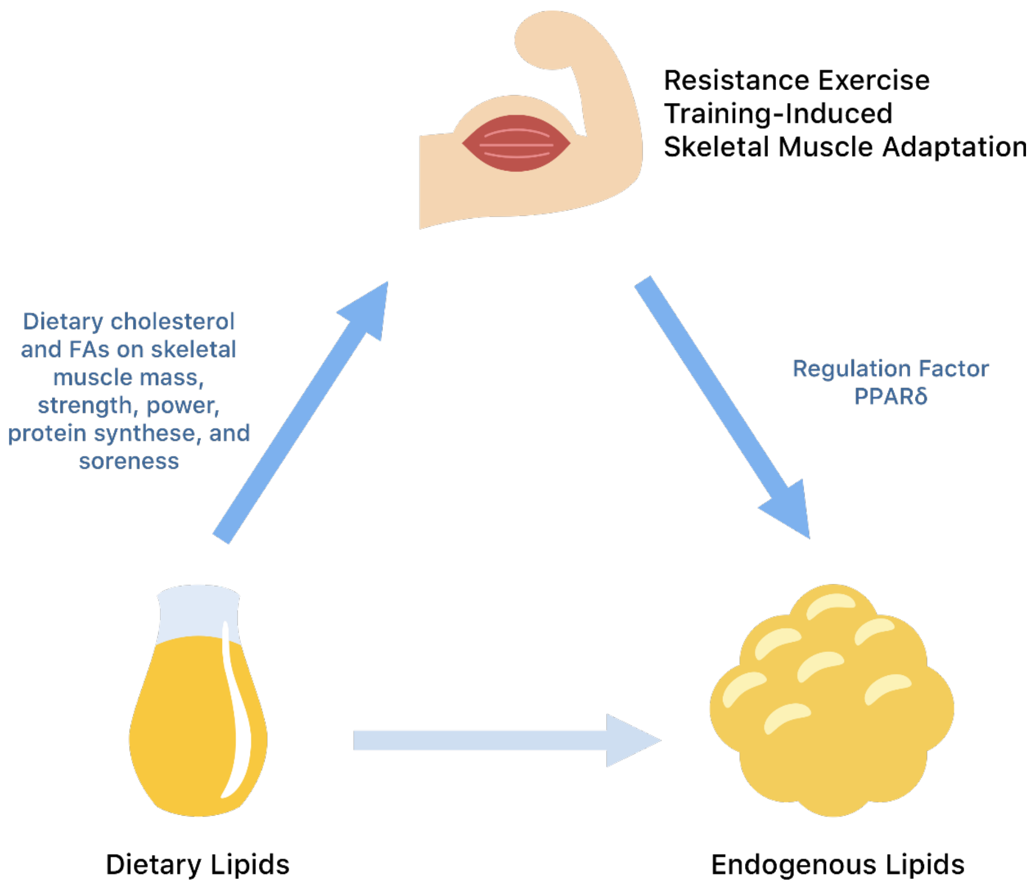

Figure 1. The Proposed Relationship among Dietary Lipids, Resistance Exercise-induced Skeletal Muscle Adaptation, and the Regulation of Endogenous Lipids.

Three studies were conducted to test this hypothesis:

- *Study 1 (Chapter II)*: A randomized, placebo controlled trial was conducted to test the effects of dietary cholesterol on the changes in skeletal muscle mass, strength, and peak power with a 12-week whole-body progressive resistance exercise training program. Additionally, FA intake variability was examined as a potential contributor to and these adaptations. It was hypothesized that dietary cholesterol enhanced the training-induced skeletal muscle adaptation, and dietary FAs were positively associated with the adaptation;
- *Study 2 (Chapter III)*: Effects of high and low cholesterol intake on muscle protein synthesis, soreness levels, and PPARδ protein content following a high-intensity short-term resistance exercise were examined. Additionally, dietary FA intake variability was examined as a potential contributor to these adaptations. It was hypothesized that the exercise-induced muscle protein synthesis would increase and soreness levels would decrease with high cholesterol intake, and that the exercise would increase muscle PPARδ protein content;
- *Study 3 (Chapter IV)*: The effects of a 10-week whole-body progressive resistance exercise training on skeletal muscle PPARδ protein content and serum lipid profile were examined. Additionally, the association between the changes in muscle PPARδ protein content and lipid profile following the training were analyzed. It was hypothesized that resistance exercise would acutely and chronically increase muscle PPARδ protein content, and that the increase would be associated with improved serum lipid profile.

Resistance Exercise and Fatty Acid Oxidation

Exercise is defined as "a subset of physical activity that is planned, structured, and repetitive, and has as a final or an intermediate objective the improvement or maintenance of physical fitness" (22). Endurance exercise is defined as a type of exercises performed to improve endurance. Performing endurance exercise training increases mitochondrial density, FA oxidation, and decreases the use of muscle glycogen and blood glucose at absolute intensities (63). On the other hand, resistance exercise utilizes resistance to induce muscle contraction and enhances strength, anaerobic power, and skeletal muscle mass. Unlike endurance exercise, resistance exercise is generally considered less related to lipid metabolism. Furthermore, some early studies suggested that muscle hypertrophy induced by resistance exercise reduced the oxidative capacity of skeletal muscle due to the dilution of mitochondria. Alway et al. (3) revealed that resistance exercise-induced muscle hypertrophy did not increase mitochondria proportionally with contractile protein, while cytoplasm, sarcoplasmic reticulum, and lipid components did. MacDougall et al. (91) also reported the reduction in the mitochondrial volume density following a 6-month intensive resistance exercise training program and concluded that resistance exercise training attenuated endurance performance. Tesch et al. (145) compared the activities of mitochondrial enzymes in resistance exercise-trained (more than four years of training) to sedntary subjects and found decreased activities in citrate synthase (CS) and 3-OH-

acyl-CoA dehydrogenase as well as lower density of capillary and mitochondria in the trained subjects and thus concluded that resistance training decreased aerobic capacity. In the study of Chilibeck et al. (27), 12 weeks of resistance training reduced the activity of succinate dehydrogenase (SDH). The decrease in SDH activity was associated with the reduced mitochondrial volume density caused by muscle hypertrophy, and therefore the authors suggested that the muscle oxidative capacity was reduced by the resistance exercise training.

These assumptions of the decreased oxidative capacity following resistance exercise training were mainly based on the reduction of mitochondrial volume density following resistance exercise training. Although Tesch and Chilibeck investigated some oxidative enzymes, such as CS, 3-OH-acyl-CoA dehydrogenase, and SDH, the results were inconsistent and other important oxidative enzymes remained to be examined (142).

Tang et al. (142) argued that it is problematic in the previous studies to assume the cause of lower oxidative potential to be merely training-induced hypertrophy, but not other independent factors. They also found inconsistent results of mitochondrial enzyme activities with different protocols of resistance exercise training. In their study, mitochondrial enzyme activities were examined in 12 untrained men before and after performing a 12-week, high-intensity, whole-body resistance exercise training. Their results showed increased activities of CS, β-hydroxyacyl-CoA dehydrogenase (β-HAD),

and Hexokinase (HK). There was no change in the activity of 6-phosphofructokinase (6-PFK), the rate-limiting enzyme in glycolysis, implying a limited effect of resistance exercise training on glycolysis. With the increases in CS and β-HAD activities, the authors concluded that the resistance exercise training enhanced muscle oxidative potential. Furthermore, the increase in HK and the unchanged 6-PFK activities may favor glycogenesis. Whereas resistance exercise causes glycogen depletion, resistance exercise training increases the ability of glycogen synthesis in the skeletal muscle (143).

Resistance exercise has also been shown to acutely utilize fat as an energy source. Goto et al. (50) found increases in glycerol in the blood stream after a bout of resistance exercise, suggesting hydrolysis of triglyceride (TG) during the exercise. They also observed the enhanced FA oxidation in the endurance exercise 20 minutes after resistance exercise. In a review article by Ozaki et al. (109), VO_2max was shown to increase following resistance exercise training in young and old individuals.

Besides exercise, nutrition and its metabolism are major factors that affect skeletal muscle biology and whole-body metabolism. Dietary lipids provide essential energy, not only for daily physical activities but also for energy expenditure during exercise (122). When combining with exercise, saturated FAs improve cardiac health by increasing brachial artery dilation (111). Monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) help maintain blood cholesterol and lipid levels,

lower blood pressure, and therefore reduce cardiovascular risk (5, 96). They may also decrease the risk of type 2 diabetes by enhancing insulin sensitivity (85, 117, 146). Cholesterol may have several important roles in muscle hypertrophy induced by exercise through provision of building blocks for cell repair (cell membrane), facilitating inflammation to repair exercised muscle, forming lipid rafts for cell signaling, and being the precursor to endogenous steroids (121).

Resistance Exercise and Skeletal Muscle

Resistance exercise is a type of physical activity specializing in the use of resistance to induce muscle contraction. It involves the sequence cascade: 1. activation of muscle fibers, 2. cell signaling stimulated by muscle contraction, hormones, and immune/inflammatory responses, 3. muscle protein synthesis, and 4. hypertrophy (134).

During resistance exercise, skeletal muscle fibers are activated by α motor neurons to create force. The regulation of the force produced is determined by the frequency of neural stimulation and the number of motor units recruited. Higher frequency of neural stimulation produces larger force. The recruitment of muscle fiber is determined by the need of force to perform a movement. These determination factors include exercise

load, the rate of force development, and muscle fatigue. According to the size principle, the smaller motor units (mainly Type I) are recruited first, followed by the larger units (mainly Type II) until the need of force is fulfilled (19, 61). While performing resistance exercise, a large force is produced, and therefore high-threshold motor units (Type II) are recruited. When performing an explosive exercise, the large force is induced by the high acceleration (force = mass x acceleration), and therefore the motor units with higher threshold are recruited. The recruitment of motor units may also be affected by fatigue and failure (116). For instance, when performing a prolonged exercise, higher-threshold motor units may be recruited to compensate the force needed while the lower-threshold units start to fatigue.

Resistance exercise activates several cell signaling pathways that induce muscle protein synthesis via muscle contraction, hormones, and inflammatory responses (Figure 2).

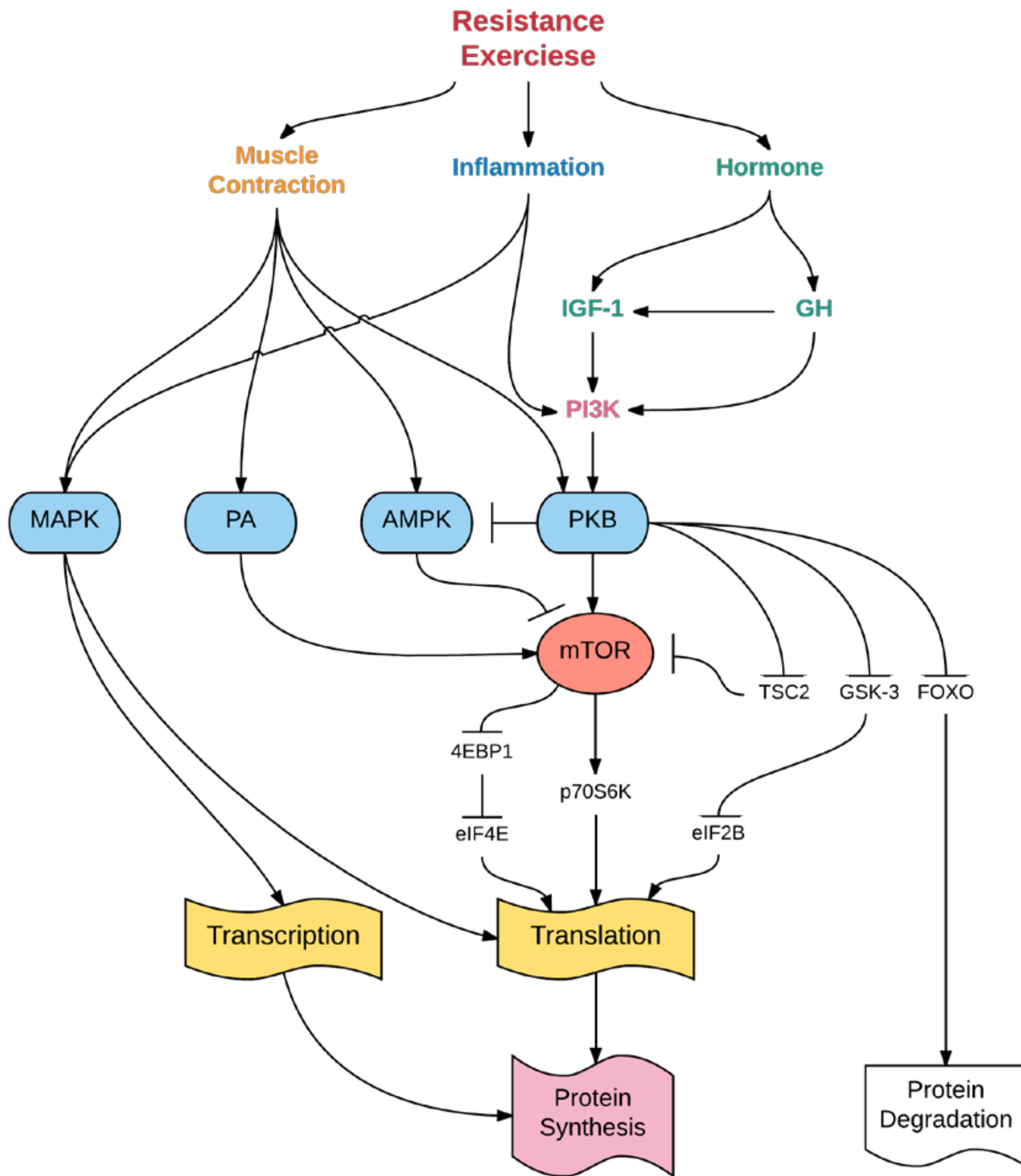

Figure 2. Resistance Exercise-induced Cell Signaling. ***IGF-1****: Insulin-like growth factor 1;* ***GH****: Growth hormone;* ***PI3K****: Phosphoinositide 3-kinase;* ***MAPK****: Mitogen-activated protein kinase;* ***PA****: Phosphatidic acid;* ***AMPK****: 5' adenosine monophosphate-activated protein kinase;* ***PKB****: Protein kinase B (Akt);* ***TSC2****: Tuberous Sclerosis Complex 2;* ***mTOR****: mammalian target of rapamycin;* ***4EBP1****: Eukaryotic initiation factor 4E binding protein 1;* ***eIEF4E****: Eukaryotic initiation factor 4E;* ***p70S6K****: Ribosomal protein S6 kinase beta-1 (S6K1);* ***GSK-3****: Glycogen synthase kinase-3;* ***eIF2B****: Eukaryotic initiation factor 2B;* ***FOXO****: Fork-head box O transcription factor.*

Cell Signaling Induced by Muscle Contraction

Muscle contraction induces mechanical deformation of muscle fiber, which evokes signaling pathways independent of hormones and growth factors, including protein kinase B (PKB/Akt)-mammalian target of rapamycin (mTOR), phosphatidic acid (PA)-mTOR, adenosine monophosphate-activated protein kinase (AMPK), and mitogen-activated protein (MAPK) pathways (Figure 2) (6, 58, 67, 134, 149). Muscle contraction may also activate the mTOR-independent phosphatidylinositol-3-kinases (PI3K)-PKB cascade through PA (108).

PKBα and PKBβ are the two main PKB/AKt isoforms in skeletal muscle, while PKBγ is mainly expressed in brain, lung, and kidney (161). While PKBα is essential in the early stage of myoblast differentiation, including the embryonic, myogenic developments, and postnatal survival, PKBβ plays a critical role in the glucose homeostasis and insulin sensitivity by inducing the translocation of glucose transporter (GLUT) for glucose uptake. It may be activated by either insulin and muscle contraction alone.

Stimulation and overexpression of PKB result in muscle hypertrophy via mTOR and GSK-3β pathways (29, 134). PKB activates mTOR by directly phosphorylating it or repressing its inhibitors such as AMPK and tuberous sclerosis complex 2 (TSC2) (55). mTOR enhances translational efficiency by phosphorylating ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor 4E binding protein 1 (4EBP1). When p70S6K is

phosphorylated, it activates the S6 subunit on the 40S ribosomal subunits, which regulates translation. 4EBP1 binds to the eukaryotic initiation factor 4E (eIF4E) and suppresses its functionality on recruiting 40S ribosomal subunits and therefore inhibits the translation. When 4EBP1 is phosphorylated by mTOR, it releases eIF4E and facilitates the translation.

PKB also phosphorylates glycogen synthase kinase-3β (GSK-3β) and the fork-head box O transcription factor (FOXO) (9). GSK-3β attenuates translation by suppressing eIF2B, which regulates the translation through 40S ribosomal subunits. The phosphorylation of GSK-3β by PKB reduces its inhibitive effect on eIF2B and therefore enhances the translation. FOXO induces protein degradation by enhancing the expression of proteolytic ubiquitin ligases. Phosphorylation of FOXO by PKB attenuates its effect on protein degradation.

AMPK is the master metabolic switch in cells. When energy status is low (high AMP and low glycogen concentrations), AMPK is activated to increase energy production and reduce energy consumption. whereas AMPK inhibits mTOR activity to lower the energy consumption for muscle protein synthesis, PKB may suppress AMPK (13, 55).

MAPK are the protein kinases that are activated by stress stimuli in skeletal muscle (161). The parallel MAPK signaling pathways include extracellular signal-regulated

kinase 1/2 (ERK 1/2), extracellular signal-regulated kinase 5 (ERK5), and the stress-activated protein kinase cascades including p38 MAPK and c-Jun NH_2-ternminal kinase (158). Besides growth factors and hormones, mechanical stress and intracellular calcium induced by muscle contraction stimulate MAPK signal pathways, resulting in enhanced transcription and translation.

Cell Signaling Induced by Hormones

Resistance exercise induces several hormonal responses depending on the protocol. Two of the important muscle hypertrophy promoting hormones are growth hormone (GH) and insulin-like growth factor-1 (IGF-1) (134).

Growth hormone (GH) is a family of more than 100 hormones, including 22 kDa GH monomers, 20 kDa mRNA spice variants, disulfide-linked homodimers and heterodimers, glycosylate GH, high molecular weight oligomers, and hormone fragments from proteolysis (107, 134). Resistance exercise increases circulating GH acutely and chronically (68, 80, 107). When the circulating GH binds to its receptor on the membrane, the janus kinase 2 (JAK2) is activated. JAK2 activates phosphatidylinositol-3 Kinase (PI3K), which activates the PKB signaling pathways and increase muscle protein synthesis by enhancing transcriptional efficiency (134).

Insulin-like growth factor 1 (IGF-1) is a polypeptide protein hormone that binds to both the IGF-1 receptor (IGF1R) and the insulin receptor on the cell surface in many tissues including skeletal muscle. It stimulates cell growth and inhibits programmed cell death. Resistance exercise increases both circulating and muscular IGF-1, putatively via the increase of GH (134). IGF-1 increases muscle protein synthesis by activating the PI3K-PKB pathways and stimulating the proliferation and differentiation of satellite cells (92, 124). As described previously, PI3K stimulates PKB and activates the cell signaling cascades that induce muscle protein synthesis. PKB also facilitates satellite cell proliferation by attenuating the cell-cycle inhibitor Cyclin-dependent kinase inhibitor 1B (CDKN1B) (92). Furthermore, IGF-1 promotes muscle differentiation by enhancing the activity and expression of myogenic regulatory factors (100).

Besides GH and IGF-1, other hormones may also play roles in the muscle growth in response to resistance exercise. Testosterone is one of the most discussed hormones that promotes muscle hypertrophy. Testosterone is an anabolic steroid hormone that induces muscle protein synthesis by binding to androgen receptors and enhancing gene expression. The increase in satellite cells by testosterone is independent of testosterone quantity (134).

Cell Signaling Induced by Inflammatory Responses

Resistance exercise induces muscle damage by overstretching muscle fibers. The muscle damage causes the inflammatory response resulting in the recruitment of macrophages and neutrophils (134). They break down damaged muscle tissue and produce cytokines including interleukin-6 (IL-6) and transforming growth factor-β (TGFβ), which play important roles in the exercise-induced muscle hypertrophy (57, 77). IL-6 induces satellite cell proliferation by activating the MAPK and PI3K cell signaling cascades via the JAK signaling pathway (60, 127). TGFβ enhances muscle differentiation and regulates local collagen synthesis to build up skeletal muscle architecture (57).

The factors involved in the signaling pathways not only affect muscle protein synthesis, but they may also contribute to the regulation of energy sources during and after exercise. It will be discussed in the following sections.

Lipid

Lipids, including FAs and sterols, are essential for energy production and the regulation of metabolism. They not only provide a key dense energy source but are also the building blocks for biological components such as membranes, signaling constituents, and hormones. Plasma lipid concentrations are regulated primarily by the liver,

adipose, and muscle tissues (14, 15). Therefore, this dissertation studied the interaction, especially with exercised skeletal muscle, of dietary lipids, blood lipids, and exercise training responses.

Cholesterol

Cholesterol is a 27-carbon, four-ring sterol that is involve in the formation of the semipermeable membrane, regulation of membrane fluidity and membrane proteins, and modulation of membrane trafficking and cell signaling processes (70). It is also a component of bile and the precursor of bile acids and salts, vitamin D, and steroid hormones including testosterone and estrogen. With its regulatory roles in the plasma membrane, cell signaling, and inflammation, cholesterol may be important for skeletal muscle hypertrophy (121).

Cholesterol is abundant in the plasma membrane, Golgi complex, and endocytic recycling compartments. The sources of cholesterol in the human body are *de novo* synthesis and diet. Dietary cholesterol has been suggested to be the contributor of cardiovascular diseases. A limit of 200 mg cholesterol daily intake has been recommended by the American Heart Association (AHA) to improve the blood cholesterol concentration and reduce the risk of coronary heart disease (112). However, the elevation of blood cholesterol after dietary cholesterol consumption is inconsistent and some individuals show increases in both low-density lipoprotein (LDL)

and high-density lipoprotein (HDL) levels (122, 129). The recommendation of limiting dietary cholesterol to <300 mg per day for the general population by USDA has recently been rescinded due to the lack of evidence that higher intakes contributes to diseases (39, 66, 147).

Cholesterol biogenesis is regulated by the intracellular cholesterol levels. Sterol regulatory element binding protein-2 (SREBP-2) is the main transcriptional regulator of cholesterol homeostasis. When the sterol levels are high, cholesterol binds to the SREBP cleavage-activating protein (SCAP) on its sterol-sensing domain (SSD) and 25-hydroxycholesterol binds to the endoplasmic reticulum (ER) retention protein insulin induced gene protein (INSIG), resulting in the attachment of INSIG to the SREBP-SCAP complex. The attachment of INSIG and SREBP-SCAP complex prevents the transportation of the complex to Golgi apparatus, where the mature SREBP is released to nucleus (Figure 3). When sterol levels are low, INSIG releases the SREBP-SCAP complex, which is then transported to Golgi apparatus by the COPII vesicle from ER. At the Golgi apparatus, the site-1 protease (S1P) and site-2 protease (S2P) cleave the SREBP precursor protein and release the active part of SREBP to the nucleus. SREBP binds to the sterol regulatory element (SRE) area on the DNA and upregulates the transcription of genes involved in the synthesis of low-density lipoprotein receptor (LDLR) and 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase.

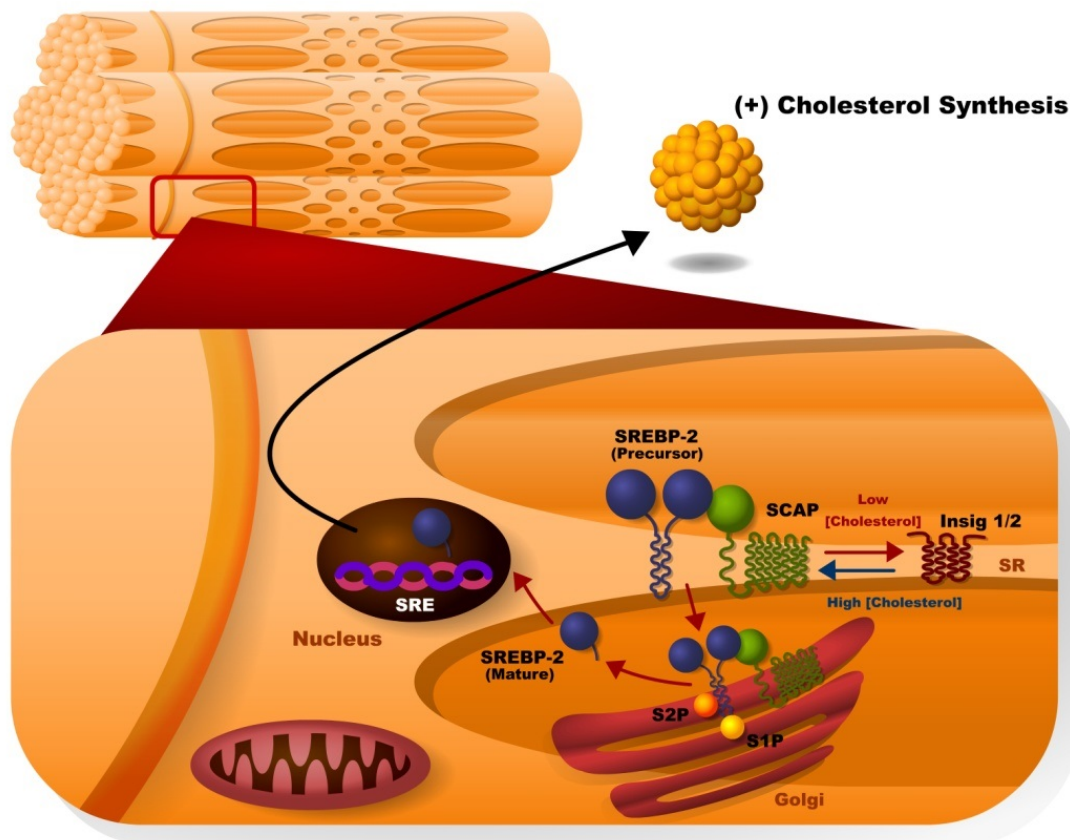

*Figure 3. Regulation of Cholesterol in Skeletal Muscle. **SREBP2**: Sterol regulatory element binding protein-2; **SCAP**: SREBP cleavage activating protein; **Insig 1/2**: Insulin induced gene protein 1/2; **S1P**: Site-1 protease; **S2P**: Site-2 protease; **SRE**: Sterol regulatory element; **SR**: Sarcoplasmic reticulum.*

HMG-CoA reductase regulates cholesterol biosynthesis and therefore it has been a target for the treatment of hypercholesterolemia (53, 72, 104). HMG-CoA is synthesized from three acetyl-CoA through thiolase and HMG-CoA synthase. HMG-CoA reductase binds to four HMG-CoAs and transforms them to mevalonate, which is then converted to squalene via multiple steps. Following the function of enzyme squalene

cyclase, squalene is then oxidized to lanosterol, which is further oxidized to synthesize cholesterol (Figure 4).

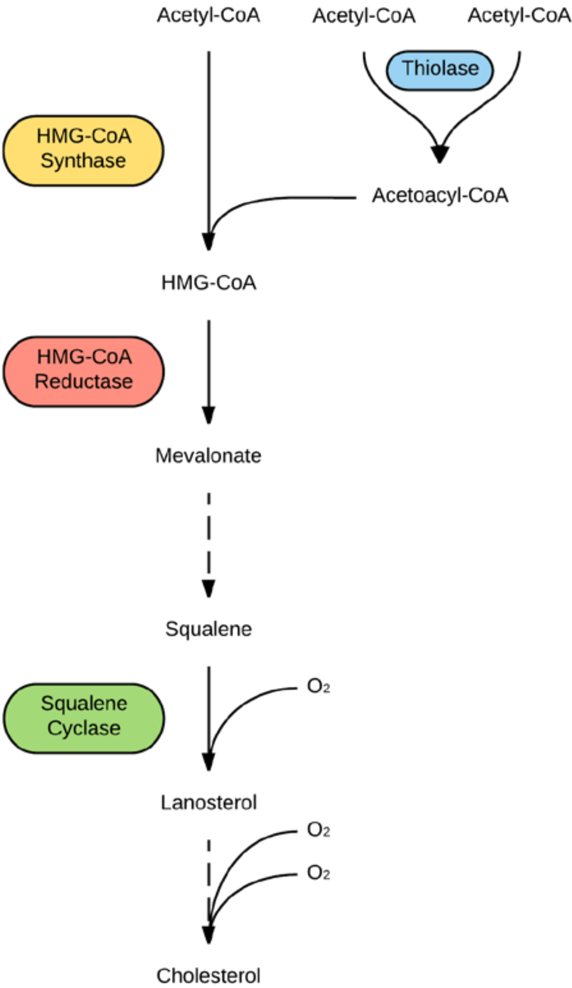

Figure 4. Cholesterol Synthesis.

Our laboratory was the first to demonstrate that dietary and serum cholesterol are associated with skeletal muscle adaptation to resistance exercise training (120). Exercise, especially resistance exercise, induces muscle damage, which causes inflammation. The mechanical deformation also promotes the release of growth factors. Muscle damage-induced inflammation is essential for the muscle growth because it causes the accumulation of nutrient, cytokines, and growth factors that are delivered by the elevated blood flow and released from neutrophils and macrophages. Cholesterol plays important roles in the process of inflammation, cell signaling, and cell stability during muscle hypertrophy. At the inflammation site, cholesterol functions as the component of the cell membrane that increases membrane viscosity and promotes the membrane stability. Cholesterol forms lipid rafts, which modulate signaling molecules, regulate membrane fluidity and membrane protein trafficking, and influence neurotransmission and receptor trafficking, with glycosphingolipids at the cell membrane (115). In skeletal muscle, the deficiency of cholesterol has been found to be associated with decreased lipid rafts, which may be restored by the cholesterol supplementation (114). By forming the lipid rafts as a platform for the molecules of cell signaling pathways, cholesterol facilitates pathways such as the PKB-mTOR and MAPK that promote muscle protein synthesis. Lipid rafts are also essential for the muscle growth pathways through the receptors of insulin, IGF-1, TNF-α, EGFR, PDGFR, IL-6, ERK-2, AKt-1 and steroid hormones.

Besides the contribution to the muscle contraction-induced cell signaling pathways, cholesterol also contributes to the formation of steroid hormones that stimulate muscle growth.

In summary, cholesterol may have important roles in muscle hypertrophy induced by exercise by providing the building blocks for cell membrane repair, facilitating inflammation, and forming lipid rafts and steroids that enhance cell signaling (121).

Fatty Acids

A FA consists of a long hydrocarbon chain and a carboxyl group (COOH) (Figure 5). They are either saturated when containing no carbon-carbon double bond or unsaturated when having one or more carbon-carbon double bonds in their structures. There are two categories of unsaturated FAs: monounsaturated FAs (MUFAs) which contain only one carbon-carbon double bond and polyunsaturated FAs (PUFAs) that have two or more carbon-carbon double bonds. Non-essential FAs (NEFAs) may be endogenously synthesized or derived from other FAs. Essential FAs (EFAs) cannot be synthesized endogenously, and therefore they must be obtained from dietary sources (33).

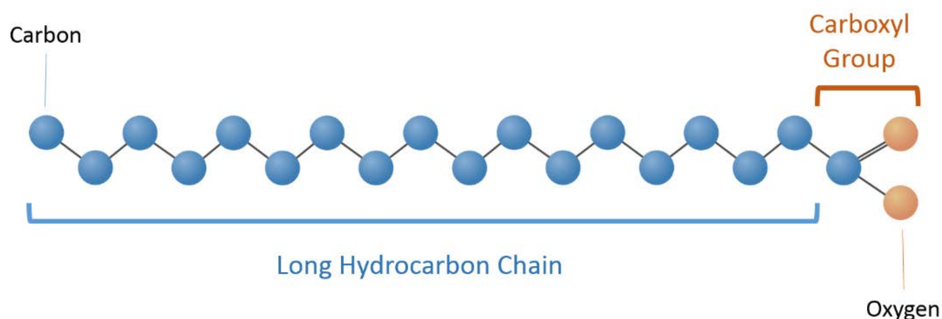

Figure 5. General Structure of Fatty Acids.

Linoleic acid (LA, 18:2 n-6) and α-linolenic acid (ALA, 18:3 n-3) are the two EFAs for animals. Endogenous n-6 and n-3 FAs are derived from LA and ALA, respectively. The n-9 FAs are derived from the desaturation of saturated FAs (SFAs) by the Δ9 desaturase (33). Δ6 desaturase converts LA to γ-linolenic acid (GLA, 18:3 n-6) which is elongated to di-homo-GLA (DGLA, 20:3 n-6) and further desaturated to arachidonic acid (AA, 20:4 n-6) by the Δ5 desaturase. DGLA is the precursor of 1 series-prostaglandins (PGs) and AA serves as the precursor of 2 series-PGs, thromboxanes (TX), and 4 series-leukotrienes (LTs). Eicosapentaenoic acid (EPA, 20:5 n-3) is derived from ALA by elongation and Δ5, Δ6 desaturation. It is further converted to docosahexaenoic acid (DHA, 22:6 n-3) by the elongation and Δ6 desaturation, and partial peroxisomal oxidation. EPA is the precursor of 3 series-PGs and 5 series-LTs while DHA is a critical component of the human brain, cerebral cortex, retina, and skin.

