FUNCTION AND ACTIVATION OF HUMAN ADIPOSE TISSUE: THE ROLE OF GENES IN THE LINK BETWEEN PHYSICAL ACTIVITY AND BROWN ADIPOSE-LIKE PHENOTYPE

PETROS NTINAS, MSc

A thesis submitted in partial fulfilment of the requirements of the University of Wolverhampton for the degree of Doctor of Philosophy

March 2017

This work or any part thereof has not previously been presented in any form to the University or to any other body whether for the purposes of assessment, publication or for any other purpose (unless otherwise indicated). Save for any express acknowledgments, references and/or bibliographies cited in the work, I confirm that the intellectual content of the work is the result of my own efforts and of no other person.

The right of Petros Ntinas to be identified as author of this work is asserted in accordance with ss.77 and 78 of the Copyright, Designs and Patents Act 1988. At this date copyright is owned by the author.

Signature
Date.................30-3-2017........................
Background: Excess white adipose tissue (WAT) in humans is considered as a harmful health index. However, increased brown adipose tissue (BAT) and brown-like adipose tissue activity are associated with increased resting energy expenditure (REE) that may help to control body weight. Exercise may enhance browning formation of WAT and reduce WAT that may lead to health improvements. Aims: a) to examine the effects of physical activity on the link between peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) and fibronectin type III domain-containing protein 5 (FNDC5) genes in muscle, circulating Irisin and uncoupling protein one (UCP1) of WAT in humans (study 1); b) to examine the relationship between UCP1 mRNA and protein expression as well as PGC-1α, peroxisome proliferator-activated receptor alpha (PPARα) and PPARγ genes with physical activity levels in WAT of healthy men (study 2); c) to examine the effects of different types of exercise and de-training on the UCP1 mRNA and protein expression (study 3), and d) on leptin mRNA in WAT of healthy men (study 4). Method: Study 1: A systematic review was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Studies 2-4: The total of 46 healthy men subjected to measurements for physical activity levels, diet, anthropometry, body composition, REE, peak oxygen consumption, 1-repetition maximum and provided subcutaneous fat biopsies to determine mRNA and protein expression of six genes in one cross-sectional study and one randomized controlled trial. Results: Study 1: No link was found between PGC-1α and FNDC5, circulating Irisin and UCP1 of WAT in response to physical activity. Study 2: The mRNA of, UCP1, PGC-1α, PPARα and PPARγ genes of WAT were not associated with physical activity levels. The UCP1 protein expression however, was negatively associated with physical activity levels. Studies 3-4: Different types of chronic exercise and de-training do not affect UCP1 mRNA and protein expression
and leptin mRNA in WAT. However, effect size analyses demonstrated increased UCP1 mRNA and protein expression, PPARγ and leptin in response to chronic exercise. **Conclusions:** There is no evidence to support the link between PGC-1α and FNDC5 in human muscle or the link between FNDC5 and circulating Irisin and UCP1 in WAT in response to exercise. There are no effects of exercise and de-training on browning formation of WAT and no link between browning formation indices and REE, body weight as well as leptin mRNA in healthy men. Further research is required to elaborate the aforementioned phenomena.
# CONTENTS

ABSTRACT .................................................................................................................. 2

LIST OF FIGURES ................................................................................................. 8

LIST OF TABLES ................................................................................................... 10

LIST OF ABBREVIATIONS ................................................................................... 11

ACKNOWLEDGMENTS ........................................................................................... 13

RESPONSIBILITIES ............................................................................................... 15

SKILLS ACQUIRED AS A RESULT OF THIS PhD .................................................. 17

1. CHAPTER 1: INTRODUCTION ........................................................................... 18

2. CHAPTER 2: REVIEW OF LITERATURE ........................................................... 20

   2.1. APPROACH .................................................................................................. 20

   2.2. THE PHENOMENON OF OBESITY .............................................................. 20

      2.2.1. Physical activity, exercise and obesity ..................................................... 23

   2.3. WHITE ADIPOSE TISSUE ......................................................................... 24

      2.3.1. Function .................................................................................................. 24

      2.3.2. White adipose tissue and inflammation ............................................... 26

      2.3.3. Leptin ..................................................................................................... 28

      2.3.4. Adiponectin .......................................................................................... 33

   2.4. BROWN ADIPOSE TISSUE ........................................................................ 36

      2.4.1. The link between brown adipose tissue and exercise ............................. 39

      2.4.2. The link between brown adipose tissue and nutrition ......................... 42

   2.5. BROWN-LIKE ADIPOSE TISSUE ............................................................... 42

      2.5.1. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) ................................................................. 45

      2.5.2. Peroxisome proliferator-activated receptor alpha (PPARα) ..................... 46

      2.5.3. Peroxisome proliferator-activated receptor gamma (PPARγ) ................. 47

      2.5.4. Brown-like adipose tissue and physical activity/exercise ..................... 48

3. CHAPTER 3: RATIONALE, AIMS & HYPOTHESES ............................................. 52

   3.1. RATIONALE .................................................................................................. 52

   3.2. AIMS ............................................................................................................. 55

   3.3. HYPOTHESES .............................................................................................. 55

4. CHAPTER 4: METHODS USED IN PhD STUDY .................................................. 57

   4.1. ETHICAL APPROVAL ................................................................................. 57
4.2. APPROACH .................................................................................................................. 57
4.3. SYSTEMATIC REVIEW METHOD ................................................................................ 57
  4.3.1. Search strategy ......................................................................................................... 57
  4.3.2. Selection criteria ....................................................................................................... 60
  4.3.3. Risk of bias assessment and quality of reporting data ............................................. 60
4.4. EXPERIMENTAL METHODS ....................................................................................... 61
  4.4.1. Study design of randomized controlled trial ................................................................. 61
  4.4.2. Experimental protocol for randomized controlled trial .............................................. 63
  4.4.3. Anthropometry ......................................................................................................... 64
  4.4.4. Assessment of resting energy expenditure ................................................................. 65
  4.4.5. Assessment of physical activity levels ....................................................................... 65
  4.4.6. Assessment of peak oxygen uptake and 1-repetition maximum ............................. 66
  4.4.7. Subcutaneous fat biopsies ........................................................................................ 66
  4.4.8. Gene and protein expression analyses ..................................................................... 67
  4.4.9. Exercise interventions for randomized controlled trial ........................................... 68
  4.4.10. Diet Assessment ..................................................................................................... 69
  4.4.11. General statistical approach .................................................................................... 70

5. CHAPTER 5: EFFECTS OF PHYSICAL ACTIVITY ON THE LINK BETWEEN
PGC-1α AND FNDC5 IN MUSCLE, CIRCULATING IRISIN, AND UCP1 OF WHITE
ADIPOCYTES IN HUMANS: A SYSTEMATIC REVIEW (1st study) ........................................ 72
  5.1. INTRODUCTION ........................................................................................................... 72
  5.2. METHODS .................................................................................................................. 73
  5.3. RESULTS ...................................................................................................................... 74
    5.3.1. Searching procedure results ..................................................................................... 74
    5.3.2. Characteristics of the included studies .................................................................... 75
    5.3.3. Risk of bias and quality of reporting data ............................................................... 81
  5.4. REPORTING OF OUTCOMES .................................................................................. 86
    5.4.1. Main results of randomized controlled trials ............................................................ 86
    5.4.2. Main results of controlled trials ............................................................................. 87
    5.4.3. Main results of single-group design studies ............................................................. 88
    5.4.4. Main results of cross-sectional studies ................................................................... 89
    5.4.5. Results for associations of Irisin with secondary outcome measures ................. 90
  5.5. DISCUSSION ............................................................................................................... 91
    5.5.1. Applicability of evidence ......................................................................................... 91
8.3.2. Secondary outcomes ................................................................. 169
8.3.3. Results from analysis within groups ........................................ 179
8.4. DISCUSSION .............................................................................. 180
8.5. CONCLUSIONS .......................................................................... 182
9. CHAPTER 9: GENERAL DISCUSSION .............................................. 184
10. CHAPTER 10: LIMITATIONS ......................................................... 195
11. CHAPTER 11: SUGGESTIONS FOR FUTURE RESEARCH ............ 197
12. CHAPTER 12: REFERENCES ......................................................... 201
13. APPENDICES .............................................................................. 223
  13.1. APPENDIX 1: Ethical approval from the University of Wolverhampton .......... 223
  13.2. APPENDIX 2: Ethical approval from the University of Thessaly .......... 224
  13.3. APPENDIX 3: Written consent form .......................................... 225
  13.4. APPENDIX 4: Medical history form (by interview) ...................... 227
  13.5. APPENDIX 5: International Physical Activity Questionnaire .......... 230
  13.6. APPENDIX 6: Physical activity readiness questionnaire ............... 232
  13.7. APPENDIX 7: Representative photographic film images of UCP1 protein expression quantification ......................................................... 233
  13.8. APPENDIX 8: Publications .......................................................... 234
LIST OF FIGURES

Figure 1: Global obesity prevalence in adults in 2014 .................................................. 22
Figure 2: Obesity and chronic inflammation ................................................................. 27
Figure 3: Leptin function .............................................................................................. 30
Figure 4: Adiponectin function ..................................................................................... 34
Figure 5: The non-shivering thermogenesis phenomenon ............................................ 37
Figure 6: The Irisin mechanism .................................................................................... 49
Figure 7: CONSORT diagram of the randomized controlled trial design ................. 63
Figure 8: Experimental protocol for randomized controlled trials ......................... 64
Figure 9: Flow diagram of study selection and identification (PRISMA guidelines) ... 75
Figure 10: Risk of bias assessment of the included studies using the Cochrane Collaboration’s tool .......................................................... 82
Figure 11: Summary of risk of bias ............................................................................. 83
Figure 12: Associations of UCP1 protein expression and physical activity levels (pedometer steps/day) .......................................................... 102
Figure 13: Associations of PGC-1α and PPARα mRNA with BMI ......................... 103
Figure 14: Associations of PGC-1α and PPARα mRNA with WHR ....................... 104
Figure 15: Associations of PGC-1α and PPARα mRNA with fat mass percentage. 105
Figure 16: Association of PGC-1α mRNA with fat-free mass .................................. 105
Figure 17: Individual data for UCP1 mRNA (A) and protein expression (B) at baseline, post exercise (week 8) and de-training (week 16) ................................. 121
Figure 18: Cohen’s d effect size in UCP1 mRNA between baseline and post-exercise (week 8) .................................................................................... 124
Figure 19: Cohen’s d effect size in UCP1 mRNA between post-exercise (week 8) and de-training (week 16) in REG, CEG and CG .............................................. 125
Figure 20: Cohen’s d effect size in UCP1 mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16) .................. 126
Figure 21: Cohen’s d effect size in UCP1 protein expression between baseline, post-exercise (week 8) and de-training (week 16) .................................................. 128
Figure 22: Cohen’s d effect size in UCP1 protein expression between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16) .... 130
Figure 23: Individual data for PGC-1α (A), PPARα (B) and PPARγ (C) mRNAs at baseline, post-exercise (week 8) and de-training (week 16) .................... 132
Figure 24: Cohen’s d effect size in PGC-1α mRNA between baseline, post-exercise (week 8) and de-training (week 16) ................................................................. 136
Figure 25: Cohen’s d effect size in PGC-1α mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16) ...................... 138
Figure 26: Cohen’s d effect size in PPARα mRNA between baseline, post-exercise (week 8) and de-training (week 16) ........................................................................ 139
Figure 27: Cohen’s d effect size in PPARα mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16) .......... 141
Figure 28: Cohen’s d effect size in PPARγ mRNA between baseline, post-exercise (week 8) and de-training (week 16) ...................................................................... 143
Figure 29: Cohen’s $d$ effect size in PPARγ mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16) .................................................. 145
Figure 30: Individual data for leptin mRNA at baseline, post exercise (week 8) and de-training (week 16) ........................................................................................................... 164
Figure 31: Cohen’s $d$ effect size in leptin mRNA between baseline, post-exercise (week 8) and de-training (week 16) ............................................................. 167
Figure 32: Cohen’s $d$ effect size in leptin mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16) .................. 168
Figure 33: Individual data for adiponectin (A) and leptin to adiponectin ratio (B) mRNAs at baseline, post exercise (week 8) and de-training (week 16) ......................... 170
Figure 34: Cohen’s $d$ effect size in adiponectin mRNA between baseline, post-exercise (week 8) and de-training (week 16) ........................................................................ 174
Figure 35: Cohen’s $d$ effect size in adiponectin mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16) .......... 176
Figure 36: Cohen’s $d$ effect size in leptin to adiponectin ratio mRNA between baseline, post-exercise (week 8) and de-training (week 16) .............................................. 178
Figure 37: Cohen’s $d$ effect size in leptin to adiponectin ratio mRNA between control and exercise groups at de-training (week 16) ........................................................ 179
# LIST OF TABLES

Table 1: Body mass index categories ................................................................. 21
Table 2: Effects of acute and chronic exercise on leptin ................................ 32
Table 3: Effects of acute and chronic exercise on adiponectin ...................... 35
Table 4: Main differences between Brown Adipose Tissue and White Adipose Tissue ........................................................................................................ 36
Table 5: Effects of exercise on brown adipose tissue .................................... 41
Table 6: Searching algorithm for PubMed ......................................................... 58
Table 7: Searching algorithm for EMBASE ...................................................... 59
Table 8: Exercise protocols for randomized controlled trials ......................... 69
Table 9: Characteristics of the studies included in the systematic review ....... 76
Table 10: Results of the quality of the reporting of the results using the Consolidated Standards of Reporting Trials checklist ........................................ 84
Table 11: Results of the quality of the reporting of the results using the Strengthening the Reporting of Observational Studies in Epidemiology checklist ........ 85
Table 12: Characteristics of the participants (n=46) ........................................ 101
Table 13: Baseline characteristics of the participants (n=32) ......................... 117
Table 14: Number and percentage of the low and high responders for UCP1 mRNA and protein expression .............................................................. 123
Table 15: Number and percentage of the low and high responders for PGC-1α, PPARα and PPARγ mRNAs ........................................................................ 134
Table 16: Mean and standard deviation of outcome variables in AEG (n=9) .... 146
Table 17: Mean and standard deviation of outcome variables in REG (n=8) ...... 148
Table 18: Mean and standard deviation of outcome variables in CEG (n=8) .... 149
Table 19: Mean and standard deviation of outcome variables in CG (n=7) ...... 150
Table 20: Baseline characteristics of the participants (n=32) ............................ 162
Table 21: Number and percentage of the low and high responders for leptin mRNA ........................................................................................................ 165
Table 22: Number and percentage of the low and high responders for adiponectin and leptin to adiponectin ratio mRNAs .................................................. 172
Table 23: Mean and standard deviation of leptin, adiponectin and L/A mRNAs ..... 179
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2D</td>
<td>Type 2 diabetes</td>
</tr>
<tr>
<td>WAT</td>
<td>White adipose tissue</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BAT</td>
<td>Brown adipose tissue</td>
</tr>
<tr>
<td>REE</td>
<td>Resting energy expenditure</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist to hip ratio</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>VO₂max</td>
<td>Maximal oxygen uptake</td>
</tr>
<tr>
<td>Kcal</td>
<td>Kilocalories</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>Peroxisome proliferator-activated receptor gamma coactivator 1-alpha</td>
</tr>
<tr>
<td>UCP1</td>
<td>Uncoupling protein one</td>
</tr>
<tr>
<td>NST</td>
<td>Non-shivering thermogenesis</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>PPARα</td>
<td>Peroxisome proliferator-activated receptor alpha</td>
</tr>
<tr>
<td>NRF-1</td>
<td>Nuclear Factor One</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>PPARβ</td>
<td>Peroxisome proliferator-activated receptor beta</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA (mRNA expression)</td>
</tr>
<tr>
<td>FNDC5</td>
<td>Fibronectin type III domain-containing protein 5</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trials</td>
</tr>
<tr>
<td>CT</td>
<td>Controlled trials</td>
</tr>
<tr>
<td>SGS</td>
<td>Single group design studies</td>
</tr>
<tr>
<td>CONSORT</td>
<td>Consolidated Standards of Reporting Trials</td>
</tr>
<tr>
<td>CSS</td>
<td>Cross sectional studies</td>
</tr>
<tr>
<td>STROBE</td>
<td>Strengthening the Reporting of Observational Studies in Epidemiology</td>
</tr>
<tr>
<td>AEG</td>
<td>Aerobic exercise group</td>
</tr>
<tr>
<td>REG</td>
<td>Resistance exercise group</td>
</tr>
<tr>
<td>CEG</td>
<td>Combined exercise group</td>
</tr>
<tr>
<td>CG</td>
<td>Control group</td>
</tr>
<tr>
<td>1RM</td>
<td>One repetition maximum</td>
</tr>
<tr>
<td>AE</td>
<td>Aerobic exercise</td>
</tr>
<tr>
<td>RE</td>
<td>Resistance exercise</td>
</tr>
<tr>
<td>CE</td>
<td>Combined exercise</td>
</tr>
<tr>
<td>CG</td>
<td>Control group</td>
</tr>
<tr>
<td>VO₂peak</td>
<td>Peak oxygen uptake</td>
</tr>
<tr>
<td>PAR-Q</td>
<td>Physical Activity Readiness Questionnaire</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>RPLP0</td>
<td>Ribosomal Protein, Large, P0</td>
</tr>
<tr>
<td>PRISMA</td>
<td>Preferred Reporting Items for Systematic Reviews and Meta-Analyses</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>M</td>
<td>Males</td>
</tr>
<tr>
<td>F</td>
<td>Females</td>
</tr>
<tr>
<td>AB</td>
<td>Aviscera Bioscience</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>CNS</td>
<td>Code not specified</td>
</tr>
<tr>
<td>PP</td>
<td>Phoenix Pharmaceuticals</td>
</tr>
<tr>
<td>C-RCT</td>
<td>Cross-over randomized controlled trial</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic Syndrome</td>
</tr>
<tr>
<td>PA</td>
<td>Physical activity</td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostatic model assessment</td>
</tr>
<tr>
<td>LBM</td>
<td>Lean body mass</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>METs</td>
<td>Metabolic equivalent</td>
</tr>
<tr>
<td>SNPs</td>
<td>Single nucleotide polymorphisms</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>L/A</td>
<td>Leptin to adiponectin</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

This PhD study was made possible by support from the European Union 7th Framework Programme [FP7-PEOPLE-2012-IRSES (FUEGO grant no. 612547), and 319010FP7-PEOPLE-2013-IRSES (U-GENE grant no. 319010)].

I would like to thank my supervisory committee. My sincere thanks go to my Director of studies, Professor George Metsios, who taught me the value of research and offered me the opportunity to work in a high quality research environment. I also offer my sincerest gratitude to my mentor, co-supervisor and the Director of FAME Laboratory, Dr. Andreas Flouris. I feel very lucky I had Andreas as a mentor who taught me science and passion for life. Finally, I express my sincere gratitude to my co-supervisor, Professor Yiannis Koutedakis, who supported me and inspired me to undertake this PhD.

I would like to thank my colleagues of our research group: Dr. Andres Carrillo and Ms. Natalie Davies from the Chatham University, Mrs. Georgia Ntina and Mrs. Paraskevi Gkiata from the FAME Laboratory, as well as Dr. Ian Lahart from the University of Wolverhampton. I would also like to thank Dr. Vasileios Chachopoulos and Dr. Chasioti Fani for their valuable help in collecting human biological tissue, as well as Associate Professor Athanasios Jamurtas, Assistant Professor Giorgos Sakkas and Mrs. Chara Deli, who generously supplied me with scientific equipment during my PhD experiments. I also thank Ms. Katherine Zacharopoulou, Ms. Dimitra Psychou and Mr. Lefteris Vogiatzis for their help in supervising the exercise sessions of the participants.

My special thanks also go to Mrs. Angelica Valente from the FAME Laboratory and Dr. Antonis Stavropoulos-Kalinoglou from the University of Thessaly for their time to discuss several aspects of this PhD study. A special thank also goes to Mr. Paul Grant for the proofreading of this PhD thesis.
Many thanks also go to my brother, Athanasios Ntinas, and my cousin, Athanasios Tsiavaliaris, for their administration work during my PhD research. I also thank my brother-in-law, Giorgos Mavropoulos, who financially supported me to complete this PhD research, as well as my brother, Giorgos Ntinas, and my brother-in-law, Efthimios Mavropoulos who supported me spiritually.

I would like to thank my ex-wife, Katherine, for her understanding and for her extra role to take care of our son, Lefteris, during my PhD research.