Dietary n-3 FAs such as DHA and EPA have been shown to decrease inflammation by enhancing the production of less pro-inflammatory PGs, LTs, and TXs, including PGE3, PGF3α, LTB5, LTC5, LTD5, and TXA3 (33). n-6 FAs have been recommended to be reduced in the diet because some PGs and LTs produced from AA, such as PGE2, PGF2α, TXA2, LTB4, LTC4, and LTD4, are essential for inflammation and known to be involved in atherosclerosis (33). However, anti-inflammatory factors such as prostacyclin, lipoxin A4, and epoxyeicosatrienoic acids are also derived from AA. In a review of AHA Science Advisory (56), n-6 FAs were indicated to reduce coronary heart disease. Studies have shown that n-6 FAs play roles in the regulation and suppression of excess inflammation by increasing anti-inflammatory markers such as TGFβ and suppressing the production of adhesion molecules, chemokines, and interleukins (23, 40).

AA, DHA, and EPA all are capable of producing lipoxins and resolvins, the anti-inflammatory factors that aid the cellular debris clearance and leukocyte infiltration at the inflammation site (33, 56). Linking FAs to the cardiovascular diseases based on its potential of producing pro-inflammatory factors without considering its anti-inflammatory effects may be inappropriate. Further studies are needed to identify the factors that drive the derivation of FAs to either the pro- or anti-inflammatory routes.

Dietary FAs provide energy not only for fasting metabolism and daily physical activity, but also for bulk energy expenditure of exercise. They contribute to the recovery after exercise by providing energy, serving as building blocks for membrane and hormones, and facilitating the cell signaling and membrane trafficking by forming lipid rafts with cholesterol. When combined with exercise, SFAs improve cardiac health by increasing brachial artery dilation. Padilla et al. (111) reported that after performing a single aerobic exercise after a meal, participants consuming a high-SFA meal showed bigger changes in brachial artery diameter than those who consumed a low fat meal. MUFA and PUFA have shown to help maintain blood cholesterol and lipid levels, lower blood pressure, and therefore reduce the risk of cardiovascular disease. Mensink et al. (96) reported that diets rich in MUFA and PUFA both lowered blood LDL cholesterol. Appel et al. (5) found that diet containing high unsaturated FAs decreased systolic blood pressure, lowered blood TGs, and increased HDL cholesterol (noting that these studies were outside the context of exercise). Furthermore, MUFA and SFA may modulate resting testosterone concentration independent of exercise (152).

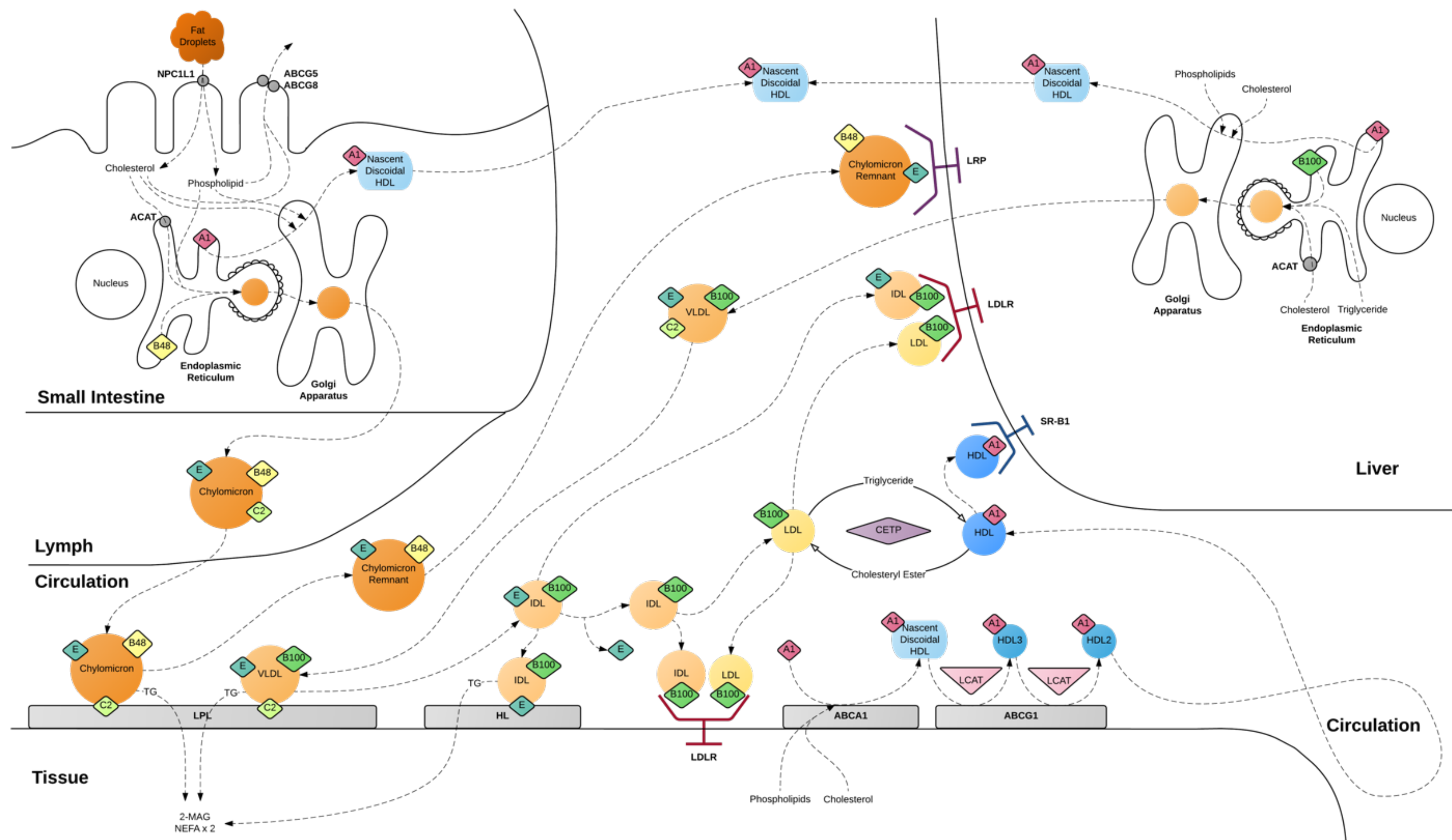

*Figure 6. Lipid Transport and Metabolism. **NCP1L1**: Niemann-pick C1-like 1, **ABCG5/G8**: ATP-binding cassette sub-family G member 5 and 8; **ACAT**: Acyl-CoA cholesterol acyltransferase; **LPL**: Lipoprotein lipase; **HL**: Hepatic lipase; **ABCA1**: APT-binding cassette transporter; **ABCG1**: ATP-binding cassette sub-family G member 1; **TG**: Triglyceride; **2-MAG**: sn-2-monoacylglycerol; **NEFA**: Non-esterified fatty acid; **CETP**: Cholesteryl ester transfer protein; **VLDL**: Very-low-density lipoprotein; **IDL**: Intermediate-density lipoprotein; **LDL**: Low-density lipoprotein; **HDL**: High-density lipoprotein; **LDLR**: LDL receptor; **LRP**: Low-density lipoprotein receptor-related protein; **SR-B1**: Scavenger receptor class B member 1.*

Lipid Transport and Metabolism

Figure 6 shows the overall lipid transport and metabolism. The small intestine plays a critical role in the lipid homeostasis and is the primary site where lipids are absorbed (1). When the lipid consumed from the diet enters the small intestine, cholecystokinin at the intestinal mucosal cell is activated. Cholecystokinin evokes the secretion of pancreatic lipase and colipase from pancreas as well as the release of bile from the gallbladder. The release of pancreatic lipase may also be activated or inhibited by bile acid at low concentration or high concentration, respectively. The inhibitory reaction of high concentration bile acid on pancreatic lipase may be reduced by the binding of colipase to the micelles.

Pancreatic lipase hydrolyzes TG into two FAs and one *sn*-2-monoacylglycerol (2-MAG). The 2-MAG may be further broken down into a free fatty acid and glycerol by MAG lipase. Bile containing bile acid is produced in the liver and stored in the gallbladder. Bile acid induces the emulsification of TGs, FAs (long-chain FAs), 2-MAG, phospholipids (mostly lysolecithin), and cholesterol to form micelles. Micelles travel across the small intestine until the FAs, 2-MAG, phospholipid, and cholesterol dissociate from micelle at the enterocytes.

Phospholipid and 2-MAG are absorbed by diffusion through the brush-border membrane. FAs may diffuse through the brush-border membrane or be absorbed by

the FA translocase (FAT), also known as cluster of differentiation 36 (CD36), on the brush-border membrane. Cholesterol is absorbed or transferred by the Niemann-Pick C1-Like 1 (NPC1L1) on the brush-border membrane of the small intestine. There may be other NPC1L1-independent pathways for sterol transportation (1). Once reaching the ER, cholesterol may be esterified with the FAs to form cholesterol ester (CE) by acyl-CoA cholesterol acyltransferase (ACAT). The FAs, 2-MAG, and phospholipids are then resynthesized to TGs in the ER and released to the Golgi complex to be packed into chylomicrons with CEs.

FAT/CD36, NPC1L1 and ATP-binding cassette sub-family G member 5 and 8 (ABCG5/G8) regulate lipid absorption at the enterocyte. Besides the small intestine, FAT/CD36 is abundant in the heart, skeletal muscle, adipose tissue, and capillary endothelium where FA uptake is essential for the energy production (1). Although the contribution of FAT/CD36 to the net fat absorption in the small intestine is insignificant, FAT/CD36 is important for the chylomicron formation by facilitating the packaging of FAs and CEs into chylomicron. However, the mechanism is unclear. NPC1L1 is expressed in human small intestine, liver, ovary, lung, and muscle. It binds to cholesterol and other sterols at the brush border membrane and intracellularly transports it to the ER. At ER, the sterols are esterified by ACAT (1). The expression of NPC1L1 are shown to be decreased by PPARα and PPARδ (71, 148, 150, 153). The ABCG5/G8 complex is the sterol export

pump that releases cholesterol and other sterols back to the gut at the brush-border membrane (51).

Chylomicrons are formed in ER packing TG, phospholipid, cholesterol, and CE with apolipoprotein-B48 (ApoB48), which is transported to Golgi complex via the COPII vesicle. They are then delivered by the Golgi vesicle to the cell membrane, extracted to the lacteals, and then transported to the subclavian vein via lymphatics. Chylomicrons contain ApoB48, E, and C2. When ApoC2 binds to lipoprotein lipase (LPL), the TG in chylomicrons is hydrolyzed to release two FAs and one 2-MAG by LPL. The chylomicron remnants with less TGs, ApoC2, and higher cholesterol density are then released into the circulation and are eventually taken up by the low-density lipoprotein receptor-related protein (LRP) through ApoE at the surface of the liver. Although the LDLR may also attract and take up ApoE, it is not the main receptor for chylomicron remnant (10).

In liver, the lipids received from lipoproteins, including chylomicron remnants, Intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), are packed with ApoB100 to form very-low-density lipoprotein (VLDL) in the ER. VLDL is transported to the Golgi complex via the COPII vesicle and then released into the circulation. VLDL containing ApoB100, ApoC2, and ApoE is then taken up by LPL at the tissues via the ApoC2. TG in VLDL is hydrolyzed to release two FAs and 2-MAG at the tissue. VLDL then becomes IDL with higher cholesterol density.

IDL loses its ApoE and deposits the cholesterol to tissues in the circulation, causing the increase in density. It may either be hydrolyzed by attaching to hepatic lipase (HL) via ApoE at the tissue when the ApoE is abundant or taken up by the LDLR via Apo B100 at the tissue and liver. After losing the ApoE and releasing cholesterol in the circulation, IDL becomes LDL, which travels to tissues and is taken up by the LDLR via ApoB100 at extrahepatic tissues and liver.

Nascent discoidal HDL is synthesized in the liver, small intestine, and extrahepatic tissues. In the liver and small intestine, ApoA1 at the ER is relocated to Golgi complex, where a small amount of cholesterol and phospholipid are packed with ApoA1 to form nascent discoidal HDL. Discoidal HDL is then released into the circulation. At the tissue, the circulating ApoA1 is picked up by ATP-binding cassette transporter (ABCA1) which transports cholesterol and phospholipid in the cell membranes to the apolipoprotein to form nascent discoidal HDL. The nascent discoidal HDL then picks up cholesterol and phospholipid at the ATP-binding cassette sub-family G member 1 (ABCG1) on the surface of tissues. While ABCG1 transports cholesterol and phospholipid from the tissue to the discoidal HCL, the lecithin:cholesterol acyltransferase (LCAT) esterifies the free cholesterol into CE. After picking up the cholesterol in the tissue, discoidal HDL becomes a more globular HDL3 and then HDL2 with the increase of cholesterol content. The circulating HDL and LDL exchange lipids via the plasma cholesteryl ester transfer protein (CETP), where the CE in HDL is transported to LDL, and the TG in LDL is

delivered to HDL. HDL circulates through the body and collects excess cholesterol from tissues. The mature HDL is eventually taken up by the scavenger receptor class B member 1 (SR-B1). This cholesterol efflux pathway driven by HDL is known as the reverse cholesterol transport (70).

Resistance Exercise and Lipid Metabolism

During exercise, working muscle takes up and uses the FAs mainly from the blood, and not the stored intramuscular TG. However, during prolonged exercise leading to glycogen depletion, intramuscular TG may be utilized (154). FAs are also used during recovery from fatiguing exercise and glycogen depletion.

As shown in Figure 7, at the onset of exercise, the adrenal gland releases epinephrine (EPi) and cortisol, while the sympathetic nervous system (SNS) releases norepinephrine (NEP) (2, 50, 81, 95). The catecholamines increase heart rate, stroke volume, and cardiac output. They also induce vasoconstriction, limiting the blood flow to the non-working tissues. For example, NEP works on precapillary sphincter and causes the arteriole vasoconstriction. At the active muscles, muscle contraction causes the release of local metabolites such as adenosine, ATP, hypoxia, hydrogen ion, nitric oxide, potassium, prostanoids, and endothelium-derived hyperpolarizing factor (30). These

factors are vasodilators that overweigh the effects of the catecholamines and cause vasodilation. Therefore, blood flow is elevated at the working muscle and reduced at the non-working muscle. This reaction is known as functional sympatholysis.

Glucagon released from the pancreas is activated by sympathetic nerve stimulation, specifically by the NEP (132). Hormone-sensitive lipase (HSL) in adipose and muscle tissues is activated by the catecholamines and glucagon via the adenylate cyclase-cAMP system. The activated HSL hydrolyzes stored TG to release 2-MAG and two free FAs (FFAs). 2-MAG may be further broken down into a FA and glycerol by MAG lipase. Glycerol in adipose tissue and muscle cells cannot be oxidized or reused because adipose tissue and muscle lack the enzyme glycerol kinase, which phosphorylates glycerol to glycerol-3-phosphate, the active form of glycerol for TG synthesis. Therefore, glycerol in the bloodstream is considered the indicator of lipolysis in adipose tissue and muscle. Glycerol was found in the blood after a bout of resistance exercise, suggesting the use of FAs during resistance exercise or recovery (50). GH is released from the anterior pituitary gland 10-15 minutes after the onset of exercise and continues to activate HSL to release FFAs for prolonged exercise. FFAs are not water soluble, and therefore it is carried by albumin in the bloodstream (133). Each albumin carries three FAs. When it arrives at the working muscle, the FA translocase/FA binding protein (FAT/FABP) complex at the sarcolemma transports the FAs into the muscle (15).

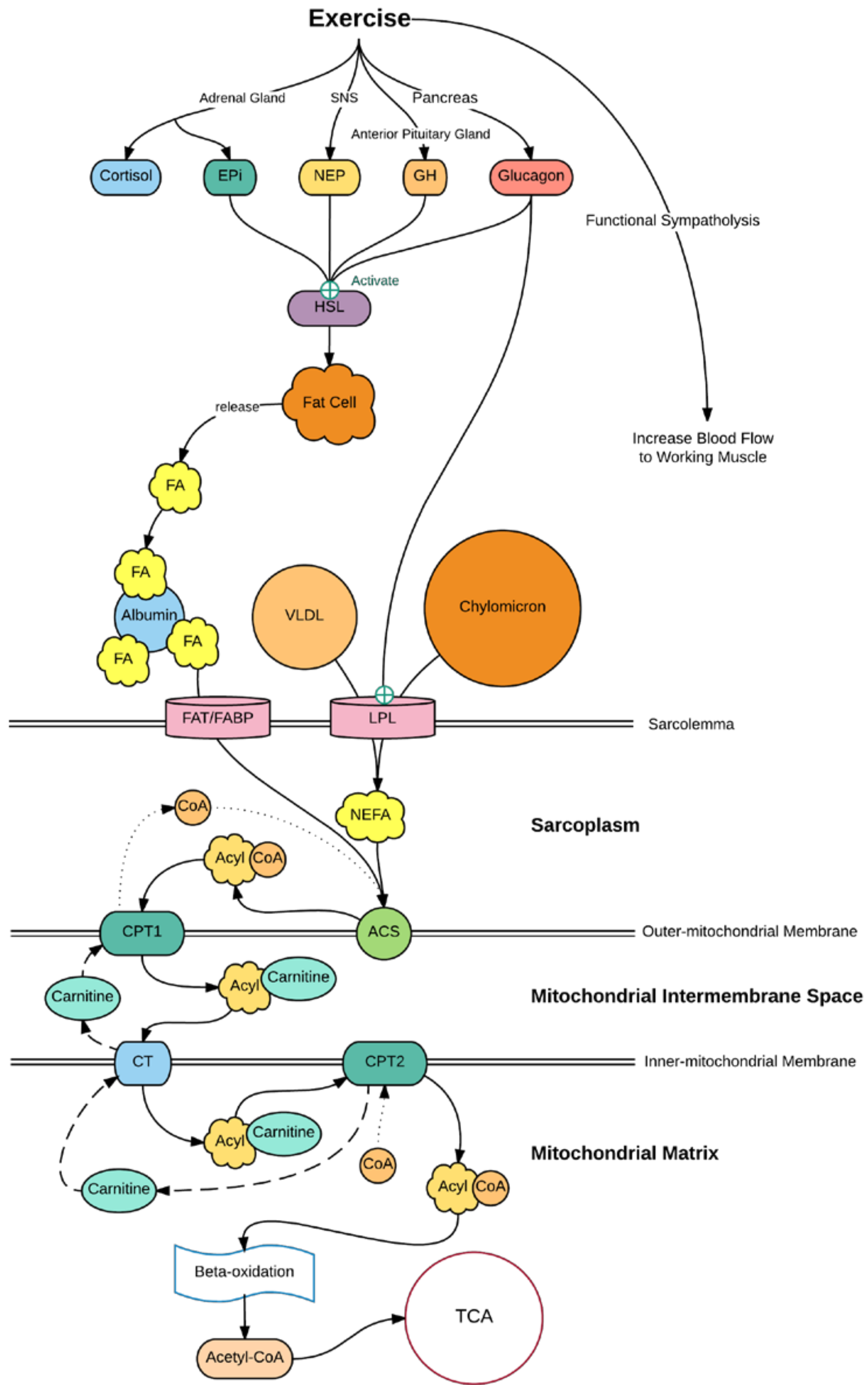

Figure 7. Exercise-induced Lipid Metabolism.

Glucagon also stimulate LPL. LDL-receptor (LDLR) on the muscle tissue picks up LDL in the blood stream. The catecholamines activate the TG lipase via the cAMP-PKA pathway (73).

The FAs must be converted to fatty acyl-CoA for further use in the mitochondria. Acyl-CoA synthase (ACS) at the outer membrane of mitochondria converts the FAs to fatty acyl-CoA with the use of ATP. Then the fatty acyl-CoA is transported to the inter-mitochondrial space by the carnitine palmitoyltransferase 1 (CPT1) at the outer-mitochondrial membrane. During this process, coenzyme A is replaced by carnitine to form acyl-carnitine, allowing it to be transported between the outer- and inner-mitochondrial membranes. The acyl-carnitine is picked up by the carnitine transporter (CT) at the inner-mitochondrial membrane and delivered into the mitochondria matrix. When the acyl-carnitine enters the mitochondria matrix, CPT2 replaces the carnitine with coenzyme A, forming acyl-CoA. Acyl-CoA are then oxidized to acetyl-CoA, NADH, and FADH2 via beta-oxidation. Acetyl-CoA enters TCA cycle and releases NADH, FADH2, and GTP. NADH and FADH2 enter the electron transport chain (ETC) at the inner mitochondrial membrane to produce ATPs. Each NADH may produce three ATPs while one FADH2 creates two ATPs through the ETC.

Peroxisome Proliferator-Activated Receptors

Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription factors that belong to the nuclear receptor superfamily. In animal, there are 48 known transcription factors, including endocrine and metabolite-activated receptors (105). Three PPARs have been identified so far: PPARα (NR1C1), PPARδ/β (NR1C2), and PPARγ (NR1C3). While the three PPARs share similar function and structure, human PPARα, PPARδ and PPARγ are encoded by gene PPARA on chromosomal region 22q12-q13.1 with eight exons, gene PPARD on chromosomal region 6p21.2-p21.1 with nine exons, and PPARG on chromosomal region 3p25 with nine exons, respectively (37).

Structure and Distribution

PPARs consist of four functional domains: A/B, C, D and E/F (Figure 8). Domain A/B contains the ligand-independent activation function (AF-1) at the N-terminal and its activity is almost insignificant. PPARα and PPARγ share respectively 23% and 12% of similarity on the A/B domain to PPARδ. Domain C is structured with α-helical and two zinc finger-like configuration. It accommodates the DNA binding motif and is highly conserved among the PPARs with 86% and 85% similarity in PPARα and PPARγ to PPARδ, respectively. Domain D is the hinge region that interacts with cofactors and preserves the functional structure by connecting the domains C and E/F. PPARα and PPARγ share 57% and 40% similarity to PPARδ on the domain D, respectively. Domain

E/F is the ligand-binding domain that contains the ligand-dependent activation function (AF-2) at the COOH-terminus and forms a heterodimer with Retinoid X Receptor (RXR). It consists of 13 α-helices and one small four-stranded β sheet, which forms a large hydrophobic Y-shape area with a flexible binding pocket. This Y-shape area is larger than other nuclear receptors, and therefore it can bind to many endogenous and synthetic lipophilic ligands. Beside the ligand binding area, domain E/F also contains a folded region that forms hydrogen bonds with carbohydrate groups on cofactors. It is well conserved among PPARs with 76% and 73% similarity in PPARα and PPARγ to PPARδ, respectively (8, 37, 105).

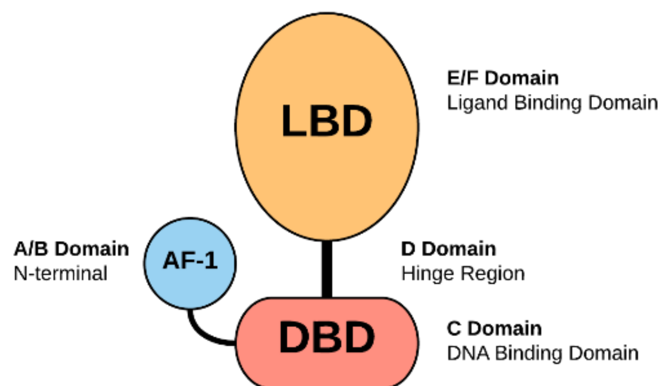

Figure 8. The Structure of PPARs.

The distribution of PPARs is tissue-specific (37). PPARγ promotes adipogenesis and lipid storage and is expressed mostly in adipose tissues and immune cells such as macrophages (52). PPARs -α and -δ are expressed mainly in high-metabolic tissues. PPARα is primarily expressed in liver, kidneys, heart and skeletal muscle. While PPARδ is also seen in liver and kidney at relatively low levels, it is the most abundant isoform in skeletal muscle and is the key regulator of FA catabolism and metabolism in skeletal muscle. In skeletal muscle, expression of PPARδ is higher in type I than type II fibers (156).

Activation of PPARs

PPARs are activated by ligand binding and phosphorylation. Ligands that activate PPARs include endogenous long chain FAs and their derivatives and specific synthetic compounds. The endogenous ligands include eicosanoids, leukotrienes, leukotriene B4, prostaglandins, 8(s)-hydroxyeicosatetraenoic acid, the PPARγ specific activators 9-hydroxy-10, 12-octadecadienoic acid, 13-hydro-9, 11- octadecadienoic acid, 15Δ-deoxy-12, 14-postaglandin J2, and the PPARδ specific activator retinoic acid. The synthetic ligands include fibrates that activate PPARα, thiazolidinediones that activate PPARγ, and derivatives of phenoxyacetic acid, L-165041, GW501516, GW0742, and MBX-8052 that activate PPARδ. However, there is no synthetic ligand for PPARδ in clinic use (8, 37, 105).

PPARδ has the smallest cavity on its ligand binding domain among the PPAR isoforms. The size of the cavity determines its capability and variety of ligand binding. The ligands need to be delivered to the nucleus to activate the PPARs that bind to DNA. FABPs transport the ligands to specific PPARs. FABP3, FABP4, and FABP5 deliver the ligands to PPARα, PPARγ, and PPARδ, respectively (8, 105).

RXR is also a member of nuclear hormone receptor superfamily and is activated by 9-cis-retinoic acid (RA). Three isoforms, RXRα, RXRβ, and RXRγ, form heterodimers with PPARα, PPARδ, and PPARγ, respectively. PPARs heterodimerize with RXR and attach to the PPRE (Peroxisome Proliferator Response Element) in the regulatory region of a gene without ligand binding (Figure 9). PPRE contains a DR-1 motif that includes two direct repeats of the consensus sequence AGGTCA, separated by a single nucleotide. The DNA binding sites on PPARs and RXR bind to the AGGTCA sections. The PPAR/RXR heterodimer may be activated by either RA or PPAR ligands while the combination of both activators yields a stronger effect. When the heterodimer is activated, it promotes the transcription of proteins related to lipid and lipoprotein metabolism, lipid transportation, inflammation, wound healing, mitochondrial respiration, cell proliferation and differentiation, and thermogenesis (105).

PPARs may also be regulated by phosphorylation (20). The activities of PPARα and γ are regulated by the phosphorylation via AMPK, MAPK, and PKA. While the

phosphorylation of PPARδ is less studied, PPARδ may also be activated by AMPK, MAPK, and PKA (20, 34).

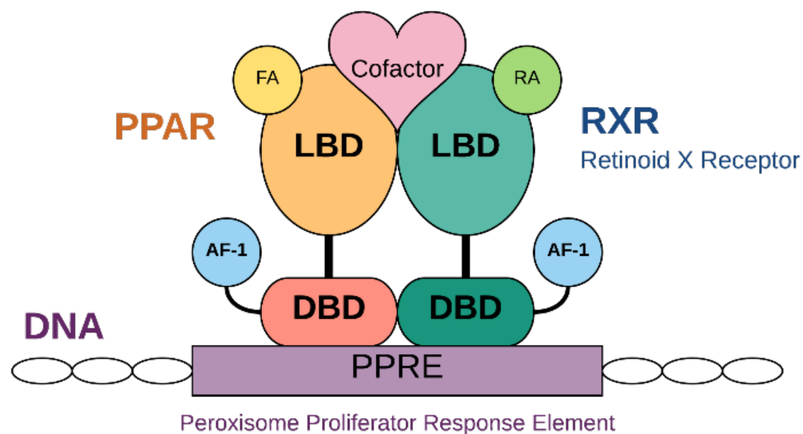

Figure 9. The Heterodimer of PPAR and RXR.

Cofactors

Cofactors influence the transcriptional activity by changing the chromatin structures. There are two kinds of cofactors: coactivator and corepressor. Coactivators of PPARs containing one or more LXXLL amphipathic α-helix consensus sequence interact with the Ligand-binding domain (E/F) of the PPARs to enhance the target gene expression. Coactivators are recruited when the agonists bind to the receptors. Two groups of coactivators have been identified. The first group alters the chromatin structure by

maintaining the histone acetyl-transferase and methyltransferase activities. The changes in the chromatin structure induce the transcription. The second group forms multiprotein complexes connecting the nuclear receptors and the basal transcriptional machinery. Steroid hormone receptor coactivator family and PPARγ coactivator 1α (PGC1α) are coactivators that belong to the second group. PGC1α co-activates most nuclear receptors, including PPARs. Overexpression of PGC1α and PPARδ have been shown to have similar effects on muscle metabolism. Furthermore, activation of PPARδ has been shown to increase PGC1α contents (37, 79). Corepressors inhibit gene transcription by influencing histone deacetylase (HDAC) and other enzyme activities to maintain a solid chromatin structure. PPARs interact with the silencing mediator for retinoic acid, thyroid hormone receptor (SMRT), and the nuclear receptor corepressor in the absence of ligands or receptor-interacting protein 140 (RIP140) when binding to ligands. In skeletal muscle, RIP140 has been shown to suppress the expression of PPARδ-dependent genes that affect mitochondrial activity and fiber type determination. When binding to DNA, PPARδ may interact with SMRT, HDAC, and their associated repressor proteins to inhibit PPARα and PPARγ-activated transcriptions.

Regulation of PPARδ Expression in Skeletal Muscle

Among three PPARs, PPARδ is the most abundant isoform in skeletal muscle and has higher expression in oxidative than glycolytic muscle fibers. Short-term exercise,

endurance training, and short-term muscle unloading have been shown to increase PPARδ expression (93).

Exercise and fasting elevate the FFA levels, a physiological signal that triggers the increase in PPARδ protein content (37). mRNA expression of PPARδ and PGC1-α are shown to increase three hours after a bout of high-intensity cycling exercise (93). Similar PPARδ mRNA expression has been observed to increase after endurance exercise performed at either elevated or depressed FA levels, suggesting that muscle contraction, not the nutritional condition, may be the main stimulator of the elevated PPARδ mRNA expression induced by exercise (37, 157). However, no evidence of increasing muscle PPARδ protein content by resistance exercise in humans has been reported. Resistance exercise induces both muscle contraction and the increase of FFAs (50). Both are the stimulators of PPARδ expression. Therefore, we hypothesized that muscle contraction induced by resistance exercise would result in a similar elevation of PPARδ protein content induced by the endurance exercise.

Most previous studies regarding PPARδ expression have been focusing on the PPARδ mRNA content. However, the mRNA levels do not necessarily predict its protein content (54). The protein abundance in humans may be affected by transcription, mRNA decay, translation, and protein degradation (151). In this dissertation, the

changes in PPARδ protein content in response to resistance exercise is determined as a contributor to the mechanism by which resistance exercise induces lipid metabolism.

Regulation of Skeletal Muscle Fiber Types by PPARδ

Skeletal muscle can be classified into three fiber types: Type I, Type IIa, and Type IIx. Type I fiber has the highest mitochondrial density and is the most oxidative. It is also identified as the slow red oxidative fiber based on its physical appearance and contractile properties. Type IIa fiber is the fast red oxidative fiber that has a higher capability for glycolytic oxidation. Compared to the Type IIx fiber, Type IIa fiber is more oxidative and therefore is more resistant to fatigue. Type IIx fiber is referred as the fast white glycolytic fiber. It contains few mitochondria and is very susceptible to fatigue.

Resistance exercise training induces hypertrophy in all muscle types. The proportion of Type IIx fibers decrease while Type IIa fibers increase with the resistance training, suggesting the conversion of muscle fiber type from IIx to IIa (137, 138). Because Type IIa fibers exhibit a more oxidative potential, the increase in the Type IIa fiber proportion implies a conversion of the preference for energy source induced by resistance exercise training. PPARδ is a key regulator of muscle fiber type (37). The reduction of PPARδ's corepressor RIP140 and the increase of its coactivator PGC1α enhance the formation of Type I fibers. PGC1α is one of the first muscle fiber type regulators to be identified and it promotes the conversion of fast type II to slow type I fibers. In skeletal muscle, it is

PPARδ that regulates the activation and expression of PGC1α, not PPARα (65, 126). Other fiber-type regulators such as calcineurin may interfere with the PPARδ/PGC1α pathway. However, while these studies were mainly conducted on animals, the switch between type II and Type I fibers has not been demonstrated in human skeletal muscles. The effects of PPARδ and PGC1α on muscle fiber type in humans were suggested in the studies that presented higher expressions of PPARδ and PGC1α in the human muscle samples with a higher portion of oxidative fibers (37).

Regulation of Lipid Metabolism and Fuel Utilization by PPARδ in Skeletal Muscle

Skeletal muscle is the largest tissue for lipid metabolism, and therefore whole body homeostasis is affected by the factors that regulate skeletal muscle lipid metabolism. The importance of PPARδ in the regulation of whole-body metabolism was revealed by the studies showing that PPARδ mRNA expression declines with age and is higher in the adults with heavier birth weight (37). The number of mitochondria in a cell may indicate its ability to utilize lipid as fuel. The activation and expression of PGC1α, the master regulator of mitochondrial biogenesis and quantity, is regulated by PPARδ in skeletal muscle (65, 126). Therefore, activation of PPARδ may increase the amount of mitochondria via PGC1α (37).

PPARδ upregulates genes involved in lipid and FA metabolism proteins in skeletal muscle. These proteins include cholesterol metabolism proteins ABCA1, FA and TG

synthetic gene regulator sterol regulatory element-binding protein 1c (SREBP-1c), FA uptake proteins LPL, FAT/CD36 and FABP, essential mitochondrial membrane FA transport proteins ACS and CPT1, β oxidation enzymes long-chain acyl-CoA dehydrogenase and acetyl-CoA acyltransferase, and other FA oxidation facilitating proteins including malonyl-CoA decarboxylase, pyruvate dehydrogenase kinase 2 (PDK2), and PDK4 (35, 38, 75, 99, 123). As discussed in previous sections, these proteins play important roles in the lipid utilization and FA oxidation during and after resistance exercise.

SREBP-1c is a transcription factor that regulates the expression of genes involved in FA and TG synthesis. In muscle, adipose tissue, and liver, SREBP-1c expression is enhanced by insulin. Exercise training also increases the expression of SREBP-1c (69, 101). It has been identified to be the possible cause for the elevated intramuscular TG in skeletal muscle of exercise-trained individuals (101).

Pyruvate dehydrogenase complex (PDC) is the rate-limiting step in muscle glucose oxidation. The deactivation of PDC by PDK2 and PDK4 results in the switching of fuel utilization toward FA oxidation. PPARδ enhances both PDK2 and PDK4 in skeletal muscle. In fact, these proteins and enzymes interplay with PPARδ to keep the utilization of FA favorable (37, 102). The upregulation of FAT/CD36 expression by PPARδ increases the uptake of FAs, which then activates PPARδ and further promotes the expression of

FAT/CD36 and other FA metabolism proteins, PDK2, and PDK4. PDK2 and PDK4 inhibit glucose oxidation and favors FA utilization. Therefore, when blood FA concentration is increased, PPARδ is activated and alter the fuel utilization towards lipid oxidation. It explains how fasting and exercise, the conditions when blood FFA is elevated, may enhance lipid metabolism.

Although glucose oxidation may be attenuated with the increased PDK expression, glucose uptake may be enhanced by PPARδ (83). When PPARδ is activated, the increased FA oxidation lowers the FFA levels in the blood, and therefore a negative feedback is induced to increase glucose uptake by the effect of insulin (82). Insulin sensitivity may also be improved by PPARδ (86). The increased glucose uptake and suppressed glucose oxidation by PPARδ cause the accumulation of glucose in cells. The accumulated glucose may either be metabolized via glycolysis or be used to synthesize glycogen. Although PPARδ increases both glycogen synthase-2 mRNA expression and content in adipose tissue, whether it has effects on glycogen synthase-1 in skeletal muscle remains to be investigated (94). Taken together, PPARδ increases the availability of glucose in the muscle cell. The elevated glucose may be further oxidized as energy or synthesized into glycogen.

AMPK has been identified as an energy sensor that plays a major role in energy homeostasis. It is activated when energy is low to promote energy production and

suppress energy consuming processes. AMPK increases glucose uptake and FA oxidation, while suppresses FA synthesis by phosphorylating (inhibiting) Acetyl-CoA carboxylase (ACC) and depresses protein synthesis by inhibiting mTOR (13, 17, 160). Since AMPK mainly promotes energy production and inhibits energy consumption, it has been shown to inhibit glycogen synthesis by suppressing glycogen synthase through the activation of glycogen synthase kinase-3α and β (GSK-3α/β), the glycogen synthase inhibitor (64, 74). However, studies also showed that the chronic activation of AMPK might increase glycogen in skeletal muscle because of the accumulation of glucose (78). Activation of PPARδ has been shown to be coincident with enhanced expression and phosphorylation of AMPK. Unlike PPARδ, AMPK does not seem to directly regulate the transcription of lipid metabolism genes (82). However, AMPK may affect the transcriptional activity of PPARδ by a protein-to-protein interaction in the skeletal muscle (103). Moreover, AMPK activity has been shown to increase with activated PPARδ (37, 82, 103). In summary, there seems to be an interaction between PPARδ and AMPK that enhances the activity of transcription of PPARδ target genes, but the mechanism remains to be clarified.

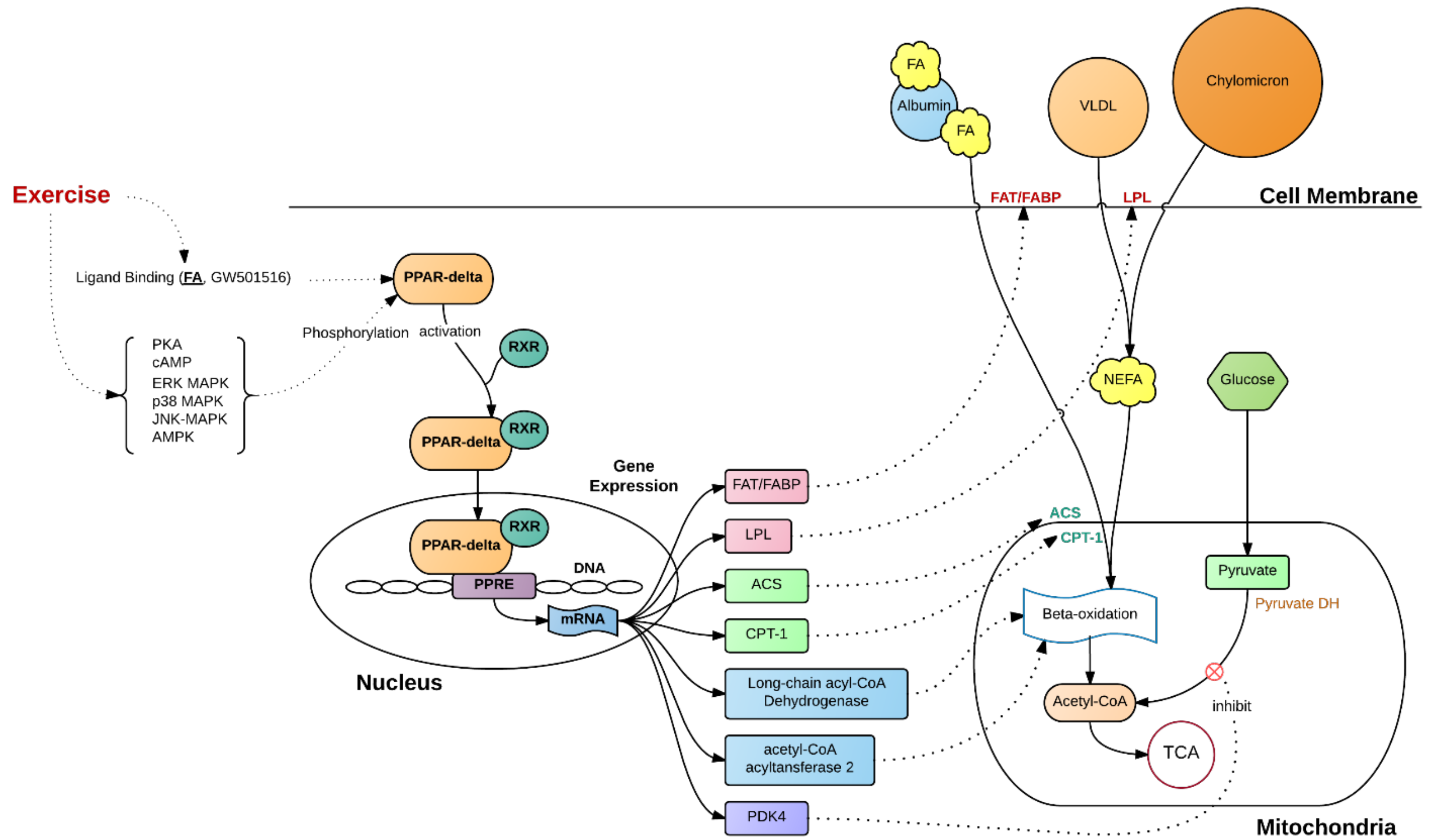

Figure 10. The Effects of Exercise on Lipid Regulation via PPARδ.

The Effects of Exercise on Lipid Regulation via PPARδ

Exercise activates PPARδ and upregulates its mRNA expression (Figure 10). The exercise-induced PPARδ expression is primarily via AMPK and MAPK phosphorylation induced by muscle contraction, but not the elevated FFAs (93, 157). The activated PPARδ promotes the expression of lipid transport and metabolism proteins, and thus enhances the lipid metabolism in skeletal muscle.

The Role of PPARδ in Muscle Protein Synthesis

Besides the regulation of energy source and the determination of muscle type, PPARδ may also affect protein synthesis in skeletal muscle. Resistance exercise induces mechanical deformation (muscle contraction) and the increase in IGF-1 and GH (134). These factors activate and increase the expression of PPARδ activators including AMPK, MAPK, and PKA (20, 34). PPARδ may increase muscle growth via the forkhead box class O transcription factor 1 (FOXO1). It activates and promotes the expression of FOXO1, which inhibits the early stage of myoblast proliferation and enhance the later stage of fusion of differentiated myocytes into myotubes (125). The combination of the effects of PPARδ on the different stages of skeletal muscle regeneration has been shown to have a positive effect on muscle growth (4).

Summary

Resistance exercise induces cell signaling pathways that promote muscle protein synthesis via muscle contraction, inflammation, and hormones. Lipids facilitate these mechanisms by providing energy and building blocks for cell membrane regeneration, regulating membrane permeability for hormones, and forming lipid rafts for the signaling molecules. Exercise increases the oxidative capacity by upregulating the expression of proteins responsible for uptake, transport, and oxidation of FAs in skeletal muscle. Exercise increases FFAs and mobilizes several kinases, the factors that activate PPARδ, which has been identified as a major regulator of lipid metabolism in skeletal muscle and a therapeutic target for metabolic syndrome. While PPARδ has been studied with endurance exercise, the effects of resistance exercise on muscle PPARδ protein content remain unclear.

The following chapters tested the hypotheses that dietary lipids would be associated with enhanced skeletal muscle mass, strength, and peak power following resistance exercise training and that resistance exercise would improve lipid profiles and increases the PPARδ protein content as biomarkers of enhanced lipid metabolism in skeletal muscle.

CHAPTER II

THE EFFECTS OF DIETARY CHOLESTEROL AND FATTY ACIDS ON SKELETAL MUSCLE RESPONSES TO RESISTANCE EXERCISE TRAINING

Overview

Cholesterol and FAs may be essential for the resistance exercise-induced skeletal muscle adaptation through the provision of an energy source and cell signaling regulators (FAs), providing the building blocks for the cell membrane, forming steroid hormones and lipid rafts for cell signaling molecules, and regulating inflammation (cholesterol). Thirty-seven generally healthy, untrained older adults (50-65 years old) were recruited and randomly assigned to three cholesterol intake groups (LC: zero additional cholesterol per day, n=13; MC: 3.5 mg additional cholesterol/kg lean/day, n=11; HC: 10.5 mg additional cholesterol/kg lean/day, n=13) and performed a 12-week whole-body progressive resistance exercise training program. Changes in skeletal muscle mass, strength, peak power, and muscle quality by cholesterol intake as well as the association with dietary FA intake were analyzed. The results showed that skeletal muscle mass, strength, peak power, and muscle quality increased with the training in all cholesterol intake groups and both gender, but the increases were not affected by

cholesterol intake levels. There were associations between specific dietary FAs and gains in skeletal muscle mass, strength, peak power, and muscle quality in different cholesterol intake groups and gender. In conclusion, although skeletal muscle adaptations to resistance exercise training were not altered by cholesterol intake, the exploratory analyses of dietary FA suggested potential effects on the adaptations.

Introduction

Obesity and physical inactivity are major risk factors for cardiovascular disease (42, 159). While obesity may be caused by overconsumption of energy and insufficient physical activity, nutritional control and exercise may be the countermeasure to prevent obesity.

Overconsumption of fat and cholesterol has been linked to the increased risk of cardiovascular diseases (84, 128). However, lipids, including FAs and sterols, are essential for energy production and maintenance of metabolism. Cholesterol is abundant in the plasma membrane, Golgi complex, and endocytic recycling compartments. It controls membrane fluidity, regulates membrane proteins, and modulates membrane trafficking and cell signaling (70). Dietary cholesterol is considered the contributor to cardiovascular diseases, and a limit of 200 mg daily

intake is recommended by AHA to reduce the risk of coronary heart disease (112). However, the elevation of blood cholesterol after dietary cholesterol consumption is inconsistent and some individuals show increases in both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels (122, 129). MUFA and PUFA have been shown to help maintain blood cholesterol and lipid levels, lower blood pressure, and therefore may reduce the cardiovascular risk (5, 96). Studies have shown that n-6 FAs play important roles in the regulation and suppression of excess inflammation by increasing the anti-inflammatory factors and prevent coronary heart disease (23, 40, 56). Dietary n-3 FAs such as DHA and EPA have been shown to regulate inflammation by enhancing the production of less pro-inflammatory PGs, LTs, and TXs (33). The n-3 and n-6 FAs are both capable of producing anti-inflammatory factors such as lipoxins and resolvins that aid the cellular debris clearance and leukocyte infiltration at the inflammation site.

Physical activity has been shown to reduce the risk of cardiovascular diseases by influencing lipid and lipoprotein metabolism (36). Skeletal muscle accounts 40-50% of total body mass in non-obese individuals and is responsible for more than 50% of resting metabolism. It may further increase metabolism by 10-fold with exercise. Skeletal muscle plays a major role in the regulation of electrolytes, acidity, glucose and lipid metabolism. Therefore, the impact of calorie, fat, glucose, and cholesterol intake on disease is associated with skeletal muscle activity (121). In fact, skeletal muscle has been recognized as the determinant of resting metabolic rate (16). Therefore,

resistance exercise, which increases skeletal muscle mass, has the potential to reduce the risk of cardiovascular disease and is suggested to be included in the diseases-prevention exercise program by American College of Sports Medicine, American Heart Association, and American Diabetes Association (16). Dietary and serum cholesterol has been observed to be associated with skeletal muscle adaptation to resistance exercise training (120). Furthermore, when combined with exercise, dietary cholesterol and FAs may have beneficial effects. Dietary SFAs have been shown to improve cardiac health by increasing brachial artery dilation (111). Cholesterol, MUFA, and SFA intake may modulate testosterone which affects the adaptation of skeletal muscle to resistance exercise (121, 152).

Exercise-induced cell signaling pathways for muscle protein synthesis may be induced by muscle contraction, hormones, and inflammation (134). Muscle contraction causes mechanical deformation of muscle fiber, which promotes the release of growth factors and evokes signaling pathways including PKB-mTOR, PA-mTOR, PA-PI3K, AMPK, and MAPK, independent of hormones and growth factors (Figure 2) (6, 58, 67, 108, 134, 149). Cholesterol facilitates the cell signaling pathways by forming lipid rafts as the platform for transporting the signaling molecules through the membrane trafficking. It also contributes to the formation of steroid hormones such as testosterone that stimulates muscle growth (121).

Resistance exercise induces skeletal muscle damage that causes inflammation. At the inflammation site, macrophages and neutrophils are recruited to break down damaged muscle tissue and produce cytokines including IL-6 and TGFβ (57, 77, 134). IL-6 induces satellite cell proliferation by activating the MAPK and PI3K cell signaling cascades via JAK signaling pathway (60, 127). TGFβ enhances muscle differentiation and regulates local collagen synthesis to build up skeletal muscle architecture (57). At the inflammation site, FAs and cholesterol provide building blocks for the regeneration of muscle membrane, increase membrane viscosity and promote membrane stability, and facilitates cell signaling pathways by forming lipid rafts.

Studies have been investigating the effects of resistance exercise on lipid metabolism by examining its influence on the lipoproteins. However, the effects of dietary lipid on the skeletal muscle adaptation to resistance exercise remain to be clarified. The purpose of this study is to investigate the effects of dietary cholesterol and FAs on muscle mass, strength, peak power, and muscle quality (strength and power adjusted to mass) in response to the 12-week whole-body progressive resistance exercise training program.

Methods

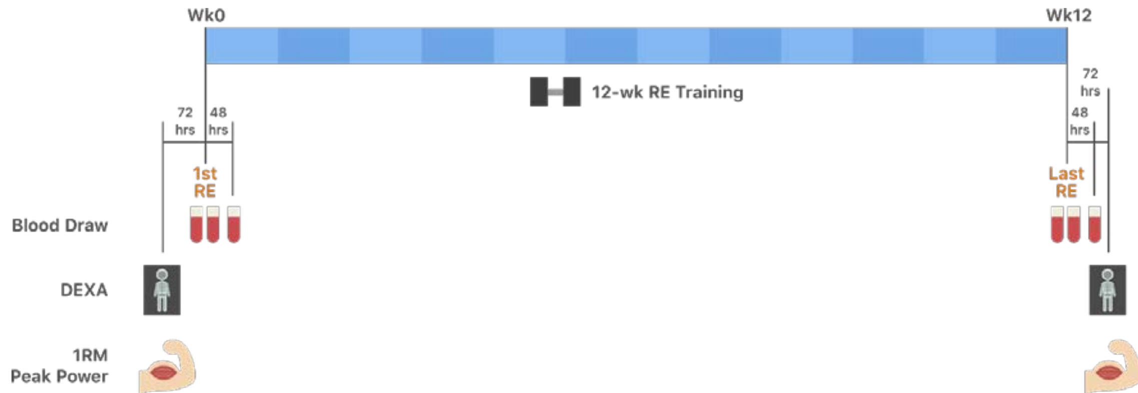

Figure 11. Timeline of Study 1.

Subjects

Thirty-seven, 50 to 65 years old, generally healthy men (n=15) and women (n=22) were recruited via flyers and advertisements in local newspapers. Smokers and individuals with any of the following conditions were excluded: hypertension (Blood pressure > 160 Systolic/100 Diastolic mmHg), cardiac arrhythmias, cancer, hernia, aortic aneurysm, kidney disease, diabetes, lung disease, and blood cholesterol >240 mg/dl or <160 mg/dl, taking cholesterol lowering medications, and participating in one hour or more of resistance exercise training (RET) in the previous year. Women were postmenopausal for more than two years. The eligible participants were randomly assigned into one of three cholesterol intake groups in a double-blind manner: Low Cholesterol Intake Group (LC, n=13, 5 men and 8 women, zero additional cholesterol

per day), Mediam Cholesterol Intake Group (MC, n=11, 5 men and 6 women, 3.5 mg per kilogram lean mass of additional cholesterol per day), and High-Cholesterol Intake Group (HC, n=13, 5 men and 8 women, 10.5 mg per kilogram lean mass of additional cholesterol per day). Figure 11 shows the general protocol of this study. This study was approved by Texas A&M University Institutional Review Board (IRB2015-0175M), and all the participants provided written informed consent before participating in the study.

Orientation

Participants attended two sessions of nutrition education and four sessions of exercise familiarization during the 2 weeks before the training. Each nutrition education session lasted for two hours and was performed by a registered dietitian (RD). The participants learned about proper nutrient intake, calorie and portion control, study specific diet guidelines, and the operation of a nutrition software (Nutribase; version 7; Client Intake Module; Cybersoft Inc., Phoenix, AZ) to maintain diet records throughout the study. The exercise familiarization provided the participants with the information about the benefits of regular exercise and principles of resistance exercise. Proper exercise techniques were demonstrated to help participants become familiar with the resistance exercise protocol by practicing the techniques with light weight on the Keiser 300 series pneumatic exercise machines (Keiser, Palo Alto, CA). The intensity was gradually increased to 40% of their estimated maximum strength (4/10 on the Omnibus-RE Scale [OMNI-RES] ratings of perceived exertion [RPE]) (48). The purpose of the exercise

familiarization was to standardize strength measures while minimizing skeletal muscle adaptations, estimate maximum strength (1RM) before testing, and reduce the possibility of exercise-induced injury.

Testing

Following the orientation and at least 72 hours before the first resistance exercise training session, 1RM, peak power, body composition, and resting metabolic rate (RMR) were measured. 1RM's for all the exercises in the RET program were determined by gradually increasing exercise weights until the maximum resistance, at which only one repetition could be completed with proper form in full range of motion, was reached using the Keiser machines. Following a three-minute warm-up on a cycle ergometer (Schwinn Fitness, Inc., Denver, CO) and stretching, participants performed four warm-up repetitions with the weight corresponding to 55% of an estimated 1RM obtained during the exercise orientation. The weight was then increased to 75% of a re-estimated 1RM (based on RPE) to perform only one repetition. After 60 seconds of rest, the weight was increased again to 90% of a re-estimated 1RM to perform one repetition. Additional attempts for 1RM were made after 60 seconds of rest until the true 1RM value was obtained, in a manner that the total number of 1RM attempts was minimized. The same procedure was performed for all exercises and in the same order for all participants.

The power (force x velocity) output for each exercise was be measured during 1RM tests. Participants were instructed to perform the concentric phase of repetitions at their maximal speed. The Keiser machines calculated the power output for each repetition. The peak power was recorded as the value of the power outputs at 100% 1RM.

Body composition was assessed by a dual energy X-ray absorptiometry (DEXA) Lunar Prodigy machine (General Electric, Fairfield, CT). RMR was measured with ParvoMedics TrueMax 2400 Metabolic Measurement System (Sandy, UT) in the morning after an overnight fast to determine the total calorie required for the nutrition control.

Fasted (12 hours, overnight) blood samples were collected from antecubital veins immediately before, immediately after, and 48 hours after the first and the last resistance exercise. Blood serum samples were drawn from an antecubital vein into vacutainer tubes containing a serum clotting factor (Becton Dickinson and Company, Rutherford, NJ) with the participant seated at rest. The serum samples were immediately isolated by centrifugation at 1500x g for 30 minutes at 4°C and then stored at -80°C for later analysis. The blood lipid panels were analyzed with standard methods at St. Joseph Regional Health Center's CDC certified laboratory (Bryan, TX) to examine the effects of dietary cholesterol intake and resistance exercise training on blood lipid profiles.

All tests were repeated at the completion of the 12-weeks resistance exercise training program. 1RM and body composition were measured 48 and 72 hours after the last exercise, respectively. Blood samples were collected immediately before, immediately after, and 48 hours after the last exercise (Figure 11).

Resistance Exercise Training

Participants performed a full body resistance exercise training program on 3 non-consecutive days per week for 12 weeks on the Keiser 300 series exercise machines. The resistance exercise training program consisted of 10 minutes of warm-up on a cycle ergometer (Schwinn Fitness, Inc., Denver, CO), 5 minutes of dynamic stretching, seated chest press, lat pull down, leg press, calf raises, seated leg curls, knee extension, biceps curls, and triceps extension exercises. Participants performed three sets of 8-12 repetitions with resistance set at 70% of 1RM. They were instructed to perform as many repetitions as possible until they reached 12 repetitions or muscle failure on each set. When a participant was able to complete 12 repetitions on all three sets of an exercise, the weight was increased by 3-5% of 1RM in the next exercise session so that only eight repetitions would be possible. Rest periods between sets and exercises were restricted to one and two minutes, respectively. All exercise sessions were supervised by Exercise Physiology graduate students, and the participants were instructed to

maintain their regular physical activities at the pre-study level and not to perform any additional resistance exercise.

Nutrition Control

Participants were instructed to consume 50% of total calories from carbohydrate, 30% from fat, 20% from protein, and <10% from saturated fat to meet daily caloric consumption goals as determined by RMR test. They were also instructed to consume >1.0 g/kg/day of protein, 25-30 g/day of fiber, and <200 mg/day of cholesterol, as recommended by the AHA (112). Participants were required to maintain 24-hour diet logs at least four times per week (three weekdays and one weekend day) during the study period. Feedback on the diet logs was provided weekly, and adjustments were made as necessary to ensure adherence to the study dietary guidelines. All nutrition data were recorded and analyzed using the NutriBase 7 software.

Supplement

The Low Cholesterol Intake Group (LC, n=13) consumed zero additional cholesterol per day, the Medium Cholesterol Intake Group (MC, n=11) consumed 3.5 mg additional cholesterol/kg lean/day, and the High Cholesterol Intake Group (HC, n=13) consumed 10.5 mg additional cholesterol/kg lean/day in the supplement consisting of egg white and yolk powder and/or peanut oil. Egg white powder and peanut oil were used to achieve equivalent amounts of protein and fat content for each group's supplement,

and the supplement provided additional 0.9 g/kg lean/day of carbohydrate and 0.3 g/kg lean/d of fat equally for all groups. Peanut oil was used because its fat content best matched the fat in eggs among the edible oils. To minimize any potential effect that the variability of protein consumption may have, participants consumed protein supplements (0.4 g/kg lean mass/supplement; MET-Rx protein [MET-Rx USA Inc., Boca Raton, FL] + egg protein) every 12 hours throughout the study period.

Composite Strength (CS)

Composite strength was defined as the combination of 1RMs of chest press and leg press assessed by the Keiser exercise machine.

$$CS\ (kg) = Chest\ Press\ 1RM\ (kg) + Leg\ Press\ 1RM\ (kg)$$

Thigh Strength (TS)

Thigh strength was defined as the 1RM of leg press assessed by the Keiser exercise machine.

$$TS\ (kg) = Leg\ Press\ 1RM\ (kg)$$

Composite Peak Power (CP)

Composite peak power was defined as the combination of peak powers of chest press and leg press assessed by the Keiser exercise machine.

$$CP\ (W) = Chest\ Press\ Peak\ Power\ (W) + Leg\ Press\ Peak\ Power\ (W)$$

Thigh Peak Power (TP)

Thigh peak power was defined as the peak power of leg press assessed by the Keiser exercise machine.

$$TP\ (W) = Leg\ Press\ Peak\ Power\ (W)$$

Whole Body Muscle Quality

Whole body muscle quality-strength (WBMQ-S) was defined as composite strength adjusted to total lean mass. Whole body muscle quality-peak power (WBMQ-P) was defined as composite peak power adjusted to total lean mass.

$$WBMQ\text{-}S = Composite\ Strength\ (kg)\ /\ Total\ Body\ Lean\ Mass\ (kg)$$

$$WBMQ\text{-}P = Composite\ peak\ power\ (W)\ /\ Total\ Body\ Lean\ Mass\ (kg)$$

Thigh Lean Mass and Thigh Muscle Quality

Total thigh lean mass was determined through the construction of a four-sided polygon encompassing the entire region of each thigh and combining lean mass of both thighs together. As shown in Figure 12, the first line segment of the polygon consisted of point (a) inferior to the pubic bone immediately below any flesh as a reference point with point (b) positioned as to obliquely transverse the intertrochanter crest of the femur bone. The next line segment (c-d) transected the tibiofemoral joint. Two more line segments were drawn to enclose the entire thigh tissue (b-c, a-d). Lean mass values located inside the polygon were calculated with DEXA and defined as thigh lean mass. Inter- and intra-rater coefficient of variability was <2%.

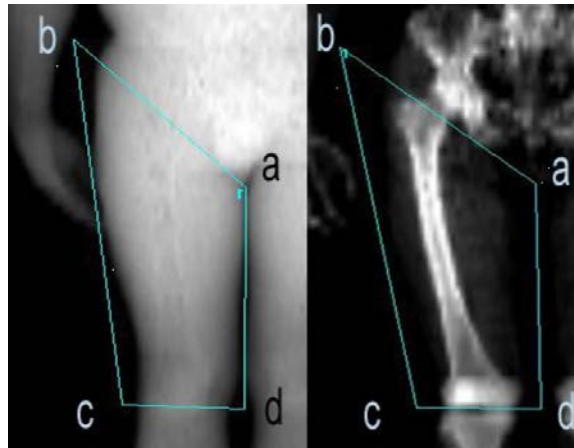

Figure 12. Example of DEXA Scan Image of Right Thigh. A four-sided polygon is constructed with one point below the pubic bone (a) that is connected to another point (b) as to obliquely cross the intertrochanter crest of the femur bone. Points (c) and (d) are positioned as to be traversing the tibio-femoral joint. Points (b)-(c) and (a)-(d) are connected to ensure the entire thigh was encompassed.

Thigh muscle quality-strength (TMQ-S) was defined as leg press 1RM adjusted to total thigh lean mass for both legs. Thigh muscle quality-peak power (TMQ-P) was defined as leg press peak power adjusted to total thigh lean mass for both legs.

TMQ-S = leg press 1RM (kg) / total thigh lean mass (kg)

TMQ-P (W/kg) = leg press peak power (W) / total thigh lean mass (kg)

Statistics

The assumption of normal distribution was checked using Shapiro-Wilk test. Differences in baseline values (age, BMI, initial body fat percentage, TG, TC, HLD, LDL, total cholesterol intake, total FA intake, n-3 FA intake, n-6 FA intake, MUFA intake, and PUFA intake) among groups and between genders were tested with One-way ANOVA and Student's t-test, respectively. One sample t-test was performed to compare the proportion of calories from protein, carbohydrate, and fat to the required nutrition control value. Differences of the proportion of calories from protein, carbohydrate, and fat on two weeks before and the first week of the training were detected by paired sample t-test. Changes in lean mass, strength, peak power, and muscle quality before and after training were detected by Mixed-ANOVA with gender and cholesterol intake group as the between-subject factors to detect the effects of gender and dietary cholesterol on skeletal muscle adaptation to resistance exercise, respectively.

Correlations of fatty acids to percentage changes in lean mass, strength, peak power, and muscle quality were analyzed by Pearson Correlation and Linear Regression.

Data were expressed as means ± SEM. The comparison-wise error rate, α, was set to be 0.05 for all statistical tests. All data were analyzed using Statistical Package for Social Science software (SPSS version 24; IBM, New York, NY).

Results

Baseline and Dietary Lipids among Cholesterol Intake Groups and Gender

Baseline measurements were presented in Table 1 and Table 2. Age, body weight, body fat percentage, and BMI were not significantly different among cholesterol intake groups while men had significantly higher body weight and lower body fat percentage before training. Women consumed more FA and less cholesterol than men.

Table 1. Baseline Measurement by Cholesterol Intake Groups.

	LC	MC	HC	Sig.
N	13	11	13	
Age (years)	58.77 ± 1.87	60.66 ± 1.39	60.11 ± 1.46	0.700
Pre-train Body Weight (kg)	82.11 ± 4.40	80.81 ± 6.90	79.85 ± 4.27	0.950
Pre-train Lean Mass (kg)	47.52 ± 2.86	46.89 ± 3.26	45.24 ± 3.10	0.858
Pre-train Body Fat %	38.92 ± 2.93	37.61 ± 2.83	40.92 ± 1.98	0.651
Pre-Train BMI (kg/m^2)	29.07 ± 1.64	28.13 ± 1.71	27.95 ± 1.22	0.851
Cholesterol Intake (mg/kg)	147.29 ± 10.91	141.82 ± 9.96	137.05 ± 13.30	0.817
Total FA Intake (g/kg)	1.45 ± 0.15	1.16 ± 0.06	1.35 ± 0.13	0.271
n-3 FA Intake (g/kg)	0.008 ± 0.001	0.005 ± 0.001	0.007 ± 0.001	0.133
n-6 FA Intake (g/kg)	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.065
MUFA Intake (g/kg)	0.25 ± 0.03	0.20 ± 0.02	0.33 ± 0.06	0.140
PUFA Intake (g/kg)	0.13 ± 0.03	0.09 ± 0.01	0.15 ± 0.03	0.210

Cholesterol and protein did not include supplements. * No significant difference on the baseline measurements between groups. Data are Mean ± SEM.

Table 2. Baseline Measurement by Gender.

	Men	Women	Sig.
N	15	22	
Age (years)	60.77 ± 1.63	59.15 ± 1.08	0.393
Pre-train Lean Mass (kg)	57.06 ± 1.95	39.35 ± 0.90 *	>0.001
Pre-train Body Weight (kg)	90.72 ± 3.90	74.25 ± 3.45 *	0.004
Pre-train Body Fat %	33.04 ± 1.54	43.25 ± 1.61 *	0.000
Pre-Train BMI (kg/m^2)	29.07 ± 1.35	27.94 ± 1.13	0.525
Cholesterol Intake (mg/kg)	179.12 ± 8.37	116.81 ± 4.27 *	>0.001
Total FA Intake (g/kg)	1.13 ± 0.05	1.46 ± 0.11 *	0.025
n-3 FA Intake (g/kg)	0.006 ± 0.001	0.007 ± 0.001	0.359
n-6 FA Intake (g/kg)	0.06 ± 0.01	0.08 ± 0.01	0.197
MUFA Intake (g/kg)	0.23 ± 0.02	0.29 ± 0.04	0.262
PUFA Intake (g/kg)	0.10 ± 0.01	0.14 ± 0.02	0.149

Cholesterol and protein did not include supplements. * P<0.05, significantly different compared to male. Data are Mean ± SEM.

Nutrition Control

The proportion of nutrition intake two weeks before (week -2), on the first week (week 0), and throughout the 12-week training (week 0-12) are presented in Table 3. Participants initially (week -2) consumed fewer calories from carbohydrate and more calories from fat comparing to the required proportion. They also consumed more than 200 mg of cholesterol per day two weeks before the training. At the start (week 0) and through the 12 weeks (week 0-12) of training, participants only consumed fewer calories from protein and maintained less than 200 mg of daily cholesterol intake through the training period.

Table 3. Daily Proportion of Nutrients at Two Weeks before, at the beginning of, and throughout the Training.

	Required	Week -2	Week 0	Week 0-12
% Calories from Carbohydrate	50	45.54 ± 1.35 *	48.08 ± 1.18	49.04 ± 0.67
% Calories from protein	20	17.98 ± 1.05	18.04 ± 0.52 *	17.39 ± 0.40 *
% Calories from fat	30	34.33 ± 1.11 *	30.66 ± 1.21 †	31.32 ± 0.73
Cholesterol (mg)	<200	247.77 ± 28.58	137.65 ± 8.82 *†	142.07 ± 6.58 *

Cholesterol and calories from protein did not include supplements. * P<0.05, significantly different compared to required value. † P<0.05, significantly different between week -2 and week 0. Data are Mean ± SEM.

Adaptation to Resistance Exercise Training

The response to the 12-week resistance exercise training among cholesterol intake groups and between genders are presented in Table 4. Body fat percentage decreased, total lean mass, thigh lean mass, composite strength (CS), thigh strength (TS), whole body muscle quality-strength (WBMQ-S), and thigh muscle quality-strength (TMQ-S) increased in all three cholesterol intake groups and both genders. The increase of CS in men was significantly higher than the increase in women (P=0.049).

Body weight, body mass index (BMI), composite peak power (CP), thigh peak power (TP), whole body muscle quality-peak power (WBMQ-P), thigh muscle quality-peak power (TMQ-P), and resting serum lipid profile, including triglyceride, total cholesterol, HDL, and LDL, did not change after the training.

Besides the higher CS in men than women, no significant difference was observed on the changes in these adaptations among cholesterol intake groups and between gender.