Last, but not the least, I would like to thank my family. To my lovely wife, Maria-Rozina, and our sons, Christos and Yiannis, who always stand by me and support me unconditionally. To my son, Lefteris, who supported me spiritually to achieve my goals during my PhD experiments. To my parents, Christos and Loukia, as well as my parents-in-law, Yiannis and Panagiota, who believed in me and supported me spiritually. To my family I dedicate this thesis.
RESPONSIBILITIES

My responsibilities in the four studies that constitute this thesis were, to:

- Undertake and present a bibliographic systematic review
- Structure the methodology for the original research projects
- Gain ethical approval from the University of Thessaly, Greece, and the University of Wolverhampton, UK
- Recruit participants for the experiments
- Assess the participants, including assisting in the collection of subcutaneous fat biopsies
- Analyse the collected data. (The experimental methodological design - Randomized Controlled Trial - made it impossible for me to be involved in the analysis of mRNA, nutritional data and physical activity data)
- Input and interpret data. (My supervisors, Professor Metsios, Dr. Flouris and Professor Koutedakis, helped me significantly in this respect)
- Write, present and publish the results and related papers of my PhD in scientific congresses and peer-reviewed journals. These can be found in the table below.

Peer-reviewed journals

**Petros C. Dinas**, Ian M. Lahart, James A. Timmons, Andreas D. Flouris, Yiannis Koutedakis, Per-Arne Svensson, and George S. Metsios. The effects of physical activity on the link between PGC-1α and FNDC5 in muscle, circulating irisin and UCP1 of white adipocytes in humans: A systematic review. *F1000Research*, *submitted*.


Dinas P., Lahart I., Flouris A.D., Koutedakis Y., Svensson P., Boguszewski C., and Metsios G. The effects of physical activity on PGC-1α and FNDC5 in muscle, circulating irisin, and UCP1 of white adipocytes in humans: A systematic review. 5th Conference of Exercise Biochemistry and Exercise Physiology, 6-7th Nov 2015, Athens, Greece. (Second prize for the best poster presentation).


SKILLS ACQUIRED AS A RESULT OF THIS PhD

- Increased knowledge regarding a systematic review process following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, while improved skills in formatting a systematic review according to the Cochrane Library guidelines.

- Augmented knowledge regarding the settings of the research methodological designs in the area of human physiology (i.e. cross-sectional studies, controlled trials, randomized controlled trials etc).

- Increased knowledge in general principles of human genes and protein functions

- Increased experience in synthesis and data statistical analyses

- Augmented experience in recruiting human participants for a research project including randomization and allocation skills

- Increased experience in problem solving regarding a research project in human physiology including problems with the participants and equipment

- Improved interpersonal and leadership skills given that I had to supervise my assistants in this PhD study as well as undergraduate and postgraduate students

- Improved skills in preparing short written materials and write effectively scientific articles

- Improved oral presentations skills given that I have presented some of the outcomes of this PhD study in scientific conferences

- Increased teaching experience

- Increased experience of different European countries ethical committees considerations and processes
Excessive adipose tissue is defined as an obesity state in humans and has been recognized as a harmful health index [1]. Specifically, obesity can lead to compromises in health, such as cardiovascular disease, type 2 diabetes (T2D), and hypertension [2-5]. About 700 million adults are obese worldwide, while ~20 million children and ~2.3 billion adults are overweight [6]. Excessive adipose tissue may disrupt biological processes such as immune function, insulin sensitivity, and blood pressure [7, 8] increasing the risk for disease in humans. Therefore, by increasing our knowledge on how to reduce the excessive adipose tissue in humans, it may help to lessen the risk of weight gain and disease.

Exercise regulates hormonal activity within adipose tissue [9-13] and may reduce adipose tissue mass [14], particularly when this is in combination with caloric restriction [15]. Exercise also significantly reduces total and abdominal fat in obese individuals with T2D [16] and minimizes the risk for cardiovascular disease [15, 17]. On the other hand, physical inactivity is a risk factor for developing T2D [18] and has been linked to greater risks of morbidity and mortality than increased adipose tissue mass [19]. It is essential, therefore, to identify the exercise mechanisms that can reduce adipose tissue mass.

Researchers have observed two types of human adipose tissue: brown and white [20-22]. Both tissues store energy and are considered as endocrine organs, however, they display different functions [23]. The main difference is that white adipose tissue (WAT) produces energy in the form of adenosine triphosphate (ATP) [24], whereas brown adipose tissue (BAT) uses energy to produce heat and maintain body temperature [25]. In other words, WAT stores calories as a “stock energy room”, while BAT spends energy for thermogenesis [26]. It was believed however, that BAT is present only in infants [27]. In 2009 it was confirmed that BAT is also active in
healthy adults after 2-hour of exposure to 16°C [21, 22], while increased BAT activity was associated with elevated daily resting energy expenditure (REE) [21]. Also, increased BAT activity has been associated with reduced body mass index (BMI) [28], age [29], and fat mass [21]. Finally, a recent study has found a positive association of BAT activity with increased physical activity levels in humans [29].

Recent research suggests that a third type of adipose tissue – the brown-like or beige adipose tissue – can play a significant role in human metabolism by increasing REE [30], and that exercise can change WAT to function in a brown-like mode. This was attributed to a newly discovered protein called Irisin, which is a fragment protein released by skeletal muscle during exercise [30]. However, current research shows conflicting results [31-34] and more investigation is needed into the role of Irisin in the human body, as well as the role of exercise in the browning process of WAT. This is because the methods used for circulating Irisin identification were questioned [35]. Also, the role of exercise on browning formation of WAT has not been extensively investigated in humans. Given also that adipose tissue has a significant impact on human metabolism, more research is needed that could direct potential therapeutic pathways through exercise on humans’ health and wellbeing. In this light, the current PhD study includes the first systematic review to date to examine the role of exercise on the browning formation of WAT in humans. Also, this PhD includes the first randomized controlled trial (RCT) to date to examine the effects of exercise training on a part of the mechanism of the browning formation of WAT, while it examined a specific mechanism of WAT related to appetite levels in humans. Finally, this PhD study examined for the first time the effects of de-training on the browning formation of WAT in humans.
2. CHAPTER 2: REVIEW OF LITERATURE

Parts of the review of literature have been published in the peer-reviewed journal *ISRN Physiology*, vol. 2014, Article ID 964627, 11 pages, 2014. doi:10.1155/2014/964627, (Appendix 8) where the author of this Thesis also appears as the leading author.

2.1. APPROACH

The current review of literature will offer an overview of the function and activation of human adipose tissue. First, it will describe the phenomenon of obesity and the effects of exercise, highlight the function of WAT and the role of leptin and adiponectin in metabolism as well as their relationship to exercise. This will also display the role of WAT in human health. Secondly, it will describe the function and activation of BAT and its link with exercise and nutrition while a description of the function of brown-like adipose tissue and the role of four genes that indicate a browning process of WAT will be given. Finally, the role of exercise in brown-like adipose tissue formation will be discussed.

2.2. THE PHENOMENON OF OBESITY

Obesity is characterized by the presence of excessive weight primarily in the form of excessive adipose tissue [36]. Clinical practise indicates that an individual is defined as obese when he/she displays a Body Mass Index (BMI) ≥ 30 kg/m$^2$. Increased BMI is a major risk factor for heart disease, stroke, T2D, musculoskeletal disorders and endometrial, breast and colon cancers [37-40]. BMI is used to describe general body mass and to estimate adiposity. It generally displays small margin errors and is suitable for identifying trends within sedentary or overweight populations [41], by simply dividing weight (kg) by height (m) squared [6]. The BMI categories can be found in Table 1.
Table 1: Body mass index categories

<table>
<thead>
<tr>
<th>Category</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;19 kg/m²</td>
</tr>
<tr>
<td>Normal weight</td>
<td>19-24.9 kg/m²</td>
</tr>
<tr>
<td>Overweight</td>
<td>25-29.9 kg/m²</td>
</tr>
<tr>
<td>Class I obese</td>
<td>30-34.9 kg/m²</td>
</tr>
<tr>
<td>Class II obese</td>
<td>&gt;35 kg/m²</td>
</tr>
</tbody>
</table>

kg: kilograms; m: meters

Another index of increased fat accumulation in humans is the waist to hip ratio (WHR). This is defined as the ratio of the smallest diameter of the waist circumference and the largest diameter of the hip circumference. WHR >1 in men and >0.85 in women is considered a risk of abdominal obesity [42] and an independent risk factor for cardiovascular disease [43]. However, neither BMI nor WHR actually describe the composition of the human body and should be used with that limitation in mind. They are both useful for showing general correlations regarding obesity whereas it is BMI that has been used extensively by clinical practise and by the World Health Organization to record obesity statistics [44].

In the absence of an underlying pathology, obesity is mainly caused by an energy imbalance between calories consumed and calories expended [45, 46]. It may also be the result of genetic predisposition [47], medical conditions, or psychiatric illness [48]. Research indicates that most of the obesity cases worldwide are due to increased consumption of high-fat foods in conjunction with decreased physical activity [49, 50]. In developed countries, some argue that access to plenty of food [51] and reliance on vehicles for transport [52, 53] contribute to the observed increased obesity trends. This raises the debate as to whether the increased obesity rates are due to physical inactivity or poor diet. Some support the latter given that physical activity rates have not been changed significantly in recent times [54]. For instance, excess sugar consumption increases by 11-fold the prevalence of T2D independent of physical activity levels [55]. Other evidence indicates that up to 40% of people with
a normal BMI will develop hypertension, dyslipidaemia, and cardiovascular disease, conditions associated with obesity [56, 57] while the “western diet”, stress and sleep deprivation increase insulin resistance, leading to increased food intake [58-60].

Contrary to the above, it has been claimed that daily physical activity has declined over the last decades [61], due to the increase in sedentary jobs [62] and that there is no firm data that people eat more calories per day nowadays than in previous decades [63]. This view suggests that physical activity cannot be taken out of the equation as a cause of human obesity. Evidence suggests that obesity increases with age [64-66], with ~700 million adults being obese now [6]. The global obesity prevalence in adults in 2014 is displayed in Figure 1.

**Figure 1:** Global obesity prevalence in adults in 2014

Europe, North America, Australia and North Asia display high prevalence of obesity cases (20-20.9%) in adults, while the United States of America displays the highest prevalence in obesity cases in adults worldwide (>30%). Africa (<10%) and South Asia (<10%) display the lowest prevalence in obesity cases in adults worldwide. Figure retrieved from the World Health Organization website: http://gamapserver.who.int/mapLibrary/app/searchResults.aspx
Physical inactivity should be considered one of the main reasons of obesity [67] and the fourth highest risk factor for mortality worldwide [68]. In 2010, 23% of the adult and 80% of the adolescent population were physically inactive [68]. Insufficient physical activity levels may lead to cardiovascular disease, cancer and T2D [69]. Excessive body fat mass is associated with T2D in 64% of the cases in men and in 77% of the cases in women [70]. Being physically active in combination with smoking cessation reduces the risk for coronary heart disease by 59%, the risk for mortality due to cardiovascular disease by 77% and the risk for all-cause mortality by 69% in men [71]. Increased fat mass is also associated with osteoarthritis, cancer, cardiovascular disease, non-alcoholic fatty liver disease [72-75], insulin resistance and pro-inflammatory [76] and pro-thrombotic states [77].

2.2.1. Physical activity, exercise and obesity

Physical inactivity is defined as very low or no bodily movement that requires energy expenditure. On the other hand, physical activity is defined as the total of the daily movements produced by skeletal muscles causing energy expenditure including programmed exercise [78].

Overweight and obese individuals are usually recommended to eat less and be more physically active. These lifestyle changes are important as only ~25–30% of lifespan variation can be attributed to genetic factors [79, 80]. However, it remains difficult to prescribe an exact amount of exercise to prevent weight gain for obese and overweight populations. This is partly because each person requires an individual plan to maximize the benefits gained from exercise training. Nevertheless, evidence suggests that exercise has a dose-dependent relationship with health and that any
amount of exercise can be effective in weight management for overweight and obese individuals [81, 82].

Exercise has also been shown to reduce adipose tissue mass and result in weight loss [83, 84]. Evidence suggests, however, that exercise-induced adaptations could occur independent of weight loss. Thirteen weeks of supervised aerobic exercise [60 minutes at 60% peak oxygen uptake (VO\textsubscript{2peak})] significantly reduced total and abdominal fat in obese individuals with T2D [16], as well as minimized the presence of cardiovascular disease risk factors [17]. In addition, a 12-week exercise intervention [walking or light jogging on a treadmill, 5 times/week, 60% maximum oxygen uptake (VO\textsubscript{2max}), 60 min/session] reduced waist circumference in obese men with or without T2D [85].

A major concern for overweight and obese individuals is weight regain following a weight loss intervention. Hunter and colleagues [86] examined weight regain in healthy premenopausal overweight women after one year of following a dietary intervention in which the participants lost 12.3±2.5 kg. The results revealed that both aerobic and resistance training exercisers regained 3.5 kg after one year compared to 6.4 kg of weight regained by the non-exercisers [86]. Thus, exercise has a critical role in the maintenance of body weight that should be included in lifestyle modification programmes.

2.3. WHITE ADIPOSE TISSUE

2.3.1. Function

WAT stores energy in the form of lipids and provides insulation. It is also considered an endocrine organ [87, 88]. WAT has three main functions: a) lipids metabolism that includes storage of triglycerides and fatty acids, b) catabolism of triglycerides to
produce glycerol and fatty acids that are involved in glucose metabolism of several tissues and c) secretion of adipokines, including hormones that are involved in biological functions [89]. WAT also produces hormones and cytokines to regulate energy metabolism including, estrogen, resistin, tumor necrosis factor alpha (TNFα), leptin and adiponectin [87, 88, 90]. WAT is also involved in angiogenesis, adipogenesis, steroid metabolism, in haemostasis and the function of the immune system [91].

The size of white adipocytes is increased during positive energy balance (increased energy intake) and decreased when energy expenditure is more than the energy intake [87]. WAT can undergo hyperplasia mainly in the growth stage of an organism and also hypertrophy, which designates the regulation of the capacity of the adipocytes [92]. Subcutaneous adipose tissue (located underneath the skin) shows the different body composition of men and women and is mainly responsible for the thermal insulation [93]. Visceral adipose tissue (located between organs) maintains the integrity of organs [93]. These different locations designate its different functions. For instance, in the absence of a pathological condition in gluteal area, white adipocytes are larger in women than in men, which may indicate larger lipolysis rate in women [94, 95]. Under energy deprivation subcutaneous adipose tissue may be triggered faster than the visceral one, in order to release fatty acids for energy production [96]. However, excessive visceral and subcutaneous fat mass are associated with metabolic abnormalities: visceral mass and adipocyte size are positively correlated with insulin resistance [97, 98] while excessive subcutaneous adipose tissue may also be responsible for insulin resistance, hypertension and cardiovascular disease [99]. In humans, an overall percentage body fat of ≥ 25% in
men and ≥ 35% in women classifies obesity [100], which also strongly correlates with chronic inflammation that may lead to disease [101].

2.3.2. White adipose tissue and inflammation

Individuals suffer of chronic inflammation may experience macrophage infiltration of adipose tissue that leads to the production of cytokines, such as interleukin-6 (IL-6) that activate the immune system, through the acute phase response [87, 102]. Additionally, chronically high levels of circulating inflammatory cytokines are associated with impaired insulin sensitivity that may indicate a high risk for T2D [76] as well as atherosclerosis [103]. Furthermore, increased levels of the pro-inflammatory biomarker TNFα appear to be associated with reduced circulating adiponectin concentrations that increase the risk for inflammation [104], while a potential mechanism for the increase in adiponectin is a reduction in circulating concentrations of TNFα [105]. A summary of the association of obesity with chronic inflammation can be found in Figure 2.
Abdominal obesity causes the production of tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), which may increase C-reactive protein (CRP) that enhances insulin resistance, hyperinsulinemia, hypertriglyceridemia and decreases high-density lipoprotein (HDL). This process may promote cardiovascular disease.

During an inflammatory condition macrophages release IL-6 and other cytokines that trigger the acute phase response. Subsequently, the liver synthesizes and releases C-reactive protein (CRP) in the bloodstream. CRP is then overexpressed leading to further increase of cytokines [106]. A recent systematic review and meta-analysis shows that exercise reduces CRP and IL-6 in T2D patients [107]. Regarding the acute effects of exercise, one study showed that after a single bout of exercise (on the treadmill at 55% of VO₂max), IL-6 was significantly reduced in WAT in a group of obese men. However, there was no corresponding reduction in circulating IL-6, which may indicate a local adipose tissue anti-inflammatory response to acute exercise [17]. Evidence concerning the chronic effects of exercise shows a significant reduction in IL-6 in obese adolescent men following participation in a 12-week aerobic exercise programme [108]. Also, a 6-month aerobic exercise programme exerted anti-
inflammatory effects in overweight individuals with T2D [109]. However, no changes in IL-6 and TNFα in overweight and obese post-menopausal women have been found following participation in 6 months of exercise training [110]. Broadly, IL-6 may be increased in response to exercise most likely because of the demand of energy from the muscle contraction and may act as a messenger for glucose production from the liver [111]. After exercise, however, IL-6 may have a suppressive effect on the immune system by triggering anti-inflammatory cytokines that may designate an anti-inflammatory role of IL-6 [112].

There is also evidence showing that a 6-month exercise programme reduced both CRP and fat mass in patients with rheumatoid arthritis [113]. This may indicate that the anti-inflammatory effects of exercise may also occur due to changes in body composition. Indeed, evidence showed that obese individuals reduced inflammatory markers, such as IL-6, TNFα and CRP, in response to long-term exercise [114]. Exercise may also improve endothelial dysfunction [112] that can produce IL-6 and promote inflammation [115]. These markers act peripherally and are positively correlated with the endothelial dysfunction [116]. In summary, exercise training appears to exert anti-inflammatory effects via the stimulation of anti-inflammatory cytokines (IL1Ra, sTNFr, IL-10) and the suppression of inflammatory cytokines (TNFα, IL1b) [117] that partly may be due to reductions in body weight, total body fat and visceral fat [118].

2.3.3. Leptin
The understanding of the function of WAT changed when leptin (a protein mainly secreted by WAT) was discovered and its effects on central and peripheral organs were noted [47]. This helped determine the mechanisms of energy balance and
various physiological processes. Indeed, WAT is homeostatically controlled by leptin that regulates food intake and metabolism [119]. Leptin is an adipokine that is characterized as a satiety peptide because of its role in food intake regulation [120]. Leptin is formed by the ob gene that is thought to be responsible for the development of obesity [87, 121]. The mRNA of the ob gene reflects rates of leptin production [87], while the production of leptin in WAT is positively associated with the amount of body fat [122]. Fasting may inhibit the mRNA of ob gene in WAT that may lessen leptin concentrations in the bloodstream. This, however, is inverted in the re-feeding process [123, 124]. In order to regulate hunger, circulating leptin is detected by receptors in the hypothalamus, where it inhibits appetite [120]. Despite having greater concentrations of circulating leptin, obese individuals may experience a condition referred to as leptin resistance that leads to inadequate communication with the hypothalamus [120]. This may occur due to: a) general changes in leptin receptor signaling, b) alterations of leptin during the development phase, and c) changes in the way that leptin crosses the blood brain barrier that separates the circulating blood from the brain extracellular fluid [125]. Evidence suggests that leptin sensitivity affects lipolysis in adipose tissue and hepatic fatty acid uptake and oxidation [126]. Recent data, however, show that increased inflammatory cytokines and free fatty acid levels were associated with impaired leptin sensitivity in mice [127]. Indeed, circulating leptin in humans has been associated with greater levels of systemic inflammation and fat mass [120]. Thus, increased circulating leptin, potentially indicating leptin resistance, may be involved in enhancing adipose tissue accumulation [120].

Leptin may also be involved in other physiological processes, such as the inhibition of insulin production by the pancreas, sugar transport and platelet aggregation [128-130]. Studies have shown that exposure to cold may reduce
circulating leptin via the suppression of the ob gene [123, 131-133]. While leptin is suppressed in response to cold exposure, which is accompanied by a reduction of fat mass [134], non-shivering thermogenesis (NST) is increased [134-136]. This shows an inverse relationship of leptin with NST that exclusively occurs in BAT. Cold exposure and excessive food intake activate the sympathetic nervous system (SNS) that, via norepinephrine, increases BAT activity [25]. The SNS is the main regulator of leptin (it reduces the production of the protein) [137] and norepinephrine suppresses leptin production [122, 123]. Overall, the interaction of SNS with leptin occurs when leptin stimulates SNS in WAT [138] and when there are signals from WAT to leptin sensors [139]. The Figure 3 summarizes leptin's function.

**Figure 3: Leptin function**

*In obesity state leptin develops resistance with its receptor in the hypothalamus, which lead to adiposity signals, inflammatory overactivity, food intake and decreased energy expenditure. This leads to insulin resistance.*
The extent to which exercise may influence leptin concentrations depends on (a) the leptin circadian rhythm, (b) energy spent during an exercise session, (c) resting leptin levels, (d) gender, and (e) postprandial time prior to exercise [120]. Acute exercise (i.e. treadmill exercise at 90% VO$_2$max for 5 min, or 100% VO$_2$max for 2 min) does not modify circulating leptin concentrations in well-trained adult men (age: 28.71±2.91 years) [140]. Similarly, a VO$_2$max test did not affect circulating leptin concentrations in healthy sedentary individuals [141]. Also, while acute exercise did not affect leptin concentrations immentiatelly after the exercise bout it reduced leptin concentrations 24 hours post exercise in healthy male who had negative energy balance (caloric restriction) [142]. The same study also showed that acute exercise increased leptin concentrations 24 hours post exercise when the participants had positive energy balance [142]. Furthermore, acute exercise at 95% VO$_2$max in male adolescents showed that leptin concentrations did not change after 30 minutes of cycling, even though they had consumed a meal 2-3 hours prior to exercise [143].