Table 4. Changes before and after Training.

		Group			Gender	
		LC	MC	HC	Men	Women
Body Fat Percentage	Pre Train	38.92 ± 2.92	37.61 ± 2.38	40.92 ± 1.98	33.04 ± 1.54	43.25 ± 1.61
(%)	Post Train	37.99 ± 3.07 *	35.66 ± 2.60 *	39.02 ± 1.98 *	31.59 ± 1.52 *	41.61 ± 1.79 *
Body Weight	Pre Train	82.11 ± 4.40	80.81 ± 6.90	79.85 ± 4.27	90.72 ± 3.90	74.25 ± 3.45
(kg)	Post Train	83.09 ± 4.50	81.66 ± 7.08	80.40 ± 4.41	91.67 ± 3.96	74.94 ± 3.59
BMI	Pre Train	29.07 ± 1.64	28.12 ± 1.71	27.95 ± 1.22	29.07 ± 1.35	27.94 ± 1.13
(kg/m^2)	Post Train	29.41 ± 1.63	28.42 ± 1.77	28.13 ± 1.26	29.38 ± 1.36	28.19 ± 1.15
Total Lean Mass	Pre Train	47.52 ± 2.86	46.89 ± 3.26	45.24 ± 3.10	57.06 ± 1.95	39.35 ± 0.90
(kg)	Post Train	48.85 ± 3.14 *	48.31 ± 3.06 *	47.18 ± 3.09 *	58.88 ± 1.87 *	40.75 ± 0.94 *
Thigh Lean Mass	Pre Train	9.92 ± 0.60	9.67 ± 0.63	9.38 ± 0.72	11.88 ± 0.36	8.14 ± 0.26
(kg)	Post Train	10.25 ± 0.63 *	10.16 ± 0.61 *	9.85 ± 0.71 *	12.30 ± 0.36 *	8.57 ± 0.25 *
CS	Pre Train	254.29 ± 29.50	216.82 ± 13.84	212.04 ± 21.72	290.81 ± 22.65	185.68 ± 9.04
(kg)	Post Train	316.82 ± 28.34 *	297.27 ± 18.21 *	292.81 ± 31.94 *	388.03 ± 19.92 *	244.30 ± 11.16 *†
TS	Pre Train	219.19 ± 25.51	183.13 ± 11.66	177.56 ± 19.03	239.29 ± 22.07	162.86 ± 8.27
(kg)	Post Train	273.03 ± 24.44 *	256.94 ± 15.41 *	249.16 ± 26.04 *	325.38 ± 18.29 *	215.19 ± 10.44 *
WBMQ-S	Pre Train	5.21 ± 0.35	4.76 ± 0.33	4.71 ± 0.37	5.13 ± 0.39	4.74 ± 0.22
	Post Train	6.40 ± 0.29 *	6.22 ± 0.27 *	6.06 ± 0.36 *	6.60 ± 0.30 *	5.98 ± 0.21 *
TMQ-S	Pre Train	21.57 ± 1.52	19.70 ± 1.63	19.25 ± 1.58	20.27 ± 1.83	20.15 ± 0.90
	Post Train	26.37 ± 1.36 *	25.71 ± 1.36 *	24.86 ± 1.31 *	26.45 ± 1.35 *	25.09 ± 0.90 *
CP	Pre Train	724.73 ± 151.99	1112.50 ± 171.97	753.09 ± 152.48	796.36 ± 110.38	862.95 ± 134.72
(W)	Post Train	809.73 ± 162.32	865.88 ± 175.94	710.09 ± 105.24	872.91 ± 151.13	739.11 ± 98.73
TP	Pre Train	561.08 ± 116.61	864.11 ± 125.35	586.69 ± 111.67	583.14 ± 77.46	698.65 ± 105.50
(W)	Post Train	647.42 ± 139.92	764.78 ± 137.43	545.08 ± 72.02	661.86 ± 109.10	623.60 ± 86.34
WBMQ-P	Pre Train	16.36 ± 4.10	25.55 ± 5.53	17.96 ± 11.53	14.14 ± 1.77	22.44 ± 3.62
(W/kg)	Post Train	18.10 ± 4.55	18.27 ± 10.39	15.96 ± 6.47	15.01 ± 2.38	18.71 ± 12.40
TMQ-P	Pre Train	61.38 ± 16.63	99.64 ± 19.90	64.73 ± 12.51	49.33 ± 6.63	89.21 ± 14.26
(W/kg)	Post Train	69.19 ± 19.23	79.61 ± 14.14	57.01 ± 7.19	54.35 ± 8.88	76.35 ± 12.11
Triglyceride	Pre Train	99.40 ± 10.52	117.10 ± 15.45	123.00 ± 24.82	103.77 ± 10.24	120.35 ± 16.22
(mmol/L)	Post Train	95.50 ± 11.40	122.20 ± 19.59	114.70 ± 21.97	102.38 ± 9.80	117.24 ± 16.81
Total Cholesterol	Pre Train	183.10 ± 9.04	203.90 ± 11.52	179.70 ± 9.26	186.46 ± 8.96	190.76 ± 8.09
(mmol/L)	Post Train	178.30 ± 5.22	206.30 ± 9.41	185.40 ± 10.51	190.54 ± 10.36	189.59 ± 5.37
HDL	Pre Train	54.40 ± 3.19	55.20 ± 3.29	52.10 ± 3.78	51.00 ± 3.14	56.12 ± 2.35
(mmol/L)	Post Train	53.80 ± 3.07	54.80 ± 3.00	54.80 ± 4.08	51.69 ± 3.06	56.59 ± 2.37
LDL	Pre Train	108.70 ± 7.38	115.30 ± 6.35	103.10 ± 9.18	114.77 ± 8.35	104.65 ± 4.38
(mmol/L)	Post Train	105.30 ± 5.51	127.00 ± 9.82	107.70 ± 10.19	118.38 ± 10.54	109.47 ± 4.57

LC: Low cholesterol intake group; **MC**: Median cholesterol intake group; **HC**: High cholesterol intake group; **Pre Train**: Before training; **Post Train**: After Training; **BMI**: Body mass index; **CS**: Composite strength; **TS**; Thigh strength; **WBMQ-S**: Whole body muscle quality-strength; **TMQ-S**: Thigh muscle quality-strength; **CP**: Composite peak power; **TP**: Thigh peak power; **WBMQ-P**: Whole body muscle quality-peak power; **TMQ-P**: Thigh muscle quality-peak power. * P<0.05, significantly different after training. ‡ P<0.05, significantly different between. † P<0.05, significantly different between the increase between genders. Data are Mean ± SEM

Correlation of Dietary Fatty Acids to Skeletal Muscle Adaptation

Table 5 and Figure 13-25 shows the correlation between dietary FAs and the skeletal muscle adaptation to resistance exercise training. Note that a large number of correlation analyses were performed and the possibility of type 1 error was high. These exploratory analyses were intended to provide a preliminary reference for future studies.

In the analysis that included all participants, only thigh lean mass gain had positive correlations to several dietary n-6 FAs. In men, gains in thigh lean mass, TS, and TMQ-S had correlations with several dietary FAs. In women, thigh lean mass gain was positively correlated to dietary n-6/n-3 ratio, and total lean mass gain was negatively correlated to DHA intake (Figure 17). After removing the two outliers, the correlation between total lean mass gain and DHA intake in women was deceased (R^2=0.030, P=0.466). In the LC group, thigh lean mass was correlated to GLA intake, while peak power gains, including CP, TP, WBMQ-P, and TMQ-P, were correlated to several dietary FAs. In MC and LC groups, thigh lean mass gain was correlated to dietary MUFA and n-6 FAs, respectively.

Table 5. Correlation of Dietary Fatty Acids to Skeletal Muscle Adaptation to Resistance Exercise Training.

		Total Fat	n-3	n-6	Sc-SFA	PUFA	MUFA	SFA	n-6/n-3	DHA	LA	GLA
All	Total Lean Mass (kg)	0.004	0.001	0.002	0.002	0.011	0.023	0.017	0.003	0.023	0.001	0.037
Participants	Thigh Lean Mass (kg)	0.090	0.001	0.117 *	0.025	0.105 *	0.163 *	0.065	0.162 *	0.002	0.061	0.136 *
	CS (kg)	0.001	0.029	0.007	0.011	0.000	0.000	0.031	0.010	0.038	0.079	0.002
	TS(kg)	0.000	0.042	0.011	0.010	0.002	0.001	0.026	0.011	0.032	0.061	0.000
	WBMQ-S	0.000	0.028	0.008	0.010	0.000	0.000	0.027	0.012	0.031	0.084	0.000
	TMQ-S	0.002	0.046	0.022	0.005	0.007	0.008	0.015	0.024	0.026	0.044	0.005
	CP (W)	0.000	0.047	0.004	0.002	0.028	0.011	0.020	0.027	0.006	0.047	0.025
	TP (W)	0.001	0.005	0.000	0.003	0.003	0.001	0.002	0.017	0.002	0.020	0.006
	WBMQ-P (W/kg)	0.001	0.047	0.004	0.003	0.025	0.010	0.020	0.029	0.011	0.046	0.021
	TMQ-P (W/kg)	0.002	0.004	0.000	0.003	0.001	0.000	0.001	0.022	0.001	0.013	0.002
Men	Total Lean Mass (kg)	0.102	0.213	0.083	0.216	0.131	0.252	0.121	0.166	0.015	0.000	0.209
	Thigh Lean Mass (kg)	0.224	0.013	0.013	0.582 *	0.025	0.117	0.356 *	0.009	0.001	0.048	0.568 *
	CS (kg)	0.100	0.072	0.029	0.028	0.038	0.014	0.044	0.011	0.038	0.225	0.000
	TS(kg)	0.096	0.089	0.032	0.017	0.051	0.021	0.004	0.006	0.037	0.287 *	0.002
	WBMQ-S	0.086	0.086	0.033	0.015	0.047	0.022	0.033	0.006	0.039	0.223	0.001
	TMQ-S	0.078	0.093	0.034	0.005	0.056	0.029	0.027	0.005	0.038	0.267 *	0.010
	CP (W)	0.212	0.067	0.054	0.024	0.006	0.059	0.060	0.101	0.004	0.067	0.138
	TP (W)	0.030	0.003	0.000	0.037	0.021	0.000	0.003	0.041	0.021	0.003	0.011
	WBMQ-P (W/kg)	0.197	0.053	0.047	0.014	0.003	0.048	0.050	0.094	0.002	0.052	0.118
	TMQ-P (W/kg)	0.026	0.004	0.001	0.043	0.023	0.000	0.005	0.038	0.022	0.004	0.005
Women	Total Lean Mass (kg)	0.000	0.058	0.000	0.086	0.002	0.008	0.003	0.014	0.230 *	0.001	0.009
	Thigh Lean Mass (kg)	0.042	0.001	0.115	0.017	0.094	0.147	0.028	0.183 *	0.089	0.054	0.053
	CS (kg)	0.000	0.002	0.000	0.008	0.036	0.008	0.032	0.005	0.091	0.095	0.015
	TS(kg)	0.001	0.005	0.001	0.009	0.024	0.005	0.027	0.004	0.089	0.062	0.006
	WBMQ-S	0.000	0.000	0.000	0.001	0.040	0.006	0.035	0.010	0.050	0.121	0.014
	TMQ-S	0.005	0.005	0.001	0.005	0.007	0.000	0.017	0.026	0.059	0.040	0.001
	CP (W)	0.000	0.058	0.004	0.000	0.060	0.013	0.022	0.011	0.006	0.085	0.016
	TP (W)	0.000	0.039	0.002	0.005	0.032	0.006	0.015	0.006	0.005	0.059	0.014
	WBMQ-P (W/kg)	0.001	0.065	0.003	0.002	0.056	0.013	0.024	0.013	0.018	0.085	0.014
	TMQ-P (W/kg)	0.001	0.039	0.000	0.007	0.023	0.003	0.013	0.010	0.010	0.046	0.009

Table 5 Continued.

		Total Fat	n-3	n-6	SCFA	PUFA	MUFA	SFA	n-6/n-3	DHA	LA	GLA
LC	Total Lean Mass (kg)	0.001	0.105	0.053	0.161	0.006	0.053	0.007	0.006	0.021	0.025	0.025
	Thigh Lean Mass (kg)	0.130	0.004	0.011	0.017	0.112	0.048	0.231	0.041	0.032	0.284	0.385 *
	CS (kg)	0.077	0.040	0.004	0.043	0.048	0.008	0.139	0.037	0.120	0.237	0.232
	TS(kg)	0.057	0.057	0.016	0.055	0.018	0.000	0.105	0.032	0.110	0.168	0.160
	WBMQ-S	0.102	0.023	0.001	0.021	0.063	0.020	0.178	0.047	0.116	0.243	0.239
	TMQ-S	0.038	0.064	0.027	0.054	0.006	0.001	0.070	0.026	0.106	0.116	0.102
	CP (W)	0.004	0.398 *	0.325	0.261	0.506 *	0.481 *	0.192	0.129	0.008	0.550 *	0.666 *
	TP (W)	0.013	0.071	0.163	0.007	0.382 *	0.334 *	0.099	0.021	0.035	0.492 *	0.506 *
	WBMQ-P (W/kg)	0.006	0.487 *	0.358	0.368 *	0.518 *	0.531 *	0.219	0.136	0.027	0.529 *	0.646 *
	TMQ-P (W/kg)	0.005	0.074	0.145	0.010	0.332 *	0.309	0.070	0.033	0.026	0.421 *	0.424 *
MC	Total Lean Mass (kg)	0.437	0.074	0.000	0.323	0.022	0.211	0.169	0.000	0.070	0.007	0.106
	Thigh Lean Mass (kg)	0.353	0.163	0.089	0.164	0.194	0.536 *	0.164	0.026	0.048	0.065	0.161
	CS (kg)	0.004	0.069	0.003	0.119	0.041	0.125	0.065	0.206	0.042	0.007	0.064
	TS(kg)	0.000	0.048	0.000	0.111	0.029	0.095	0.061	0.224	0.052	0.002	0.058
	WBMQ-S	0.001	0.056	0.004	0.090	0.035	0.008	0.039	0.216	0.062	0.011	0.043
	TMQ-S	0.005	0.023	0.001	0.059	0.008	0.034	0.029	0.246	0.066	0.000	0.024
	CP (W)	0.139	0.014	0.002	0.143	0.023	0.003	0.032	0.128	0.064	0.082	0.102
	TP (W)	0.071	0.028	0.015	0.141	0.050	0.004	0.032	0.109	0.097	0.070	0.090
	WBMQ-P (W/kg)	0.121	0.017	0.048	0.159	0.022	0.003	0.027	0.127	0.068	0.079	0.118
	TMQ-P (W/kg)	0.050	0.031	0.015	0.146	0.056	0.008	0.025	0.106	0.110	0.068	0.093
HC	Total Lean Mass (kg)	0.022	0.110	0.008	0.032	0.122	0.062	0.026	0.000	0.043	0.284	0.007
	Thigh Lean Mass (kg)	0.220	0.009	0.320 *	0.019	0.209	0.259	0.018	0.470 *	0.000	0.014	0.059
	CS (kg)	0.008	0.112	0.039	0.000	0.028	0.023	0.015	0.025	0.037	0.188	0.172
	TS(kg)	0.005	0.140	0.041	0.000	0.041	0.027	0.029	0.022	0.037	0.275	0.176
	WBMQ-S	0.010	0.125	0.039	0.001	0.037	0.028	0.013	0.024	0.046	0.220	0.174
	TMQ-S	0.012	0.143	0.062	0.000	0.059	0.044	0.024	0.042	0.036	0.262	0.188
	CP (W)	0.116	0.051	0.006	0.005	0.004	0.009	0.080	0.020	0.039	0.000	0.095
	TP (W)	0.093	0.006	0.003	0.025	0.010	0.009	0.117	0.010	0.045	0.001	0.045
	WBMQ-P (W/kg)	0.117	0.002	0.069	0.004	0.055	0.009	0.182	0.018	0.040	0.000	0.094
	TMQ-P (W/kg)	0.102	0.006	0.006	0.027	0.013	0.012	0.117	0.015	0.046	0.002	0.051

LC: Low cholesterol intake group; **MC**: Median cholesterol intake group; **HC**: High cholesterol intake group; **CS**: Composite strength; **TS**; Thigh strength; **WBMQ-S**: Whole body muscle quality-strength; **TMQ-S**: Thigh muscle quality-strength; **CP**: Composite peak power; **TP**: Thigh peak power; **WBMQ-P**: Whole body muscle quality-peak power; **TMQ-P**: Thigh muscle quality-power; **n-3**: Omega 3 fatty acids; **n-6**: Omega 6 fatty acids, **SCFA**: Short-chain fatty acids; **PUFA**: Polyunsaturated fatty acids; **MUFA**: Monounsaturated fatty acids; **SFA**: Saturated fatty acids; **n-6/n-3**: Omega 6 to omega 3 fatty acid ratio; **DHA**: Docosahexaenoic acid; **LA**: Linoleic acid; **GLA**: gamma-linoleic acid. * $P<0.05$, significant correlation. Data are R-square (R^2).

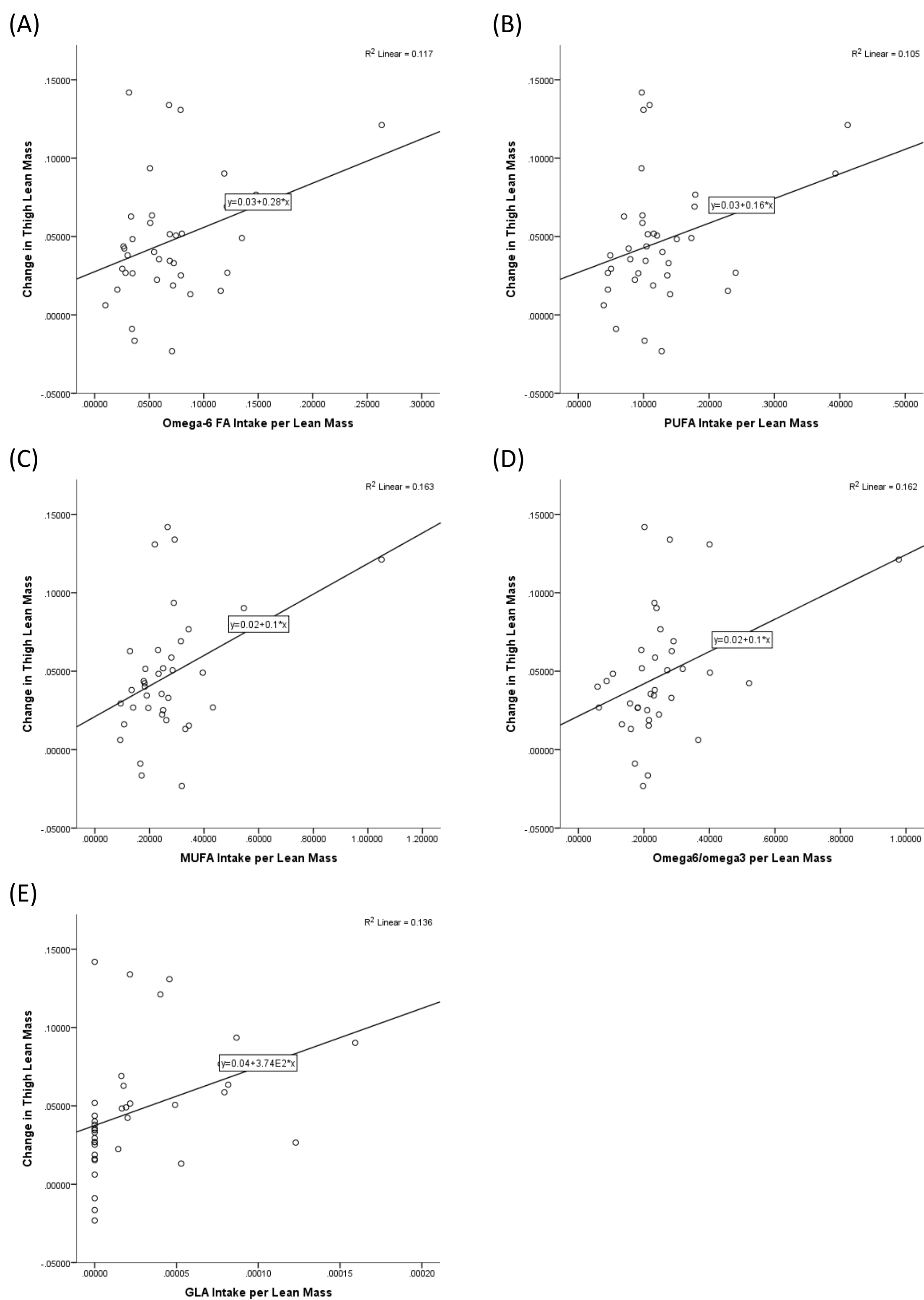

Figure 13. Correlation of Change in Thigh Lean Mass Gain to Dietary (A) Omega 6 Fatty Acids, (B) PUFA, (C) MUFA, (D) Omega 6 to Omega 3 Fatty Acids Ratio, and (E) GLA, per KG Lean Mass, in All Participants.

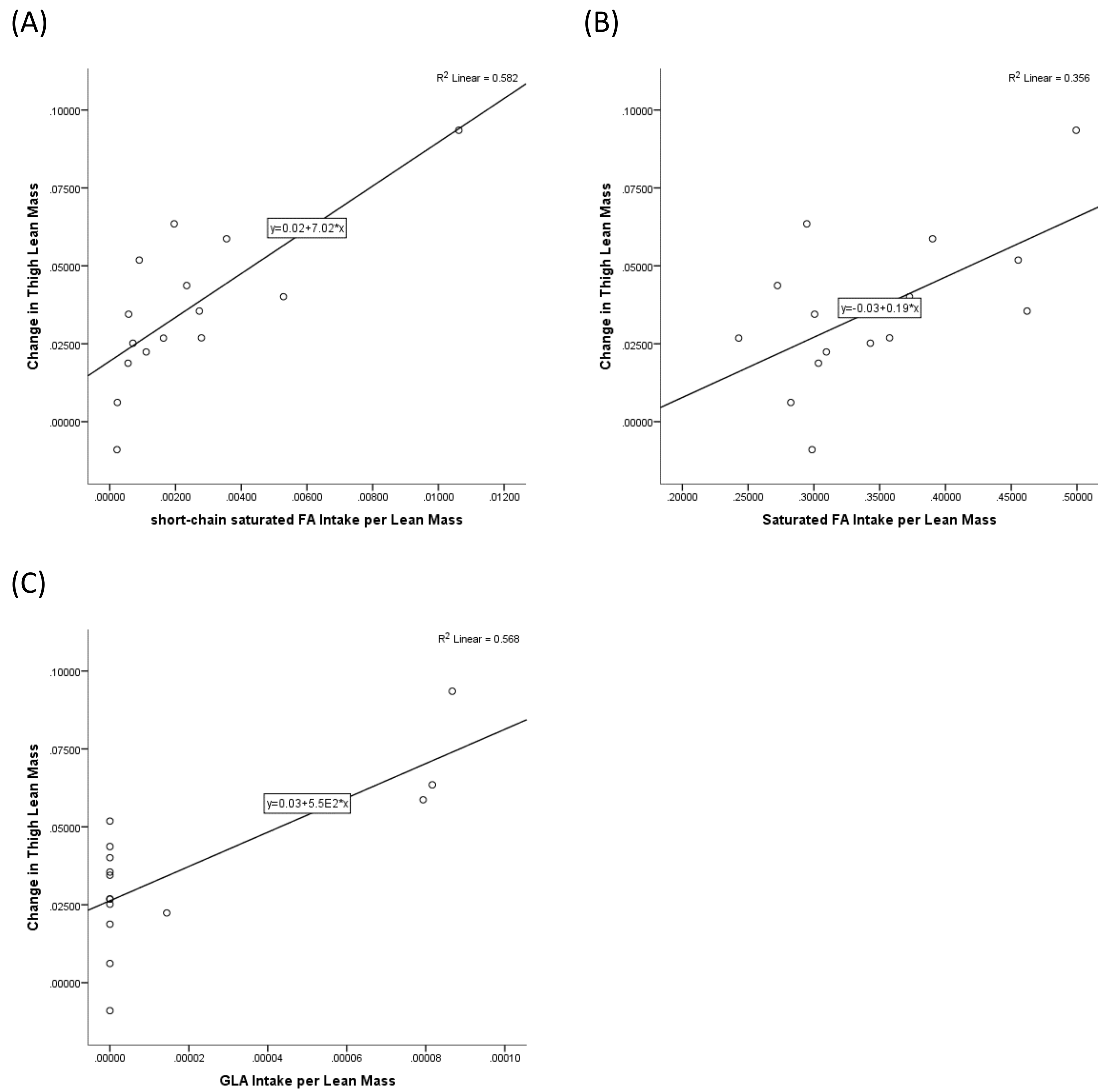

Figure 14. Correlation of Change in Lean Mass Gain to Dietary (A) Short-chain Saturated Fatty Acids, (B) Saturated Fatty Acids, and (C) GLA per KG Lean Mass in Men.

.

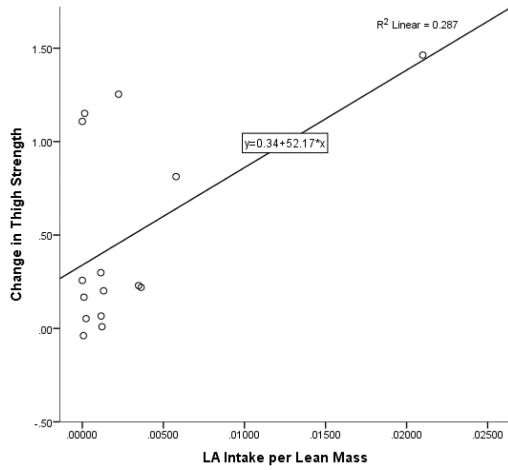

Figure 15. Correlation of Change in Thigh Strength (TS) to Dietary LA in Men.

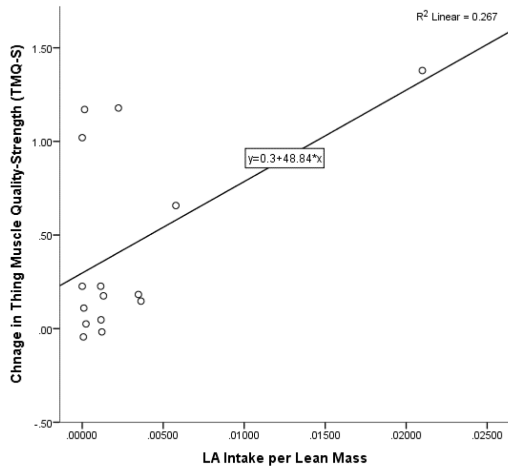

Figure 16. Correlation of Change in Thigh Muscle Quality- Strength (TMQ-S) to Dietary LA in Men.

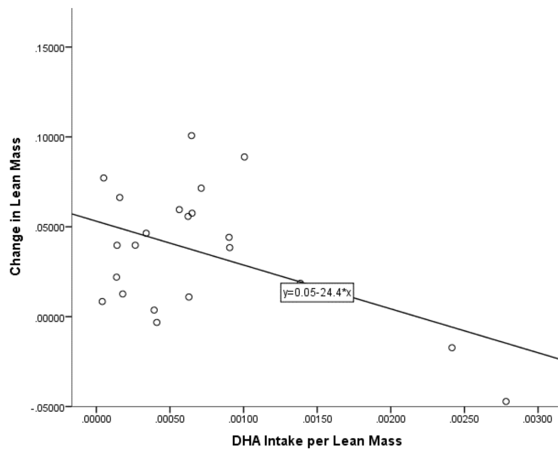

Figure 17. Correlation of Change in Lean Mass Gain to Dietary DHA per KG Lean Mass in Women.

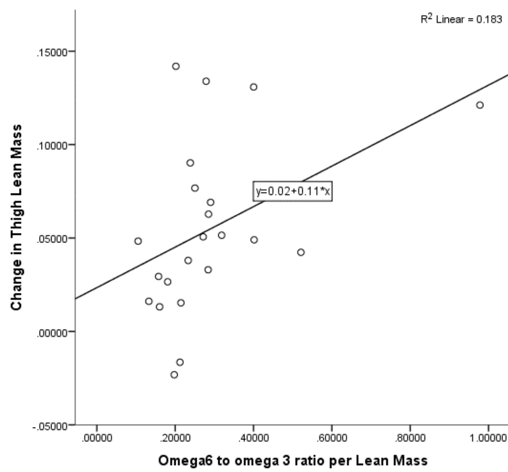

Figure 18. Correlation of Change in Thigh Lean Mass to Dietary Omega-6 to Omega-3 Fatty Acids Intake Ratio per KG Lean Mass in Women.

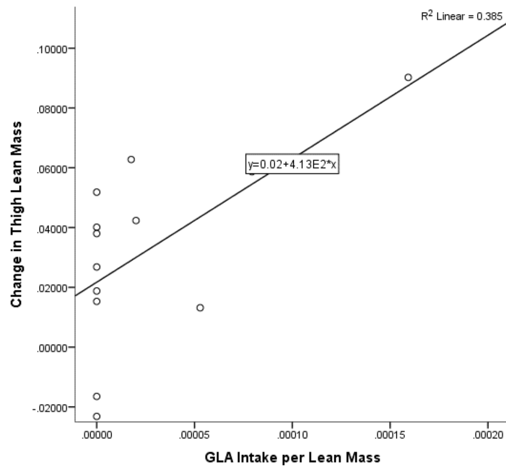

Figure 19. Correlation of Change in Thigh Lean Mass to Dietary GLA per Lean Mass in LC.

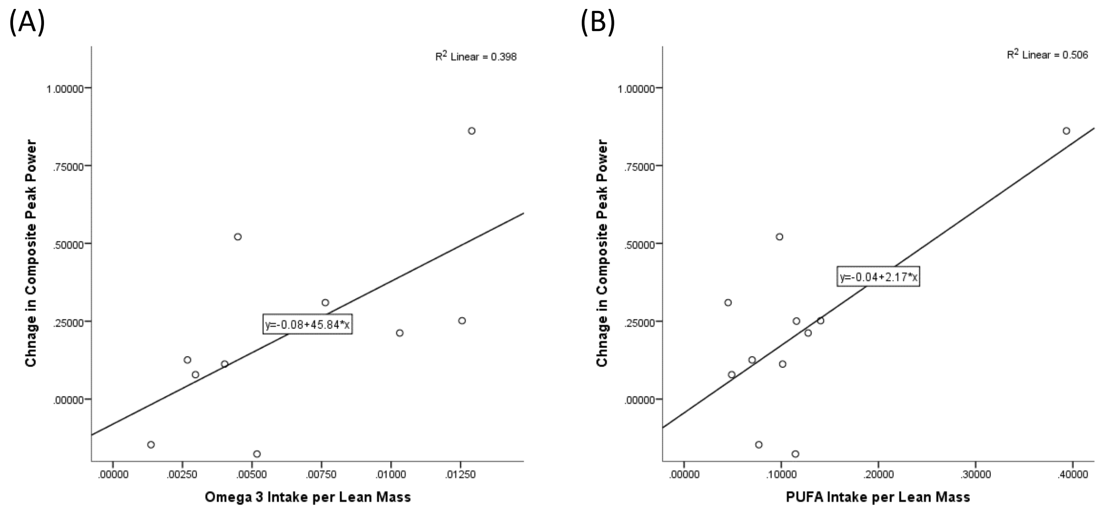

Figure 20. Correlation of Change in Composite Peak Power (CP) to Dietary (A) Omega-3 Fatty Acids, (B) PUFA, (C) MUFA, (D) LA, and (E) GLA per KG Lean Mass in LC.

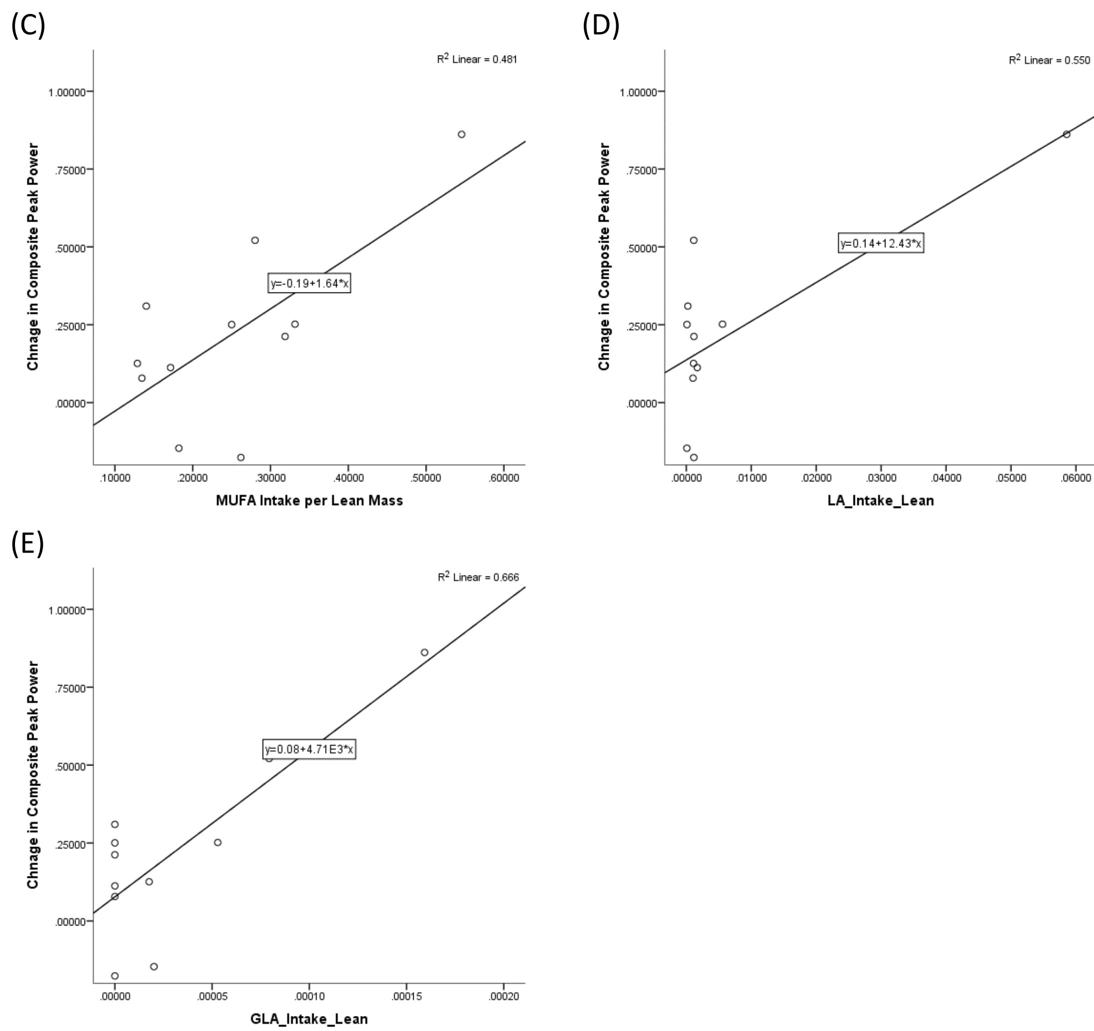

Figure 20 Continued.

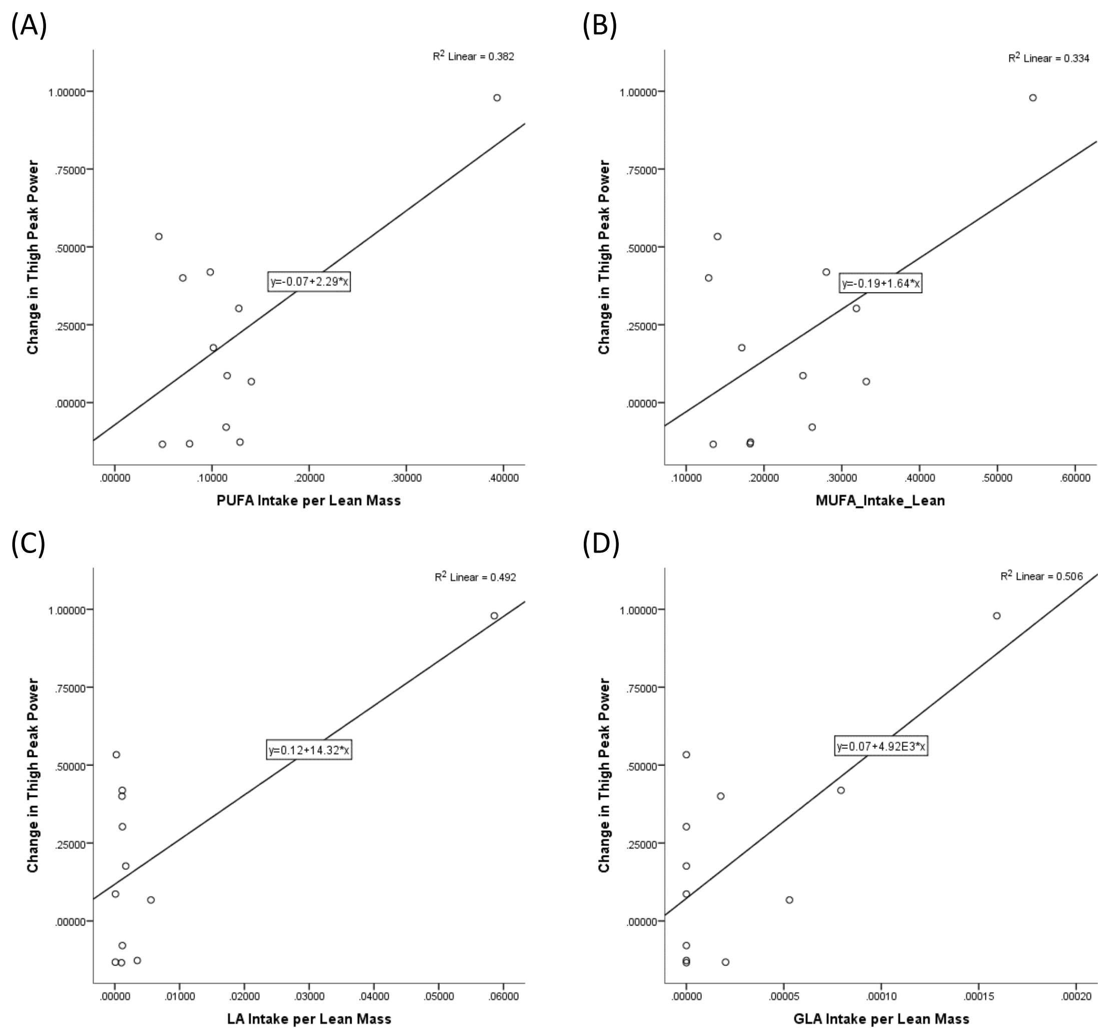

Figure 21. Correlation of Change in Thigh Peak Power (TP) to Dietary (A) PUFA, (B) MUFA, (C) LA, and (D)GLA per KG Lean Mass in LC.

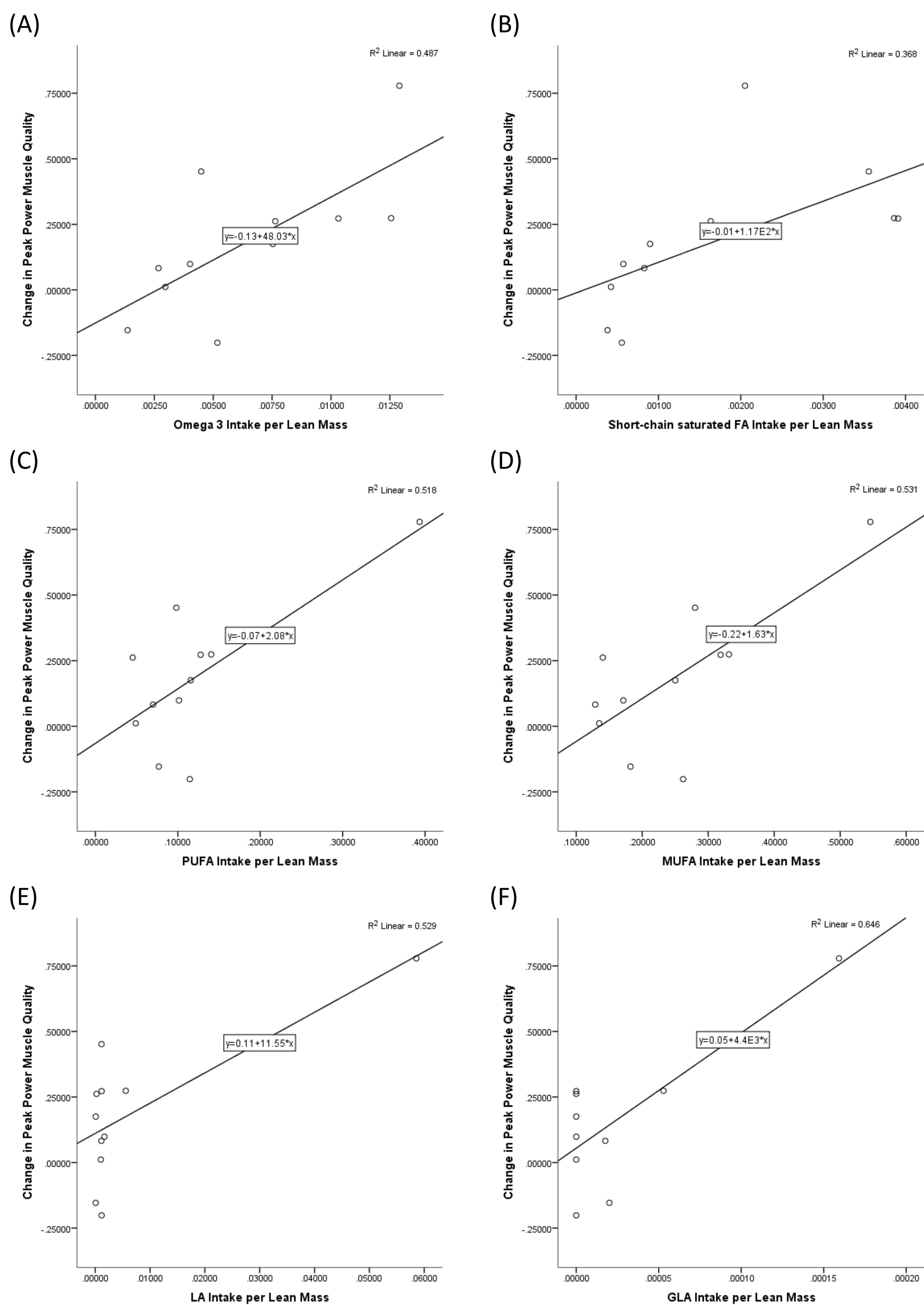

Figure 22. Correlation of Change in Whole Body Muscle Quality-Peak Power (WBMQ-P) to Dietary (A) Omega-3 Fatty Acids, (B) Short-chain Saturated Fatty Acids, (C) PUFA, (D) MUFA, (E) LA, and (F) GLA per KG Lean Mass in LC.

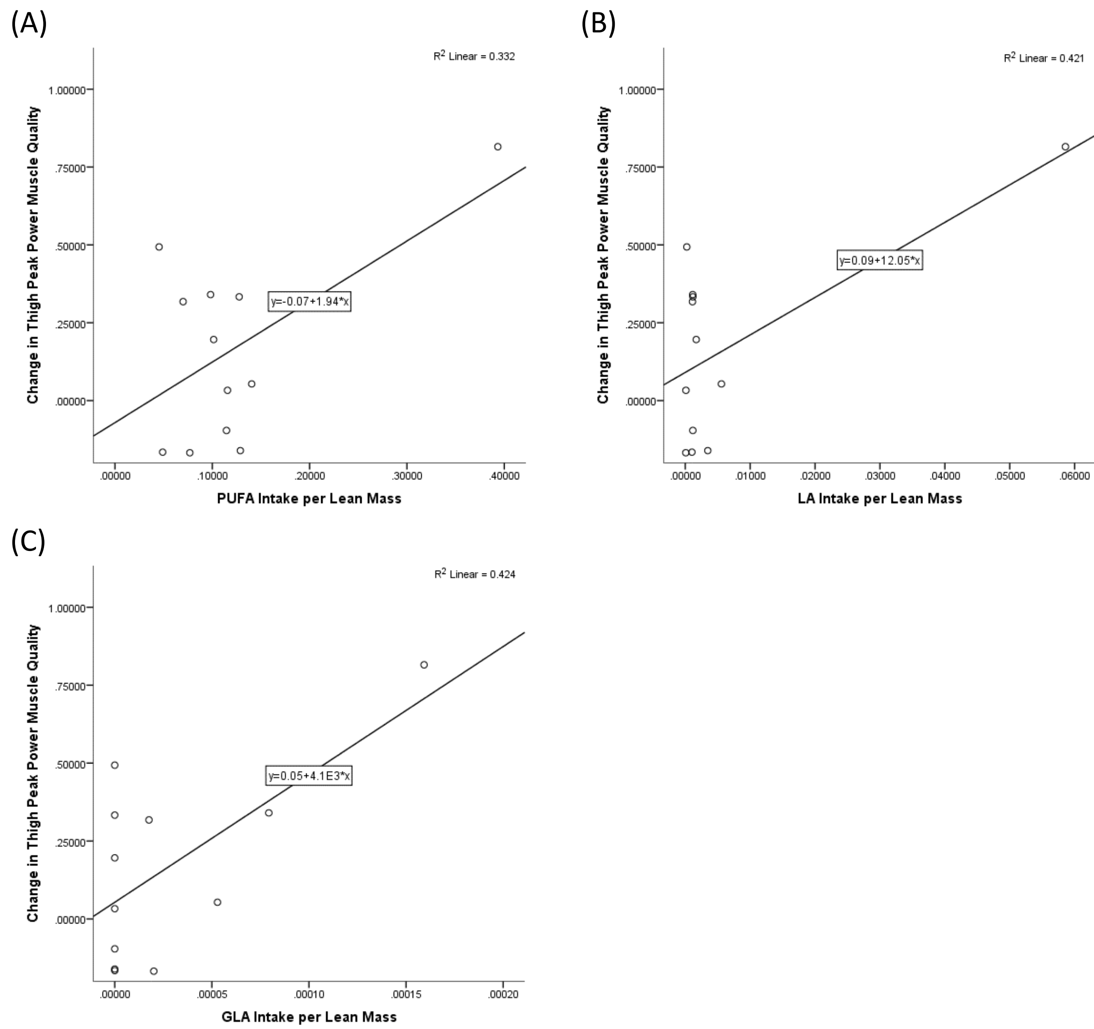

Figure 23. Correlation of Change in Thigh Muscle Quality-Peak Power (TMQ-P) to Dietary (A) PUFA, (B) LA, and (C) GLA per KG Lean Mass in LC.

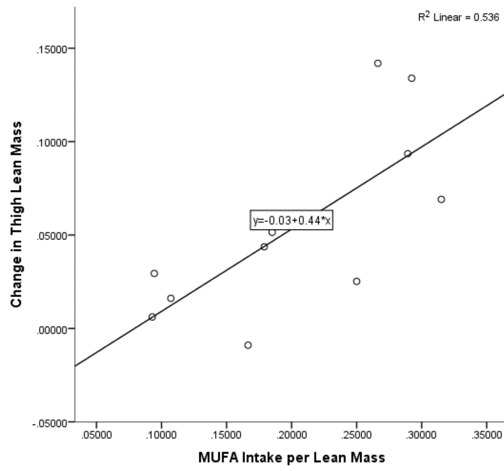

Figure 24. Correlation of Change in Thigh Lean Mass to Dietary MUFA per Lean Mass in MC.

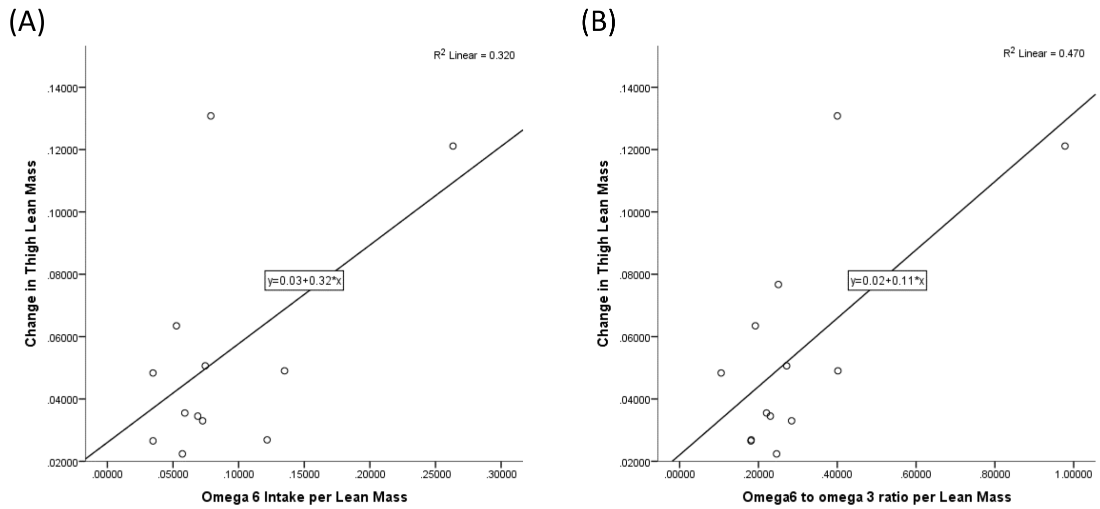

Figure 25. Correlation of Change in Thigh Lean Mass to Dietary (A) Omega-6 Fatty Acids and (B) Omega-6 to Omega-3 Fatty Acids Ratio per Lean Mass in HC.

Discussion

The results in this study showed that the 12-week whole-body progressive resistance exercise training program decreased body fat percentage, increased skeletal muscle mass, strength, and quality. These changes were not affected by different levels of cholesterol intake. The exploratory analyses suggested potential effects of dietary FAs on the skeletal muscle adaptation to resistance exercise training.

Resistance exercise training has been shown to increase lean mass and strength in order adults in previous studies (113, 139). In this study, after 12 weeks of the whole-body resistance exercise training, lean mass, strength, and muscle quality-strength were increased, and body fat percentage was decreased in all three cholesterol intake groups and both gender. These increases were consistent with previous studies and expected. However, the increases were not significantly different among groups and between gender. Although we expected dietary cholesterol to enhance the adaptation of skeletal muscle with resistance exercise training, higher cholesterol intake was not shown to have the effects.

Total cholesterol, LDL, and HDL did not change after the training, and there was no significant difference in the lipid profile among cholesterol intake groups and between gender. It may indicate that when the body is capable of maintaining stable cholesterol

levels, cholesterol supplementation is not necessary for enhancing the skeletal muscle adaptation to resistance exercise training. Furthermore, with resistance exercise training, the excess cholesterol intake did not increase serum cholesterol. A study by Crouse et al. (32) showed that plasma volume decreased immediately and increased 24 hours after a high intensity exercise (80% VO_2 max). In the present study, the serum lipid levels were not adjusted to plasma volume. Therefore, the interpretation on the changes in lipid profile in this study warranted caution.

There was no change in skeletal muscle peak power after the 12-week resistance exercise training program. Cholesterol intake and gender showed no effects on the peak power with the training. In a previous study by Fielding et al. (41) comparing the changes in peak power between high- and low-velocity resistance exercise training, the high-velocity training showed a significantly higher increase in peak power than the low-velocity training. Their study showed the need of incorporating velocity in training to improve the peak power. Since the training protocol in the present study did not include high velocity, the results were expected.

Although the proportion of fat intake was controlled in this study, the type of FAs consumed from the diet was not modulated. The association of different FAs to the resistance exercise training-induced skeletal muscle adaptation were analyzed. Although the associations between some fatty acids to gains in lean mass, strength, and

muscle quality in different groups and gender were identified, the large number of analyses (a necessary Type I error adjustment) prevents any conclusions from this data. However, as preliminary data, these results may help direct more focused studies on the effects of certain dietary FAs on the skeletal muscle adaptation to resistance exercise.

After 12 weeks of whole-body resistance exercise training, both men and women had decreases in body fat percentage and increases in lean mass, which were not significantly different between gender, identifying a similar response to the resistance exercise training in body composition between gender. These results suggest that the lean mass gain induced by resistance exercise training is more dependent of starting lean mass than gender. The common perception that men gain more muscle than women is not supported by this study.

Within two weeks of adjustment before the training, participants were able to adapt their proportions of dietary carbohydrate and fat to the required value while keeping daily cholesterol intake lower than 200 mg, and these adjustments were maintained throughout the 12 weeks of training. The two sessions of nutrition education and two weeks of adjustments were efficient for the nutritional control.

Protein is essential for the skeletal muscle adaptation to resistance exercise on lean mass and strength (24). Therefore, it is important for the participants to consume sufficient protein during the study. Although the proportion of dietary protein (without supplement) were lower than advised, the protein supplements were provided to not only ensure the sufficiency but also minimize the potential effect of the variability of protein consumption.

Conclusion

This study showed no significant difference among different levels of cholesterol intake on the skeletal muscle adaptation to the 12-week whole-body progressive resistance exercise training. While serum cholesterol remained the same after the training, excess dietary cholesterol may not be necessary for enhancing the adaptation, and the dietary cholesterol did not affect serum cholesterol level when combined with the resistance exercise training. This study suggested a potential association between dietary FAs and resistance exercise training-induced skeletal muscle gain in mass, strength, and muscle quality. Further studies are warranted to investigate the effects of FAs on skeletal muscle adaptation to resistance exercise training by controlling selected FAs, which may be suggested by the results of this study.

CHAPTER III

DIETARY LIPIDS, PPARδ, AND SHORT-TERM HIGH-INTENSITY RESISTANCE EXERCISE

Overview

Resistance exercise-induced muscle damage promotes inflammation and muscle protein synthesis. Cholesterol and FAs are essential for the inflammation and cell signaling through energy and immune cell proliferation and membrane fluidity regulation. The purpose of this study is to investigate the effects of cholesterol on muscle protein synthesis and the association between lipids and the exercise-induced muscle soreness. PPARδ, the key lipid metabolism regulator in skeletal muscle, was also measured to investigate the effects of resistance exercise and dietary cholesterol on the regulation of lipid metabolism. Sixteen untrained, healthy young men and women were randomly assigned to a high cholesterol (HC: 10.5 mg additional cholesterol/kg lean/day, n=9) or a low cholesterol intake group (LC: 0 mg additional cholesterol, n=7) and instructed to perform two bouts of high-intensity eccentric resistance exercise (RE, 85% 1RM) consisting of five sets of unilateral leg presses and extensions to fatigue on day 0 and day 9. Two light exercise (LE, 50% 1RM) consisting of three sets and ten

repetitions per set was performed on day 3 and 6. Muscle biopsies were performed on both exercised (EX) and non-exercised (CON) legs 22 hours after the second RE. Deuterium oxide (^2H_2O, heavy water) was used to measure muscle protein synthesis. PPARδ protein content was analyzed via Western Blotting. The results showed no effect of dietary cholesterol on skeletal muscle protein synthesis in respond to the training. Soreness levels were lower with high cholesterol intake. Despite the expectation of the negative correlation, there were positive correlations of n-3 FAs (DHA, DPA, and EPA) to muscle soreness. PPARδ protein content increased 22 hours after the exercise in the high cholesterol intake group. In conclusion, the present study revealed potential effects of dietary lipids on skeletal muscle recovery after resistance exercise. It also showed a short-term effect of resistance exercise on PPARδ protein content with high cholesterol intake.

Introduction

Resistance exercise-induced muscle damage causes inflammation, which results in the recruitment of macrophages and neutrophils which break down damaged muscle tissue and produce cytokines including interleukin-6 (IL-6) and transforming growth factor-β (TGFβ) (28, 57, 77, 134). IL-6 induces satellite cell proliferation by activating the MAPK and PI3K cell signaling cascades via JAK signaling pathway (60, 127). TGFβ enhances

muscle differentiation and regulates local collagen synthesis to build up skeletal muscle architecture (57). They are essential for muscle hypertrophy in response to resistance exercise.

Cholesterol is a part of the semipermeable membrane, regulates membrane fluidity and functions of membrane proteins, and modulates membrane trafficking and cell signaling processes (70). It is involved in the process of inflammation, cell signaling, and cell stability during muscle hypertrophy. At the inflammation site, cholesterol provides the component of the cell membrane that increases membrane viscosity and promotes stability. With or without inflammation, cholesterol facilitates the cell signaling pathways that promote muscle protein synthesis, including PKB-mTOR, PA-mTOR, and MAPK pathways, by forming lipid rafts as platforms for transporting cell signaling molecules (121). Lipid rafts are also essential for the muscle growth through the pathways activated by insulin, IGF-1, TNF-α, EGFR, PDGFR, IL-6, ERK-2, AKt-1 and steroid hormones. Furthermore, cholesterol contributes to the formation of the steroid hormone such as testosterone that stimulates muscle growth (121).

Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription factors that belong to the nuclear receptor superfamily. PPARα, PPARδ, and PPARγ are three PPARs that have been identified. PPARα is primarily expressed in liver, kidneys, heart and skeletal muscle. PPARγ promotes adipogenesis and lipid storage and is expressed

mostly in adipose tissues and immune cells such as macrophages (52). PPARδ is the most abundant PPAR isoform in skeletal muscle and is the key regulator of FA catabolism and metabolic adaptation in skeletal muscle (156). It may be activated by ligand binding (FAs) or phosphorylation. Short-term exercise and endurance training have been shown to increase PPARδ expression (93).

Exercise elevates FFA levels, a physiological signal that triggers the increase in PPARδ protein content (37). mRNA expression of PPARδ and PGC1-α were shown to increase three hours after a bout of high-intensity cycling exercise (93). Similar PPARδ mRNA expression has been observed after the endurance exercise performed at either elevated or depressed FA levels, suggesting that muscle contraction, not the nutritional condition, may be the main contributor to the elevated PPARδ mRNA expression induced by exercise (37, 157). Muscle contraction activates several kinases, including MAPK, AMPK, and PKA, which may phosphorylate PPARδ (20, 34). Resistance exercise induces muscle contraction and the release of FFAs (50). Both are the stimulators of PPARδ expression. However, the effect of resistance exercise on PPARδ protein content in humans has not been studied.

The purpose of this study is to investigate the effects of dietary cholesterol and FAs on muscle protein synthesis and soreness after the short-term high-intensity resistance

exercise. PPARδ protein content will also be analyzed to test the effects of the resistance exercise on skeletal muscle lipid regulation.

Methods

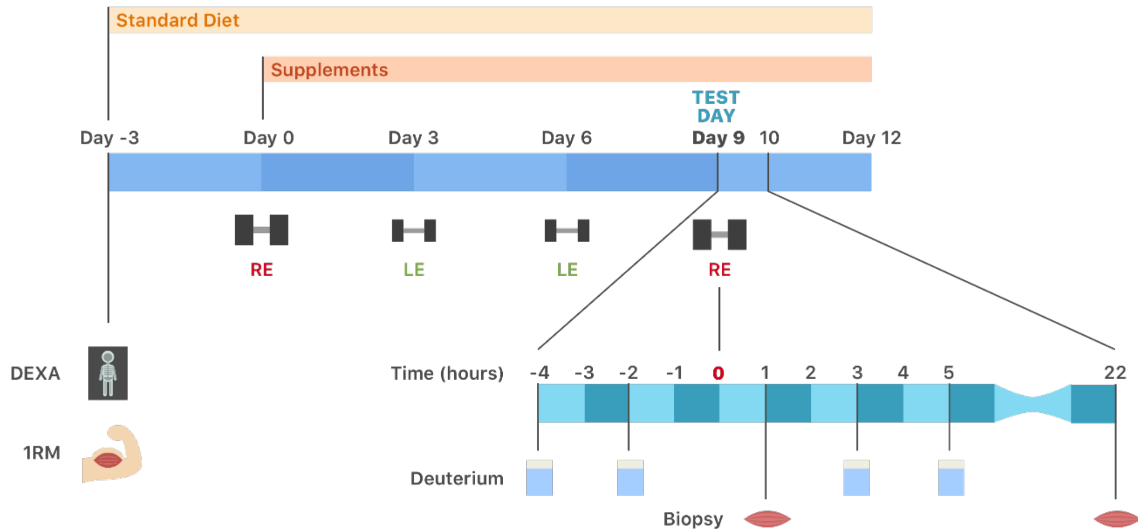

Figure 26. Timeline of Study 2.

Subjects

Sixteen untrained, healthy young men (n=12) and women (n=4), 20 to 35 years old, were recruited through advertisements and flyers on the campus of Texas A&M University. Participants were required to be generally healthy, non-smoking, and able to perform exercise testing and training. They were excluded if any of the following condition was detected via pre-screen testing and questionnaire: high blood pressure (>160/100), cardiac arrhythmias, cancer, hernias, aortic aneurysms, diabetes, kidney disease, and lung disease. Furthermore, to limit variances in training history, those who participated in one or more hours of resistance exercise per week were disqualified.

Participants were randomly assigned to a Low Cholesterol Intake Group (LC, n=7, 5 men and 2 women, zero additional cholesterol per day) or High Cholesterol Intake Group (HC, n=9, 7 men and 2 women, 10.5 mg per kilogram lean mass of additional cholesterol per day) in a double-blind manner. Figure 26 shows the general protocol of this study. This study was approved by Texas A&M University Institutional Review Board (IRB2015-0835M), and all the participants provided written informed consent before participating in the study.

Orientation

All participants attended a session of nutrition education instructed by a registered dietitian (RD) and an exercise orientation before the start of the study. During the 2-hour nutrition education, the participants learned about proper nutrient intake, calorie and portion control, and study specific diet guidelines as well as the use of the Nutribase nutrition software (Nutribase; version 9; Client Intake Module; Cybersoft Inc., Phoenix, AZ). The Nutribase software was used to maintained diet logs by the participants throughout the study. During the exercise orientation, participants were provided the information and knowledge on the resistance exercise, and the proper exercise techniques were demonstrated. The participants became familiarized with the resistance exercise by practicing the techniques with light weight on the Keiser 300 series pneumatic exercise machines (Keiser, Palo Alto, CA) and the intensity was gradually increased to 40% of their estimated maximum strength (4/10 on the

Omnibus-RE Scale [OMNI-RES] ratings of perceived exertion [RPE]) (47). The purpose of the exercise orientation was to allow rapid motor learning while minimizing skeletal muscle adaptations to standardize strength measures, estimate maximum strength (1RM) before testing, and reduce the possibility of exercise-induced injury.

Testing

Following the orientation and at least 72 hours before the first resistance exercise session, 1RM, peak power, body composition, and RMR were measured. 1RM was determined by gradually increasing exercise weights until the maximum resistance, at which only one repetition could be completed with proper form in full range of motion, was reached using the Keiser machines. Following a three-minute warm-up on a cycle ergometer (Schwinn Fitness, Inc., Denver, CO) and stretching, participants performed four warm-up repetitions with an exercise weight corresponding to 55% of an estimated 1RM obtained during the exercise orientation. The weight was then increased to 75% of a re-estimated 1RM (based on RPE) for only one repetition being performed. After 60 seconds of rest, the weight was increased again to 90% of a re-estimated 1RM for one repetition being performed. Additional attempts for 1RM was made after 60 seconds of rest until the true 1RM value was obtained, in a manner that the total number of 1RM attempts was minimized.

Body composition was assessed by a dual energy X-ray absorptiometry (DEXA) Lunar Prodigy machine (General Electric, Fairfield, CT). RMR was measured with ParvoMedics TrueMax 2400 Metabolic Measurement System (Sandy, UT) in the morning after an overnight fast to determine the total calorie required for the nutrition control.

Resistance Exercises

Participants performed a short-term high-intensity resistance exercise protocol consisting of unilateral leg press and extension with five sets and repetitions until failure per set at 85% 1RM performed on the Keiser 300 series pneumatic exercise machines (Keiser, Palo Alto, CA) in the Resistance Exercise (RE) sessions. During the RE session, participants were asked to concentrate on the 3-second eccentric contraction. The Light Exercise (LE) sessions consisted of unilateral leg press and extension with three sets and ten repetitions per set at 50% 1RM performed on the Keiser machine. One leg (EX) performed two REs and two LEs while the other leg served as non-exercise control (CON) throughout the study. The RE was performed on the starting day (day 0) and the test day (day 9) while LE was performed on day 3 and 6 (Figure 26).

Nutrition Control

Participants were instructed to consume 50% of total calories from carbohydrate, 30% from fat, 20% from protein, and <10% from saturated fat to meet daily caloric consumption goals as determined by RMR test. They were also instructed to consume

>1.0 g/kg/day of protein, 25-30 g/day of fiber, and <200 mg/day of cholesterol, as recommended by the AHA (112). Nutrition control started three days before the resistance exercise program. Participants were required to maintain 24-hour diet logs three times per week during the study period. Feedback on the diet logs was provided, and adjustments were made as necessary to ensure adherence to the study dietary guidelines. All nutrition data were recorded and analyzed using the NutriBase 9 software.

On the test day (Day 9), caloric-standardized meals were provided. Caloric requirements were calculated using the Harris-Benedict equation and meals were provided following a macronutrient ratio of 55% carbohydrates, 20% fat, and 25% protein. Meals consisted of a Lean Cuisine Culinary Collection (Wilkes-Barre, PA) frozen meal for breakfast and dinner and any additional caloric needs were provided throughout the day with Boost High Protein (Fremont, MI) drinks.

Supplement

Low Cholesterol Intake Group (LC) consumed zero additional cholesterol per day, and High Cholesterol Intake Group (HC) consumed 10.5 mg/kg lean/day of additional cholesterol in the supplement consisting of egg yolk, egg white, peanut oil, and Boost High Protein (Fremont, MI) drinks. Egg white and peanut oil were used to achieve equivalent amounts of protein and fat content for each group’s supplement, and the

supplement additionally provided 0.9 g/kg lean/day of carbohydrate and 0.3 g/kg lean/d of fat equally for all groups. Peanut oil was used because its fat content best matched the fat in eggs among the edible oils. To minimize any potential effect that the variability of protein consumption might have, participants consumed protein supplements (0.4 g/kg lean mass/supplement; MET-Rx protein [MET-Rx USA Inc., Boca Raton, FL] + egg protein) every 12 hours throughout the study period.

Soreness Assessment

The soreness levels were assessed on day 0 (before the first RE), day 1, day 2, day 3 (before the first LE), day 6 (before the second LE), day 9 (before the second RE), day 10 (before the biopsy), day 11, and day 12. As shown in Figure 27, a soreness visual analogue scale adapted from a previous study (25) with the top as “extremely sore” and bottom as “no soreness at all” was used to obtain the soreness levels. Participants were asked to mark their soreness levels on the scale based on how sore they felt on their EX legs. To identify their soreness level, the distance between the marked point and the bottom of the scale bar was measured and adjusted to the total length of the scale bar.

Directions: Draw a horizontal line on the vertical line that corresponds to your current state of **muscle** soreness.

Right now my **muscles** feel……

Extremely Sore………………..I don't want to move at all. Maximal soreness I can imagine.

Pretty Sore…………..Soreness is clearly present. It is likely that the soreness will interfere with activity.

Somewhat Sore……… I feel some soreness especially stretching/moving. It won't likely interfere with my activity.

No Soreness At All…………..I have no sensations of muscle ache at rest or when I move or exercise.