Chronic exercise, may reduce circulating leptin particularly when exercise is accompanied by a reduction in fat mass [141, 144, 145]. When a chronic exercise programme did not reduce body weight, leptin concentrations did not change [146]. Caloric restriction in conjunction with a 2-month exercise programme showed decreased circulating leptin as opposed to either caloric intake restriction or exercise alone in obese adolescents [147]. In adolescent female athletes, however, leptin concentrations were increased at the fourth and seventh weeks of the chronic exercise programme, probably due to hemoconcentration that may have affected the protein concentrations [148]. Therefore, even though leptin concentrations are dependent on energy balance, exercise may play an important role in circulating leptin concentrations. Also, leptin may be increased in response to caloric consumption while
caloric intake restriction may disrupt leptin action independently of exercise [149].

Overall, the evidence indicates that, particularly when accompanied by weight loss, exercise training reduces circulating leptin concentrations and enhance leptin sensitivity. It can be also suggested that caloric intake restriction in combination with exercise may be effective in decreasing circulating leptin concentrations. These findings are confirmed by a systematic review which concluded that chronic exercise decreases circulating leptin concentrations in obese individuals [150]. However, a more recent systematic review failed to accumulate available data of the effects of combined exercise and diet interventions on circulating leptin, due to the small number of available trials and limitations in the methodology of the existing trials [151]. Finally, leptin may be increased in 12 hours (short-term) and in 2-8 weeks after overfeeding [152, 153]. Leptin may not however, be regulated by short-term exercise (<60 minutes) while it might be decreased by long-term exercise (1-3 hours), yet this is dependent on the energy balance [154]. The effects of acute and chronic exercise on leptin in humans can be found in Table 2.

<table>
<thead>
<tr>
<th>Table 2: Effects of acute and chronic exercise on leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
</tr>
<tr>
<td>Pérusse et. al. 1997</td>
</tr>
<tr>
<td>Kraemer et. al. 2003</td>
</tr>
<tr>
<td>van Aggel-Leijssen et. al. 1999</td>
</tr>
<tr>
<td>Pomerants et. al. 2006</td>
</tr>
<tr>
<td>Study</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Halle et al. 1999</td>
</tr>
<tr>
<td>Kohrt et al. 1996</td>
</tr>
<tr>
<td>Kelly et al. 2007</td>
</tr>
<tr>
<td>Elloumi et al. 2009</td>
</tr>
<tr>
<td>Kraemer et al. 2001</td>
</tr>
</tbody>
</table>

wk:Week; VO$_2$max: Maximal oxygen consumption.

2.3.4. Adiponectin

Adiponectin, encoded by the ADIPOQ gene [155], is an adipokine that affects energy homeostasis and insulin sensitivity. High levels of circulating adiponectin may reduce free fatty acids after a meal [156], while it reduces the blood glucose production by the liver that may affect insulin sensitivity [156]. Low levels of adiponectin have been associated with increased appetite and impaired insulin sensitivity [157]. In the presence of hyperinsulinemia and dyslipidaemia, adiponectin is decreased in skeletal muscle and this is thought to be associated with insulin resistance [158]. Increased adiponectin may increase fatty acid oxidation in skeletal muscle in the presence of hyperinsulinemia [159] while TNFα may decrease circulating adiponectin [104] due to TNFα and adiponectin may inhibit each other’s production in WAT [160]. However, when TNFα is inhibited adiponectin directly increases nitric oxide and adenosine monophosphate–activated kinase that may indirectly reduce CRP and IL-6 that indicates decreased inflammation [161]. Reduced circulating adiponectin is associated with the development of cardiovascular disease and metabolic syndrome [162], T2D and increased IL-6 [163]. The Figure 4 displays adiponectin’s function.
Figure 4: Adiponectin function

Circulating adiponectin concentrations are negatively associated with body mass index and inflammatory status, cardiovascular disease, insulin resistance and type 2 diabetes. Circulating adiponectin concentrations are positively associated however, with type 1 diabetes while it is expressed more in females than in males.

Recent research suggests that a 120-minute aerobic exercise bout at 50% of VO$_2$max did not affect circulating adiponectin concentrations immediately after the exercise bout in healthy lean males [164]. Also, a 60-minute of aerobic exercise at 65% of VO$_2$max did not alter circulating adiponectin concentrations immediately after the exercise bout in healthy males and females [165]. Circulating adiponectin did not also change immediately after a 75-minute brisk walking session in children with type 1 diabetes [166]. Another study showed that 60 minutes of cycling at 50% of VO$_2$max did not alter circulating adiponectin neither immediately after the exercise bout nor 30 minutes post-exercise in adult healthy men [167]. In contrast, 20 minutes of high intensity rowing significantly reduced circulating adiponectin immediately and 30 minutes after the exercise bout in athletes [12]. In general, acute exercise has been shown not to influence circulating adiponectin while evidence from a systematic review
shows that short-term high intensity resistance and aerobic exercise that changes body composition may increase adiponectin [161].

The effects of chronic exercise on adiponectin however, seems to be different than the effects of acute exercise. Indeed, a chronic aerobic exercise intervention (7 months cycling, 60-70% VO$_2$max, 4-5 days/week) in obese Japanese individuals increased adiponectin concentrations that was relative to the amount of weight loss [13]. Similarly, circulating adiponectin was increased in response to a school-based exercise programme in “normal weight” American adolescents [168] and in obese adolescents boys after a 2-month exercise intervention in combination with caloric restriction [147]; the improvement in adiponectin concentrations was accompanied by an improvement in body composition, lipid oxidation and insulin sensitivity [147]. In obese adolescents girls, however, more training than in boys is needed to achieve similar responses for adiponectin [169]. Finally, a 12-week exercise programme did not increase circulating adiponectin in overweight and obese adolescent girls, even though an improvement in insulin sensitivity was found [170]. Overall, chronic exercise may increase circulating adiponectin that may be accompanied by increased insulin sensitivity and weight loss. The effects of acute and chronic exercise on adiponectin in humans can be found in Table 3.

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Exercise mode</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punyadeera et. al. 2005</td>
<td>Healthy lean males</td>
<td>120 min of aerobic exercise at 50% of VO$_2$max</td>
<td>No changes in adiponectin</td>
</tr>
<tr>
<td>Ferguson et al. 2004</td>
<td>Healthy males and females</td>
<td>60 min of aerobic exercise at 65% of VO$_2$max</td>
<td>No changes in adiponectin</td>
</tr>
<tr>
<td>DRCN group 2008</td>
<td>Children with type1 diabetes</td>
<td>75 min of brisk walking</td>
<td>No changes in adiponectin</td>
</tr>
<tr>
<td>Numao et. al. 2008</td>
<td>Adult healthy men</td>
<td>60 min of cycling at 50% of VO2max</td>
<td>No changes in adiponectin</td>
</tr>
</tbody>
</table>
Jürimäe et al. 2005  Athletes rowers  20 min at maximum intensity  ↑ adiponectin
Kondo et. al. 2006 Obese Japanese  7 months cycling, 60-70% VO₂max, 60 min/day, 4-5 days/week  ↑ adiponectin
Carrel et al. 2009 American adolescents  School-based exercise programme  ↑ adiponectin
Elloumi et al. 2009 Obese adolescent  2-month, 4 days/wk, 90 min of warm-up and games  ↑ adiponectin with caloric restriction
Park et al. 2007 Obese adolescents girls  12-wk of lifestyle & exercise intervention  No changes in adiponectin
Nassis et al. 2005 Overweight & obese adolescent girls  12-wk, 40 min, 3 days/wk, >150 beats/min  No changes in adiponectin

VO₂max: Maximal oxygen consumption; DRCN: Diabetes Research in Children Network (DirecNet) Study Group; wk:Week.

2.4. BROWN ADIPOSE TISSUE

BAT displays different functions and morphological properties to WAT. BAT displays more mitochondria than WAT while it highly expresses the co-activator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) [25] that is responsible for mitochondria biogenesis in response to external stimuli [171, 172]. Also, BAT is characterized by increased expression of the uncoupling protein one (UCP1) that is the only known contributor in NST [173], the main function of BAT.

Table 4 displays the main differences between BAT and WAT.

<table>
<thead>
<tr>
<th></th>
<th>WAT</th>
<th>BAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Ivory or yellow</td>
<td>Brown</td>
</tr>
<tr>
<td>Function</td>
<td>Storage of energy</td>
<td>Storage of energy</td>
</tr>
<tr>
<td>Mitochondrial number</td>
<td>Small number</td>
<td>High number</td>
</tr>
<tr>
<td>UCP1</td>
<td>Absent</td>
<td>High</td>
</tr>
<tr>
<td>PCG-1α</td>
<td>Present</td>
<td>High</td>
</tr>
<tr>
<td>SNS innervation</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

WAT: White adipose tissue, BAT: Brown adipose tissue, UCP1: Uncoupling protein one, PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, SNS: Sympathetic nervous system
BAT in humans is located in small amounts in the neck and thorax (specifically in the area of the lower neck and collarbone), as well as along the spine and abdomen [21]. Lipolysis of brown adipocytes produces heat to maintain body temperature as a response to cold exposure [25]. This mechanism is termed NST and occurs through the release of norepinephrine from SNS that activates adrenergic receptors on the surface of brown adipocytes [25]. Subsequently, in the mitochondria the unique protein UCP1 is involved in the respiratory chain process and bypasses the ATP synthase entry by uncoupling protons and brings them back into the mitochondrial matrix allowing the energy to be dissipated as heat [25]. The NST phenomenon is displayed in Figure 5.

**Figure 5:** The non-shivering thermogenesis phenomenon.

The neurotransmitter norepinephrine (NE) that released from the sympathetic nervous system (SNS), binds to β-adrenergic receptor on the surface of brown adipocytes. This results in the activation of lipases which hydrolyze triacylglycerols (TG) in free fatty acids (FFA). The FFA are then bounded to fatty acids binding proteins and transferred into mitochondria where they undergo β-oxidation and enter Krebs cycle. This leads to respiratory chain process in which protons pumped across the mitochondrial inner
membrane. In a normal pathway in the eukaryotic cells the latter would cause an electrochemical gradient formation during the entrance of protons in the mitochondrial matrix and subsequently would generate adenosine triphosphate (ATP) synthase. The presence of uncoupling protein one (UCP1) in brown adipocytes however, bypasses the ATP synthase entrance and return protons to the mitochondrial matrix through UCP1. As a result, the energy of the electrochemical gradient is dissipated as heat.

SNS: Sympathetic nervous system; NE: Norepinephrine; TG: triacylglycerol; FFA: Free fatty acids; ATP: Adenosine triphosphate; UCP1: Uncoupling protein one.

Under cold exposure NST occurs that may consume up to 30% of daily REE [174, 175] compared to diet-induced thermogenesis that consumes approximately 5-10% of daily REE in adults [176]. Nevertheless, shivering thermogenesis cannot occur over prolonged periods [176]. It is well known that BAT is highly expressed in infants (approximately 1% of their body weight) [177]. However, it was believed that after the first decade of human life, BAT becomes inactive and it is only found in the area of kidneys, the supraclavicular area, the aorta and the mediastinum [27]. This changed in 2009, when two studies confirmed that BAT is activated in the area beyond the chest in adults after 2-hour exposure at 16-19°C, and that it is positively associated with the daily REE of the participants [21, 22]. It was also hypothesized that approximately 100 gr of BAT might produce energy equal to the activity of 3-4 kg of WAT [22], due to the higher oxidative capacity of brown adipocytes [178].

Following these discoveries, BAT activity has been extensively investigated for its potential role in mammals’ metabolism. Animal studies have shown that increased BAT activity reduces weight gain, up-regulates insulin sensitivity, lowers free fatty acid levels in serum, as well as the risk for T2D and other metabolic disorders [179-183]. Studies in humans revealed that BAT activity is significantly lower in overweight and obese individuals than in lean, healthy males [21], while BMI [28], age [29], and fat mass [21] are inversely associated with BAT activity. BAT was found to be more active in young individuals and women than in older men, respectively [28].
2.4.1. The link between brown adipose tissue and exercise

There are limited scientific data on the links between exercise and classical BAT in humans that located beyond the chest and along the spine. To the best of our knowledge, only one such study is available and this was in cancer patients [29]. This study revealed that individuals with higher physical activity levels display more BAT activity, and that BAT activity was inversely associated with BMI and age [29]. Similarly, animal studies suggest that chronic exercise may increase BAT activity. Indeed, exercise may increase PGC-1α and peroxisome proliferator activated receptor delta as well as nuclear respiratory factor 1 that enhance NST [184]. Similarly, genetically modified obese mice [B6.Cg-m Lepr<sup>db</sup>/++/J(db/db)] on a six week swimming programme of 2 hours/day at 35°C water temperature significantly improved UCP1 mRNA and protein expression in BAT [185]. However, this study was conducted on genetically obese mice [185], that might not represent an increase of energy metabolism. Indeed, obese mice generally display different eating habits and metabolic efficiency in relation to wild type mice [186], which may influence BAT activity. On the other hand, evidence also showed that chronic exercise did not increase the mRNA of UCP1 in BAT of wild type mice [187]. Similarly, an eight week running programme (90 minutes/day) showed no differences in UCP1 mRNA in BAT in pathogen-free rats [188], while a two week swimming programme (30 minutes/day) in wild type mice [189] and a twelve week’s swimming for 90 minutes/day in wild type high-fat mice [30] reported similar results. By contrast, an eight-week treadmill running programme increased UCP1 mRNA in BAT in normal diet mice, compared to inactive mice [190].

A high-fat diet in combination with chronic exercise did not change BAT mass [191-193] in wild type rats. Another study reports that exercise in combination with
high-sugar diet reduced UCP1 mRNA in BAT [193], probably because the diet-induced thermogenesis that increased by the high-sugar diet. A seven-day running programme (60 minutes/day) in diet-induced mice, given ephedrine, increased levels of UCP1 mRNA in BAT [194]. Other evidence showed that chronic exercise increased BAT mass in genetically modified animals (Otsuka Long Evans Tokushima fatty rats characterized by early-onset hyperphagia-induced obesity and Male Wistar obese insulin-resistant rats) [191, 195]. However, these modified animals usually display different metabolic efficiencies compared with wild type mice [196]. Interestingly, a 4-week treadmill running programme augmented UCP1 mRNA in BAT in mice, but only when both exercise and cold stimuli were present [184].

The mRNA measurements have been extensively used to determine BAT functions however, the mRNA of genes even though they usually indicate the protein production sometimes they do not predict it [197-199]. This is because one mRNA transcription may encode the protein more than one time [197-199]. Therefore, in several experiments measurements of protein expression may also be needed in order to determine the physiological mechanisms. Indeed, in vitro measurements revealed increased UCP1 protein expression in BAT in exercised high-fat diet mice compared to sedentary high-fat diet mice [190]. There is also evidence that exercise increased BAT mass in wild type mice with or without high-fat diet and this was further confirmed by increased UCP1 protein expression in BAT cells [190]. In general, the studies to date show contrasting results mostly in UCP1 mRNA measurements, mainly because of the different experimental approaches, different types of animals and/or different types of exercise. The effects of exercise on BAT can be found in Table 5.
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants/animals</th>
<th>Exercise mode</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinas et. al. 2015</td>
<td>Cancer patients</td>
<td>Associations between habitual physical activity levels and BAT</td>
<td>Positive association of physical activity levels with BAT</td>
</tr>
<tr>
<td>Seebacher et al. 2010</td>
<td>Wild rats</td>
<td>4-wk wheel running (30 min/day) at 22°C or 12°C</td>
<td>† UCP1 with combined exercise and cold</td>
</tr>
<tr>
<td>Oh et al. 2007</td>
<td>Obese genotype: “B6.Cg-m Lepr\textsuperscript{db}/+ +/J” mice; Lean genotype: “B6.Cg-m Lepr\textsuperscript{db}/+ /J” mice</td>
<td>6-wk swimming (2 h/day) at 35°C</td>
<td>† UCP1 in obese female † UCP1 in lean male</td>
</tr>
<tr>
<td>De Matteis et al. 2013</td>
<td>Sprague Dawley rats</td>
<td>1-wk (acute) and 6-wk (chronic) treadmill running, 5 days/week, at 18 m/min (~ 60% VO\textsubscript{2max}) 60 min/day at ~ 22°C</td>
<td>No changes in UCP1</td>
</tr>
<tr>
<td>Boss et al. 1998</td>
<td>Pathogen free rats</td>
<td>8-wk treadmill running (10-90 min/day) at 22±2°C</td>
<td>No changes in UCP1</td>
</tr>
<tr>
<td>Tsuboyama-Kasaoka et al. 1998</td>
<td>C57BL/6 mice</td>
<td>2-wk swimming (30 min/day) at 35°C</td>
<td>No changes in UCP1</td>
</tr>
<tr>
<td>Boström et al. 2012</td>
<td>C57BL/6 mice</td>
<td>3-wk free wheel running (8 km/night)</td>
<td>No changes in UCP1</td>
</tr>
<tr>
<td>Xu et al. 2011</td>
<td>C57BL/6 mice</td>
<td>8-wk treadmill running (5 day/wk, 40 min/day) at 25°C</td>
<td>† UCP1 in normal diet † UCP1 in high fat diet</td>
</tr>
<tr>
<td>de Queiroz et al. 2012</td>
<td>Wistar rats</td>
<td>8-wk running (5 days a week, 30-60 min/day) at 23±1°C</td>
<td>No changes in BAT mass. † UCP1 either exercise or high-sugar diet alone ‡ UCP1 with combined exercise and high-sugar diet</td>
</tr>
<tr>
<td>Slocum et al. 2013</td>
<td>Sprague Dawley rats</td>
<td>1-wk (acute) and 6-wk (chronic) treadmill running, 5 days/week, at 18 m/min (~ 60% VO\textsubscript{2max}) 60 min/day at ~ 22°C</td>
<td>No changes in BAT mass and UCP1.</td>
</tr>
<tr>
<td>Bueno et al. 2011</td>
<td>Male Wistar rats &amp; Male Wistar obese insulin-resistant rats</td>
<td>4-wk swimming at 32–36 °C, 1.5 h/day</td>
<td>No changes in BAT mass in Male Wistar rats. † BAT mass in Male Wistar obese insulin-resistant rats</td>
</tr>
</tbody>
</table>
2.4.2. The link between brown adipose tissue and nutrition

Studies in animals revealed that low protein diet may increase REE by involving activation of BAT [200, 201]. Also, studies showed that high-fat diets enhanced cold tolerance and survival of animals [202-204] and increased animals’ mitochondria in BAT [205]. More recently, it was found that a ketogenic diet (high-fat, adequate-protein and low-carbohydrate) increased the number of mitochondria and doubled the UCP1 protein expression in BAT, while it increased the UCP1 protein expression in epididymal WAT of mice independently of physical activity levels [206]. Furthermore, a ketogenic diet intervention in mice increased by 40% the size of mitochondria and 1.5-3 fold the UCP1 protein expression in BAT compared to chow-fed diet [207]. The explanation of the latter findings is that diet-induced thermogenesis is fully dependent on UCP1 activity while it is also controlled by the UCP1 even though it is unclear if this mechanism can significantly increase NST [208]. Overall, UCP1 may boost diet-induced thermogenesis in the BAT of mice, while its absence may increase obesity in mice fed with high-fat or normal diet [208].

2.5. BROWN-LIKE ADIPOSE TISSUE

Brown-like adipocytes are cells that display properties similar to brown adipocytes and mainly occur within WAT depots. A number of theories explain their origin. These include: a) brown-like adipocytes are the result of the maturation of brown adipocytes...
b) brown-like adipocytes are split from a precursor or from a mature white adipocyte [178, 210-212] and c) brown-like adipocytes are the result of a precursor of muscle cells [213]. It should also be noted that the classical brown adipocytes come from the stem cell originator “myogenic factor 5”, the same as the skeletal muscle cells [214]. However, the brown-like adipocytes are not positive in myogenic factor 5 protein [214]. The main index that characterizes the brown-like adipocytes is the presence of the UCP1 [215]. During the 1980s it was first reported that some animal white adipocytes expressed UCP1 [216-219]. The UCP1 protein acts in mitochondria of brown adipocytes to dissipate chemical energy to produce heat [220] and is the only known contributor for NST that occurs in BAT [25]. Therefore, its presence in a white fat cell may indicate brown-like function. In this regard, the majority of the white fat cells that display UCP1 show brown-like fat properties and not brown fat properties [221].