ID______ Time______
Date______ Score______

Figure 27. Soreness Visual Analogue Scale.

Muscle Biopsy

Muscle samples were taken from both EX and CON legs at 1 and 22 hours after the second RE on day 9. Muscle biopsies were obtained from the vastus lateralis using a 5-mm needle with local anesthetic (1% Xylocaine HCL). Visible fat, connective tissue and blood were removed from all muscle samples which were immediately frozen in liquid

nitrogen (-190°C) and stored at -80°C until analysis. Participants were able to return to regular activities in a few hours after the procedure.

Muscle Protein Synthesis Rate

Muscle protein synthesis rate was accessed by analyzing the myofibrillar Fractional Synthetic Rate (FSR) using deuterium oxide (^2H_2O, heavy water). Deuterium oxide (^2H_2OCambridge Isotopes, Andover, MA) was provided in four servings of 6.5 ml of 70% ^2H_2O/kg lean body mass at the following time points: four hours before RE, two hours before RE, three hours after RE, and five hours after RE. The administration of deuterium oxide was aiming for approximately 0.8% of total body water enrichment. The ^2H rapidly equilibrated with body water leading to the intracellular ^2H labeling of alanine via transamination reactions (47). The gas chromatography-mass spectrometry (GC-MS, Agilent 7890 GC/5975 MSD, Santa Clara, CA) was used to determine ^2H labeled alanine incorporated into skeletal muscle and plasma as the marker of muscle protein synthesis (47).

To determine Plasma ^2H ratio, 2 µl of 10 N NaOH and 4 µl of 5% (vol:vol) acetone in acetonitrile solution was added to 20 µl of each plasma sample and incubate for 24 hours. The reaction was stopped by the addition of 0.6 ml of chloroform and 0.5 g of Na_2SO_4 (a drying agent) to remove the acetone. A 1 µl of sample was injected and

separated by size (using helium) in the GC-MS. Samples were then be injected into the mass spectrometer to differentiate between deuterated and non-deuterated acetone. To determine myofibrillar FSR, 30 mg of tissue was homogenized with 360 µl Norris buffer and 40 µl 10% Triton on ice for a minimum of 1 hour and then centrifuged at 14,000 rpm for 30 min at 4°C. After discarding the supernatant, 0.3 ml of 10% TCA was added to the pellet which was then homogenized with a Polytron homogenizer and centrifuged at 3800 rpm for 15 minutes at 4°C. The supernatant was discarded and 0.3 ml of 10% TCA was added to the pellet and vortexed. This process was repeated for three additional times to remove all free amino acids in the sample. Each sample was then incubated in 0.4 ml of 6 N HCl for 24 hours at 100°C to hydrolyze the proteins into free amino acids. A solution with 3:2:1 ratio of Methyl-8 (Fish Scientific, Waltham, MA), methanol, and acetonitrile was used to derivatize the samples before analysis. After heated at 70°C for one hour, a 1 µl of the aliquot was injected with a split ratio of 10:1 into the GC-MS to determine the ratio of protein-bound ^{2}H-Alaine to unlabeled alanine. The rate of protein synthesis was calculated as using the following equation:

$$\textit{Protein Synthesis Rates} = EA \cdot [EBW \times 3.7 \times t]^{-1} \times 100$$

EA indicated the quantity of protein bound ^{2}H-labeled alanine (mole % excess), *EBW* represented the quantity of $^{2}H_2O$ in body water from plasma (mole % excess), and *t*

represented time in hours or days. The constant number *3.7* was the average number of H on protein-bound alanine that was exchanged with ^2H from ^2H_2O in body water.

PPARδ Protein Analysis

PPARδ protein content was analyzed via western blotting. Muscle tissue was weighted and pulverized at low temperature with liquid nitrogen and then homogenized in cold buffer (Norris Buffer (pH 7.4) + 10% TritonX100). The Norris buffer consisted of 5 mM β-glycerophosphate, 200 µM ATP, 25 mM Hepes, 1.5 ml Protease Inhibitor cocktail, 25 mM Benzamidine, 2 mM PMSF, 4 mM EDTA, 10 mM MgCl2 and water to 250 ml. The homogenate was centrifuged at 14000 RPM, 4°C for 30 minutes. The bicinchoninic acid protein assay (BCA) was then performed to determine the protein concentration of the supernatant.

The amount of supernatant used in the Sodium dodecyl sulfate (SDS) gel electrophoresis was determined by its protein content obtained from BCA. An equal amount of protein in the supernatant was diluted in the buffer (125 mM Tris, 4% SDS, 20% glycerol, 0.002% bromophenol blue, and 200 mM DTT, pH 6.8) at the ratio of 1 buffer to 3 supernatant. Proteins were separated across a polyacrylamide gel by electrophoresis and then transferred to nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ). After transfer, membranes were incubated in blocking buffer (5% nonfat dried milk in Tris-buffered saline) at room temperature for one hour,

followed by incubation with rabbit anti-PPARδ solution (antibody : blocking buffer = 1 : 500) (Santa Cruz Biotechnology, Santa Cruz, CA) for 8 hours at 4°C. After washing with Tris-buffered saline, the membranes were then incubated with goat anti-rabbit 1gG coupled to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour at room temperature and then developed and visualized via enhanced chemiluminescence (Alpha Innotech, FluorChem SP, San Leandro, CA). A protein standard (obtained from rat quadriceps) was loaded on each gel to normalize the absorbance. Absorbance units (AU) were used to express the normalized absorbance.

Statistics

The assumption of normal distribution was checked using Shapiro-Wilk test. Differences in soreness levels and baseline values (age, BMI, and body fat percentage) between groups and genders were tested with Student's t-test. Differences in muscle protein synthesis (FSR) between EX and CON legs were detected by Mixed-ANOVA with cholesterol intake group as the between-subject factor to detect the effects of dietary cholesterol on skeletal muscle FSR after the resistance exercise. The difference in PPARδ protein content between the EX and CON legs was analyzed by Mixed ANOVA with body fat percentage as the between subject factor. Simple main effect F test was then performed to detect the difference between CON and EX legs in HC and LC groups. Correlations of fatty acids to soreness levels were analyzed by Pearson Correlation and Linear Regression.

Data were expressed as means ± SEM. The comparison-wise error rate, α, was set to be 0.05 for all statistical tests. All data were analyzed using Statistical Package for Social Science software (SPSS version 24; IBM, New York, NY).

Results

Baseline

Table 6 shows the baseline measurements in LC and HC groups. There were no differences in age (P=0.444), body weight (P=0.467), lean mass (P=0.805), and body fat percentage (P=0.185) between HC and LC.

Table 6. Baseline Measurements in LC and HC Groups.

	LC	HC
Age (years)	22.96 ± 1.13	22.02 ± 7.48
Body Weight (kg)	70.67 ± 7.48	77.16 ± 5.00
Lean Mass (kg)	49.82 ± 5.00	51.35 ± 3.70
Body Fat %	26.06 ± 2.42	30.66 ± 2.22

No significant difference on the baseline measurements between groups. Data are Mean ± SEM.

Dietary Cholesterol and Skeletal Muscle Protein Synthesis

In the LC group, the FSRs were 0.85±0.23 in the CON and 0.55±0.05 %/h in the EX leg (P=0.255). In the HC group, the FSRs were 0.46±0.05 in the CON and 0.74±0.17 %/h in the EX leg (P=0.182). Cholesterol intake had no significant effect on the thigh skeletal muscle FSR with the short-term high-intensity resistance exercise (p=0.057, adjusted to lean mass) (Figure 28).

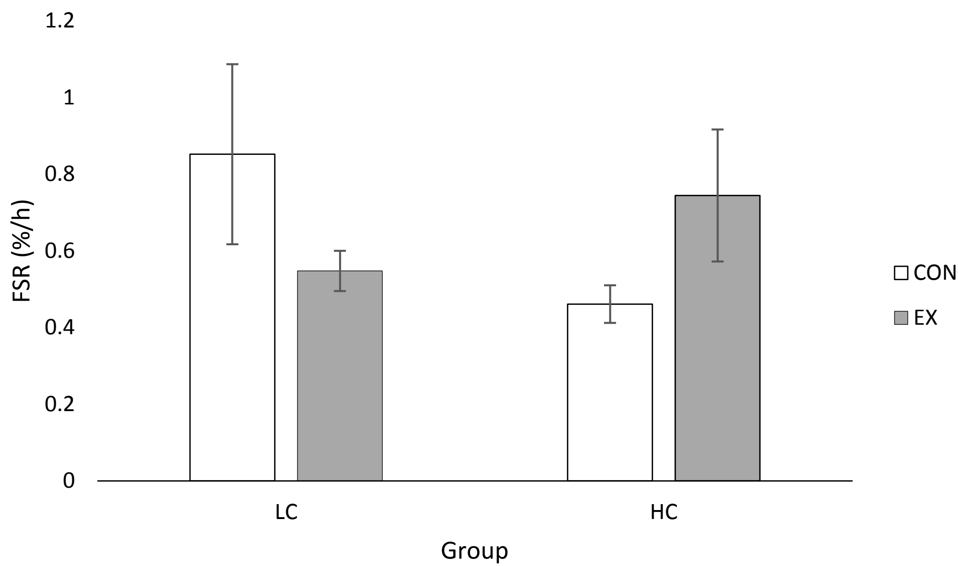

Figure 28. Dietary Cholesterol and FSR. Data are Mean ± SEM.

Dietary Cholesterol and Soreness

Figure 29 shows the average of soreness in HC and LC groups. The overall soreness levels in LC was 91.6±3.6% higher than HC (LC: 3.25±0.35, HC: 2.41±0.24, P=0.044). Two days after the first RE (Day 2), soreness levels reached the highest level and were significantly higher in LC (7.20±0.48) than HC (5.38±0.54) (P=0.028). Before the second RE on Day 9, participants reported almost no soreness in both groups (LC: 0.69±0.37, HC: 0.49±0.38, P=0.708). One day after the second RE (Day 10), the soreness levels increased to 4.39±0.79 in LC and 3.16±0.58 in HC (P=0.216). The soreness in LC on Day 10 was significantly lower than Day 2 (P=0.021), while there was no significant difference between the soreness on Day 2 and Day 10 in HC.

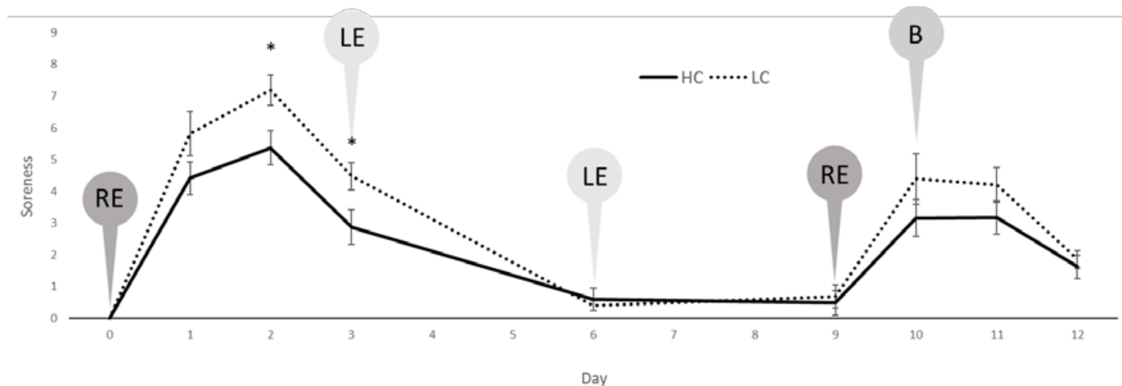

*Figure 29. Cholesterol and Soreness. B: Biopsy. * P<0.05, significant difference between HC and LC groups on the day. Data are Mean ± SEM.*

Correlation between Dietary Fatty Acids and Skeletal Muscle Adaptation

Overall soreness levels were positively correlated to dietary EPA (R^2=0.310, P=0.031), DPA (R^2=0.395, P=0.012), and DHA (R^2=0.344, P=0.022) (Figure 30).

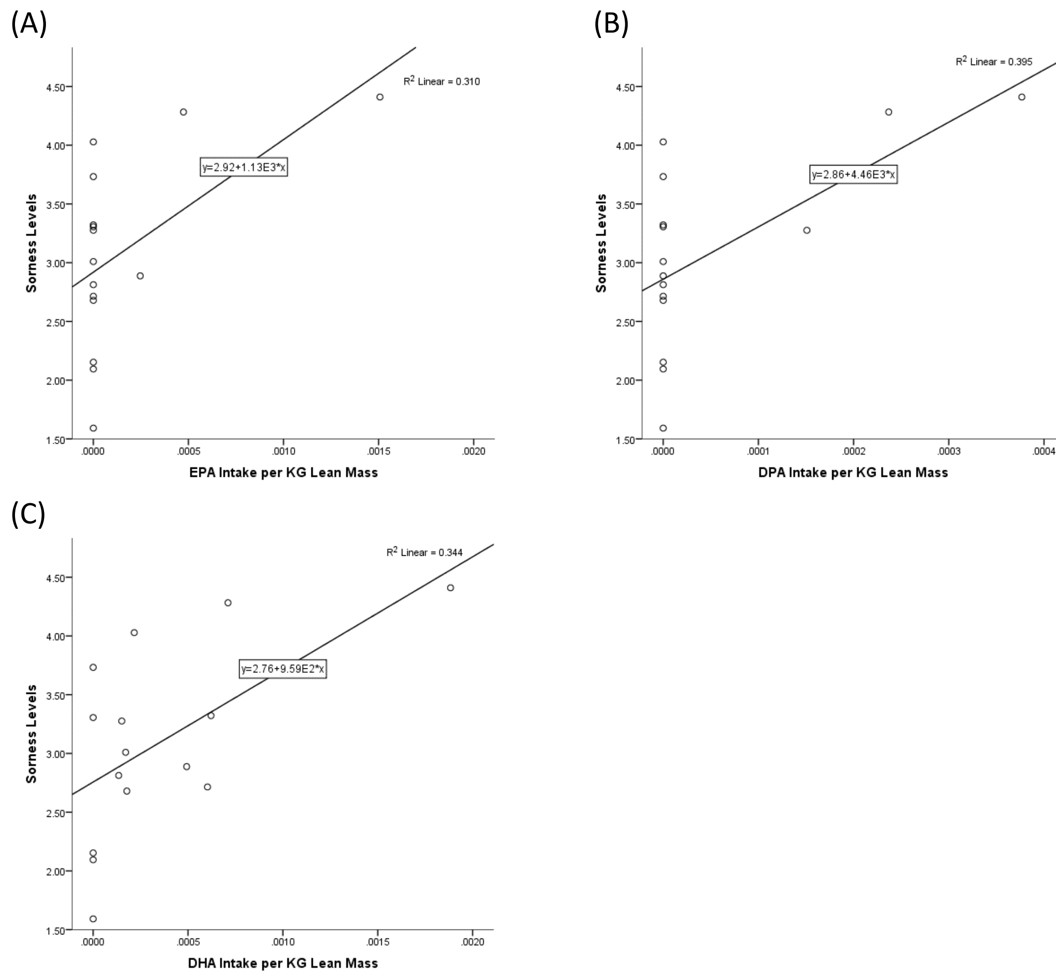

Figure 30. Correlation of Overall Soreness to Dietary (A) EPA, (B) DPA, and (C) DHA. EPA: Eicosapentaenoic acid, DPA: Docosapentaenoic acid, DHA: Docosahexaenoic acid.

The Effects of Short-tern High-intensity Resistance Exercise on PPARδ Contents

Dietary cholesterol has effects on the acute PPARδ protein content with the short-tern high-intensity resistance exercise (p=0.047) (Figure 31). In the LC group, the PPARδ protein content was not different between CON (1.64±0.44 AU) and EX legs (1.36±0.31 AU) (P=0.446). In the HC group, PPARδ protein content was significantly higher in the EX (1.81±0.44 AU) than the CON leg (1.04±0.15 AU) by 38.9±24.1% (P=0.029).

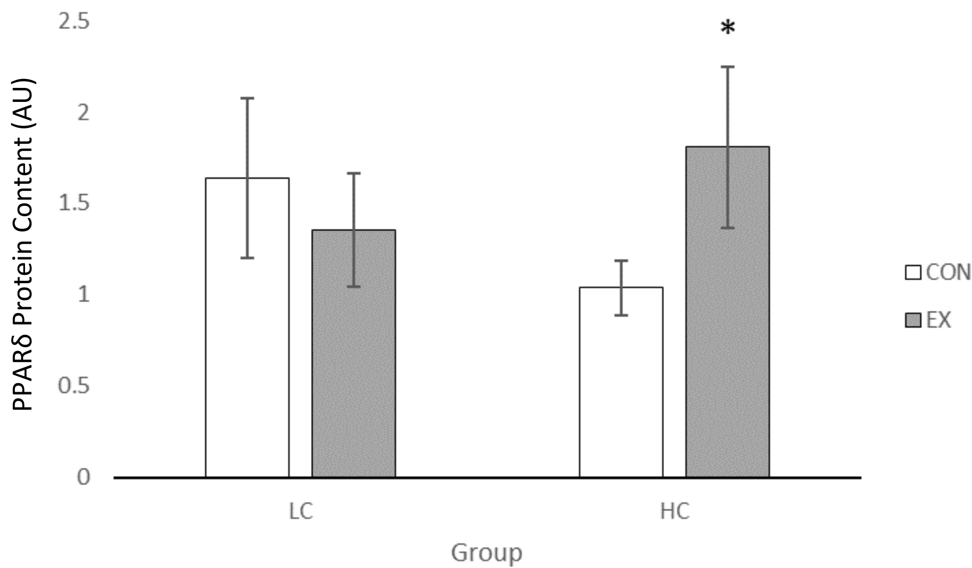

Figure 31. PPARδ Protein Content in CON and EX Legs in LC and HC Groups. Data are Mean ± SEM.

Discussion

The results of this study showed that while high cholesterol intake did not affect resistance exercise-induced muscle protein synthesis, it decreased soreness levels in the exercised leg. PPARδ protein content increased after the short-term high-intensity resistance exercise with high cholesterol intake.

Deuterium oxide (^2H_2O, heavy water), a relatively non-invasive and safe procedure, was used to determine the FSR. This method of measuring muscle protein synthesis has been effectively implemented in several studies and is comparable to a ^{2}H-Phenylalanine tracer (45, 46, 47, 106). It was used in this study due to its ability to replicate a free-living state. With the present study, the cumulative muscle protein synthesis in the 22 hours after exercise was analyzed. There were no differences in FSR between CON and EX legs during 22 hours after the resistance exercise in both HC and LC groups. Although in the HC group, the absolute FSR value in EX leg (0.74±0.17 %/h) is higher when comparing to the CON leg (0.46±0.05 %/h), the difference is insignificant (P=0.182). Cholesterol intake levels did not affect the difference of FSR between the EX and CON legs (P=0.058, when adjusted to lean mass). Unlike other studies, the present study compared the FSRs between exercised (EX) and non-exercised (CON) legs instead of the pre- and post-exercised statuses on the same leg. Participants might contract their CON legs to hold their bodies in position during the exercise, causing the

undesired isometric contraction that stimulated muscle protein synthesis on the CON legs. A different design of testing protocol may be conducted to detect the change in FSR in response to resistance exercise with different levels of cholesterol intake in future studies.

The soreness level peaked at two days after the first resistance exercise in both groups, which is consistent with the concept of delayed onset muscle soreness (DOMS). DOMS may be a sign of inflammation in skeletal muscle (26, 130). The overall soreness in the HC was lower than the LC group, suggesting a lower level of inflammation in the HC group. Cholesterol is essential at the inflammation site by regulating membrane fluidity, providing building blocks for muscle membrane, and facilitating cell signaling by forming lipid rafts (121). The higher level of dietary cholesterol might promote a more efficient tissue repair process to the damaged tissue via inflammation and thus lowered the soreness levels. However, whether the inflammation was higher or lower in the muscle of the HC group was uncertain by the observation of soreness levels. The analysis of the biomarkers such as creatine kinase (CK) and C-reactive protein (CRP) should be conducted in future studies to further investigate the effects of cholesterol on the exercise-induced inflammation.

n-3 FAs have been shown to suppress inflammation (98). Therefore, we expected a negative correlation of soreness levels to n-3 FAs. However, the results of the present

study showed positive correlations between soreness levels and three n-3 FAs: DHA, DPA, and EPA. However, note that in Figure 30, most participants had zero level of EPA and DPA consumption and a significant higher DHA intake might serve as an outlier that drove the positive correlation.

PPARδ expression in skeletal muscle has been shown to increase in response to endurance exercise training (37, 93, 157). However, the effect of resistance exercise and dietary cholesterol intake on PPARδ protein content has not been investigated in humans. In the present study, PPARδ protein content was higher in the RE than the CON legs in the HC but not in the LC group. A study conducted by Kannisto et al. (76) showed a significantly higher PPARδ mRNA expression in the high fat-fed exercised rats than the exercised-only and high fat-fed-only rats, implying a combined effects of fat intake and exercise on PPARδ expression. The data in the present study showed a combined effect of dietary cholesterol and resistance exercise. PPARδ has been shown to regulate serum cholesterol (105). Whether dietary cholesterol may affect PPARδ expression is still unknown. Our results showed a potential effect of dietary cholesterol and resistance exercise on skeletal muscle PPARδ protein content.

Conclusion

The results in this study showed no effect of dietary cholesterol on skeletal muscle protein synthesis in response to a short-term high-intensity resistance exercise. However, the exercise induced-soreness levels were lower with high cholesterol intake, implying a potential effect of dietary cholesterol on the muscle recovery after exercise. Despite the expectation of negative correlations, our data showed positive correlations of muscle soreness to omega 3 fatty acids (DHA, DPA, and EPA). The majority of zero DPA and EPA consumption and an outlier of DHA intake might cause the unexpected results. The increase of PPARδ protein content 22 hours after the exercise in the HC group indicating a potential upregulation of lipid metabolism stimulated by resistance exercise and dietary cholesterol.

These results showed that lipids might affect the skeletal muscle recovery after resistance exercise. To the best of our knowledge, the present study is the first to reveal the effects of resistance exercise and dietary cholesterol on PPARδ protein content in humans.

CHAPTER IV

THE EFFECT OF RESISTANCE EXERCISE ON SKELETAL MUSCLE PPARΔ PROTEIN CONTENT

Overview

PPARδ is the key regulator of lipid metabolism in skeletal muscle. It may be activated by FAs and phosphorylation induced by muscle contraction. Exercise increases FFAs and mobilizes several kinases that activate PPARδ. However, unlike endurance exercise, the effects of resistance exercise on skeletal muscle PPARδ remain unclear. The purpose of this study was to investigate the acute and training effects of resistance exercise on PPARδ protein content. Fifteen healthy young men (n=8) and women (n=7) were recruited to perform a 10-week whole-body progressive resistance exercise training. Body composition was measured before, at the midpoint, and after the training. Muscle biopsies were obtained 24 hours before and after the first resistance exercise before the training and 24 hours after the last exercise after the training. The results showed that PPARδ increased acutely after one bout of resistance exercise when adjusted to body fat percentage, and the change was inversely proportional to the body fat percentage. Furthermore, PPARδ protein content increased after 10 weeks of

resistance exercise training independent of body fat percentage. In conclusion, our study showed acute and chronic increases in PPARδ protein content with resistance exercise training, implying a potential mechanism for the regulation of lipid metabolism in skeletal muscle induced by resistance exercise.

Introduction

Peroxisome proliferator-activated receptors (PPARs) are a family of transcription factors that regulate the transcription of genes involving in metabolic enzymes and FA transport proteins. They have been shown to increase insulin sensitivity and improve the lipid profile (109, 140). Three PPARs have been identified: PPARα, PPARδ (also known as PPARβ) and PPARγ. PPARγ is expressed mostly in adipose tissues and immune cells such as macrophages. PPARs -α and -δ are expressed mainly in high metabolic tissues. PPARα is primarily expressed in liver, kidneys, heart and skeletal muscle. While PPARδ is also seen in liver and kidney at relatively low levels, it is the most abundant isoform in skeletal muscle and is a key regulator of FA catabolism and metabolic adaptation in skeletal muscle (52). PPARδ increases the expression of several important lipid metabolism proteins, including SREBP1, ABCA1, LPL, FAT/CD36, FATP/FABP, ACS, CPT1, Acyl-CoA Dehydrogenase and Thiolase (8, 35, 37, 75, 119, 140, 136). It is also the regulator of the PGC-1α expression, the key modulator of muscle fiber type switching

(65, 126). Activation of PPARδ by a synthetic agonist (GW501516) has been shown to switch fuel preference from glucose to FA and increase FA oxidation in rat and human skeletal muscles (19, 136). Furthermore, PPARδ improves blood lipid profile by increasing HDL and decreasing TG and LDL levels (109). Therefore, PPARδ has been identified as a potential therapeutic target for the treatment of metabolic syndrome (52, 88, 119, 155).

Skeletal muscle accounts for 40-50% of whole body mass and metabolism in non-obese individuals. Therefore, activities involved in muscle contraction significantly increases energy expenditure and result in the elevated usage of glucose and fat (121). While being the most abundant PPAR isoform that regulates metabolism and energy expenditure in skeletal muscle, PPARδ expression has been shown to increase with exercise (89, 93, 136). Exercise increases serum FA concentration and mobilizes several kinases that activate PPARδ (7, 20, 49, 154).

Compared to endurance exercise, resistance exercise is generally considered to be less effective at altering lipid metabolism. Despite earlier studies that suggested negative effects of resistance exercise on oxidative capacity due to the decreased mitochondrial volume density and some oxidative enzyme activities, recent studies revealed that resistance exercise training enhanced skeletal muscle oxidative potential (3, 27, 50, 90, 91, 142, 145). These resent studies showed the elevated activity of oxidative enzymes

and enhanced endurance performance after resistance exercise training. Increased blood glycerol and FFA were observed after a bout of resistance exercise and during recovery (50). The acute increase in blood glycerol, TG, and FFA after resistance exercise demonstrated the use of FA for the resistance exercise and the need for FA during recovery. These findings showed a potential role of resistance exercise in lipid metabolism.

PPARδ plays a critical role in the skeletal muscle adaptation to exercise by increasing oxidative capacity through upregulating the gene expression of key enzymes and transporters involved in lipid metabolism (52, 89). While the effects of endurance exercise on PPARδ expression have been studied, the effects of resistance exercise on PPARδ protein content in humans remain unclear (89, 136). The purpose of this study is to investigate the effects of resistance exercise on PPARδ protein content and the association between PPARδ protein content and blood lipid profile in response to resistance exercise training in humans.

Methods

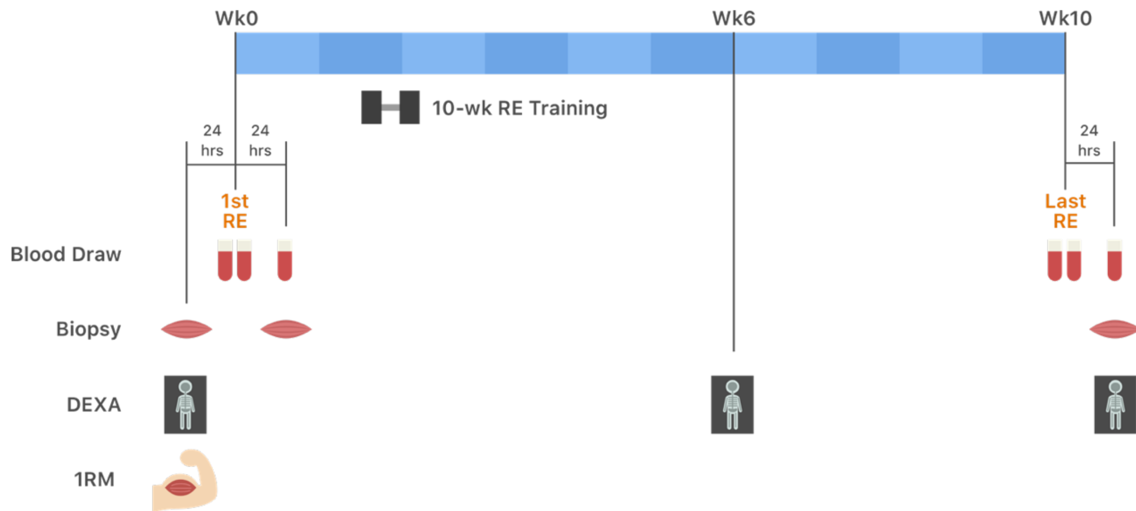

Figure 32. Timeline of Study 3.

Subjects

Fifteen healthy young men (n=8) and women (n=7), 20 to 35 years old, were recruited via broad advertisements and flyers. Before selection, each potential candidate was given a physical activity questionnaire to assess compatibility with the research protocol. participants were chosen if they were non-smokers and not currently training (performing resistance exercise < 1 hour/week during the past year). Furthermore, participants were excluded if any of the following conditions was identified in their medical history or physical exam: blood pressure > 160/100, cardiac arrhythmias, cancer, hernia, aortic aneurysm, kidney disease, lung disease. Figure 32 shows the

general protocol of this study. This study was approved by Texas A&M University Institutional Review Board (IRB2012-0245F), and all the participants provided written informed consent before participating in the study.

Orientation

All participants attended a nutrition and an exercise orientation before the start of the study. During the 1-hour nutrition orientation, the participants learned about the operation of the Nutribase nutrition software (Nutribase; version 9; Client Intake Module; Cybersoft Inc., Phoenix, AZ). The Nutribase software was used to maintain diet logs by the participants throughout the study. The exercise orientation provided the participants with information and knowledge on the resistance exercise. Proper exercise techniques were demonstrated, and the participants became familiarized with the exercise by practicing the techniques with light weight. The intensity was gradually increased to 40% of their estimated maximum strength (4/10 on the Omnibus-RE Scale [OMNI-RES] ratings of perceived exertion [RPE]) (48). The purpose of the exercise orientation was to allow rapid motor learning while minimizing skeletal muscle adaptations to standardize strength measures, estimate maximum strength (1RM) before testing, and reduce the possibility of exercise-induced injury.

Testing

Following the orientation and at least 72 hours before the first resistance exercise session, 1RM, peak power, and body composition were measured. 1RM was determined by gradually increasing exercise weights until the maximum resistance, at which only one repetition could be completed with proper form in full range of motion, was reached using the Keiser machines. Following a three-minute warm-up on a cycle ergometer (Schwinn Fitness, Inc., Denver, CO) and stretching, participants performed four warm-up repetitions with an exercise weight corresponding to 55% of an estimated 1RM obtained during the exercise orientation. The weight was then increased to 75% of a re-estimated 1RM (based on RPE) for only one repetition being performed. After 60 seconds of rest, the weight was increased again to 90% of a re-estimated 1RM for one repetition being performed. Additional attempts for 1RM were made after 60 seconds of rest until the true 1RM value was obtained, in a manner that the total number of 1RM attempts was minimized.

Body composition was assessed by a dual energy X-ray absorptiometry (DEXA) Lunar Prodigy machine (General Electric, Fairfield, CT) at 24 hours before the first resistance exercise session in the first week (wk 0), 24 hours after the 18th resistance exercise session in the sixth week (wk 6), and 24 hours after the last resistance exercise session in the last week (wk 10).

Fasted (12 hours, overnight) blood samples were collected from antecubital veins immediately before, immediately after, and 24 hours after the first and the last exercises. Blood serum samples were drawn from an antecubital vein into vacutainer tubes containing a serum clotting factor (Becton Dickinson and Company, Rutherford, NJ) with the participant seated at rest. The serum samples were immediately isolated by centrifugation at 1500x g for 30 minutes at 4°C and then stored at -80°C for later analysis. The blood lipid panels were obtained with standard methods at St. Joseph Regional Health Center's CDC certified laboratory (Bryan, TX) to examine the effects of dietary cholesterol intake and RET on blood lipid profiles.

Resistance Exercise Training

Participants performed a 10-week whole-body progressive resistance exercise training program on 3 non-consecutive days per week for 10 weeks using the Keiser 300 series exercise machines. The program consists of 10 minutes of warm-up on a cycle ergometer (Schwinn Fitness, Inc., Denver, CO), five minutes of dynamic stretching, seated chest press, lat pull down, leg press, calf raises, seated leg curls, knee extension, biceps curls, and triceps extension exercises. Participants performed three sets of 8-12 repetitions with resistance set at 75% of 1RM. They were instructed to perform as many repetitions as possible until they reached 12 repetitions or muscle failure on each set. When a participant was able to complete 12 repetitions on all three sets of an exercise, the weight was increased by 3-5% of 1RM in the next exercise session so that

only eight repetitions were possible. Rest periods between sets and exercises were restricted to 1 and 2 minutes, respectively. All exercise sessions were supervised by Exercise Physiology graduate students, and the participants were instructed to maintain their regular physical activities at the pre-study level and not to perform any additional resistance exercise.

Supplement

To minimize any potential effect that the variability of protein consumption might have, participants consumed protein supplements (MuscleTech Premium 100% Whey Protein, Iovate Health Sciences Inc, Oakville, ON, Canada) immediately following the resistance exercise and every 12 hours during the 10-week training period. Each supplementation protein was adjusted to 0.4 g of protein/kg lean mass.

Muscle Biopsy

Muscle biopsy samples were taken from the vastus lateralis under local anesthetic (1% Xylocaine HCl) using a 5-mm needle at 24 hours before (Pre-EX), 24 hours after the first resistance training (Post-EX) and 24 hours following the last resistance training after the 10-week training (Post-Train) in the Human Countermeasures Laboratory at Texas A&M University. After removing visible fat, connective tissue, and blood, muscle samples were immediately frozen in liquid nitrogen (-190°C) and then stored at -80°C until

analysis. Participants were able to return to regular activities in a few hours after the procedure.