It is suggested that brown-like adipocytes are metabolically active to reduce body weight and consequently lessen obesity [222]. Brown-like adipocytes have a role in metabolism regulation in humans, even though the mechanisms are not well established [223]. Women display more active brown-like adipocytes than men [28]. Their expression is low in obese individuals, but this increases after weight loss and bariatric surgery [224]. There are also food components that may cause a browning process of WAT in animals that include the reduction of amino-acids and high fat diet [225]. This browning process of WAT is also indicated by a study that showed that exercise increased the positive UCP1 white adipocytes in exercised high-fat diet mice, but not in sedentary high-fat diet mice [190]. It also showed that the PGC-1α mRNA within the white adipocytes decreased in sedentary high-fat diet mice compared to
sedentary normal-diet mice [190], probably indicating a role of diet in browning formation of WAT.

Apart from UCP1, the presence of other genes within white adipocytes may also indicate a brown-like function of human WAT. These include PGC-1α [220] and peroxisome proliferator-activated receptor gamma (PPARγ) [215], which are involved in the development of brown fat cells. PGC-1α is responsible for mitochondrial biogenesis [171] of the external stimuli [172] and it is mainly expressed in skeletal muscle and BAT but, it is also present in WAT [226]. Mice with transgenic expression of PGC-1α in the muscle displayed more brown-like than white adipocytes in subcutaneous adipose tissue [30]. PPARγ is also necessary for the function of both brown and white adipocytes and displays similar pathways in both tissues. When PPARγ is mutated (P465L mutation) brown adipocytes show a reduction in thermogenic capacity through the reduction of UCP1, but mutated (P465L mutation) PPARγ can still contribute in the development of white adipocytes [227]. This may indicate a key role of PPARγ in WAT that may be related to increased UCP1 mRNA [209, 228], which may specify brown-like function. Finally, evidence suggests that PPARα activates UCP1 within the white adipocytes in response to exercise [30], leading to brown-like properties of the cells. A specific PPARα antagonist (GW6471) treatment in vitro decreased UCP1 in white adipocytes while PPARα was found to be driven by an exercise-dependent protein (FNDC5/Irisin) [30]. However, PPARα may not be the only activator of UCP1 even though the other mechanisms remains to be established [30, 229]. Overall, there is only limited evidence concerning the transformation of white into brown-like adipocytes, especially in response to exercise. Given that the formation of brown-like adipocytes via exercise may improve glucose homeostasis and thus may reduce metabolic diseases [30], a further exploration of
this formation is needed. However, the role of physical activity and exercise on the UCP1, PGC-1α, PPARα and PPARγ mRNAs of WAT in humans has not been extensively investigated to date.

2.5.1. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)

PGC-1α is a transcriptional co-activator that regulates genes involved in energy metabolism. It is responsible for several functions, including the control of the cellular response to oxidative stress [230]. It is also responsible for mitochondrial biogenesis [171] of the external stimuli [172]. It does this by activating the production of the Nuclear Respiratory Factor One (NRF-1) and NRF-2, stimulators of the mitochondrial transcription factor A, which duplicates mitochondrial DNA [231]. PGC-1α is mainly expressed in tissues that demand energy, such as skeletal muscle and cardiac muscle, but it is also present in WAT [226]. Evidence indicates that PGC-1α is inversely associated with age in mice [231], while mitochondrial dysfunction in mice is positively associated with depressed PGC-1α levels [232]. PGC-1α deficiency in mice leads to insulin resistance, while increased PGC-1α mRNA in skeletal muscle leads to increased mitochondrial density [233]. PGC-1α is also highly expressed in BAT [234] and it co-activates with PPARγ to stimulate genes involved in brown adipocytes differentiation [234, 235].

Evidence suggests that PGC-1α mRNA is increased in response to both acute [31, 33, 236, 237] and chronic exercise [32, 34, 236, 238] in human skeletal muscle. This may be due to increased demands of ATP and, therefore, increased demands of mitochondria [239]. However, studies that measured PGC-1α protein expression in skeletal muscle show a large range of the protein molecular weight from 70 to >110
Kda [240-242] that may reflect both the antibodies used to measure the protein and the stimuli itself (i.e. exercise). A theory suggests that the link between PGC-1α and exercise may be the result of free radicals that are produced in response to exercise stress, which may trigger a signal to increase mitochondrial biogenesis [231, 243]. PGC-1α increases the expression of catalase and superoxide dismutases that may protect against oxidative stress caused by free radicals [244] while antioxidant supplements may inhibit mitochondria production during exercise via the reduction of PGC-1α [243]. This indicates that the production of free radicals during exercise may increase mitochondrial biogenesis and thus, may increase PGC-1α. Indeed, PGC-1α may be increased in WAT in response to exercise [245].

2.5.2. Peroxisome proliferator-activated receptor alpha (PPARα)

PPARα is a transcriptional factor that regulates genes to increase lipid metabolism [246] and is involved in mitochondrial fatty acid beta oxidation [247]. PPARα is a member of the PPAR family that also includes, PPARβ, PPARγ and PPARδ that are nuclear receptors for fat vitamins, steroid hormones, and sterols [247, 248]. Also, PPARα increases fatty acid oxidation in liver and the production of hepatic glucose to normalize responses to fasting [247]. While PPARα mRNA was found to be higher in the skeletal muscle of trained than untrained individuals [249], it was also increased in the skeletal muscle of mice after a chronic high-intensity interval exercise programme [250]. Another animal study suggested that chronic exercise can prevent weight gain, adiposity and hypertrophy of adipocytes, in part, via the activation of PPARα [251].

Recent evidence also suggests that chronic activation of PPARα via a pharmaceutical method increased the brown-like cells in subcutaneous WAT of mice, which was also accompanied by the elevation of PGC-1α and UCP1 [252].
Specifically, high-fat diet animals were administered with Fenofibrate, which increased the mRNA of PPARα in subcutaneous WAT. This caused a decrease in body weight and enhanced insulin sensitivity. The increased mRNA of PGC-1α and UCP1 within the white adipocytes indicates a predominant role of PPARα in browning formation of WAT in animals [252]. Evidence also suggests that PPARα plays a role in increasing UCP1 in human white adipocytes in response to chronic exercise [30]. However, this suggestion is based on a human white adipocytes pharmacological treatment that inhibited PPARα and subsequently UCP1 [30]. In general, PPARα may play a role in browning formation of WAT, a role that should be further explored.

2.5.3. Peroxisome proliferator-activated receptor gamma (PPARγ)

Another member of the PPAR genes family is the transcriptional factor PPARγ, discovered to be active in BAT [253]. Its main function is to regulate fatty acid storage and glucose metabolism [254]. PPARγ demonstrates the highest expression of the PPAR family genes in adipose tissue, particularly in BAT [255]. PPARγ has also an adipogenic effect in BAT of animals through its effect on various genes involved in lipid synthesis [255, 256]. A chronic treatment of animals with PPARγ led to increased brown adipocytes within BAT [257]. PPARγ may also be responsible for both brown and white adipocytes’ survival [255]. Evidence also shows that PPARγ knockout mice increased insulin resistance [258] while they failed to generate adipose tissue when fed a high-fat diet [259].

Previous research has also investigated whether PPARγ can trigger a transformation of white into brown-like adipocytes via the UCP1 mRNA. PPARγ may increase the UCP1 mRNA in subcutaneous white fat of mice [228]. Indeed, a treatment of white adipocytes with PPARγ ligands for several days showed that PPARγ may
cause a browning effect of WAT by enhancing UCP1 mRNA [209]. Chronic exercise also increased PPARγ mRNA in WAT of mice [260]. A recent study showed that a nine week aerobic exercise programme in mice increased the PPARγ mRNA in epididymal WAT compared to control counterparts [261]. Interestingly, PPARγ mRNA in epididymal WAT was significantly increased in a group of chronic exercised rats after a four-week de-training period compared to a group of sedentary rats probably because an adipogenic effect that was triggered during the de-training period [262]. However, other evidence indicates that the protein levels of PPARγ of subcutaneous fat of mice did not alter in response to an eight-week exercise programme [263]. Finally, given that PPARγ may play a role in the browning process of WAT and probably responds to exercise stimuli in WAT, further research is needed to designate this.

2.5.4. Brown-like adipose tissue and physical activity/exercise

A study conducted in both humans and mice revealed a novel mechanism regarding the role of exercise in brown-like adipocytes functions. Given that exercise increases the PGC-1α mRNA in skeletal muscle, it was suggested that PGC-1α up-regulates the fibronectin type III domain-containing protein 5 (FNDC5) [30]. Subsequently, the FNDC5 protein is cleaved and released into the bloodstream as Irisin – a newly discovered protein – that is attached, via an unknown receptor, on the surface of white adipocytes to generate a cascade of events changing the function of white adipocytes into brown-like adipocytes [30]. The latter was confirmed by the presence of UCP1 mRNA in the white adipocytes, which probably was increased in part by the PPARα involvement [30]. UCP1 is the only known contributor in NST in brown adipocytes and its presence in a white fat cell indicates brown-like fat cell function [223]. The Irisin mechanism is displayed in Figure 6.
Exercise increases the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), which causes the production of the fibronectin type III domain-containing protein 5 (FNDC5) within the skeletal muscle. FNDC5 is then cleaved and released into the bloodstream as Irisin. Irisin is then attached on the surface of white adipocytes that may increase the peroxisome proliferator-activated receptor alpha (PPARα) and uncoupling protein one (UCP1). This may increase energy expenditure, weight loss while it improves glucose homeostasis as well as insulin sensitivity. These are properties of a brown adipose-like phenotype.

Furthermore, when Irisin was administered in obese mice it improved glucose homeostasis and caused weight loss [30]. Given that brown-like adipocytes may have antidiabetic effects on mice [264] and humans display white adipocytes that contain UCP1 [265], it was suggested that Irisin may have a therapeutic role in humans. The therapeutic role of Irisin has also been investigated in humans showing that circulating Irisin concentrations are inversely correlated with blood glucose, insulin and cholesterol indicating a positive effect on metabolism [266].

The potential therapeutic effect of Irisin on humans has also been considered with caution. For instance, FNDC5 mRNA in muscle is not significantly increased by exercise and Irisin probably is not regularly activated by exercise in humans [267].
Timmons et al. (2012) also showed that FNDC5 mRNA is 30% greater in older compared to younger adults, indicating that this gene is not equally expressed in humans [267]. Several studies have also investigated the effects of exercise on circulating Irisin concentrations and UCP1 in WAT with disparate results. While some show that acute exercise increases circulating Irisin [268, 269], others show no such effects [33, 270]. There is also evidence that circulating Irisin increased in response to chronic exercise [271] or not altered at all [33]. A recent study indicates that chronic exercise increases PGC-1α and FNDC5 mRNA in muscle, while the UCP1 mRNA in subcutaneous WAT was increased yet, not significantly [236]. Another study demonstrated for the first time that adults with high habitual physical activity levels display more BAT activity in supraclavicular and spinal areas compared to adults with low physical activity levels [29]. The latter may be of interest for further research, given that brown-like adipocytes are present within classical brown fat depots in the supraclavicular and spinal areas in adult humans [272, 273].

Given the existing evidence, the question is whether the Irisin mechanism is functional and causes a brown-like function in white adipocytes. Recent evidence indicates that the start codon of the FNDC5 gene is mutated in humans [274]. This suggested that lead to a short-length Irisin production into the bloodstream, which misses almost 50% of its sequence. The same evidence shows no effect of FNDC5 and Irisin on human white adipocytes and thus, the function of Irisin observed in mice may be lost in humans [274]. However, concerns have been raised about this due to the type of antibody used for Irisin detection [275] and the recent revelation that the studies examining circulating Irisin that used commercial antibodies may not be accurate [35]. This is because the available antibodies may attract other proteins, which suggests that the circulating Irisin value measured also includes cross-reacting
proteins [35]. Recent evidence also shows that when Irisin is measured via mass spectrometry [270, 276], the Irisin concentrations appeared to be ~10-100 times lower than the values displayed when commercial antibodies were used. Finally, given that several animal studies showed that exercise may promote brown-like properties of subcutaneous WAT [245, 277-279], the Irisin mechanism should be further explored. A first step could be a systematic review of the existing literature.
3. CHAPTER 3: RATIONALE, AIMS & HYPOTHESES

3.1. RATIONALE

Recent evidence indicates that exercise may change the mode of white adipocytes into brown-like mode [30]. As noted above, UCP1 is only responsible for heat production in the human body through its function in BAT and its presence in a white adipocyte may indicate brown-like function [229]. As such, research studies hypothesized that UCP1 may prevent the development of obesity [208] given that UCP1 activity triggers NST that increases REE, which may reduce weight gain through the development of brown-like adipocytes in mice [179, 280]. These UCP1 properties indicate its positive effect on the metabolism and its importance in improving health outcomes. UCP1 may be increased in WAT through exercise that ameliorates PGC-1α mRNA and FNDC5 protein within the skeletal muscle [30]. Subsequently, the FNDC5 protein is cleaved and released into the bloodstream as Irisin that is attached, via an unknown receptor, on the surface of white adipocytes to increase UCP1 within white adipocytes [30]. However, this mechanism displays several limitations and the evidence to date is controversial especially in humans while the evidence regarding the effects of exercise on UCP1 in WAT in humans is very limited. The current PhD study examined for the first time the aforementioned mechanism in humans via a systematic review process according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines using tools from the Cochrane Library. The current PhD study also examined for the first time the effects of exercise training and de-training on the UCP1 mRNA of WAT in healthy adults in a parallel RCT design.

There are also other genes that may indicate browning formation of WAT. PGC-1α regulates the expression of several genes and indicates brown-like function via its interactions with PPARα and PPARγ (even though the mechanisms are not fully
defined) [234, 281, 282]. PGC-1α is highly expressed in BAT [283] and it co-activates with PPARγ to stimulate genes involved in brown adipocytes differentiation [234, 235]. It is also increased in WAT in response to exercise [245], which may indicate an increased demand of WAT energy during exercise. Recent evidence also suggests that chronic activation of PPARα via a pharmaceutical method increased the brown-like adipocytes in subcutaneous WAT of mice, which was also accompanied by the elevation of PGC-1α and UCP1 [252]. However, these findings have not been confirmed by human studies. Indeed, a study showed that PPARα may increase UCP1 in human white adipocytes in response to chronic exercise, but this was in vitro observation [30]. PPARγ may also increase the UCP1 mRNA in subcutaneous white fat of mice [228, 260]. Indeed, a treatment of white adipocytes with PPARγ ligands for several days showed that PPARγ may enhance UCP1 mRNA that causes a browning effect of WAT [209]. Therefore, it could be hypothesized that physically active individuals display more the expression of the aforementioned genes in WAT and subsequently display a browning process of WAT. Nevertheless, the relationship of the expression of the aforementioned genes (UCP1, PGC-1α, PPARα, PPARγ) in WAT with physical activity levels in humans has not been investigated to date. The current PhD study examined for the first time this relationship in healthy adults in a cross-sectional design study.

It is well established that leptin is a key factor in the regulation of fat mass in humans. Increased circulating leptin concentrations are associated with increased risk of inflammation and increased fat mass [120]. Leptin is suppressed in response to cold exposure that may be accompanied by the reduction of fat mass [134]. SNS is the main regulator of leptin and reduces the production of this protein [137]. Evidence suggests that norepinephrine suppresses leptin production [122, 123], while
norepinephrine is necessary in BAT function [25]. This evidence indicates an inverse relationship between leptin and BAT activity. Data show that increased leptin sensitivity stimulates lipolysis in adipose tissue and hepatic fatty acid uptake and oxidation [126]. Recent data, however, show that increased inflammatory cytokines and free fatty acid levels were associated with impaired leptin sensitivity in mice [120, 127]. Actually, circulating leptin in humans has been associated with greater levels of systemic inflammation and fat mass [120]. On the other hand, decreased circulating adiponectin concentrations are associated with increased circulating levels of free fatty acids [156] and increased glucose production from the liver [156, 284]. Chronic exercise, however, may reduce circulating leptin particularly when exercise training is accompanied by a reduction in fat mass [120, 141, 144, 145]. It may also reduce circulating leptin concentrations and increase circulating adiponectin concentrations in overweight and obese individuals [285]. Nevertheless, the link between different types of chronic exercise and de-training with leptin and adiponectin mRNA of WAT has not been extensively investigated in healthy individuals. Therefore, this PhD study examined the effects of different types of exercise and de-training on leptin and adiponectin mRNA of WAT in healthy adults in a parallel RCT design.

The general aim of this PhD study was to investigate both the relationship of physical activity to browning formation and the effects of different exercise programmes on browning formation of subcutaneous WAT in humans using genes identified from the literature to be involved in this process. It also aimed to examine the effects of chronic exercise on leptin and adiponectin genes of WAT in humans. Given the above the specific aims and hypotheses of the current PhD study were:
3.2. AIMS

*Study 1:* To systematically investigate the literature of the effects of physical activity on the link between PGC-1α and FNDC5 in muscle, circulating Irisin and UCP1 of WAT in humans.

*Study 2:* To examine the relationship between the UCP1 mRNA and protein expression, as well as the mRNA of PGC-1α, PPARα and PPARγ genes of subcutaneous WAT in healthy men with their physical activity levels in a cross-sectional design study. A secondary aim was to examine the associations of the UCP1 mRNA and protein expression, as well as the mRNA of PGC-1α, PPARα and PPARγ genes with REE, age, fat mass, fat free mass, energy intake, WHR and BMI.

*Study 3:* To examine the effects of different types of exercise and de-training on the UCP1 mRNA and protein expression of subcutaneous WAT in healthy untrained men, in a parallel RCT design. A secondary aim was to examine the effects of different types of exercise and de-training on the mRNA of PGC-1α, PPARα and PPARγ genes.

*Study 4:* To examine the effects of different types of exercise and de-training on the leptin mRNA of subcutaneous WAT in healthy untrained men, in a parallel RCT design. A secondary aim was to examine the effects of different types of exercise and de-training on the adiponectin and leptin to adiponectin (L/A) ratio mRNA.

3.3. HYPOTHESES

*Study 1:* Physical activity may increase PGC-1α that increases FNDC5 in muscle, circulating Irisin and UCP1 within WAT in humans.

*Study 2:* mRNA and protein expression of UCP1, as well as mRNA of PGC-1α, PPARα and PPARγ genes of subcutaneous WAT of healthy men will be positively associated with increased physical activity levels and that the mRNA and protein expression of
UCP1, as well as mRNA of PGC-1α, PPARα and PPARγ genes will be positively associated with REE, fat free mass and negatively associated with energy intake, WHR, BMI, age and fat mass.

**Study 3:** Different types of chronic exercise will increase and the following de-training period will decrease the UCP1 mRNA and protein expression in the subcutaneous WAT of healthy untrained men and that the mRNA of PGC-1α, PPARα and PPARγ genes will be increased in response to exercise and decreased in response to de-training.

**Study 4:** Different types of chronic exercise will decrease and the following de-training period will increase the leptin mRNA in subcutaneous WAT of healthy untrained men. Also, different types of chronic exercise will increase and the following de-training period will decrease the adiponectin mRNA while different types of chronic exercise will decrease and the following de-training period will increase L/A ratio mRNA in subcutaneous WAT of healthy untrained men.
4. CHAPTER 4: METHODS USED IN PhD STUDY

4.1. ETHICAL APPROVAL

This PhD study conformed to the standards set by the Declaration of Helsinki and was approved from the Ethics Committees of the University of Wolverhampton (protocol number 92944/13-6-2013) and the University of Thessaly (protocol number 698/13-3-2013) (Appendices 1 and 2, respectively).

4.2. APPROACH

The current PhD is consisted of four studies. a) A systematic review to identify evidence regarding the effects of physical activity on the brown-like formation of WAT in humans. Also, 46 healthy men were recruited to conduct b) a cross-sectional study to identify associations of the mRNAs and protein expression of genes that indicate browning formation of subcutaneous WAT with physical activity levels. From the 46 healthy men a subset of 32 individuals were recruited to conduct an RCT. This RCT was split into c) a study to examine the effects of exercise and de-training on the UCP1 mRNA and protein expression, and d) a study to examine the effects of exercise and de-training on the leptin mRNA of subcutaneous WAT.

4.3. SYSTEMATIC REVIEW METHOD

4.3.1. Search strategy

Using the PRISMA guidelines [286-288] two databases [(PubMed and EMBASE (1980-2015))] were searched up until the 14th September 2015. Two investigators independently conducted two identical searches in both databases using the terms that appear in Table 6 (PubMed) and Table 7 (EMBASE). Finally, the reference lists of the included articles were reviewed in order to identify publications that were relevant to the topic under review.
Table 6: Searching algorithm for PubMed
1. PGC1 alpha[Title/Abstract]
2. PGC-1 alpha[Title/Abstract]
3. PGC-1a[Title/Abstract]
4. PGC1a[Title/Abstract]
5. PGC-1alpha[Title/Abstract]
6. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha[Title/Abstract]
7. Peroxisome proliferator-activated receptor gamma coactivator 1-a[Title/Abstract]
8. Peroxisome proliferator-activated receptor gamma coactivator 1 alpha[Title/Abstract]
9. Peroxisome proliferator-activated receptor gamma coactivator 1a[Title/Abstract]
10. Peroxisome proliferator-activated receptor gamma coactivator 1alpha[Title/Abstract]
11. PGC1alpha[Title/Abstract]
12. (#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11)
13. FNDC5[Title/Abstract]
14. Fibronectin type III domain-containing protein 5[Title/Abstract]
15. Irisin-encoding gene[Title/Abstract]
16. (#13 OR #14 OR #15)
17. Irisin[Title/Abstract]
18. PGC1alpha-dependent myokine[Title/Abstract]
19. (#17 OR #18)
20. Uncoupling protein one[Title/Abstract]
21. Uncoupling protein 1[Title/Abstract]
22. UCP1[Title/Abstract]
23. (#20 OR #21 OR #22)
24. (#12 OR #16 OR #19 OR #23)
25. exercise[MeSH Terms]
26. exercise[Title/Abstract]
27. motor activity[MeSH Terms]
28. sports[MeSH Terms]
29. sport*[Title/Abstract]
30. resistance training[MeSH Terms]
31. training[Title/Abstract]
32. fitness[Title/Abstract]
33. physical activity[Title/Abstract]
34. physical activities[Title/Abstract]
35. physical activity intervention*[Title/Abstract]
36. exercise intervention*[Title/Abstract]
37. active[Title/Abstract]
38. aerobic[Title/Abstract]
39. (#25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38)
40. (#24 AND #39)
41. animals[MeSH Terms]
42. humans[MeSH Terms]
43. (#41 NOT #42)
44. (#40 NOT #43)

**Table 7: Searching algorithm for EMBASE**

1. PGC1 alpha.ti,ab.
2. PGC-1 alpha.ti,ab.
3. PGC-1a.ti,ab.
4. PGC1a.ti,ab.
5. PGC-1alpha.ti,ab.
6. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha.ti,ab.
7. Peroxisome proliferator-activated receptor gamma coactivator 1-a.ti,ab.
8. Peroxisome proliferator-activated receptor gamma coactivator 1 alpha.ti,ab.
9. Peroxisome proliferator-activated receptor gamma coactivator 1a.ti,ab.
10. Peroxisome proliferator-activated receptor gamma coactivator 1alpha.ti,ab.
11. PGC1alpha.ti,ab.
12. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11
13. FNDC5.ti,ab.
14. Fibronectin type III domain-containing protein 5.ti,ab.
15. Irisin-encoding gene.ti,ab.
16. 13 OR 14 OR 15
17. Irisin.ti,ab.
18. PGC1alpha-dependent myokine.ti,ab.
19. 17 OR 18
20. Uncoupling protein one.ti,ab.
21. Uncoupling protein 1.ti,ab.
22. UCP1.ti,ab.
23. 20 OR 21 OR 22
24. 12 OR 16 OR 19 OR 23
25. exercise/
26. exercise.ti,ab.
27. motor activity/
28. sport/
29. sport*.ti,ab.
30. resistance training/
31. training.ti,ab.
32. fitness.ti,ab.
33. physical activity.ti,ab.
34. physical activities.ti,ab.
35. physical activity intervention*.ti,ab.
36. exercise intervention*.ti,ab.
37. active.ti,ab.
38. aerobic.ti,ab.
39. 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35
   OR 36 OR 37 OR 38
40. 24 AND 39
4.3.2. Selection criteria
We included studies that met at least one of the following eligibility criteria, a) measurements of PGC-1α in conjunction with measurements of FNDC5, b) measurements of FNDC5, and/or circulating Irisin concentrations and/or UCP1 in WAT, along with the following criteria c) measurements of physical activity levels and/or exercise interventions, and d) human participant study. No other eligibility criteria were set (e.g., language, date of publication). From the included studies, we retrieved outcomes regarding the effects of a) physical activity on PGC-1α in conjunction with FNDC5 in muscle, b) FNDC5 in muscle, c) Irisin in the bloodstream and d) UCP1 in WAT. We report the studies’ design, the participants’ characteristics and the main outcomes. Also, when the Irisin peptide was investigated in any of the studies included, we reported the methods of analysing Irisin (Table 9). We have also recorded the secondary associations in the included studies, i.e. associations between FNDC5 and/or circulating Irisin and several health related phenotypes (e.g. REE, blood pressure, WHR, BMI).

4.3.3. Risk of bias assessment and quality of reporting data
Two independent reviewers evaluated the risk of bias of the studies included in the systematic review via the “Cochrane Collaboration’s tool for assessing risk of bias” [289]. Conflicts in the risk of bias assessment were resolved by a third reviewer. Also, two reviewers evaluated independently the quality of the reporting data of the included RCTs, controlled trials (CT) and single group design studies (SGS) using the Consolidated Standards of Reporting Trials (CONSORT) checklist [290]. This is a 25-item checklist and a score for each study was provided. Also, two reviewers evaluated independently the quality of the reporting data of the included cross sectional studies.
(CSS) using the 22-item checklist of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and we also provided a score for each study included [291]. Disagreements on studies’ CONSORT and STROBE scores were resolved though the agreement refereed by a third reviewer.

4.4. EXPERIMENTAL METHODS

Written consent form (Appendix 3) was obtained from 46 healthy men who were recruited for a cross-sectional study in order to examine the associations of the mRNAs and protein expression of genes that indicate browning formation of subcutaneous WAT with physical activity levels. A subset of 32 out of the 46 individuals undertook an exercise programme followed by a de-training period in a RCT design to assess the effects of exercise and de-training on browning formation and on leptin mRNA of WAT. The measurements in these studies included anthropometry (WHR, BMI, blood pressure and body composition), indirect calorimetry for REE, physical activity levels, subcutaneous fat biopsies to assess mRNA and protein expression of the genes examined, VO₂peak, 1-repetition maximum (1RM) and diet recalls. The reporting of the results of the RCT based on the CONSORT guidelines [290] and of the cross-sectional study based on the STROBE guidelines [291].

4.4.1. Study design of randomized controlled trial

A parallel RCT of an 8-week of exercise training followed by an 8-week of de-training period was performed. The participants were recruited by advertisements in a local newspaper in the area of Trikala, Thessaly, Greece; the experimental protocol started in July 2013 and ended in June 2014. The participants were asked to call the researcher (PhD student) for an initial interview in order to identify eligibility. The
inclusion criteria were: healthy adult men not participated in a regular exercise programme, non-smoker, no chronic disease and/or being under medication treatment. Information of the health status was retrieved through a medical history questionnaire via interview (Appendix 4). Information of the physical activity levels was retrieved via the International Physical Activity Questionnaire (Appendix 5) [292]. Participants, who were healthy, not participated in a regular exercise programme and displayed low or moderate physical activity levels were eligible to participate in the study. If a candidate was eligible to participate in the study he was given a period of seven days to decide his participation. Upon inclusion, a participant was randomly assigned to a group using an online computer software [293]. The initial allocation and enrol of the participants to an experimental group was completed by the researcher (PhD student) while the supervision of the exercise interventions was completed by three assistants, one for each exercise group who were blinded to the aim of the study. The selection process along with reasons for exclusions as well as participant drop outs in the RCT design appear in Figure 7.
Figure 7: CONSORT diagram of the randomized controlled trial design

4.4.2. Experimental protocol for randomized controlled trial

The RCT involved four groups: an aerobic exercise group (AEG), a resistance exercise group (REG), a combined exercise group (aerobic+resistance) (CEG) and a non-exercise control group (CG). Measurements for anthropometry, body composition, REE, and subcutaneous fat biopsy were obtained at baseline, after the exercise (week 8) and the de-training (week 16) periods while physical activity and nutritional data were randomly collected for two weekdays and one weekend day at baseline, 8th and 16th week. The data collection for physical activity and diet was conducted by an
independent investigator who was blinded to the aim of the study. VO\textsubscript{2}peak and 1RM were measured at baseline, 4\textsuperscript{th}, 8\textsuperscript{th} and 16\textsuperscript{th} week. The details of the experimental protocol are displayed in Figure 8.

**Figure 8:** Experimental protocol for randomized controlled trials

![Experimental Protocol](image)

VO\textsubscript{2}peak: Peak oxygen consumption; 1RM: One repetition maximum

### 4.4.3. Anthropometry

The participants visited the laboratory between 07:00 and 09:00 am and the following anthropometry measures took part: Height was measured using a Seca (Hamburg, Germany) device and weight using a scale (KERN & Sohn GmbH, Version 5.3, Germany) while WHR was measured using a tape measure and blood pressure through an acoustic method using an Aneroid sphygmomanometer. Percent body fat and fat-free mass were measured via bioelectrical impedance using a body composition monitor (Fresenius Medical Care AG & Co. KGaA D-61346 Bad Hamburg, Germany).
4.4.4. **Assessment of resting energy expenditure**

REE assessments were conducted between 07:00 to 09:00 am following a 12-hour fast, while participants refrained from exercise, alcohol, and passive smoking in 72-hour prior to the measurements [294, 295]. REE was measured using an automated gas analyser (Vmax, CareFusion, USA) that was attached to the participants to record respiratory variables every 20 seconds in a supine position for 30 minutes in a quiet room of 22-24°C. From the 30 minutes of the collected data, the first and last five minutes were removed [295]. Finally, the remaining 20 minutes of the collected data were averaged to obtain the final REE (kcal) value [295].

4.4.5. **Assessment of physical activity levels**

Physical activity levels were measured through a Digi-Walker SW–200, Yamax, United Kingdom pedometer that was given to the participants immediately after they confirmed their participation in the study. Previous studies have identified the Yamax pedometers as valid and reliable [296-298]. Physical activity levels were measured at baseline, at 8th and at 16th weeks on three separate days (two weekdays, one weekend day). The selection of the three separate days was random, and it was generated by an assistant that was blinded to the aim of the studies of this PhD. The participants were contacted by the assistant to report the outcome of the measurements via telephone. The values of the three days were then averaged to obtain the final value (steps/day). This was also done by an assistant that was blinded to the aim of the studies of this PhD.
4.4.6. Assessment of peak oxygen uptake and 1-repetition maximum

A pre-screening was performed for each participant to identify their eligibility to undertake a VO\(_2\)peak test using the Physical Activity Readiness Questionnaire (PAR-Q) (Appendix 6) [299]. To ensure the familiarization of the participants with the VO\(_2\)peak test a detailed verbal description of the protocol was given in advance. The protocol involved a 5-minute warm-up and a familiarization period of a cycling in a Monark Ergomedic 839E, Vansbro, Sweden. Consequently, the test involved a 3-minute of pedalling at 60 rpm at 60 Watts followed by an increment of 30 Watts/minute until volitional exhaustion [300]. An automated gas analyser (Vmax, CareFusion, USA) was attached to the participants to record respiratory variables every 20 seconds. The highest oxygen uptake (L/min) for any 20-second interval was recorded as the final VO\(_2\)peak value [300].

The 1RM of leg extension and chest press were tested following an indirect method as previously described [301]. A suitable weight was adjusted for each participant in order to not be able to lift it for no more than 10 repetitions. The number of the repetitions were then calculated and a norm was used to predict the 1RM using the weight in kg that each participant lifted and the number of the repetitions that were performed [301].

4.4.7. Subcutaneous fat biopsies

All biopsies were executed by an experienced surgeon following a previous methodology [302] via a non-diathermy method. The participants underwent a subcutaneous fat biopsy after at least an 8-hour fast [303] and they were instructed to refrain from exercise, alcohol, and passive smoking in 72-hour prior the biopsy procedure in order to minimize the risk for misleading results [294, 295]. Each
participant was positioned on a surgical bed in a supine position. The site of the incision was disinfected and a 10 ml of xylocaine 2%-no adrenaline was injected in the region of the incision for local anaesthesia. An incision on the skin and subcutaneous tissue until adipose tissue was revealed, was executed approximately 3-5 cm nearby the navel while the incision length was approximately 2-2.5 cm. Subsequently, the subcutaneous tissue was removed with an operating scissors and when the adipose tissue became visible nearly 500 mg of adipose tissue was captured and removed. The collected adipose tissue was immediately immersed in liquid nitrogen of -190°C. For the final deposition the samples were placed in Eppendorfs and they were deposited in a freezer at -80°C until analyses.

4.4.8. Gene and protein expression analyses

The investigators that performed the gene and protein expression analyses were blinded to the aim of the studies of this PhD research.

Gene expression analysis

Total RNA was extracted from adipose tissue biopsies using RNeasy Lipid Tissue mini kit (QIAGEN) following the manufacturer’s protocol. First-strand cDNAs were synthesized from equal amounts of total RNA using random primers and M-MLV reverse transcriptase (Promega). Quantitative real time polymerase chain reaction for the UCP1, PGC-1α, PPARα, PPARγ, leptin and adiponectin genes was performed using Sybr Green fluorophore. The change in fluorescence at every cycle was monitored and a threshold cycle above background for each reaction was calculated. A melt curve analysis was performed following every run to ensure a single amplified product for every reaction. All reactions were carried out in at least duplicated for every sample. 18S rRNA gene was constantly expressed under all experimental conditions.
and was then used as a reference gene for normalization given that this gene was suggested as the most appropriate one for normalization of UCP1 mRNA [304].

**UCP1 protein expression analysis**

Subcutaneous adipose tissue was homogenized in RIPA Lysis Buffer with protease inhibitors (Sigma-Aldrich, Milan, Italy), centrifuged at 800g for 10 minutes at 4°C and then the middle layer was collected. Equal amounts (50µg) of proteins were separated on 10% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane. UCP1 from subcutaneous adipose tissue samples were detected by primary antibodies, respectively rabbit polyclonal anti-human UCP1 (1:1000, Sigma-Aldrich, Milan, Italy) and mouse monoclonal anti-human β-actin (1:5000, Sigma-Aldrich, Milan, Italy). Secondary antibodies were peroxidase-conjugated anti-rabbit IgG for UCP1 and anti-mouse IgG for β-actin. Human adipocytes that treated with 100µM menthol were used as positive controls for UCP1 protein expression according to previous methodology [305]. Western blotting analysis was performed using Immobilon Western Chemiluminescent HRP Substrate (Millipore) and detection was made using photographic films. The images have been analysed by densitometry, which evaluates the relative amount of protein staining and quantifies the results in terms of optical density. Representative images of the photographic films are shown in Appendix 7.

### 4.4.9. Exercise interventions for randomized controlled trial

The exercise programmes (Table 8) for each group based on a previous study [306]. The supervised exercise interventions could not be blinded to the participants but to minimize risk of bias the exercise assistants were blinded to the aim and the design of the study. Also, the three different exercise programmes were performed in three different local Gyms to avoid participant cross contamination. To ensure the
attendance of the participants in the exercise sessions, a diary was created in each
Gym where notes were kept for the presence of each participant.

Table 8: Exercise protocols for randomized controlled trials

<table>
<thead>
<tr>
<th>Aerobic exercise group</th>
<th>Resistance exercise group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1&lt;sup&gt;st&lt;/sup&gt; week</strong></td>
<td>Resistance exercises: leg press, chest press, leg extension, leg curls, crunches, biceps curls, triceps extension, and lat pulldowns</td>
</tr>
<tr>
<td>30 minutes of bicycle at 65% of VO&lt;sub&gt;2&lt;/sub&gt;peak, 3 days/week</td>
<td>A familiarization with resistance exercises and practice the movements with light weight for 4 training sessions</td>
</tr>
<tr>
<td><strong>2&lt;sup&gt;nd&lt;/sup&gt; week</strong></td>
<td>65% of 1RM, 2 sets, 8-10 repetitions/set to failure, 4 days/week</td>
</tr>
<tr>
<td>45 minutes of bicycle at 65% of VO&lt;sub&gt;2&lt;/sub&gt;peak, 3 days/week</td>
<td><strong>3&lt;sup&gt;rd&lt;/sup&gt; week</strong></td>
</tr>
<tr>
<td>45 minutes of bicycle at 65% of VO&lt;sub&gt;2&lt;/sub&gt;peak, 5 days/week</td>
<td>65% of 1RM, 3 sets of 8-10 repetitions/set to failure 4 days/week</td>
</tr>
<tr>
<td><strong>4&lt;sup&gt;th&lt;/sup&gt; – 8&lt;sup&gt;th&lt;/sup&gt; week</strong></td>
<td><strong>4&lt;sup&gt;th&lt;/sup&gt; – 8&lt;sup&gt;th&lt;/sup&gt; week</strong></td>
</tr>
<tr>
<td>60 minutes of bicycle at 65% of VO&lt;sub&gt;2&lt;/sub&gt;peak, 5 days/week</td>
<td>65% of 1RM, 4 sets, 8-10 repetitions to failure, 4 days/week</td>
</tr>
<tr>
<td><strong>Combined exercise group (aerobic + resistance)</strong></td>
<td><strong>Combined exercise group (aerobic + resistance)</strong></td>
</tr>
<tr>
<td><strong>1&lt;sup&gt;st&lt;/sup&gt; week</strong></td>
<td>15 minutes of bicycle at 65% of VO&lt;sub&gt;2&lt;/sub&gt;peak. Familiarization with resistance exercises and practices the movements with light weight. 3 days/week</td>
</tr>
<tr>
<td>15 minutes of bicycle at 65% of VO&lt;sub&gt;2&lt;/sub&gt;peak. Familiarization with resistance exercises and practices the movements with light weight. 3 days/week</td>
<td><strong>2&lt;sup&gt;nd&lt;/sup&gt; week</strong></td>
</tr>
<tr>
<td>23 minutes of cycling at 65% of VO2peak. 65% of 1 RM, 1 set, 8-10 repetitions to failure. 3 days/week</td>
<td>23 minutes of cycling at 65% of VO2peak. 65% of 1 RM, 2 sets, 8-10 repetitions to failure. 4 days/week</td>
</tr>
<tr>
<td><strong>3&lt;sup&gt;rd&lt;/sup&gt; week</strong></td>
<td><strong>4&lt;sup&gt;th&lt;/sup&gt; – 8&lt;sup&gt;th&lt;/sup&gt; week</strong></td>
</tr>
<tr>
<td>23 minutes of cycling at 65% of VO2peak. 65% of 1 RM, 2 sets, 8-10 repetitions to failure. 4 days/week</td>
<td>30 minutes of cycling at 65% of VO2peak. 65% of 1 RM, 2 sets, 8-10 repetitions to failure. 5 days/week</td>
</tr>
</tbody>
</table>

VO<sub>2</sub>peak: Peak oxygen uptake; 1RM: One repetition maximum

4.4.10. Diet Assessment

The participants were randomly contacted by telephone – by an independent assistant who was blinded to the aim of this PhD study – on three separate days (two weekdays, one weekend day) during a one-week period for the completion of a three-day diet record. All food and beverages consumed on the day of contact were recorded. Three-day diet records were collected at baseline, 8<sup>th</sup> and 16<sup>th</sup> week of the intervention. All diet records were analysed by another trained assistant that was blinded to the aim of this PhD study using Nutritionist Pro, Version 5.4.0, Axxya Systems (Redmond, WA,
USA). This software (i.e. Nutritionist Pro) has been previously used for research purposes [307, 308]. For the analysis, a search was conducted in Nutritionist Pro for each food and beverage consumed. Details regarding the amount and preparation of each food and/or beverage were also included. Once all the relevant information regarding the food or beverage was entered into the software, a corresponding list of macro and micronutrient content was provided and subsequently saved. This process was repeated for all food and beverages listed on the diet record. If a particular food or beverage was not found within the Nutritionist Pro database, the investigator manually entered the macro and micronutrient content of that particular food and saved it to the database for future use. The feedback provided by the software for each food or beverage included the following dietary variables: total energy intake (kcal), weight of food (g), protein (g), carbohydrate (g), total fat (g), total sugar (g) and caffeine (mg).

4.4.11. General statistical approach

All variables were tested for normal distribution using Kolmogorov-Smirnov. The associations between the variables were tested using Spearman correlation coefficient and linear regression analyses. Given the non-normal distribution of the variables examined in the RCTs, delta scores were calculated and they were tested for normal distribution using Kolmogorov-Smirnov. The delta scores displayed normal distribution. The effects of exercise and de-training on the delta scores of the variables were tested using analyses of variance (ANOVA) with Bonferroni post-hoc t-tests. Following previous methodology, the 50th percentile of each variable was used to define the low and high responders in order to test the effects of exercise and de-training on the high responders [309]. Chi square tests were used to assess
differences between the experimental groups and the variables examined based on low and high responders status. Kruskal–Wallis analysis of variance with post-hoc Mann-Witney U tests were used to assess the effects of exercise and de-training of high responders on the variables examined. Effect sizes of the variables between and within groups were calculated using pooled Cohen’s d effect size analyses [310, 311]. Finally, Wilcoxon Signed Rank tests were used to calculate differences within groups between time points given the abnormal distribution of some variables. All analyses were conducted with SPSS Inc., Chicago, IL, USA, version 22 and a P≤0.05 level of significance.
5. CHAPTER 5: EFFECTS OF PHYSICAL ACTIVITY ON THE LINK BETWEEN PGC-1α AND FNDC5 IN MUSCLE, CIRCULATING IRISIN, AND UCP1 OF WHITE ADIPOCYTES IN HUMANS: A SYSTEMATIC REVIEW (1st study)

This systematic review was submitted for publication to the peer-reviewed journal F1000Research where the author of this Thesis also appears as the leading author.