PPARδ Protein Analysis

PPARδ protein content was analyzed via western blotting. Muscle tissue was weighted and pulverized at low temperature with liquid nitrogen and then homogenized in cold buffer (Norris Buffer (pH 7.4) + 10% TritonX100). The Norris buffer consisted of 5 mM β-glycerophosphate, 200 µM ATP, 25 mM Hepes, 1.5 ml Protease Inhibitor cocktail, 25 mM Benzamidine, 2 mM PMSF, 4 mM EDTA, 10 mM MgCl2 and water to 250 ml. The homogenate was centrifuged at 14000 RPM, 4°C for 30 minutes. The bicinchoninic acid protein assay (BCA) was then performed to determine the protein concentration of the supernatant.

The amount of supernatant used in the Sodium dodecyl sulfate (SDS) gel electrophoresis was determined by its protein content obtained from BCA. An equal amount of protein in the supernatant was diluted in the buffer (125 mM Tris, 4% SDS, 20% glycerol, 0.002% bromophenol blue, and 200 mM DTT, pH 6.8) at the ratio of 1 buffer to 3 supernatant. Proteins were separated across a polyacrylamide gel by electrophoresis and then transferred to nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ). After transfer, membranes were incubated in blocking buffer (5% nonfat dried milk in Tris-buffered saline) at room temperature for one hour,

followed by incubation with rabbit anti-PPARδ solution (antibody : blocking buffer = 1 : 500) (Santa Cruz Biotechnology, Santa Cruz, CA) for 8 hours at 4°C. After washing with Tris-buffered saline, the membranes were then incubated with goat anti-rabbit 1gG coupled to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour at room temperature and then developed and visualized via enhanced chemiluminescence (Alpha Innotech, FluorChem SP, San Leandro, CA). A protein standard (obtained from rat quadriceps) was loaded on each gel to normalize the absorbance. Absorbance units (AU) were used to express the normalized absorbance.

Statistics

The assumption of normal distribution was checked using Shapiro-Wilk test. Differences in baseline values (age, BMI, initial body fat percentage, TG, TC, HLD, and LDL) between genders were tested with Student's t-test. Changes in body composition and lipid profile were analyzed by Repeated Measures ANOVA with Fisher's least significant difference (LSD) Post-Hoc test to detect differences among samples. The differences in PPARδ protein content between the muscle samples obtained at Pre-EX, Post-EX, and Post-Train were analyzed by Repeated Measures ANOVA. Two-way Repeated Measures ANCOVA was performed on muscle samples obtained at Pre-EX, Post-EX and Post-Train with body fat percentage as the within-subject factor and gender as the between-subject factor to identify the effects of body fat percentage and gender on the change of PPARδ protein content, respectively. Correlation of body fat percentage to the

percentage change in PPARδ protein content was analyzed by Pearson Correlation and Linear Regression. Correlations of PPARδ protein content to serum lipid profile before and after resistance exercise training were analyzed by Pearson Correlation and Linear Regression.

Data were expressed as means±SEM. The comparison-wise error rate, α, was set to be 0.05 for all statistical tests. All data were analyzed using Statistical Package for Social Science software (SPSS version 24; IBM, New York, NY).

Results

Baseline and Changes in Body Composition with Resistance Exercise Training

Table 7 shows the subject baseline and body composition before the training (wk 0), at the midpoint (wk 6) and after the training (wk 10). Age and BMI were not significantly different between men and women. With the resistance exercise training, lean mass increased at the 6th week in all subjects and increase further at the 10th week. Fat mass and body fat percentage decreased significant at the 6th week but did not change further at the 10th week.

Table 7. Subject Baseline and the Changes in Body Composition.

		All Subjects	Men	Women
N		15	8	7
Age (year)		25.71 ± 1.38	25.43 ± 2.30	26.00 ± 1.70
BMI (kg/m^2)		26.91 ± 1.84	29.23 ± 2.70	24.59 ± 2.37
Body Weight (Kg)	wk 0	74.26 ± 5.64	89.09 ± 6.69	61.44 ± 5.40
	wk 6	75.46 ± 5.63	89.87 ± 6.52	61.04 ± 5.07
	wk 10	76.14 ± 5.76	91.10 ± 6.59	61.19 ± 5.07
Lean Mass (Kg)	wk 0	47.01 ± 3.36	58.24 ± 1.57	35.78 ± 2.10
	wk 6	48.04 ± 3.60 *	60.24 ± 1.53 *	35.84 ± 2.04
	wk 10	48.89 ± 3.59 * ‡	61.03 ± 1.27 *	36.75 ± 2.26 †
Fat Mass (Kg)	wk 0	25.37 ± 3.60	27.48 ± 6.35	23.26 ± 3.78
	wk 6	24.54 ± 3.52 *	26.33 ± 6.25	22.76 ± 3.68
	wk 10	24.41 ± 3.43	26.75 ± 6.25	22.07 ± 3.20
Body Fat %	wk 0	34.26 ± 2.74	30.07 ± 4.48	38.46 ± 2.57
	wk 6	33.23 ± 2.87 *	28.50 ± 4.48 *	37.96 ± 2.85
	wk 10	32.74 ± 2.59 *	28.61 ± 4.28 *	36.86 ± 2.24

BMI = Body mass index; **wk** = Week. * P<0.05, significantly different when comparing to wk 0 value, ‡ P<0.05, significantly different between wk 6 and wk 10, † *p* = 0.05 between wk 0 and wk 10. Data are Mean ± SEM.

This study showed a difference in lean mass gain over the 10-week training between men and women ($P=0.028$). Men had a significant increase in lean mass and decrease in body fat percentage on the 10th week. While women showed a gain in lean mass ($P=0.05$), their fat mass and body fat percentage did not significantly decrease on week 10.

The Effects of Acute Resistance Exercise on Skeletal Muscle PPARδ Protein Content

Muscle samples from 4 men and 6 women were successfully obtained and processed for the analysis of acute effects of resistance exercise on PPARδ protein content. Without accounting for covariates, PPARδ protein content did not significantly change after a bout of the whole-body resistance exercise ($P=0.146$). When incorporating body fat percentage as a covariate, the PPARδ protein content acutely increased by 49.07±28.73% ($P<0.001$) after one bout of resistance exercise before training (Figure 33). Furthermore, there was a negative correlation between the changes in PPARδ protein content and body fat percentage ($R^2=0.77$, $P=0.001$) (Figure 34).

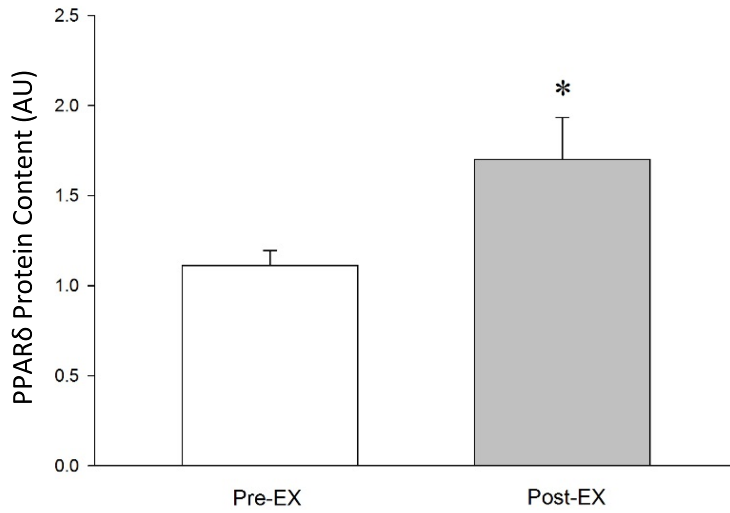

Figure 33. Acute Effect of Resistance Exercise on PPARδ Protein Content. ***Pre-EX****: Pre Exercise/Pre Training;* ***Post-EX****: Post Exercise/Pre Training. *P<0.05 when adjusted to body fat percentage, n=10. Data are Mean ± SEM.*

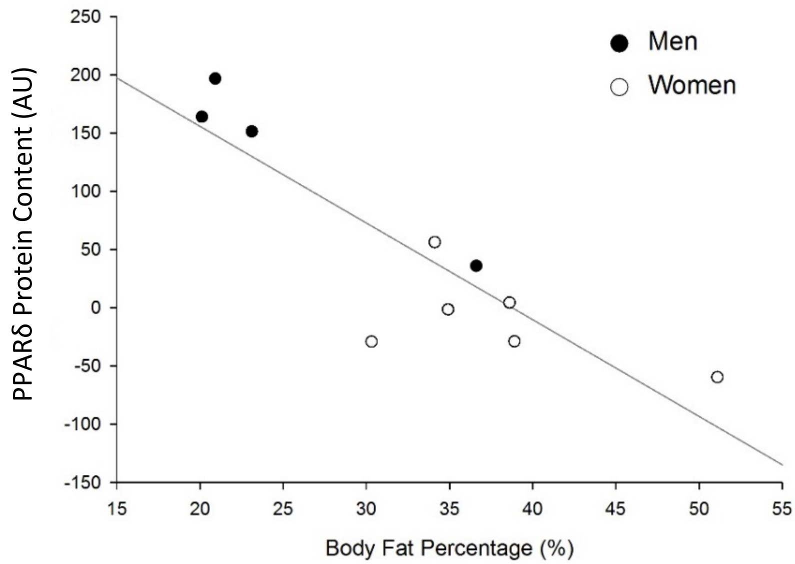

Figure 34. The Relationship between Change in PPARδ Protein Content and Body Fat Percentage. R^2*=0.77, p=0.001.*

Gender has an effect on the acute change in PPARδ protein content with resistance exercise ($P=0.008$). The data of the subjects analyzed for the acute effect of resistance exercise on PPARδ protein content showed that women had a significantly higher body fat percentage than men (women: 37.98±2.93%, men: 25.18±3.86%, $p=0.028$). To determine whether the effect of gender is dependent of body fat percentage, a Two-way Repeated Measures ANCOVA was performed, in which body fat percentage was added as a within-subject covariate with gender as the between-subject factor. As a result, the data showed an independent effect of gender on the acute increase in PPARδ protein content with resistance exercise ($P=0.046$). The effect of body fat percentage remained independent ($P=0.009$). Men showed a significant increase in PPARδ protein content ($P=0.035$) while women's PPARδ protein content stayed unchanged ($P=0.427$) (Figure 35).

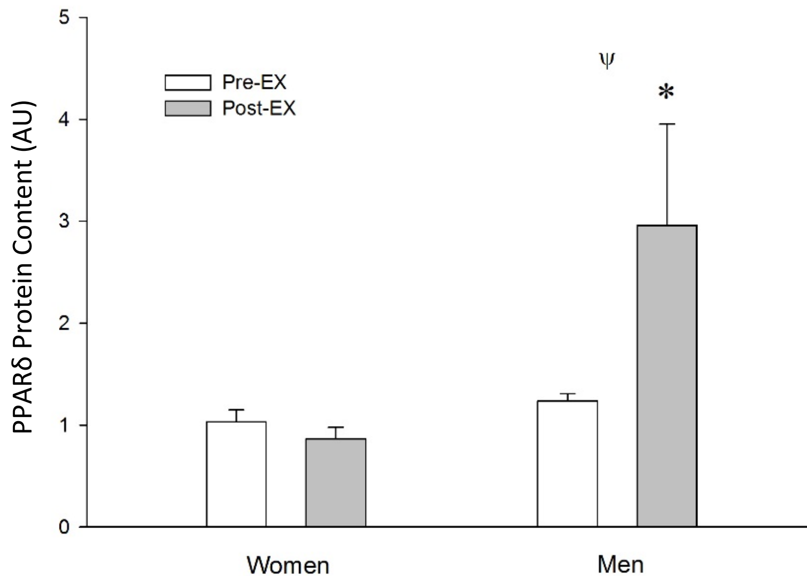

Figure 35. Changes in PPARδ Protein Content between Men and Women. ***Pre-EX****: Pre Exercise/Pre Training,* ***Post-EX****: Post Exercise/Pre Training, * P<0.05 between Pre-EX and Post-EX.* $^{\psi}$ *P<0.05 between men (n=4) and women (n=6). Data are Mean ± SEM.*

The Effects of Resistance Exercise Training on Skeletal Muscle PPARδ Protein Content

Muscle samples from 6 men and 2 women were successfully obtained and processed for the analysis of training effects of resistance exercise on PPARδ protein contents.

PPARδ protein content increased by 114.69±31.89% (P=0.015) after 10 weeks of resistance exercise training. Gender and body fat percentage did not have effects on the changes in PPARδ protein content with resistance exercise training (Figure 36).

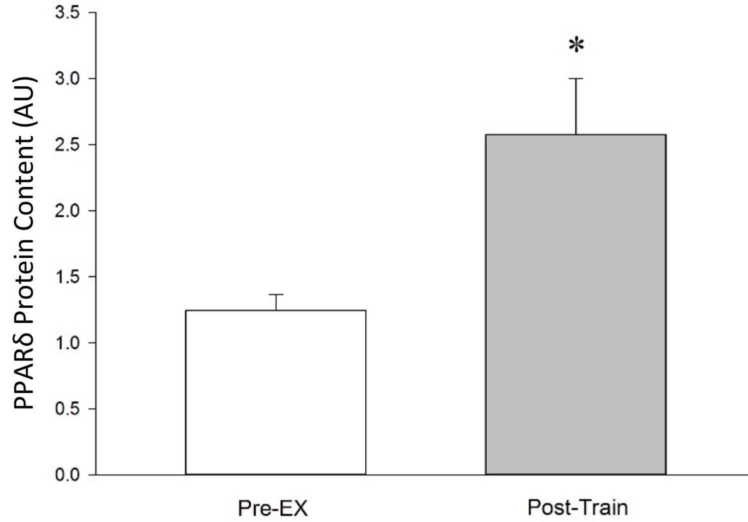

Figure 36. Effect of Resistance Exercise Training on PPARδ Protein Content. ***Pre-EX****: Pre Exercise/Pre-Training,* ***Post-Train****: Post Exercise/Post Training, * p<0.05, n=8. Data are Mean ± SEM.*

Correlation of PPARδ Content to Serum Lipid Profile before and after Resistance Exercise Training

Before the resistance exercise training, there was no correlation between PPARδ protein content and serum triglyceride, total cholesterol, HDL, and LDL (Table 8). After 10 weeks of training, PPARδ protein content was negatively correlated to serum total cholesterol (P=0.040, Figure 37 (A)) and LDL (P=0.033, Figure 37 (B))

Table 8. Coefficient of Determination of Skeletal Muscle PPARδ Protein Content to Serum Lipid Profile.

	PPARδ Protein Content (AU)	
	Before Training	After Training
Triglyceride (mg/dL)	0.023	0.086
Total Cholesterol (mg/dL)	0.027	0.534 *
HDL (mg/dL)	0.025	0.033
LDL (mg/dL)	0.012	0.557 *

* P<0.05, significant correlation. Data are R-square (R^2).

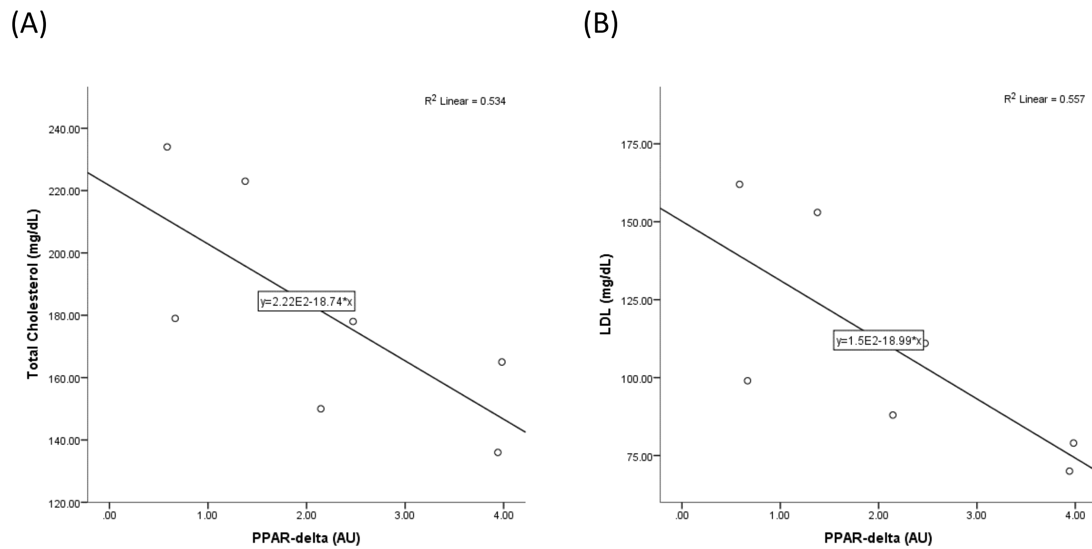

Figure 37. Correlation of PPARδ Protein Content to (A) Total Cholesterol and (B) LDL after Resistance Exercise Training.

Changes in Lipid Profile before and after Resistance Exercise Training

Table 9 presents lipid profile immediately before, after, and 24 hours after the resistance exercise before and after the resistance exercise training. Before the training, TG decreased at 24 hours after the exercise. Total cholesterol, HDL, and LDL increased immediately after exercise and returned to the pre-exercise levels 24 hours after the exercise. After training, TG, total cholesterol, HDL, and LDL levels stayed unchanged immediately and 24 hours after the exercise.

Table 9. Lipid Profile before and after Resistance Exercise Training.

	Before Training			After Training		
	Immediately Before RE T1	Immediately After RE T2	24 hrs after RE T3	Immediately Before RE T4	Immediately After RE T5	24 hrs after RE T6
Triglyceride (mg/dL)	106.30 ± 13.50	107.60 ± 13.99	91.40 ± 12.56†	99.20 ± 11.01	101.80 ± 12.76	110.40 ± 19.80
Total Cholesterol (mg/dL)	168.70 ± 12.89	179.70 ± 13.42*	166.50 ± 12.31‡	174.10 ± 10.82	178.60 ± 12.53	178.90 ± 11.00
HDL (mg/dL)	49.50 ± 4.66	54.30 ± 4.71*	49.80 ± 4.37‡	52.10 ± 4.11	54.20 ± 3.62	52.10 ± 4.19
LDL (mg/dL)	97.80 ± 11.17	104.10 ± 11.92*	98.40 ± 10.70	102.10 ± 10.41	104.00 ± 11.13	104.90 ± 9.32

RE: Resistance Exercise. * $P<0.05$, significant difference between T1 and T2; ‡ $P<0.05$, significant difference between T2 and T3; † $P<0.05$, significant difference between T1 and T3. Data are Mean ± SEM.

Discussion

To the best of our knowledge, this present study is the first to investigate the effects of resistance exercise training on skeletal muscle PPARδ protein content in humans. Acute resistance exercise increased PPARδ protein content when adjusted for body fat percentage and the change in PPARδ protein content was inversely proportional to body fat percentage. Ten weeks of resistance exercise training increased PPARδ protein content independent of body fat percentage and gender. The results showed that for untrained individuals, higher body fat percentage might blunt the effect of acute resistance exercise on PPARδ protein content. However, the negative effect of higher body fat on the exercise-induced increase in PPARδ protein content may not persist with 10 weeks of resistance exercise training.

While men showed a significant increase in PPARδ protein content after a bout of resistance exercise, the change was not observed in women. In general, women have a higher body fat percentage than men. The present study showed a gender effect independent of body fat percentage, indicating the different response between gender was not caused solely by body composition. However, the small sample size and the significant difference in body fat percentage between gender warrant caution in this interpretation.

Six weeks of resistance exercise training was enough to increase lean mass and decrease fat mass and body fat percentage with additional increase in lean mass seen after 10 weeks. Although a lower body fat percentage was not observed in women after the 10-week training in this study, it was shown to decrease with a 14-week resistance exercise training in a study by Prabhakaran et al. (118). A longer training period might be needed for women to see the decrease in body fat percentage.

PPARδ protein content were not correlated with blood lipid profile before the training. However, there were negative correlations of PPARδ protein content to total cholesterol and LDL after the training. The results imply that resistance exercise training may enhance the regulation of blood lipid profile via PPARδ.

Before the training, total cholesterol, HDL, and LDL increased acutely and then returned to their original levels 24 hours after one bout of resistance exercise. Blood TG did not change acutely and decreased 24 hours after the exercise. The results indicated the need of cholesterol and the use of fat after the acute resistance exercise. However, after 10 weeks of resistance exercise training, the changes were not observed. The effects of acute resistance exercise on blood lipid profile were blunted after the training. Although the training did not alter the resting lipid profile, our participants were healthy young adults whose blood lipid profiles were within the AHA recommended levels (131). A study by Crouse et al. (32) showed that plasma volume

decreased immediately and increased 24 hours after a high intensity exercise (80% VO_2 max). In the present study, the serum lipid levels were not adjusted to plasma volume. Therefore, the interpretation on the changes in lipid profile in this study warranted caution.

Earlier studies suggested that muscle hypertrophy induced by resistance exercise might reduce the oxidative capacity of skeletal muscle due to the dilution of mitochondria and decrease in some oxidative enzymes such as 3-OH-acyl-CoA dehydrogenase and SDH (3, 27, 91, 145). However, it is problematic to assume the cause of lower oxidative potential to be merely training-induced hypertrophy and the lower levels of some oxidative enzymes (142). For example, SDH is not considered to be a good indicator for the oxidative capacity in skeletal muscle, and the activities of citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (β-HAD) have been shown to increase after resistance exercise training (31, 142). Furthermore, VO_2max either remained unchanged or increased after resistance exercise training, indicating a positive effect of resistance exercise training on the oxidative capacity (21, 44, 62, 90). PPARδ is a major regulator of lipid metabolism in skeletal muscle (52). It increases FA oxidation by upregulating the lipid transporter and oxidative enzymes such as FAT/CD36, FATP/FABP, ACS, CPT1, Acyl-CoA Dehydrogenase and Thiolase in skeletal muscle. Although these enzymes were not analyzed in the present study, the increase of PPARδ protein content suggested a potential for their expression. Further studies are

recommended to analyze these PPARδ target proteins to provide a better understanding of the regulation of lipid metabolism by resistance exercise training.

Conclusion

Recent studies showed that resistance exercise training improved oxidative capacity. While these studies revealed the increase in oxidative enzymes following resistance exercise training, the regulatory mechanism remained unclear. PPARδ is a key regulator of the lipid metabolism in skeletal muscle. The results of the present study showed that resistance exercise increased PPARδ protein content both acutely and chronically. The changes in body composition showed that resistance exercise exhibited the benefits similar to endurance exercise. The changes in lipid profile and the increases in PPARδ protein content provided evidence for the utilization of lipids and enhanced lipid metabolism when performing resistance exercise.

CHAPTER V

CONCLUSION

In debating the meaning of “healthy food”, general thought amongst the population emphasizes foods with low fat and cholesterol content, such as chicken breast, fat-free milk, and egg white. Dietary lipids are suggested to be avoided because they are high in calorie and elevated blood lipid levels have been observed in the individuals with metabolic syndrome and cardiovascular disease. However, lipids not only provide sufficient energy for physical activities, but they are essential for maintaining the normal physiological function.

Beneficial results of combining dietary FAs and exercise have been revealed by studies investigating the interaction of exercise and dietary fats. Resistance exercise induces skeletal muscle mass and strength gains through cell signaling pathways activated by muscle contraction, hormones, and inflammation. Lipids regulate membrane permeability and fluidity for hormones, form lipid rafts for cell signaling molecules, and provide building blocks and energy for the regeneration of tissues. Mechanisms have been identified for the enhanced oxidative capacity and lipid metabolism by endurance exercise. However, not until recent decades has resistance exercise been revealed to improve lipid profile and enhance oxidative potential, while the mechanism remains

unclear. PPARδ has been identified as a key regulator of lipid metabolism in skeletal muscle. While endurance exercise increases PPARδ expression, the effects of resistance exercise on PPARδ protein content has not been investigated in humans. Therefore, we conducted three studies to test the hypotheses that lipids, including FAs and cholesterol, would enhance skeletal muscle adaptation to resistance exercise and that resistance exercise would stimulate the regulation of lipid metabolism as demonstrated by changes in the lipid metabolism regulator PPARδ.

Dietary Lipids and Resistance Exercise

In study 1 and 2 (Chapter II and III), the adaptation of skeletal muscle on short- and long-term resistance exercise training were investigated with different levels of cholesterol intake. While higher cholesterol intake did not affect the gains in muscle mass and strength, it attenuated muscle soreness induced by resistance exercise. With the training, the blood cholesterol levels were not chronically affected by the high cholesterol intake. Although our expectations for the effects of cholesterol on resistance exercise-induced muscle adaptations were not well reflected by the results, these studies provided a potential effect of dietary cholesterol on the inflammation induced by resistance exercise.

The analyses of the association between dietary FAs and the resistance exercise-induced muscle adaptation showed potential effects of fat intake on the gains in muscle mass, strength, peak power, and quality. Although these exploratory analyses were not statistically significant, they might provide guidance regarding several FAs to be investigated in future studies.

Resistance Exercise and PPARδ

In study 2 and 3 (Chapter III and IV), PPARδ protein content was analyzed to examine the acute and training effects of resistance exercise on lipid regulation. In this dissertation, PPARδ was demonstrated to change in response to resistance exercise. PPARδ is a biomarker of enhanced lipid metabolism based on its roles in the upregulation of enzymes and proteins for FA oxidation and lipid transport in skeletal muscle. PPARδ increased acutely after resistance exercise in both studies under specific conditions. In study 2, in which participants performed unilateral high-intensity leg exercise, PPARδ protein content was compared between exercised and non-exercised legs. PPARδ protein content was higher in the exercised-legs with high cholesterol intake. In study 3, PPARδ protein content was compared before and after a bout of whole-body resistance exercise. The acute increase of PPARδ protein content was inversely proportional to body fat percentage.

Ten weeks of resistance exercise increased skeletal muscle PPARδ protein content. PPARδ has been shown to regulate other known regulators of lipid metabolism in skeletal muscle, including PGC-1α and AMPK. These results provided evidence that resistance exercise had effects on lipid metabolism, and the regulation might be driven by the increase of PPARδ.

Final Conclusion

Exercise is medicine. With resistant exercise, high cholesterol intake was not shown to alter resting blood lipid profile in healthy young and old individuals with resistance exercise training. This dissertation also provided the evidence that resistance exercise increased PPARδ protein content, the therapeutic target in metabolic diseases, indicating a therapeutic effect of resistance exercise on the diseases.

While poor nutrition and physical inactivity are primary contributors to preventable death in the United States, an active lifestyle and regular exercise that may counteract the potential negative effects of excess lipid intake and improve lipid metabolism may be the solution.

REFERENCE

1. **Abumrad NA**, **Davidson NO**. Role of the gut in lipid homeostasis. *Physiol Rev* 92: 1061–85, 2012.

2. **Ahtiainen JP**, **Pakarinen A**, **Kraemer WJ**, **Hakkinen K**. Acute hormonal responses to heavy resistance exercise in strength athletes versus nonathletes. *Can J Appl Physiol* 29: 527–543, 2004.

3. **Alway SE**, **MacDougall JD**, **Sale DG**, **Sutton JR**, **McComas AJ**. Functional and structural adaptations in skeletal muscle of trained athletes. *J Appl Physiol* 64: 1114–1120, 1988.

4. **Angione AR**, **Jiang C**, **Pan D**, **Wang Y-X**, **Kuang S**. PPARδ regulates satellite cell proliferation and skeletal muscle regeneration. *Skelet Muscle* 1: 33, 2011.

5. **Appel LJ**, **Sacks FM**, **Carey VJ**, **Obarzanek E**, **Swain JF**, **Miller ER**, **Conlin PR**, **Erlinger TP**, **Rosner BA**, **Laranjo NM**, **Charleston J**, **McCarron P**, **Bishop LM**. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. *JAMA* 294: 2455–64, 2005.

6. **Atherton PJ**, **Babraj J**, **Smith K**, **Singh J**, **Rennie MJ**, **Wackerhage H**. Selective activation of AMPK-PGC-1α or PKB-TSC2-mTOR signaling can explain specific adaptive responses to endurance or resistance training-like electrical muscle stimulation. *FASEB J* 19: 786–788, 2005.

7. **Barbier O**, **Pineda Torra I**, **Duguay Y**, **Blanquart C**, **Fruchart J-C**, **Glineur C**, **Staels B**. Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. *Arterioscler Thromb Vasc Biol* 22: 717–726, 2002.

8. **Barish GD**, **Narkar VA**, **Evans RM**. Review series PPARδ : a dagger in the heart of the metabolic syndrome. *J Clin Invest* 116: 590–597, 2006.

9. **Bassel-Duby R**, **Olson EN**. Signaling pathways in skeletal muscle remodeling. *Annu Rev Biochem* 75: 19–37, 2006.

10. **Beisiegel U**, **Weber W**, **Ihrke G**, **Herz J**, **Stanley KK**. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature* 341: 162–164, 1989.

11. **Berthon PM**, **Howlett RA**, **Heigenhauser GJF**, **Spriet LL**. Human skeletal muscle carnitine palmitoyltransferase I activity determined in isolated intact mitochondria. *J Appl Physiol* 85: 148–153, 1998.

12. **Bird SP**, **Tarpenning KM**, **Marino FE**. Independent and combined effects of liquid carbohydrate/essential amino acid ingestion on hormonal and muscular adaptations following resistance training in untrained men. *Eur J Appl Physiol* 97: 225–238, 2006.

13. **Bolster DR**, **Crozier SJ**, **Kimball SR**, **Jefferson LS**. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. *J Biol Chem* 277: 23977–80, 2002.

14. **Bonen A**, **Dyck DJ**, **Ibrahimi A**, **Abumrad NA**. Muscle contractile activity increases fatty acid metabolism and transport and FAT/CD36. *Am J Physiol - Endocrinol Metab* 276: E642-649, 1999.

15. **Bonen A**, **Miskovic D**, **Kiens B**. Fatty acid transporters (FABPpm, FAT, FATP) in human muscle. *Can J Appl Physiol* 24: 515–523, 1999.

16. **Braith RW**, **Kerry J. Stewart**. Resistance exercise training: its role in the prevention of cardiovascular disease. *Circulation* 113: 2642–2650, 2006.

17. **Breen DM**, **Sanli T**, **Giacca A**, **Tsiani E**. Stimulation of muscle cell glucose uptake by resveratrol through sirtuins and AMPK. *Biochem Biophys Res Commun* 374: 117–122, 2008.

18. **Brunmair B**, **Staniek K**, **Dörig J**, **Szöcs Z**, **Stadlbauer K**, **Marian V**, **Gras F**, **Anderwald C**, **Nohl H**, **Waldhäusl W**, **Fürnsinn C**. Activation of PPAR-delta in isolated rat skeletal muscle switches fuel preference from glucose to fatty acids. *Diabetologia* 49: 2713–2722, 2006.

19. **Burke RE**, **Edgerton VR**. Motor unit properties and selective involvement in movement. *Exerc Sport Sci Rev* 3: 31–81, 1975.

20. **Burns KA**, **Vanden Heuvel JP**. Modulation of PPAR activity via phosphorylation. *Biochim Biophys Acta - Mol Cell Biol Lipids* 1771: 952–960, 2007.

21. **Campos GE**, **Luecke TJ**, **Wendeln HK**, **Toma K**, **Hagerman FC**, **Murray TF**, **Ragg KE**,

Ratamess NA, **Kraemer WJ**, **Staron RS**. Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones. *Eur J Appl Physiol* 88: 50–60, 2002.

22. **Caspersen CJ**, **Powell KE**, **Christenson GM**. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep* 100: 126–131, 1985.

23. **De Caterina R**, **Liao JK**, **Libby P**. Fatty acid modulation of endothelial activation. *Am J Clin Nutr*. 71: 213S–23S, 2000.

24. **Cermak NM**, **Res PT**, **Groot LC De**, **Saris WHM**, **Loon LJC Van**. Protein supplementation augments the adaptive response of skeletal muscle to resistance type exercise training a meta analysis. *Am J Clin Nutr* 96: 1454–1464, 2012.

25. **Chen TC**, **Nosaka K**, **Sacco P**. Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect. *J Appl Physiol* 102: 992–999, 2006.

26. **Cheung K**, **Hume PA**, **Maxwell L**. Delayed onset muscle soreness. *Sport Med* 33: 145–164, 2003.

27. **Chilibeck PD**, **Syrotuik DG**, **Bell GJ**. The effect of strength training on estimates of mitochondrial density and distribution throughout muscle fibres. *Eur J Appl Physiol Occup Physiol* 80: 604–609, 1999.

28. **Clarkson PM**, **Hubal MJ**. Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil* 81: S52–S69, 2002.

29. **Cleasby ME**, **Reinten TA**, **Cooney GJ**, **James DE**, **Kraegen EW**. Functional studies of Akt isoform specificity in skeletal muscle in vivo; maintained insulin sensitivity despite reduced insulin receptor substrate-1 expression. *Mol Endocrinol* 21: 215–228, 2007.

30. **Clifford PS**, **Hellsten Y**. Vasodilatory mechanisms in contracting skeletal muscle. *J Appl Physiol* 97: 393 LP-403, 2004.

31. **Costill DL**, **Fink WJ**, **Getchell LH**, **Ivy JL**, **Witzmann FA**. Lipid metabolism in skeletal muscle of endurance-trained males and females. *J Appl Physiol* 47: 787–791, 1979.

32. **Crouse SF**, **O'Brien BC**, **Rohack JJ**, **Lowe RC**, **Green JS**, **Tolson H**, **Reed JL**. Changes

in serum lipids and apolipoproteins after exercise in men with high cholesterol: influence of intensity. *J Appl Physiol* 79: 279 LP-286, 1995.

33. **Das UN**. Essential fatty acids: biochemistry, physiology and pathology. *Biotechnol J* 1: 420–439, 2006.