5.1. INTRODUCTION

Recently it has been presented that acute and chronic exercise activates a brown adipose-like phenotype in WAT [30] through a number of sequential steps. Indeed, it has long been established that exercise increases the activation of the PGC-1α gene in human skeletal muscle [312]. Further, PGC-1α has been implicated in both the control of tissue mitochondrial content and the programme that results in BAT formation [313]. PGC-1α is a co-transcriptional regulator facilitating multiple transcription factors to regulate a complex network of genes [314]. While skeletal muscle properly adapts to exercise in the absence of PGC-1α [315], activation of PGC-1α was proposed to increase the FNDC5.

FNDC5, is a membrane protein expressed mainly in brain and skeletal muscle [316]. It was proposed that FNDC5 was cleaved during exercise, released into the bloodstream as Irisin – a peptide fragment of FNDC5 measured by western blotting [30]. In vitro, exposure of white adipocytes to Irisin subsequently – through an unknown receptor – led to increased expression of the PPARα which in turn increased UCP1 mRNA [30, 229]. The increase in white adipocyte UCP1 mRNA observed with Irisin treatment, presented as fold-change over control, was extremely modest as white adipocytes in culture do not usually express UCP1 mRNA and thus use of “fold increase” could be deceptive [209].

Since, UCP1 is the only contributor to NST that occurs in BAT [208] and it appears that the presence of UCP1 in a white adipocyte is accompanied by brown-
like adipose tissue properties [209, 317, 318] it was proposed that increased circulating Irisin in humans after a chronic exercise programme [30] may promote increased weight loss and improved metabolic control through induction of UCP1. This hypothesis seemed reasonable, as Irisin over-expression stimulated oxygen consumption and has been described to have an inverse association with blood glucose, insulin, total cholesterol, and a positive association with adiponectin concentrations [266]. Nevertheless, many other studies have failed to observe similar positive associations [319-321], while the ability for exercise to cause “browning” of white adipose phenotype remains unclear [33, 236, 238].

Further, a role for exercise regulating circulating Irisin concentration remains to be definitively established. Indeed, data indicate that while older adults appear to have a 30% increase in FNDC5 mRNA in muscle compared to younger adults, FNDC5 mRNA was unresponsive to six weeks of endurance training [267] despite robust increase in mitochondria [322]. Indeed, results have overall, been rather ambiguous in terms of the effects of exercise on circulating Irisin [33, 268-271] and various methodological concerns have been highlighted that may explain the various highly conflicting results [35, 323]. Given that circulating Irisin continues to be measured using diverse methods, and additional correlative studies progressed, an evaluation of the available evidence for its relationship with humans’ health is heavily warranted. Therefore, the aim of the current review was to systematically identify the effects of physical activity on the link between PGC-1α and FNDC5 in muscle, circulating Irisin as well as evidence for regulation of UCP1 in WAT in humans.

5.2. METHODS

Methods of this systematic review are described in detail in Chapter 4, pages 57-61.
5.3. RESULTS

5.3.1. Searching procedure results

The searching procedure revealed 45 studies that met the inclusion criteria and therefore, were included in this systematic review. The reference lists of these studies did not result in the identification of additional relevant articles. The searching outcome is presented in a PRISMA flow diagram in Figure 9.
Figure 9: Flow diagram of study selection and identification (PRISMA guidelines)

5.3.2. Characteristics of the included studies

The characteristics and the results of the included studies can be found in Table 9.
<table>
<thead>
<tr>
<th>First author</th>
<th>Design</th>
<th>Participants</th>
<th>Main outcome</th>
<th>Secondary outcome measures</th>
<th>Method of Irisin identification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Randomised Controlled Trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bang 2014</td>
<td>RCT</td>
<td>Seven healthy Korean M</td>
<td>An 8-week of CE (resistance) did not alter circulating Irisin</td>
<td>Exercise did not alter blood glucose and insulin levels.</td>
<td>PP, Burlingame. CNS</td>
</tr>
<tr>
<td>Greulich, 2014</td>
<td>RCT</td>
<td>COPD patients: 26 M, 14 F</td>
<td>An 8-day vibration exercise increased circulating Irisin</td>
<td>No relationship between changes in circulating Irisin with age, sex and BMI</td>
<td>AB, INC. CNS</td>
</tr>
<tr>
<td>Hecksteden, 2013</td>
<td>RCT</td>
<td>Healthy sedentary: 38 M, 64 F</td>
<td>No changes in circulating Irisin after 26-week aerobic exercise</td>
<td>No relationship between changes in circulating Irisin with age, sex and BMI</td>
<td>AB, Santa Clara, CA, CNS</td>
</tr>
<tr>
<td>Kim 2015</td>
<td>RCT</td>
<td>40 elderly healthy F</td>
<td>A 12-week resistance CE increased circulating Irisin</td>
<td>Increased circulating Irisin was positively correlated with muscle strength</td>
<td>PP, USA</td>
</tr>
<tr>
<td>Scharhag-Rosenberger 2014</td>
<td>RCT</td>
<td>37 exercised and 34 controls healthy M and F</td>
<td>A 6-month resistance training increased circulating Irisin in control but not in exercise group</td>
<td>Resting metabolic rate was increased in exercise group but was not associated with circulating Irisin</td>
<td>PP, Burlingame. CNS and Sunrise microplate reade (Tecan, Mannerdorf, Switzerland)</td>
</tr>
<tr>
<td>Huh, 2015</td>
<td>C-RCT</td>
<td>Eight healthy sedentary M, 4 M with MetS</td>
<td>AE (high density aerobic and resistance) increased circulating Irisin in healthy and metabolic syndrome patients</td>
<td>Resistance exercise was more effective in increasing circulating Irisin than endurance exercise</td>
<td>PP, EK-067-52, and EK-067-29</td>
</tr>
<tr>
<td>Nygaard, 2015</td>
<td>C-RCT</td>
<td>Two F and seven M moderately trained healthy</td>
<td>AE increased PGC-1α splice variant 1 mRNA in muscle. No changes on FNDC5 mRNA in muscle. Intense endurance AE and heavy strength training increased circulating Irisin.</td>
<td>No correlations between PGC-1α splice variant 1 and circulating Irisin.</td>
<td>PP, EK-067-29</td>
</tr>
<tr>
<td>Tsuchiya, 2014</td>
<td>C-RCT</td>
<td>Six healthy sedentary young M</td>
<td>AE (running) increased circulating Irisin greater in high intensity than in low intensity</td>
<td>Low intensity AE reduced circulating Irisin immediately after exercise compared with pre-exercise values</td>
<td>PP, EK-067-52</td>
</tr>
<tr>
<td>Tsuchiya, 2015</td>
<td>C-RCT</td>
<td>10 healthy M</td>
<td>Resistance AE increased circulating Irisin. Endurance and combined (endurance + resistance) AE did not alter circulating Irisin</td>
<td>Circulating Irisin was positively correlated with blood glucose, lactate and plasma glycerol</td>
<td>PP, EK-067-52</td>
</tr>
<tr>
<td><strong>Controlled trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aydin, 2013</td>
<td>CT</td>
<td>14 obese M, and 14 normal weight M</td>
<td>AE increased saliva Irisin. No changes in serum Irisin</td>
<td>Circulating Irisin was negatively correlated with BMI</td>
<td>PP, H-067-17</td>
</tr>
<tr>
<td>First author</td>
<td>Design</td>
<td>Participants</td>
<td>Main outcome</td>
<td>Secondary outcome measures</td>
<td>Method of Irsin identification</td>
</tr>
<tr>
<td>--------------</td>
<td>--------</td>
<td>--------------</td>
<td>--------------</td>
<td>---------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Ijiri 2015a</td>
<td>CT</td>
<td>8 M COPD patients</td>
<td>An 8-week of CE increased circulating Irisin. AE did not alter circulating Irisin</td>
<td>Circulating Irisin was not correlated with pulmonary function parameters and 6-min walk distance</td>
<td>PP, Burlingame. CNS</td>
</tr>
<tr>
<td>Kraemer, 2014</td>
<td>CT</td>
<td>Healthy: Seven M, Five F</td>
<td>AE increased circulating Irisin at 54th minute of the exercise session but decreased after the exercise session in men. In women AE increased circulating Irisin at 54th minute</td>
<td>-</td>
<td>AB, Burlingame, CA. CNS</td>
</tr>
<tr>
<td>Miyamoto-Mikami 2015</td>
<td>CT</td>
<td>16 M and nine F young/12 M and 16 F middle-aged older healthy</td>
<td>An 8-week CE programme increased circulating Irisin in middle-aged/older healthy. Exercise did not alter circulating Irisin in young healthy</td>
<td>Circulating Irisin was not correlated with visceral adipose tissue after CE. No correlation of circulating Irisin with abdominal subcutaneous adipose tissue area and whole-body fat.</td>
<td>PP, EK-067-16</td>
</tr>
<tr>
<td>Norheim, 2014</td>
<td>CT</td>
<td>13 M healthy controls, and 11 M pre-diabetic</td>
<td>AE and CE increased PGC-1α mRNA in muscle in both controls and pre-diabetic. CE increased FNDC5 mRNA in muscle of both groups. AE increased circulating Irisin levels in both groups. CE decreased Irisin levels. No effect of CE on UCP1 in subcutaneous WAT</td>
<td>PGC-1α mRNA in muscle was not correlated with circulating Irisin. Neither FNDC5 mRNA in muscle nor circulating Irisin nor UCP1 mRNA in subcutaneous WAT were correlated</td>
<td>PP, EK-067-52, and EK-067-29</td>
</tr>
<tr>
<td>Pekkala, 2013</td>
<td>CT</td>
<td>Healthy M: 17 middle-age, 10 young, 29 older</td>
<td>A 21-week CE did not alter PGC-1α and FNDC5 mRNA in muscle, and circulating Irisin. Resistance AE increased FNDC5 mRNA in muscle in young</td>
<td>Circulating Irisin and FNDC5 mRNA in muscle were not associated with HOMA, plasma glucose and serum insulin</td>
<td>PP, Inc., Burlingame, CA (16–127)</td>
</tr>
<tr>
<td>Prestes, 2015</td>
<td>CT</td>
<td>72 elderly F</td>
<td>A 16-week CE (resistance) did not increase circulating Irisin</td>
<td>-</td>
<td>MyBioSource Inc., San Diego, CA. CNS</td>
</tr>
<tr>
<td>Timmons, 2012</td>
<td>CT</td>
<td>24 young sedentary and 43 healthy M</td>
<td>A 6-week of CE (intense cycling and resistance) did not alter FNDC5 mRNA in muscle</td>
<td>FNDC5 mRNA was not linked to diabetes status</td>
<td>Not measured</td>
</tr>
</tbody>
</table>

**Single group interventional design studies**

<table>
<thead>
<tr>
<th>First author</th>
<th>Design</th>
<th>Participants</th>
<th>Main outcome</th>
<th>Secondary outcome measures</th>
<th>Method of Irsin identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alvehus, 2014</td>
<td>SGS</td>
<td>17 healthy young M</td>
<td>An 8-week of CE did not alter PGC-1α mRNA in both muscle and WAT and FNDC5 mRNA in muscle</td>
<td>-</td>
<td>Not measured</td>
</tr>
<tr>
<td>Anastasilakis, 2014</td>
<td>SGS</td>
<td>20 young healthy 10 F and 10 M</td>
<td>AE increased circulating Irisin. No association of PA levels with circulating Irisin</td>
<td>Circulating Irisin was positively correlated with LBM and glucose but it was not correlated with BMI, WHR, HOMA, insulin and leptin</td>
<td>PP, EK-067-52</td>
</tr>
<tr>
<td>First author</td>
<td>Design</td>
<td>Participants</td>
<td>Main outcome</td>
<td>Secondary outcome measures</td>
<td>Method of Irisin identification</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------</td>
<td>-------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>Besse-Patin, 2014</td>
<td>SGS</td>
<td>11 sedentary obese M</td>
<td>An 8-week of CE did not alter FNDC5 mRNA in muscle</td>
<td></td>
<td>Not measured</td>
</tr>
<tr>
<td>Blüher, 2014</td>
<td>SGS</td>
<td>65 obese children 7-18 years old M and F</td>
<td>A 12-month of PA intervention increased circulating Irisin</td>
<td>Circulating Irisin was not correlated with inflammatory markers at baseline</td>
<td>PP, EK-067-52</td>
</tr>
<tr>
<td>Boström, 2012</td>
<td>SGS</td>
<td>Eight non-diabetic M</td>
<td>A 10-week of CE increased FNDC5 mRNA in muscle, and circulating Irisin</td>
<td>Circulating Irisin did not alter oxygen consumption and weight loss in vivo</td>
<td>Western blot</td>
</tr>
<tr>
<td>Camera 2015</td>
<td>SGS</td>
<td>Eight healthy trained M</td>
<td>AE (resistance) increased PGC-1α mRNA in muscle four hours post exercise, but it did not alter FNDC5 mRNA in muscle</td>
<td></td>
<td>BCA-kit (Thermo Scientific)</td>
</tr>
<tr>
<td>Comassi, 2015</td>
<td>SGS</td>
<td>14 M ironman racers, 13 M half-ironman races</td>
<td>The half-ironman race increased circulating Irisin</td>
<td>The half-ironman race decreased body weight, BMI, LBM but not fat mass</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Daskalopoulou, 2014</td>
<td>SGS</td>
<td>Healthy: 22 M, 17 F</td>
<td>AE (treadmill) increased circulating Irisin, with greater increase in maximal workload</td>
<td>Circulating Irisin was positively correlated with lactate after maximal workload and it was greater in individuals with higher than individuals with lower VO2max. No relationship of circulating Irisin with REE post exercise</td>
<td>PP, EK-067-52</td>
</tr>
<tr>
<td>Ellefsen, 2014</td>
<td>SGS</td>
<td>18 untrained young F</td>
<td>A 12-week of CE decreased PGC-1α Slice4 mRNA in muscle and circulating Irisin</td>
<td>Circulating Irisin was positively correlated with FNDC5 mRNA in muscle while no relationship was found with fat mass after exercise</td>
<td>PP, EK-067-52</td>
</tr>
<tr>
<td>Hew-Butler, 2015</td>
<td>SGS</td>
<td>Nine F non-runners</td>
<td>A 10-week of walk/running programme did not alter circulating Irisin</td>
<td>No relationship of circulating Irisin with LBM, VO2peak and fat mass after the exercise programme</td>
<td>PP, Burlingame, CA, EK-067-52</td>
</tr>
<tr>
<td>Huh, 2012</td>
<td>SGS</td>
<td>15 healthy M</td>
<td>AE increased circulating Irisin. An 8-week of CE did not alter circulating Irisin</td>
<td>Circulating Irisin was not correlated with ATP levels after exercise</td>
<td>AB, Santa Clara, CA, CNS</td>
</tr>
<tr>
<td>Huh, 2014</td>
<td>SGS</td>
<td>Healthy: 78 m, 15 M and 15 F adolescents</td>
<td>An 8-week of CE increased PGC-1α mRNA in muscle. AE (treadmill &amp; swimming) increased circulating Irisin</td>
<td>Incubated Irisin in human skeletal muscle cells (in vitro) increased glucose and fatty acid uptake</td>
<td>PP, EK-067-52</td>
</tr>
<tr>
<td>Huh, 2014a</td>
<td>SGS</td>
<td>14 healthy F</td>
<td>AE (vibration) increased circulating Irisin. A 6-week of CE (vibration) did not change circulating Irisin</td>
<td>Circulating Irisin was positively correlated with cortisol after exercise</td>
<td>PP, EK-067-52</td>
</tr>
<tr>
<td>First author</td>
<td>Design</td>
<td>Participants</td>
<td>Main outcome</td>
<td>Secondary outcome measures</td>
<td>Method of Irisin identification</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Khodadadi, 2014</td>
<td>SGS</td>
<td>21 overweight F</td>
<td>High intense interval AE increased circulating Irisin. One session of Pilates exercise did not alter circulating Irisin</td>
<td>-</td>
<td>ELISA, Sweden</td>
</tr>
<tr>
<td>Kurtiova, 2014</td>
<td>SGS</td>
<td>Sedentary overweight/obese: 10 M, Six F</td>
<td>AE increased PGC-1α mRNA in muscle but it did not alter FNDC5 mRNA in muscle. A 12-week CE did not alter FNDC5 mRNA in muscle and circulating Irisin</td>
<td>Circulating Irisin was negatively associated with fasting glycaemia but it was not associated with VO₂max before and post exercise</td>
<td>PP, RK-067-16</td>
</tr>
<tr>
<td>Lee, 2014</td>
<td>SGS</td>
<td>Healthy: Six m, Four F</td>
<td>Submaximal AE increased circulating Irisin whereas maximal AE did not alter circulating Irisin following graded stepwise cold exposure</td>
<td>Circulating Irisin increased after cold exposure. REE was greater after maximal exercise compare to cold exposure</td>
<td>Mass spectrometry/ Western blot BCA-kit / PP, Burlingame, CA, CNS</td>
</tr>
<tr>
<td>Löffler 2015</td>
<td>SGS</td>
<td>28 healthy adults. Children 12 years and older, 48 M, 40 F</td>
<td>AE increased circulating Irisin in both adults and children. A 6-week in house CE did not alter circulating Irisin in children (n=62). Three years of low grade PA intervention in children did not alter circulating Irisin</td>
<td>Circulating Irisin was positively associated with BMI, LBM and triglycerides as well as negatively with HDL in adults</td>
<td>PP, EK-067-52</td>
</tr>
<tr>
<td>Moraes, 2013</td>
<td>SGS</td>
<td>13 M, and 13 F haemodialysis patients</td>
<td>A 6-month of CE did not alter circulating Irisin</td>
<td>Circulating Irisin was greater in haemodialysis patients than in healthy (baseline). No correlation with physical capacity, anthropometry and creatinine levels</td>
<td>PP, Burlingame. CNS</td>
</tr>
<tr>
<td>Palacios-González, 2015</td>
<td>SGS</td>
<td>85 healthy children 8-11 years old. 45 F and 40 M</td>
<td>An 8-month PA programme did not alter circulating Irisin levels</td>
<td>Circulating Irisin was positively associated with BMI before and after the PA programme as well as leptin after the PA programme</td>
<td>Cusabio Biotech.CNS</td>
</tr>
<tr>
<td>Raschke, 2013</td>
<td>SGS</td>
<td>13 healthy M</td>
<td>A 10-week of CE did not alter FNDC5 mRNA in muscle</td>
<td>The FNDC5 gene is mutated due to the non-ATG start codon and it is not activated by electrical stimulation</td>
<td>Not measured</td>
</tr>
<tr>
<td>Scalzo, 2014</td>
<td>SGS</td>
<td>Healthy: Seven m, 12 F</td>
<td>A 3-week of CE did not alter FNDC5 mRNA in muscle and it decreased circulating Irisin in M while it increased circulating Irisin in F</td>
<td>Circulating Irisin was not correlated with FNDC5 mRNA, fasting glucose, insulin and HOMA</td>
<td>PP, Burlingame, CA, CNS</td>
</tr>
</tbody>
</table>

**Cross sectional studies**

<table>
<thead>
<tr>
<th>First author</th>
<th>Design</th>
<th>Participants</th>
<th>Main outcome</th>
<th>Secondary outcome measures</th>
<th>Method of Irisin identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Daghri, 2015</td>
<td>CSS</td>
<td>35 M/48 F diabetes type 2 patients and 42 M/39 F healthy</td>
<td>Habitual PA was positively associated with circulating Irisin in healthy</td>
<td>Irisin was positively correlated with waist circumference in healthy and negatively with LBM and blood pressure in diabetes type 2 patients</td>
<td>PP, CNS</td>
</tr>
<tr>
<td>First author</td>
<td>Design</td>
<td>Participants</td>
<td>Main outcome</td>
<td>Secondary outcome measures</td>
<td>Method of Irisin identification</td>
</tr>
<tr>
<td>--------------</td>
<td>--------</td>
<td>--------------</td>
<td>--------------</td>
<td>----------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Hofmann, 2014</td>
<td>CSS</td>
<td>39 anorexic F</td>
<td>Circulating Irisin was not correlated with numbers of steps per day</td>
<td>No relationship between circulating Irisin and METs, as well as energy expenditure</td>
<td>PP, RK-067-16</td>
</tr>
<tr>
<td>Ijiri 2015b</td>
<td>CSS</td>
<td>65 M and seven F COPD patients, 24 M and three F healthy controls</td>
<td>Physical activity levels was positively associated with circulating Irisin in both COPD patients and controls</td>
<td>-</td>
<td>PP, Burlingame. CNS</td>
</tr>
<tr>
<td>Jedrychowski 2015</td>
<td>CSS</td>
<td>Four sedentary controls M and Six young healthy M</td>
<td>Circulating Irisin was detected after a 12-week high-intensity aerobic CE</td>
<td>-</td>
<td>Mass spectrometer (Thermo Fisher Scientific)</td>
</tr>
<tr>
<td>Lecker, 2012</td>
<td>CSS</td>
<td>24 M systolic heart failure patients</td>
<td>PGC-1α mRNA was positively correlated with FNDC5 mRNA in muscle.</td>
<td>-</td>
<td>Not measured</td>
</tr>
<tr>
<td>Moreno, 2015</td>
<td>CSS</td>
<td>191 M and 230 F non-diabetic</td>
<td>Circulating Irisin was higher in physically active than in sedentary individuals</td>
<td>Circulating Irisin was positively associated with weight, BMI, triglycerides, insulin and HOMA</td>
<td>SK00170-01, AB INC, Santa Clara California</td>
</tr>
<tr>
<td>Palermo 2015</td>
<td>CSS</td>
<td>65 postmenopausal F affected by osteoporosis</td>
<td>No correlation between circulating Irisin and daily PA</td>
<td>No relationship between circulating Irisin and LBM, fat mass, body mass density and METs</td>
<td>AG-45A-0046EK KI01; Adipogen AG, Liestal, Switzerland</td>
</tr>
<tr>
<td>Pardo, 2014</td>
<td>CSS</td>
<td>30 anorexic, 66 obese, 49 healthy F</td>
<td>Circulating Irisin was negatively correlated with daily PA</td>
<td>Circulating Irisin was positively correlated with resting energy expenditure, LBM and fat mass</td>
<td>PP, EK-067-52</td>
</tr>
</tbody>
</table>

**RCT:** Randomized control trial; **COPD:** Chronic obstructive pulmonary disease; **M:** males; **F:** females; **PGC-1α:** peroxisome proliferator-activated receptor-γ coactivator 1α; **AB:** Aviscera Bioscience; **CNS:** Code not specified; **BMI:** Body mass index; **PP:** Phoenix Pharmaceuticals; **C-RCT:** cross-over randomized controlled trial; **MetS:** Metabolic Syndrome; **AE:** Acute exercise; **FNDC5:** Fibronectin type III domain-containing protein 5; **CT:** Controlled trial; **CE:** chronic exercise; **PA:** Physical activity; **UCP1:** Uncoupling protein 1; **WAT:** White adipose tissue; **HOMA:** homeostatic model assessment; **SGS:** Single group design studies; **LBM:** Lean body mass; **WHR:** Waist to hip ratio; **VO2max:** Maximal oxygen uptake; **REE:** Resting energy expenditure; **VO2peak:** Maximal peak uptake; **ATP:** Adenosine triphosphate; **HDL:** High density lipoprotein; **CSS:** Cross-sectional study; **METs:** Metabolic equivalent; **VO2peak:** peak oxygen consumption; **SNPs:** Single nucleotide polymorphisms.
From the 45 eligible studies, nine (20%) were RCTs, of which four were cross-over RCTs (C-RCT), eight (17.8%) were CTs, 21 (46.6%) were SGS, and seven (15.6%) were CSS. One of the included RCTs [324] report the effect of resistance exercise training versus the effects of resistance exercise training combined with Ursolic supplementation, because for the latter group the effects of resistance exercise cannot be isolated, we will report only the results from the resistance exercise training group. Furthermore, one of the CTs [325] will be included in the results of both CTs and CSS because this study consisted of a controlled trial nested within a cross-sectional study. The 84% of the included studies (n=38) examined healthy populations, while seven included overweight/obese adults and children [33, 309, 326-330]. Ten of the 45 studies also included a clinical population: including patients with chronic obstructive pulmonary disease (COPD) [271, 325], heart failure [331], metabolic syndrome [332], haemodialysis [333], osteoporotic [334], anorexia nervosa [327, 335], pre-diabetes [236] and diabetes type 2 [336].

5.3.3. Risk of bias and quality of reporting data

The risk of bias assessment results can be found in Figure 10, while a summary is displayed in Figure 11.
Figure 10: Risk of bias assessment of the included studies using the Cochrane Collaboration’s tool

<table>
<thead>
<tr>
<th>First author</th>
<th>Random sequence generation</th>
<th>Allocation concealment</th>
<th>Blinding of participants</th>
<th>Blinding of outcome assessment</th>
<th>Incomplete outcome data</th>
<th>Selective reporting</th>
<th>Other bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bang, 2014</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Greulich, 2014</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hecksteden, 2013</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kim, 2015</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Scharhag-Rosenberger, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nygaard, 2015</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tsuchiya, 2014</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tsuchiya, 2015</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aydin, 2013</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Jiri, 2015a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kraemer, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Miyamoto-Mikami, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Norheim, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pekkala, 2013</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Prestes, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Timmons, 2012</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alvehus, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anastasiakis, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Besse-Patin, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Blüher, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Boström, 2012</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Camera, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Comassi, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Daskalopoulou, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ellefsen, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hew-Butler, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Huh, 2012</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Huh, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Huh, 2014a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Khodadadi, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kurdiouva, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lee, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Löffler, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Moraes, 2013</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Palacios-González, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raschke, 2013</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Scatzo, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Al-Daghri, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hofmann, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Jiri, 2015b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Jedrychowski, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lecker, 2012</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Moreno, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Palermo, 2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pardo, 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: +: Low risk of bias; -: High risk of bias; ?: Unclear risk of bias; RCT: Randomised controlled trials; CT: Controlled trials; SGS: Single group design studies; CSS: Cross sectional studies.
Figure 11: Summary of risk of bias

Five RCTs/C-RCTs [31, 332, 337-339] and all the included CTs, SGS as well as CSS displayed high risk of bias due to inadequate generation of a randomised sequence, while three RCTs/C-RCTs [271, 324, 340] showed low risk of bias and one RCT/C-RCT [341] showed unclear risk of bias because there was no description of the method used for allocation (even though the participants were said to be “randomly” assigned).

Five RCTs/C-RCTs [31, 271, 337, 339, 340], displayed low risk of bias for “allocation concealment”, while two [332, 341] showed unclear risk of bias because of the lack of description of the randomization allocation. Also, two RCTs/C-RCTs [324, 338] and all the included CTs, SGS as well as CSS showed high risk of bias due to the lack of concealment of allocations before assignment. In “blinding of participants and personnel” all RCTs/C-RCTs, CTs, SGS and CSS displayed high risk of bias because the exercise interventions could not be blinded to the participants.

In “blinding of outcome assessment”, three RCTs/C-RCTs displayed low risk of bias, [271, 340, 341] while four RCTs/C-RCTs [31, 332, 337, 339] and one CT [236] showed unclear risk of bias because of the lack of information regarding the blinding
of assessments. Also, two RCTs/C-RCTs [324, 338], the remaining seven CTs, all the included SGS and CSS showed high risk of bias due to the knowledge of the allocated interventions by the assessors. Five RCTs/C-RCTs [271, 324, 338, 340, 341], one CT [309] and three SGS [329, 342, 343] displayed low risk of bias while four RCTs/C-RCTs [31, 332, 337, 339], the remaining seven CTs and the remaining 18 SGS as well as all the included CSS showed unclear risk of bias for “incomplete outcome data” because of the lack of information on the participants who dropped out or exclusions in the analysis. All the included studies showed low risk of bias of “selective reporting” because they reported all the outcomes measured while all the included studies displayed low risk of bias in “other bias”.

The results of our evaluation in the quality of the reporting data showed a mean score of 14 out of 25 (55%) for the included RCTs, 11 out of 25 (42%) for the included CTs, 12 out of 25 (42%) for the included SGS and 13 out of 22 (60%) for the included CSS. Score represents the number of items (with percentage of items) on the checklist that were reported satisfactorily in each study. Therefore, a high score represents a high adherence to reporting guidelines, while a low score represents low adherence to reporting guidelines. The results of the reporting data can be found in Table 10 for the included RCTs, CTs and SGS and in Table 11 for the included CSS.

Table 10: Results of the quality of the reporting of the results using the Consolidated Standards of Reporting Trials checklist

<table>
<thead>
<tr>
<th>FIRST AUTHOR</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Randomized Controlled Trials</strong></td>
<td></td>
</tr>
<tr>
<td>1 Bang, 2014</td>
<td>(14.5/25) 58%</td>
</tr>
<tr>
<td>2 Greulich, 2014</td>
<td>(18/25) 72%</td>
</tr>
<tr>
<td>3 Hecksteden, 2013</td>
<td>(16.5/25) 66%</td>
</tr>
<tr>
<td>4 Kim, 2015</td>
<td>(14/25) 56%</td>
</tr>
<tr>
<td>5 Huh, 2015</td>
<td>(12.5/25) 50%</td>
</tr>
<tr>
<td>6 Nygaard, 2015</td>
<td>(11.5/25) 46%</td>
</tr>
<tr>
<td>7 Scharhag-Rosenberger, 2014</td>
<td>(15/25) 60%</td>
</tr>
<tr>
<td>8 Tsuchiya, 2014</td>
<td>(10.5/25) 42%</td>
</tr>
</tbody>
</table>
Score represents the number of items (with percentage of items) on the checklist that were reported satisfactorily in each study. Therefore, a high score represents a high adherence to reporting guidelines, while a low score represents low adherence to reporting guidelines.

**Table 11:** Results of the quality of the reporting of the results using the Strengthening the Reporting of Observational Studies in Epidemiology checklist

<table>
<thead>
<tr>
<th>FIRST AUTHOR</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Al-Daghri, 2015 (12.8/22) 58.18 %</td>
</tr>
<tr>
<td>2</td>
<td>Hofmann, 2014 (15.6/22) 70.9 %</td>
</tr>
<tr>
<td>3</td>
<td>Ijiri, 2015b (13.5/22) 61.36%</td>
</tr>
<tr>
<td>4</td>
<td>Jedrychowski 2015 (12/22) 54.45 %</td>
</tr>
<tr>
<td>5</td>
<td>Lecker, 2012 (13.3/22) 60.45 %</td>
</tr>
<tr>
<td>6</td>
<td>Moreno, 2015 (12.5/22) 56.81 %</td>
</tr>
<tr>
<td>7</td>
<td>Palermo, 2015 (13.5/22) 61.36</td>
</tr>
<tr>
<td>8</td>
<td>Pardo, 2014 (12.2/22) 55.45 %</td>
</tr>
</tbody>
</table>
Score represents the number of items (with percentage of items) on the checklist that were reported satisfactorily in each study. Therefore, a high score represents a high adherence to reporting guidelines, while a low score represents low adherence to reporting guidelines.

5.4. REPORTING OF OUTCOMES

5.4.1. Main results of randomized controlled trials

Acute effects of exercise

A resistance training session increased PGC-1α splice variant 1 but it did not change FNDC5 mRNA in the muscle of healthy adults 4-hour after exercise [31] while circulating Irisin increased over the following 24-hour [31] so indicating that there is no short-term association between FNDC5 and Irisin. Similarly, a running exercise session in healthy individuals [337] and an aerobic exercise session as well as a resistance exercise session in healthy individuals and in metabolic syndrome patients [332] increased circulating Irisin. Also, an acute resistance exercise session increased circulating Irisin as oppose to aerobic and combined (aerobic+resistance) sessions that did not alter circulating Irisin in healthy males [339].

Chronic effects of exercise

Overall, the results of the RCTs/C-RCTs show large inconsistency on the effects of chronic exercise on circulating Irisin while the populations examined showed large heterogeneity. An 8-week of resistance training in healthy adults did not alter circulating Irisin [324]. Also, an 8-day vibration exercise increased circulating Irisin in COPD patients [271] while a 26-week aerobic exercise program revealed no changes in circulating Irisin of healthy adults [340]. A 6-month resistance training increased circulating Irisin in healthy controls but not in the exercisers [341]. Furthermore, a 12-week resistance exercise increased circulating Irisin in elderly healthy females [338].
5.4.2. Main results of controlled trials

Acute effects of exercise

A 45-minute endurance exercise session increased PGC-1α mRNA in muscle and circulating Irisin, but it did not change FNDC5 mRNA in muscle in both healthy and pre-diabetic adults [236]. Also, a resistance exercise session increased PGC-1α mRNA in muscle of both young and older healthy adults, while it increased FNDC5 mRNA in muscle only in young healthy adults and it did not alter circulating Irisin of both young and older healthy adults [32]. These results cannot confirm the link between PGC-1α, FNDC5 and Irisin. Furthermore a 90-minute aerobic exercise session increased circulating Irisin in healthy adults [344] whereas 45 minutes of running did not alter circulating Irisin in obese healthy adults [326]. Similarly, an acute cycling session did not alter circulating Irisin in COPD patients [325]. These results indicate an inconsistency of the effects of acute exercise on circulating Irisin.

Chronic effects of exercise

A 21-week endurance and resistance combined exercise programme in healthy adults did not alter PGC-1α and FNDC5 mRNA in muscle, as well as circulating Irisin [32]. However, a 12-week of endurance and resistance combined exercise training in both healthy and pre-diabetic adults increased Exon 11 of PGC-1α and FNDC5 mRNA in muscle when normalised to 60S acidic ribosomal protein P0 while it decreased circulating Irisin and had no effect on UCP1 mRNA in subcutaneous WAT [236]. One of the included CTs [267] found no effect on PGC1α or FNDC5 mRNA in younger adults (despite detecting significant changes in ~1,000 other mRNAs and finding mitochondrial enzyme activity was increased ~25%) [345]. Overall, the aforementioned findings demonstrate an inconsistency of the effects of chronic exercise on the link between PGC-1α and FNDC5. Furthermore, an 8-week of
endurance training increased circulating Irisin only in middle-aged and not in young healthy adults [346] while an 8-week chronic exercise program in COPD patients increased circulating Irisin [325]. Finally, a 16-week of resistance exercise program in elderly women did not increase circulating Irisin [309].

5.4.3. Main results of single-group design studies

**Acute effects of exercise**

An acute resistance exercise session increased PGC-1α mRNA in muscle four hours post exercise while it did not alter FNDC5 mRNA in muscle of healthy adults [237]. Nevertheless, an acute exercise session did not alter PGC-1α mRNA in muscle [347]. Also, PGC-1α mRNA in muscle increased in response to acute exercise however, neither FNDC5 mRNA in muscle nor circulating Irisin was altered in sedentary overweight and obese adults [33]. Nine out of the 21 included SGS showed that acute exercise increased circulating Irisin in healthy populations [34, 266, 268-270, 328, 343, 348, 349]. These results appear to show some consistency of the effects of acute exercise on circulating Irisin in healthy participants.

**Chronic effects of exercise**

An 8-week sprints exercise programme increased PGC-1α and FNDC5 mRNA in muscle but it did not alter circulating Irisin in healthy adults [34] while an 8-week of resistance exercise did not alter PGC-1α or FNDC5 mRNA in muscle of young healthy adults [347]. However, Bostrom et al. (2012) showed that in eight older participants [350] chronic exercise increased both FNDC5 mRNA in muscle and circulating Irisin. Interestingly, 12 weeks of resistance training did not alter PGC-1α splice variant 1 mRNA [238] while chronic resistance training changed neither the FNDC5 mRNA in muscle nor the circulating Irisin [238].
Also, a 12-week of aerobic and resistance exercise combined programme [33] and an 8-week aerobic exercise programme [330] did not alter FNDC5 mRNA in muscle of sedentary obese adults while chronic exercise had no effect on FNDC5 mRNA in muscle of healthy adults [274]. Finally, a 3-week of sprint interval training did not alter FNDC5 mRNA in muscle and showed a gender difference in circulating Irisin, which were decreased in healthy males and increased in healthy females [351]. These results do not allow drawing conclusive inferences regarding the effects of chronic exercise on PGC-1α, FNDC5, and/or circulating Irisin.

Five SGS showed that chronic exercise did not alter circulating Irisin in healthy individuals [238, 266, 269, 342, 348] and haemodialysis patients [333]. However, a 12-month physical activity intervention increased circulating Irisin by ~13% in obese children [329]. Notably, Irisin levels were 111.0 ng/ml i.e. 20 times greater than recent mass-spectrometry based detection concentrations strongly indicating that the ELISA was detecting multiple proteins. Overall, given both the inconsistency of the findings and the absence of a control group, the results from these SGS should be considered with caution.

**5.4.4. Main results of cross-sectional studies**

We have included seven CSS and a cross-sectional analysis within an included CT [325]. Particularly, PGC-1α mRNA in muscle was positively associated with FNDC5 mRNA in muscle in a sub-set of 24 patients with heart failure [331] while stratification was ad hoc. Nevertheless, increased physical activity levels were positively associated with circulating Irisin in healthy adults but not in patients with T2D [336]. Also, one study contrasted plasma Irisin concentrations in six younger individuals following 12-week high intensity aerobic exercise with those found in a separate group of four
individuals (no pre-training samples were presented) [276]. This study used mass spectrometer (Thermo Fisher Scientific) and detected 3-4ng/ml of circulating Irisin. No details regarding training or control of hydration in the training group were reported [276].

Physical activity levels were not associated with circulating Irisin in osteoporotic women [334] and in anorexic women [335]. Also, physical activity levels were positively associated with circulating Irisin in non-diabetic active individuals, but not in non-diabetic sedentary individuals [352]. However, physical activity levels were negatively associated with circulating Irisin in groups of anorexic, obese and healthy women [327]. Finally, physical activity levels were positively associated with circulating Irisin in both COPD patients and healthy [325]. These results are disparate and cannot indicate the association of physical activity with circulating Irisin in humans.

5.4.5. Results for associations of Irisin with secondary outcome measures

The secondary results of the included studies can be found in Table 9. An important secondary finding was that the start-codon for the FNDC5 gene in human and mouse are different, raising doubts that the human gene would be efficiently translated and hence limit the potential for Irisin production [274]. Also, in 118 muscle profiles FNDC5 mRNA was modestly and positively correlated with BMI ($r^2=0.1$), rather than negatively, while FDNC5 mRNA was not related to fasting glucose or glycaemic control [267]. Furthermore, circulating Irisin was not associated with inflammatory indices [329], blood glucose [32, 351], homeostatic model assessment (HOMA) [32, 349, 351], insulin [32, 349, 351], leptin [349], lean body mass [334, 342], fat mass [238, 334, 342], WHR [349], REE [268, 341], BMI [349], and pulmonary function [325].
Additional secondary results show that circulating Irisin was positively associated with BMI [348, 352, 353], triglycerides [348, 352], fat mass [327], HOMA [352], insulin [352], blood glucose [349] and leptin [353] and negatively with high density lipoprotein cholesterol [348], all of which indicate unfavourable effects of Irisin on human health. Nevertheless, some secondary evidence suggests that circulating Irisin was positively associated with fat-free mass [327, 348] and REE [327], while Irisin that was incubated within white adipocytes in vitro increased glucose and fatty acids uptake [34]. Furthermore, circulating Irisin after a maximal workload was significantly greater in individuals with higher VO$_2$max than individuals with lower VO$_2$max [268]. However, circulating Irisin was not associated with VO$_2$peak before and post exercise in healthy female [342] and sedentary overweight and obese individuals [33].

5.5. DISCUSSION

5.5.1. Applicability of evidence
We were unable to find strong evidence that links PGC-1α and FNDC5 mRNA in muscle or that circulating Irisin increases in response to exercise training or increased physical activity levels. Notably, we located only one study that examined the effects of exercise on UCP1 in WAT, and this found no effect [236]. Despite PGC-1α being firmly placed as a central regulator of adaptation to exercise in mice [354] and humans [31, 237], numerous aspects of the literature are contradictory or incomplete. For example, there is no evidence that PGC-1α mRNA accumulates with endurance training, while studies of PGC-1α protein reflect various antibodies that measure distinct molecular entities ranging from 70 to >110 Kda [240-242]. Furthermore, mice lacking PGC-1α adapt normally to endurance exercise training, and in humans the
PGC-1α regulated gene network does not correlate with aerobic adaptation [345]. Thus, any argument that places Irisin as part of the core PGC-1α regulated exercise adaptation programme needs to reflect, on both technical and theoretical grounds [355].

When PGC-1α protein content is measured (albeit with uncertainty over protein identities) exercise training increases PGC-1α protein in skeletal muscle or causes nuclear translocation of protein [356-359]. However, the studies included in the current review only relied on measuring PGC-1α mRNA to determine the effects of exercise on PGC-1α and the time-course of mRNA and protein responses to exercise are distinct. Thus, the link between PGC-1α and FNDC5 in skeletal muscle may reflect measurement of mRNA dynamics and this may explain inconsistent findings for PGC-1α. Also, the original hypothesis and data specifically indicated that induction of PGC-1α mRNA and then protein would activate the transcription of FNDC5 and hence it would be expected if this theory was correct a strong correlation between PGC-1α mRNA and FNDC5 mRNA would exist. However, previous evidence showed that the FNDC5 mRNA in muscle is not regularly increased by exercise or differently regulated between those with and without insulin resistance [267], while FNDC5 mRNA was only modestly increased in a subset of older people following chronic exercise training [267]. Critically, this time it is known that the antibodies used in the detection of Irisin by Bostrom et al. (2012) was made to correspond against the C-terminal of human FNDC5, which is not the part of the protein that yields the Irisin peptide [360]. Thus, the original data could not possibly be Irisin. We now know that the various commercially available antibodies used in the ELISA kits yield a protein concentration that appears to be ~10-100 times greater than a more recent mass spectrometry data (data that requires independent validation) and still far above what others have found.
These technical considerations may explain part or all of the equivocal results of the included studies in this current review regarding both the effects of exercise on FNDC5 mRNA in muscle and circulating Irisin, while the evidence in terms of the effects of exercise on UCP1 of WAT is very limited.