34. **Diradourian C**, **Girard J**, **Pégorier JP**. Phosphorylation of PPARs: From molecular characterization to physiological relevance. *Biochimie* 87: 33–38, 2005.

35. **Dressel U**, **Allen TL**, **Pippal JB**, **Rohde PR**, **Lau P**, **Muscat GEO**. The peroxisome proliferator-activated receptor β/δ agonist, GW501516, regulates the expression of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells. *Mol Endocrinol* 17: 2477–2493, 2003.

36. **Durstine JL**, **Grandjean P**, **Davis P**, **Ferguson M**, **Alderson N**, **DuBose K**. Blood lipid and lipoprotein adaptations to exercise. *Sport Med* 31: 1033–1062, 2001.

37. **Ehrenborg E**, **Krook A**. Regulation of skeletal muscle physiology and metabolism by peroxisome proliferator-activated receptor δ. *Pharmacol Rev* 61: 373–393, 2009.

38. **Feng YZ**, **Nikolić N**, **Bakke SS**, **Boekschoten M V**, **Kersten S**, **Kase ET**, **Rustan AC**, **Thoresen GH**. PPARδ activation in human myotubes increases mitochondrial fatty acid oxidative capacity and reduces glucose utilization by a switch in substrate preference. *Arch Physiol Biochem* 120: 12–21, 2014.

39. **Fernandez ML**, **Blesso C**. Dietary cholesterol and the lack of evidence on heart disease risk. In: *Handbook of cholesterol*. Wageningen Academic Publishers, p. 101-116–6.

40. **Ferrucci L**, **Cherubini A**, **Bandinelli S**, **Bartali B**, **Corsi A**, **Lauretani F**, **Martin A**, **Andres-Lacueva C**, **Senin U**, **Guralnik JM**. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab* 91: 439–446, 2006.

41. **Fielding RA**, **LeBrasseur NK**, **Cuoco A**, **Bean J**, **Mizer K**, **Singh MAF**. High-velocity resistance training increases skeletal muscle peak power in older women. *J Am Geriatr Soc* 50: 655–662, 2002.

42. **Fletcher GF**, **Balady G**, **Blair SN**, **Blumenthal J**, **Caspersen C**, **Chaitman B**, **Epstein S**, **Froelicher ESS**, **Froelicher VF**, **Pina IL**, **Pollock ML**. Statement on exercise: benefits and recommendations for physical activity programs for all Americans. *Circulation* 94: 857 LP-862, 1996.

43. **Fritz T**, **Krämer D**, **Karlsson HKR**, **Galuska D**, **Engfeldt P**, **Zierath JR**, **Krook A**. Low-intensity exercise increases skeletal muscle protein expression of PPARδ and UCP3 in type 2 diabetic patients. *Diabetes Metab Res Rev* 22: 492–498, 2006.

44. **Frontera WR**, **Meredith CN**, **O'Reilly KP**, **Evans WJ**. Strength training and determinants of VO2max in older men. *J Appl Physiol* 68: 329–333, 1990.

45. **Gasier HG**, **Fluckey JD**, **Previs SF**, **Wiggs MP**, **Riechman SE**. Acute resistance exercise augments integrative myofibrillar protein synthesis. *Metabolism* 61: 153–156, 2012.

46. **Gasier HG**, **Riechman SE**, **Wiggs MP**, **Buentello A**, **Previs SF**, **Fluckey JD**. Cumulative responses of muscle protein synthesis are augmented with chronic resistance exercise training. *Acta Physiol* 201: 381–389, 2011.

47. **Gasier HG**, **Riechman SE**, **Wiggs MP**, **Previs SF**, **Fluckey JD**. A comparison of 2H2O and phenylalanine flooding dose to investigate muscle protein synthesis with acute exercise in rats. *Am J Physiol - Endocrinol Metab* 297: E252 LP-E259, 2009.

48. **Gearhart RF**, **Riechman SE**, **Lagally KM**, **Andrews RD**, **Robertson RJ**. Safety of using the adult omni resistance exercise scale to determine 1-RM in older men and women. *Percept Mot Skills* 113: 671–676, 2011.

49. **Goodyear LJ**, **Chang PY**, **Sherwood DJ**, **Dufresne SD**, **Moller DE**. Effects of exercise and insulin on mitogen-activated protein kinase signaling pathways in rat skeletal muscle. *Am J Physiol - Endocrinol Metab* 271: E403-408, 1996.

50. **Goto K**, **Ishii N**, **Sugihara S**, **Yoshioka T**, **Takamatsu K**. Effects of resistance exercise on lipolysis during subsequent submaximal exercise. *Med Sci Sport Exerc* 39: 308–315, 2007.

51. **Graf GA**, **Yu L**, **Li W-P**, **Gerard R**, **Tuma PL**, **Cohen JC**, **Hobbs HH**. ABCG5 and ABCG8 are obligate heterodimers for protein trafficking and biliary cholesterol excretion. *J Biol Chem* 278: 48275–82, 2003.

52. **Grimaldi PA**. Regulatory role of peroxisome proliferator-activated receptor delta (PPAR delta) in muscle metabolism. A new target for metabolic syndrome treatment? *Biochimie* 87: 5–8, 2005.

53. **Grundy SM**. HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. *N Engl J Med* 319: 24–33, 1988.

54. **Guo Y**, **Xiao P**, **Lei S**, **Deng F**, **Xiao GG**, **Liu Y**, **Chen X**, **Li L**, **Wu S**, **Chen Y**, **Jiang H**, **Tan L**, **Xie J**, **Zhu X**, **Liang S**, **Deng H**. How is mRNA expression predictive for protein expression? A correlation study on human circulating monocytes. *Acta Biochim Biophys Sin (Shanghai)* 40: 426–436, 2008.

55. **Hahn-Windgassen A**, **Nogueira V**, **Chen C-C**, **Skeen JE**, **Sonenberg N**, **Hay N**. Akt activates the mammalian target of rapamycin by regulating cellular ATP level and AMPK activity. *J Biol Chem* 280: 32081–32089, 2005.

56. **Harris WS**, **Mozaffarian D**, **Rimm E**, **Kris-Etherton P**, **Rudel LL**, **Appel LJ**, **Engler MM**, **Engler MB**, **Sacks F**. Omega-6 fatty acids and risk for cardiovascular disease: A science advisory from the American Heart Association nutrition subcommittee of the council on nutrition, physical activity, and metabolism; council on cardiovascular nursing; and council on epidem. *Circulation* 119: 902–907, 2009.

57. **Hawke TJ**. Muscle stem cells and exercise Training. *Exerc Sport Sci Rev* 33: 63–68, 2005.

58. **Hayashi T**, **Hirshman MF**, **Kurth EJ**, **Winder WW**, **Goodyear LJ**. Evidence for 5'AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. *Diabetes* 47: 1369 LP-1373, 1998.

59. **Hayes A**, **Cribb PJ**. Effect of whey protein isolate on strength, body composition and muscle hypertrophy during resistance training. *Curr Opin Clin Nutr Metab Care* 11: 40–44, 2008.

60. **Heinrich PC**, **Behrmann I**, **Haan S**, **Hermanns HM**, **Muller-Newen G**, **Schaper F**. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J* 374: 1-20, 2003.

61. **Henneman E**, **Clamann HP**, **Gillies JD**, **Skinner RD**. Rank order of motoneurons within a pool: law of combination. *J Neurophysiol* 37: 1338–49, 1974.

62. **Hickson RC**. Interference of strength development by simultaneously training for strength and endurance. *Eur J Appl Physiol Occup Physiol* 45: 255–263, 1980.

63. **Holloszy JO**, **Coyle EF**. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol* 56: 831–8, 1984.

64. **Holmes BF**, **Kurth-Kraczek EJ**, **Winder WW**. Chronic activation of 5'-AMP-activated protein kinase increases GLUT-4, hexokinase, and glycogen in muscle. *J Appl Physiol* 87: 1990–1995, 1999.

65. **Hondares E**, **Pineda-Torra I**, **Iglesias R**, **Staels B**, **Villarroya F**, **Giralt M**. PPARδ, but not PPARα, activates PGC-1α gene transcription in muscle. *Biochem Biophys Res Commun* 354: 1021–1027, 2007.

66. **Van Horn L**, **Carson JAS**, **Appel LJ**, **Burke LE**, **Economos C**, **Karmally W**, **Lancaster K**, **Lichtenstein AH**, **Johnson RK**, **Thomas RJ**, **Vos M**, **Wylie-Rosett J**, **Kris-Etherton P**. Recommended dietary pattern to achieve adherence to the American Heart Association/American College of Cardiology (AHA/ACC) Guidelines: a scientific statement from the American Heart Association. *Circulation* 134: e1–e25, 2016.

67. **Hornberger TA**, **Stuppard R**, **Conley KE**, **Fedele MJ**, **Fiorotto ML**, **Chin ER**, **Esser KA**. Mechanical stimuli regulate rapamycin-sensitive signalling by a phosphoinositide 3-kinase-, protein kinase B- and growth factor-independent mechanism. *Biochem J* 380: 795–804, 2004.

68. **Hymer WC**, **Kraemer WJ**, **Nindl BC**, **Marx JO**, **Benson DE**, **Welsch JR**, **Mazzetti SA**, **Volek JS**, **Deaver DR**. Characteristics of circulating growth hormone in women after acute heavy resistance exercise. *Am J Physiol - Endocrinol Metab* 281: E878-887, 2001.

69. **Ikeda S**, **Miyazaki H**, **Nakatani T**, **Kai Y**, **Kamei Y**, **Miura S**, **Tsuboyama-Kasaoka N**, **Ezaki O**. Up-regulation of SREBP-1c and lipogenic genes in skeletal muscles after exercise training. *Biochem Biophys Res Commun* 296: 395–400, 2002.

70. **Ikonen E**. Cellular cholesterol trafficking and compartmentalization. *Nat Rev Mol Cell Biol* 9: 125–138, 2008.

71. **Iqbal J**, **Hussain MM**. Intestinal lipid absorption. *Am J Physiol - Endocrinol Metab* 296: E1183-E1194, 2009.

72. **Istvan ES**, **Deisenhofer J**. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 292: 1160-1164, 2001.

73. **Jocken JWE**, **Blaak EE**. Catecholamine-induced lipolysis in adipose tissue and skeletal muscle in obesity. *Physiol Behav* 94: 219–230, 2008.

74. **Jørgensen SB**, **Jensen TE**, **Richter E a**. Role of AMPK in skeletal muscle gene adaptation in relation to exercise. *Appl Physiol Nutr Metab* 32: 904–911, 2007.

75. **Jucker BM**, **Yang D**, **Casey WM**, **Olzinski AR**, **Williams C**, **Lenhard SC**, **Legos JJ**, **Hawk CT**, **Sarkar SK**, **Newsholme SJ**. Selective PPARδ agonist treatment increases skeletal muscle lipid metabolism without altering mitochondrial energy coupling:

an in vivo magnetic resonance spectroscopy study. *Am J Physiol - Endocrinol Metab* 293: E1256–E1264, 2007.

76. **Kannisto K**, **Chibalin A**, **Glinghammar B**, **Zierath JR**, **Hamsten A**, **Ehrenborg E**. Differential expression of peroxisomal proliferator activated receptors α and δ in skeletal muscle in response to changes in diet and exercise. *Int J Mol Med* 17: 45–52, 2006.

77. **Keller C**, **Steensberg A**, **Hansen AK**, **Fischer CP**, **Plomgaard P**, **Pedersen BK**. Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle. *J Appl Physiol* 99: 2075–2079, 2005.

78. **King TD**, **Song L**, **Jope RS**. AMP-activated protein kinase (AMPK) activating agents cause dephosphorylation of Akt and glycogen synthase kinase-3. *Biochem Pharmacol* 71: 1637–1647, 2006.

79. **Kleiner S**, **Nguyen-Tran V**, **Bare O**, **Huang X**, **Spiegelman B**, **Wu Z**. PPARδ agonism activates fatty acid oxidation via PGC-1α but does not increase mitochondrial gene expression and function. *J Biol Chem* 284: 18624–18633, 2009.

80. **Kraemer WJ**, **Nindl BC**, **Marx JO**, **Gotshalk LA**, **Bush JA**, **Welsch JR**, **Volek JS**, **Spiering BA**, **Maresh CM**, **Mastro AM**, **Hymer WC**. Chronic resistance training in women potentiates growth hormone in vivo bioactivity: characterization of molecular mass variants. *Am J Physiol - Endocrinol Metab* 291: E1177-E1187, 2006.

81. **Kraemer WJ**, **Ratamess N a**. Hormonal responses and adaptations to resistance exercise and training. *Sport Med* 35: 339–361, 2005.

82. **Krämer DK**, **Al-Khalili L**, **Guigas B**, **Leng Y**, **Garcia-Roves PM**, **Krook A**. Role of AMP kinase and PPARdelta in the regulation of lipid and glucose metabolism in human skeletal muscle. *J Biol Chem* 282: 19313–19320, 2007.

83. **Krämer DK**, **Al-Khalili L**, **Perrini S**, **Skogsberg J**, **Wretenberg P**, **Kannisto K**, **Wallberg-Henriksson H**, **Ehrenborg E**, **Zierath JR**, **Krook A**. Direct activation of glucose transport in primary human myotubes after activation of peroxisome proliferator-activated receptor δ. *Diabetes* 54: 1157-1163, 2005.

84. **Kromhout D**, **Menotti A**, **Bloemberg B**, **Aravanis C**, **Blackburn H**, **Buzina R**, **Dontas AS**, **Fidanza F**, **Giaipaoli S**, **Jansen A**, **Karvonen M**, **Katan M**, **Nissinen A**, **Nedeljkovic S**, **Pekkanen J**, **Pekkarinen M**, **Punsar S**, **Rasanen L**, **Simic B**, **Toshima H**. Dietary saturated and trans fatty acids and cholesterol and 25-year mortality from coronary heart disease: the seven countries study. *Prev Med (Baltim)* 24:

308–315, 1995.

85. **Lardinois CK**. The role of omega 3 fatty acids on insulin secretion and insulin sensitivity. *Med Hypotheses* 24: 243–248, 1987.

86. **Lee C-H**, **Olson P**, **Hevener A**, **Mehl I**, **Chong L-W**, **Olefsky JM**, **Gonzalez FJ**, **Ham J**, **Kang H**, **Peters JM**, **Evans RM**. PPARδ regulates glucose metabolism and insulin sensitivity. *Proc Natl Acad Sci U S A* 103: 3444–3449, 2006.

87. **Lund S**, **Holman GD**, **Schmitz O**, **Pedersen O**. Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proc Natl Acad Sci* 92: 5817–5821, 1995.

88. **Luquet S**, **Gaudel C**, **Holst D**, **Lopez-Soriano J**, **Jehl-Pietri C**, **Fredenrich A**, **Grimaldi PA**. Roles of PPAR delta in lipid absorption and metabolism: a new target for the treatment of type 2 diabetes. *Biochim Biophys Acta* 1740: 313–7, 2005.

89. **Luquet S**, **Lopez-Soriano J**, **Holst D**, **Fredenrich A**, **Melki J**, **Rassoulzadegan M**, **Grimaldi PA**. Peroxisome proliferator-activated receptor delta controls muscle development and oxidative capability. *FASEB J* 17: 2299–301, 2003.

90. **Lüthi JM**, **Howald H**, **Claassen H**, **Rösler K**, **Vock P**, **Hoppeler H**. Structural changes in skeletal muscle tissue with heavy-resistance exercise. *Int J Sports Med* 7: 123–127, 1986.

91. **MacDougall JD**, **Sale DG**, **Moroz JR**, **Elder GC**, **Sutton JR**, **Howald H**. Mitochondrial volume density in human skeletal muscle following heavy resistance training. *Med Sci Sports* 11: 164–166, 1979.

92. **Machida S**, **Booth FW**. Insulin-like growth factor 1 and muscle growth: implication for satellite cell proliferation. *Proc Nutr Soc* 63: 337–340, 2004.

93. **Mahoney DJ**, **Parise G**, **Melov S**, **Safdar A**, **Tarnopolsky MA**. Analysis of global mRNA expression in human skeletal muscle during recovery from endurance exercise. *FASEB J* 19: 1498–500, 2005.

94. **Mandard S**, **Stienstra R**, **Escher P**, **Tan NS**, **Kim I**, **Gonzalez FJ**, **Wahli W**, **Desvergne B**, **Müller M**, **Kersten S**. Glycogen synthase 2 is a novel target gene of peroxisome proliferator-activated receptors. *Cell Mol Life Sci* 64: 1145–1157, 2007.

95. **McCaulley G**, **McBride J**, **Cormie P**, **Hudson M**, **Nuzzo J**, **Quindry J**, **Travis Triplett**

N. Acute hormonal and neuromuscular responses to hypertrophy, strength and power type resistance exercise. *Eur J Appl Physiol* 105: 695–704, 2009.

96. **Mensink RP**, **Katan MB**. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. *N Engl J Med* 321: 436–441, 1989.

97. **Mokdad AH**, **Marks JS**, **Stroup DF**, **Gerberding JL**. Actual causes of death in the United States. *Jama* 270: 2207–2212, 1993.

98. **Mori TA**, **Beilin LJ**. Omega-3 fatty acids and inflammation. *Curr Atheroscler Rep* 6: 461–467, 2004.

99. **Muoio DM**, **MacLean PS**, **Lang DB**, **Li S**, **Houmard JA**, **Way JM**, **Winegar DA**, **Corton JC**, **Dohm GL**, **Kraus WE**. Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) α knock-out mice: evidence for compensatory regulation by PPARδ. *J Biol Chem* 277: 26089–26097, 2002.

100. **Musarò A**, **Rosenthal N**. Maturation of the myogenic program is induced by postmitotic expression of insulin-like growth factor I. *Mol Cell Biol* 19: 3115–3124, 1999.

101. **Nadeau KJ**, **Ehlers LB**, **Aguirre LE**, **Moore RL**, **Jew KN**, **Ortmeyer HK**, **Hansen BC**, **Reusch JEB**, **Draznin B**. Exercise training and calorie restriction increase SREBP-1 expression and intramuscular triglyceride in skeletal muscle. *Am J Physiol - Endocrinol Metab* 291: E90-E98, 2006.

102. **Nahlé Z**, **Hsieh M**, **Pietka T**, **Coburn CT**, **Grimaldi PA**, **Zhang MQ**, **Das D**, **Abumrad NA**. CD36-dependent regulation of muscle FoxO1 and PDK4 in the PPAR delta/beta-mediated adaptation to metabolic stress. *J Biol Chem* 283: 14317–26, 2008.

103. **Narkar VA**, **Downes M**, **Yu RT**, **Embler E**, **Wang YX**, **Banayo E**, **Mihaylova MM**, **Nelson MC**, **Zou Y**, **Juguilon H**, **Kang H**, **Shaw RJ**, **Evans RM**. AMPK and PPARδ agonists are exercise mimetics. *Cell* 134: 405–415, 2008.

104. **Nawrocki JW**, **Weiss SR**, **Davidson MH**, **Sprecher DL**, **Schwartz SL**, **Lupien P-J**, **Jones PH**, **Haber HE**, **Black DM**. Reduction of LDL cholesterol by 25% to 60% in patients with primary hypercholesterolemia by atorvastatin, a new HMG-CoA reductase inhibitor. *Arterioscler Thromb Vasc Biol* 15: 678-682, 1995.

105. **Neels JG**, **Grimaldi PA**. Physiological functions of peroxisome proliferator-

activated receptor β. *Physiol Rev* 94: 795–858, 2014.

106. **Nilsson MI**, **Greene NP**, **Dobson JP**, **Wiggs MP**, **Gasier HG**, **Macias BR**, **Shimkus KL**, **Fluckey JD**. Insulin resistance syndrome blunts the mitochondrial anabolic response following resistance exercise. *Am J Physiol - Endocrinol Metab* 299: E466-E474, 2010.

107. **Nindl BC**, **Kraemer WJ**, **Marx JO**, **Tuckow AP**, **Hymer WC**. Growth hormone molecular heterogeneity and exercise. *Exerc Sport Sci Rev* 31, 2003.

108. **O'Neil TK**, **Duffy LR**, **Frey JW**, **Hornberger TA**. The role of phosphoinositide 3-kinase and phosphatidic acid in the regulation of mammalian target of rapamycin following eccentric contractions. *J Physiol* 587: 3691–701, 2009.

109. **Oliver WR**, **Shenk JL**, **Snaith MR**, **Russell CS**, **Plunket KD**, **Bodkin NL**, **Lewis MC**, **Winegar DA**, **Sznaidman ML**, **Lambert MH**, **Xu HE**, **Sternbach DD**, **Kliewer SA**, **Hansen BC**, **Willson TM**. A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. *Proc Natl Acad Sci U S A* 98: 5306–5311, 2001.

110. **Ozaki H**, **Loenneke JP**, **Thiebaud RS**, **Abe T**. Resistance training induced increase in VO2max in young and older subjects. *Eur Rev Aging Phys Act* 10: 107–116, 2013.

111. **Padilla J**, **Harris RA**, **Fly AD**, **Rink LD**, **Wallace JP**. The effect of acute exercise on endothelial function following a high-fat meal. *Eur J Appl Physiol* 98: 256–262, 2006.

112. **Pearson TA**, **Blair SN**, **Daniels SR**, **Eckel RH**, **Fair JM**, **Fortmann SP**, **Franklin BA**, **Goldstein LB**, **Greenland P**, **Grundy SM**, **Hong Y**, **Miller NH**, **Lauer RM**, **Ockene IS**, **Sacco RL**, **Sallis JF**, **Smith SC**, **Stone NJ**, **Taubert KA**. AHA guidelines for primary prevention of cardiovascular disease and stroke: 2002 update: consensus panel guide to comprehensive risk reduction for adult patients without coronary or other atherosclerotic vascular diseases. *Circulation* 106: 388–391, 2002.

113. **Peterson MD**, **Sen A**, **Gordon PM**. Influence of resistance exercise on lean body mass in aging adults: a meta-analysis. *Med Sci Sports Exerc* 43: 249–258, 2011.

114. **Petrov AM**, **Kravtsova V V**, **Matchkov V V**, **Vasiliev AN**, **Zefirov AL**, **Chibalin A V**, **Heiny JA**, **Krivoi II**. Membrane lipid rafts are disturbed in the response of rat skeletal muscle to short-term disuse. *Am J Physiol - Cell Physiol* 312: C627-C637, 2017.

115. **Pike LJ**. Lipid rafts: bringing order to chaos. *J Lipid Res* 44: 655–667, 2003.

116. **Pincivero DM**, **Gandhi V**, **Timmons MK**, **Coelho AJ**. Quadriceps femoris electromyogram during concentric, isometric and eccentric phases of fatiguing dynamic knee extensions. *J Biomech* 39: 246–254, 2006.

117. **Popp-Snijders C**, **Schouten JA**, **Heine RJ**, **van der Meer J**, **van der Veen EA**. Dietary supplementation of omega-3 polyunsaturated fatty acids improves insulin sensitivity in non-insulin-dependent diabetes. *Diabetes Res* 4: 141–147, 1987.

118. **Prabhakaran B**, **Dowling EA**, **Branch JD**, **Swain DP**, **Leutholtz BC**. Effect of 14 weeks of resistance training on lipid profile and body fat percentage in premenopausal women. *Br J Sports Med* 33: 190–195, 1999.

119. **Reilly SM**, **Lee C-H**. PPAR delta as a therapeutic target in metabolic disease. *FEBS Lett* 582: 26–31, 2008.

120. **Riechman SE**, **Andrews RD**, **MacLean DA**, **Sheather S**. Statins and dietary and serum cholesterol are associated with increased lean mass following resistance training. *Journals Gerontol Ser A Biol Sci Med Sci* 62: 1164–1171, 2007.

121. **Riechman SE**, **Lee CW**, **Chikani G**, **Chen VCW**, **Lee T V.** Cholesterol and skeletal muscle health. *World Rev Nutr Diet* 100: 71–79, 2009.

122. **Riechman SE**, **Lee T V.**, **Chen VCW**, **Lee CW**, **Bui S**. Whole egg as an athlete's training and performance superfood. In: *Handbook of eggs in human function*, edited by Watson, Ronald Ross FDM. Wageningen Academic Publishers, p. 215–230.

123. **Roberts LD**, **Murray AJ**, **Menassa D**, **Ashmore T**, **Nicholls AW**, **Griffin JL**. The contrasting roles of PPARδ and PPARγ in regulating the metabolic switch between oxidation and storage of fats in white adipose tissue. *Genome Biol* 12: R75, 2011.

124. **Rommel C**, **Bodine SC**, **Clarke BA**, **Rossman R**, **Nunez L**, **Stitt TN**, **Yancopoulos GD**, **Glass DJ**. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nat Cell Biol* 3: 1009–1013, 2001.

125. **Sanchez AMJ**, **Candau RB**, **Bernardi H**. FoxO transcription factors: their roles in the maintenance of skeletal muscle homeostasis. *Cell Mol Life Sci* 71: 1657–1671, 2014.

126. **Schuler M**, **Ali F**, **Chambon C**, **Duteil D**, **Bornert J-MM**, **Tardivel A**, **Desvergne B**, **Wahli W**, **Chambon P**, **Metzger D**. PGC1α expression is controlled in skeletal muscles by PPARβ, whose ablation results in fiber-type switching, obesity, and type 2 diabetes. *Cell Metab* 4: 407–14, 2006.

127. **Serrano AL**, **Baeza-Raja B**, **Perdiguero E**, **Jardí M**, **Muñoz-Cánoves P**. Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. *Cell Metab* 7: 33–44, 2008.

128. **Shekelle RB**, **Shryock AM**, **Paul O**, **Lepper M**, **Stamler J**, **Liu S**, **Raynor WJ**. Diet, serum cholesterol, and death from coronary heart disease. *N Engl J Med* 304: 65–70, 1981.

129. **Simons K**, **Ikonen E**. How cells handle cholesterol. *Science (80-)* 290: 1721-1726, 2000.

130. **Smith LL**. Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Med Sci Sports Exerc* 23: 542–551, 1991.

131. **Smith SC**, **Allen J**, **Blair SN**, **Bonow RO**, **Brass LM**, **Fonarow GC**, **Grundy SM**, **Hiratzka L**, **Jones D**, **Krumholz HM**, **Mosca L**, **Pasternak RC**, **Pearson T**, **Pfeffer MA**, **Taubert KA**. AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update - endorsed by the National Heart, Lung, and Blood Institute. *Circulation* 113: 2363–2372, 2006.

132. **Sorenson RL**, **Elde RP**, **Seybold V**. Effect of norepinephrine on insulin, glucagon, and somatostatin secretion in isolated perifused rat islets. *Diabetes* 28: 899-904, 1979.

133. **Spector AA**. Fatty acid binding to plasma albumin. *J Lipid Res* 16: 165–179, 1975.

134. **Spiering B**, **Kraemer W**, **Anderson J**, **Armstrong L**, **Nindl B**, **Volek J**, **Maresh C**. Resistance exercise biology. *Sport Med* 38: 527–540, 2008.

135. **Spina RJ**, **Chi MM**, **Hopkins MG**, **Nemeth PM**, **Lowry OH**, **Holloszy JO**. Mitochondrial enzymes increase in muscle in response to 7-10 days of cycle exercise. *J Appl Physiol* 80: 2250–2254, 1996.

136. **Sprecher DL**. Lipids, lipoproteins, and peroxisome proliferator activated receptor-delta. *Am J Cardiol* 100: n20–n24, 2007.

137. **Staron RS**, **Karapondo DL**, **Kraemer WJ**, **Fry AC**, **Gordon SE**, **Falkel JE**, **Hagerman FC**, **Hikida RS**. Skeletal muscle adaptations during early phase of heavy-resistance

training in men and women. *J Appl Physiol* 76: 1247–1255, 1994.

138. **Staron RS**, **Malicky ES**, **Leonardi MJ**, **Falkel JE**, **Hagerman FC**, **Dudley GA**. Muscle hypertrophy and fast fiber type conversions in heavy resistance-trained women. *Eur J Appl Physiol Occup Physiol* 60: 71–79, 1990.

139. **Taaffe DR**, **Duret C**, **Wheeler S**, **Marcus R**. Once-weekly resistance exercise improves muscle strength and neuromuscular performance in older adults. *J Am Geriatr Soc* 47: 1208–1214, 1999.

140. **Takahashi S**, **Tanaka T**, **Kodama T**, **Sakai J**. Peroxisome proliferator-activated receptor δ (PPARδ), a novel target site for drug discovery in metabolic syndrome. *Pharmacol Res* 53: 501–507, 2006.

141. **Takahashi S**, **Tanaka T**, **Sakai J**. New therapeutic target for metabolic syndrome: PPARδ. *Endocr J* 54: 347–357, 2007.

142. **Tang JE.**, **Hartman JW**, **Phillips SM**. Increased muscle oxidative potential following resistance training induced fibre hypertrophy in young men. *Appl Physiol Nutr Metab* 31: 495–501, 2006.

143. **Tesch PA**, **Colliander EB**, **Kaiser P**. Muscle metabolism during intense, heavy-resistance exercise. *Eur J Appl Physiol Occup Physiol* 55: 362–366, 1986.

144. **Tesch PA**, **Larsson L**. Muscle hypertrophy in bodybuilders. *Eur J Appl Physiol Occup Physiol* 49: 301–306, 1982.

145. **Tesch PA**, **Thorsson A**, **Essen-Gustavsson B**. Enzyme activities of FT and ST muscle fibers in heavy-resistance trained athletes. *J Appl Physiol* 67: 83–87, 1989.

146. **Tsitouras PD**, **Gucciardo F**, **Salbe AD**, **Heward C**, **Harman SM**. High omega-3 fat intake improves insulin sensitivity and reduces CRP and IL6, but does not affect other endocrine axes in healthy older adults. *Horm Metab Res* 40: 199–205, 2008.

147. **U.S. Department of Health and Human Services and U.S. Department of Agriculture**. 2015-2020 dietary guidelines for Americans. *2015-2020 Diet. Guidel. Am. (8th Ed.* (2015). doi: 10.1097/NT.0b013e31826c50af.

148. **Valasek MA**, **Clarke SL**, **Repa JJ**. Fenofibrate reduces intestinal cholesterol absorption via PPARα-dependent modulation of NPC1L1 expression in mouse. *J Lipid Res* 48: 2725–2735, 2007.

149. **Vavvas D**, **Apazidis A**, **Saha AK**, **Gamble J**, **Patel A**, **Kemp BE**, **Witters LA**, **Ruderman NB**. Contraction-induced changes in acetyl-CoA carboxylase and 5'-AMP-activated kinase in skeletal muscle. *J Biol Chem* 272: 13255–13261, 1997.

150. **van der Veen JN**, **Kruit JK**, **Havinga R**, **Baller JFW**, **Chimini G**, **Lestavel S**, **Staels B**, **Groot PHE**, **Groen AK**, **Kuipers F**. Reduced cholesterol absorption upon PPARdelta activation coincides with decreased intestinal expression of NPC1L1. *J Lipid Res* 46: 526–534, 2005.

151. **Vogel C**, **de Sousa Abreu R**, **Ko D**, **Le S**, **Shapiro BA**, **Burns SC**, **Sandhu D**, **Boutz DR**, **Marcotte EM**, **Penalva LO**. Sequence signatures and mRNA concentration can explain two-thirds of protein abundance variation in a human cell line. *Mol Syst Biol* 6: 1-9, 2010.

152. **Volek JS**, **Kraemer WJ**, **Bush JA**, **Incledon T**, **Boetes M**. Testosterone and cortisol in relationship to dietary nutrients and resistance exercise. *J Appl Physiol* 82: 49–54, 1997.

153. **Vrins CLJ**, **van der Velde AE**, **van den Oever K**, **Levels JHM**, **Huet S**, **Oude Elferink RPJ**, **Kuipers F**, **Groen AK**. Peroxisome proliferator-activated receptor delta activation leads to increased transintestinal cholesterol efflux. *J Lipid Res* 50: 2046–2054, 2009.

154. **Walker M**, **Cooper BG**, **Elliott C**, **Reed JW**, **Orskov H**, **Alberti KG**. Role of plasma non-esterified fatty acids during and after exercise. *Clin Sci* 81: 319–325, 1991..

155. **Wang N**. PPAR-δ in vascular pathophysiology. *PPAR Res* 2008: 1–10, 2008.

156. **Wang Y-X**, **Zhang C-L**, **Yu RT**, **Cho HK**, **Nelson MC**, **Bayuga-Ocampo CR**, **Ham J**, **Kang H**, **Evans RM**. Regulation of muscle fiber type and running endurance by PPARδ. *PLoS Biol* 2: 1532–1539, 2004.

157. **Watt MJ**, **Southgate RJ**, **Holmes AG**, **Febbraio MA**. Suppression of plasma free fatty acids upregulates peroxisome proliferator-activated receptor (PPAR) α and δ and PPAR coactivator 1α in human skeletal muscle, but not lipid regulatory genes. *J Mol Endocrinol* 33: 533–544, 2004.

158. **Widegren U**, **Ryder JW**, **Zierath JR**. Mitogen-activated protein kinase signal transduction in skeletal muscle: effects of exercise and muscle contraction. *Acta Physiol Scand* 172: 227–238, 2001.

159. **Wilson PWF**, **D'Agostino RB**, **Levy D**, **Belanger AM**, **Silbershatz H**, **Kannel WB**. Prediction of coronary heart disease using risk factor categories. *Circulation* 97:

1837-1847, 1998.

160. **Winder WW**, **Hardie DG**. Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. *Am J Physiol - Endocrinol Metab* 270: E299-E304, 1996.

161. **Wu M**, **Falasca M**, **Blough ER**. Akt/protein kinase B in skeletal muscle physiology and pathology. *J Cell Physiol* 226: 29–36, 2011.