Also, evidence from the included studies showed that Irisin increased one hour after the exercise session, and returned to baseline levels after 24 hours [34] while other study showed that Irisin remained elevated up to 12 hours after the exercise session [337]. Furthermore, Irisin increased three minutes after the exercise session, and returned to baseline levels within 10 minutes [268], while it showed no elevation of Irisin at one, 15 and 30 minutes after the exercise session [32]. This inconsistency may again reflect critical defects in Irisin detection technology including the polyclonal nature of the Irisin antibody [35]. However, if we focus on more reliable mRNA measures of PGC-1α and FDNC5, then the variable findings may be explained by the different characteristics of the populations examined and the different exercise protocols used. There is a trend for old muscle tissue to show a higher FDNC5 expression following exercise, which is unclear if it leads to Irisin.

An interesting aspect brought forward in the included studies showed that the start codon of the FNDC5 gene is mutated in humans due to the non-ATG start codon [274]. In humans ATG is usually the first codon to lead to efficient protein production and therefore, the latter suggests that Irisin, if produced would be done so in an inefficient manner [274]. However, this notion has been questioned by a subsequent study, which supports that human Irisin is mainly translated from its non-ATG start codon while the molecular weight of the protein is similar to that of important proteins in human body such as insulin, leptin and resistin [276] indicating a biological role of Irisin. To the best of our knowledge, two studies have measured circulating Irisin via
mass spectrometry in response to exercise in humans. In the study by Jedrychowski et al. (2015) the blood samples for Irisin identification collected only after the exercise program from a small number of participants sedentary (n=4), aerobic exercisers (n=6) [276]. In the study by Lee et al. (2014) the blood samples collected only pre and post-acute exercise without a control situation [361]. Also, Lee et al. (2014) reported an ~3-fold increase of Irisin only after submaximal and not maximal exercise. These studies may display methodological limitations and a small number of participants that indicates that future longitudinal studies of changes in Irisin will clarify if the mass spectrometry measures reflect exercise. Finally, while the three studies that utilised mass spectrometry do not agree [35, 276, 361], reflecting issues of sensitivity and methodology, the latest identification and analysis of Irisin [276, 361] indicates that Irisin may circulate in blood and probably has similar or identical to the mouse structure however, whether it has genuine biological activity remains to be elucidated.

5.5.2. Quality of evidence and limitations

Based on the included RCTs/C-RCTs we cannot obtain precise conclusions regarding the effects of acute exercise on PGC-1α in conjunction with FNDC5 mRNA in muscle given the inconsistency of the findings, the different characteristics of the populations examined and the small number of the studies retrieved. The CTs display inconsistent results of the effects of both acute and chronic exercise on FNDC5 mRNA in muscle. Also, the CTs display high risk of bias due to the absence of generation of a randomised sequence, inadequate concealment of allocations before assignment and due to knowledge of the allocated interventions by the outcome assessors as well as they display unclear risk of bias due to knowledge of the allocated interventions by the
investigators during the study. The SGS also display inconsistent results in relation to the effects of acute and chronic exercise on FNDC5 mRNA in muscle.

To the best of our knowledge this is the first systematic review that examined the effects of physical activity on the link between PGC-1α and FNDC5 in muscle, circulating Irisin and on UCP1 of WAT in humans. We compared our results with a recent meta-analysis that aimed to identify the effects of exercise on circulating Irisin [362]. This meta-analysis concluded that chronic exercise may decrease circulating Irisin concentrations in the RCTs while the non-RCTs cannot form any conclusion. However, the latter meta-analysis did not take into consideration the issues raised regarding the validity of the methods used for Irisin identification [35]. In contrast, while we considered the methods used for Irisin identification in the studies included in the current review our review had a different aim, to identify the proposed mechanism by Bostrom et al. (2012) starting from the muscle and leading to the white adipocytes. Regarding circulating Irisin we also report that we cannot form any firm conclusion of the effects of exercise on circulating Irisin. Furthermore, our results are in accordance with a previous review that showed equivocal results among studies examining circulating Irisin due to the methodological variations for Irisin detection [363]. In this review, the authors examined the commercial antibodies and ELISA used to measure circulating Irisin and concluded that the currently available antibodies should be tested for cross-reacting antigens detection [363]. Also, they did not confirm a link between Irisin and BMI, fat mass and fat-free mass which is also in accordance with our results [363].

The secondary outcomes also showed that chronic exercise may increase strength and REE in healthy, indicating the efficacy of the exercise program [341]. However, REE was not associated with circulating Irisin, while circulating Irisin was
not altered by exercise [341]. This is in accordance with previous data showed that REE is not associated with circulating Irisin in women 50-70 years old [364]. Also, an included CT in the current review revealed that a chronic exercise program reduced whole body fat and the subcutaneous WAT. However, the increased circulating Irisin after the exercise program was not associated with the whole body fat and the subcutaneous WAT [346]. These findings suggest that even though metabolic adaptations occur due to exercise, this is not related to Irisin. Furthermore, our secondary outcomes showed no association of circulating Irisin with fat mass, fat-free mass and REE. Thus, we cannot confirm any favourable effect of Irisin on human body metabolism especially due to inaccurate methods used for circulating Irisin identification.

The current review has a number of strengths. For instance, we used the PubMed and the EMBASE databases using appropriate algorithms with standardized indexing terms. Standardized indexing terms can retrieve records that may use different words to describe the same concept and information beyond that may be contained in the words of the title and abstract [365]. Furthermore, the current review used a systematic manner to identify articles according to previous methodology [286-288] while we used well-established tools [289-291] to evaluate the included studies. Also, to reduce bias two investigators worked independently on the screening of the included studies for eligibility, risk of bias assessment, as well as to provide CONSORT and STROBE scores. Also, we have not excluded studies based on language. However, we used only included published literature; we did not include grey literature searching. In this light, there is a potential of publication bias in the current review nevertheless, the inclusion of grey literature may itself introduce bias and one reason to include grey literature would be the absence of peer-review sources
Furthermore, the current review found no positive relationship between Irisin and several health indices based on the included studies, which aimed on the effects of physical activity on Irisin. However, Irisin may have a physiological role in human body independently of physical activity and therefore, the current review may not be representative to determine the relationship between Irisin and health indices.

5.6. CONCLUSIONS

We found little evidence to determine the link between PGC-1α mRNA and FNDC5 mRNA in human muscle while there was limited evidence on the effects of physical activity on UCP1 in subcutaneous WAT. Our systematic review discovered a heterogeneity in populations examined in the included studies, and a relatively small number of RCTs (n=9) with inconsistent findings that also prevent us from making a firm conclusion for the link between physical activity, PGC-1α, FNDC5, and UCP1. We cannot form any conclusion regarding circulating Irisin in response to physical activity given that the methods used for Irisin identification were inaccurate in most cases. Mass spectrometry detection of Irisin of exercise effects were compromised by the methodological limitations of the existing studies. There is evidence in this systematic review indicating via mass spectrometry that Irisin is present in human blood at levels that are ~10-100 folds lower that commercial ELISA kits, therefore, future studies could re-examine the biological role for Irisin using accurate and valid methods for its analysis.
6.1. INTRODUCTION

BAT in humans is characterized by increased mitochondrial content and its ability to uncouple cellular respiration via the action of UCP1 [25]. It is hypothesized that this phenomenon may consume up to 30% of daily REE in adult humans [176]. This hypothesis is mainly based on previous evidence in humans that showed that NST was responsible for the elevation of daily REE for 25% [366] and up to 28% [174] after chronic cold exposure. This may suggest a significant involvement of BAT in the human metabolism and a pathway to weight loss.

Physical activity results in increased energy expenditure [78]. Exercise may trigger the action of PGC-1α that is thought to cause the production of the FNDC5 protein, which is then cleaved and it is released into the bloodstream as Irisin – a newly discovered protein that is a product of the FNDC5 protein [30]. Irisin may generate a cascade of events that change the properties of white adipocytes into brown-like adipocytes and this was attributed to the presence of UCP1 within the white adipocytes [30]. This presence may occur, at least in part, by the action of PPARα [30] while PPARα may have a predominant role in browning formation of WAT in animals [252]. Also, a previous report revealed that humans display white adipocytes that contain UCP1 [367]. UCP1 is the only known contributor in NST that occurs in BAT and, therefore, its presence in WAT cells indicates brown-like adipocytes properties [223]. This suggests a transformation of white into brown-like adipocytes that could increase – similar to BAT contribution – REE in adult humans. However, the evidence to date either in vitro or in vivo is limited to support this mechanism.

Following the aforementioned findings, a more recent study that examined the UCP1 in WAT as a possible candidate for the transformation of WAT into brown-like
adipose tissue in response to exercise in humans found increased, but not significant, UCP1 mRNA [236]. Recent evidence shows that individuals with increased habitual physical activity displayed increased BAT activity in supraclavicular and spinal areas compared with individuals with low habitual physical activity levels [29].

PGC-1α is responsible for mitochondrial biogenesis and is expressed in several tissues, including WAT [226]. It co-activates with PPARγ to stimulate genes that are involved in the differentiation of brown adipocytes [234, 235] and its presence in WAT may indicate brown-like adipocytes properties [245]. PPARγ may also enhance the UCP1 mRNA in BAT [173]. Evidence in vitro showed that PPARγ may cause a browning effect in WAT by enhancing UCP1 mRNA [209]. In addition, chronic exercise increases PPARγ mRNA in the WAT of mice [260, 261]. However, relevant data from human studies are limited. Therefore, the aim of the current study was to examine the relationship between the UCP1 mRNA and protein expression as well as PGC-1α, PPARα and PPARγ mRNAs of subcutaneous WAT with the physical activity levels of healthy men. A secondary aim was to examine the associations of the UCP1 mRNA and protein expression as well as PGC-1α, PPARα and PPARγ mRNAs with REE, age, fat mass, fat-free mass, energy intake, WHR and BMI. To the best of our knowledge this is the first time that the above associations are tested.

6.2. METHODS

Height and weight were measured to calculate BMI, waist and hip circumferences to calculate WHR. Also, blood pressure and body composition via a bio-electrical impedance device were assessed. We also evaluated REE, energy intake (retrieved from diet recall), physical activity levels and we obtained subcutaneous fat biopsies to
perform mRNA and protein expression analyses. For details please see general methods in Chapter 4, pages 61-71.

6.2.1. Participants and procedure

The participants were recruited through an advertisement in a local newspaper in Trikala, Thessaly, Greece, between June 2013 and June 2014. The inclusion criteria included: adult men not involved in regular exercise programmes, non-smokers, with no chronic disease and/or being under medication treatment for any disease. The eligibility of the participants was screened through an interview and health status information was retrieved through a medical history questionnaire via interview (Appendix 4). Written consent forms were obtained from 46 healthy non-smokers men (age: 35.22±6.86, BMI: 27.26±4.17).

Once the participants had been chosen, they were given a pedometer to measure physical activity levels for one week. A diet recall was also retrieved during the same week period. The following week the participants were given appointments to visit the laboratory for measurements between 07:00 to 09:00 am. They were instructed to refrain from exercise, alcohol, and passive smoking for 72 hours and fast for 12 hours prior to their visit. This was to minimize the risk of misleading results regarding the fat biopsies and REE [294, 295] as well as to standardise the process. During the appointment, the participants underwent measurements in the following order to avoid interaction effects between measurements: 1) anthropometry (height, weight, waist and hip circumferences), 2) body composition (fat mass, fat-free mass), 3) indirect calorimetry for REE and 4) subcutaneous fat biopsy. Participants’ characteristics are displayed in Table 12.
Table 12: Characteristics of the participants (n=46)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>35.22±6.86</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>87.07±13.89</td>
</tr>
<tr>
<td>BMI</td>
<td>27.26±4.17</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.92±0.77</td>
</tr>
<tr>
<td>Resting energy expenditure (kcal)</td>
<td>1626±226</td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>1454±435</td>
</tr>
<tr>
<td>Fat mass %</td>
<td>26.9±9.08</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>54.2±6.07</td>
</tr>
<tr>
<td>Steps/day</td>
<td>7209±3335</td>
</tr>
<tr>
<td>UCP1 mRNA</td>
<td>0.19±0.18</td>
</tr>
<tr>
<td>UCP1 protein expression</td>
<td>1.05±0.68</td>
</tr>
<tr>
<td>PGC1α mRNA</td>
<td>1.38±1.11</td>
</tr>
<tr>
<td>PPARα mRNA</td>
<td>1.99±0.81</td>
</tr>
<tr>
<td>PPARγ mRNA</td>
<td>0.50±0.36</td>
</tr>
</tbody>
</table>

Kg: Kilograms; BMI: Body mass index; Kcal: kilocalories; mm/Hg: Millimetre of mercury; UCP1: Uncoupling protein one; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα: Peroxisome proliferator-activated receptor alpha; PPARγ: Peroxisome Proliferator-Activated Receptor Gamma.

6.2.2. Statistical analyses

The normal distribution of the studied variables tested via the Kolmogorov-Smirnov. Due to the non-normal distribution of some variables, non-parametric tests were used. The Spearman correlation coefficient was used to assess the associations between UCP1 mRNA, UCP1 protein expression, PGC-1α mRNA, PPARα mRNA and PPARγ mRNA with physical activity levels (pedometer steps/day) as well as with BMI, WHR, REE, age, fat mass percentage, fat-free mass (kg) and energy intake (kcal). Linear regression analyses (enter mode) were used to test whether the significant associations detected by the Spearman’s correlations coefficient persisted, using as dependent variables the UCP1 protein expression, PGC-1α and PPARα mRNAs. All analyses were conducted with SPSS Inc., Chicago, IL, USA, version 22 and a P≤0.05 level of significance.
6.3. RESULTS

There were no missing values during the data collection of this study. No significant associations were detected between mRNA of UCP1, PGC-1α, PPARα and PPARγ genes with physical activity levels (pedometer steps/day) (P>0.05). Nevertheless, a negative association of UCP1 protein expression and physical activity levels (pedometer steps/day) (rho=-0.31, P=0.04) was found (Figure 12). Finally, there was no association between UCP1 mRNA and UCP1 protein expression.

**Figure 12:** Associations of UCP1 protein expression and physical activity levels (pedometer steps/day)

UCP1: Uncoupling protein one

No significant associations were detected between the UCP1 mRNA and protein expression as well as PGC-1α, PPARα and PPARγ mRNAs with REE, energy intake (kcal) and age (P>0.05). However, an inverse association between PGC-1α mRNA and BMI (rho=-0.36, P=0.01) (Figure 13A), as well as between PPARα mRNA and BMI (rho=-0.33, P=0.03) (Figure 13B) were found.
**Figure 13**: Associations of PGC-1α and PPARα mRNA with BMI

PGC-1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα: Peroxisome proliferator-activated receptor alpha; BMI: Body mass index.

The results also revealed an inverse association between PGC-1α mRNA and WHR (ρ=-0.48, P=0.006) (Figure 14A), as well as between PPARα mRNA and WHR (ρ=-0.39, P=0.03) (Figure 14B).
Figure 14: Associations of PGC-1α and PPARα mRNA with WHR

Furthermore, inverse associations between PGC-1α mRNA ($\rho=-0.43$, $P=0.003$) (Figure 15A) and PPARα mRNA ($\rho=-0.37$, $P=0.01$) (Figure 15B) with %fat mass were detected. A positive association between PGC-1α mRNA with fat-free mass ($\rho=0.41$, $P=0.01$) (Figure 16) was also found.
**Figure 15:** Associations of PGC-1α and PPARα mRNA with fat mass percentage

![Graph A](image1)

**Figure 16:** Association of PGC-1α mRNA with fat-free mass

![Graph B](image2)

*PGC-1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα: Peroxisome proliferator-activated receptor alpha*
The association of UCP1 protein expression with physical activity levels (pedometer steps/day) as well as the associations of PGC-1α and PPARα mRNAs with BMI, WHR, fat mass and fat-free mass were tested via three multiple Linear regression analysis models using as dependent variables UCP1 protein expression, PGC-1α and PPARα mRNAs and as independent variables BMI, WHR, fat mass, fat-free mass, age, REE, energy intake and physical activity levels (steps/day). The Linear regression analysis model did not confirm the association between UCP1 protein expression and physical activity levels (pedometer steps/day) (P>0.05) as well as between PGC-1α mRNA and BMI, fat mass and fat-free mass (P>0.05). However, the inverse association between PGC-1α mRNA and WHR was confirmed [Beta=-.56, t(2.8)= -1.9, P=0.05] when age, BMI, REE, fat mass and fat-free mass were included in the model. Furthermore, the Linear regression analysis model did not confirm the associations between PPARα mRNA and BMI as well as fat mass (P>0.05). Nevertheless, the inverse association between PPARα mRNA and WHR was confirmed [Beta=-.41, t(1.8)= -2.23, P=0.03] when age was included in the model.

6.4. DISCUSSION
In recent years the formation of WAT into brown-like adipose tissue in response to exercise has attracted scientific attention [30, 32, 236]. The primary aim of the current study was to examine the associations of the UCP1 mRNA and protein expression as well as the mRNAs of PGC-1α, PPARα and PPARγ genes of subcutaneous WAT with physical activity levels in healthy men. A secondary aim was to inspect the associations of the UCP1 mRNA and protein expression as well as the mRNAs of PGC-1α, PPARα and PPARγ genes with REE, WHR, BMI, age, fat mass, fat-free
mass and energy intake. A thorough review of the available literature indicates that the current study is the first to examine the aforementioned associations in humans.

The study’s hypothesis was based on the recent suggestion that exercise may change the mode of WAT into brown-like function [30, 229] through the presence of UCP1 in WAT and therefore, it was hypothesized that individuals with increased physical activity levels may display increased UCP1. Furthermore, PGC-1α is increased in WAT in response to exercise [245], PPARα was suggested to increase UCP1 in WAT in response to exercise [30] and PPARγ may increase the UCP1 mRNA in subcutaneous WAT of mice [228] while it is also increased in response to chronic exercise in mice [260]. As such it was hypothesized that PGC-1α, PPARα and PPARγ mRNAs of subcutaneous WAT in humans may be associated with physical activity levels indicating a browning effect of WAT. However, no associations were found herein between the mRNAs of UCP1, PGC-1α, PPARα and PPARγ genes that indicate browning formation of WAT with physical activity levels. In addition, UCP1 protein expression was negatively associated with physical activity levels rather than positively in the current study. This finding does not support the hypothesis that UCP1 is increased in response to exercise within a white adipocyte that may trigger a browning formation of WAT [30]. Also, this finding may explain a recent discovery that UCP1 mRNA in subcutaneous WAT was negatively correlated with weight loss – indicating no effect of UCP1 on browning formation of WAT – while it was not altered in response to exercise in healthy women [368]. In the current study however, the UCP1 mRNA tended to display a positive correlation with physical activity levels (p=0.27, P=0.06) while measurements were performed in healthy men. The latter is in line with evidence from a controlled trial that examined the effects of chronic exercise on the UCP1 mRNA of subcutaneous WAT in healthy adults, also confirming no
significant alteration of UCP1 mRNA even though UCP1 mRNA was increased [236]. The first study of this PhD (systematic review) also failed to detect a link between physical activity/exercise with UCP1 mRNA of WAT in humans. Overall, this evidence suggests that the initial hypothesis of the current study should be rejected.

UCP1 mRNA and UCP1 protein expression were not associated in the current study. Previous evidence showed no obvious relationship between UCP1 mRNA and the functional protein under cold exposure in BAT of animals [304]. Also, there is a delay between elevated UCP1 mRNA and elevated UCP1 protein expression in BAT probably because of an unchanged translational effectiveness [304]. Furthermore, UCP1 mRNA found to be increased more in WAT than in BAT in response to cold exposure, in animals [304]. However, there is no evidence regarding the differences between WAT and BAT in UCP1 mRNA in response to physical activity/exercise. The current study detected positive white fat cells of the UCP1 gene which may indicate a browning formation process of white fat cells; this however, cannot be attributed to physical activity given the lack of relevant positive associations between physical activity levels and UCP1. The UCP1 protein expression half-life is about 3-6 days in response to cold exposure [369, 370] nevertheless, the mode of the current study did not allow us to examine potential variations in UCP1 values in response to physical activity, based on the half-life of the protein. It was previously suggested that the presence of the UCP1 in white fat cells in animals is dependent on a synergetic interactions between selective genes and variations in recessive and dominants genes that affect UCP1 gene even though the mechanisms are unknown [371]. It was also demonstrated that a subset of the precursor cells in white adipocytes may promote rise of brown-like adipocytes that express UCP1 [272]. The mode of the current study
could not highlight the latter issues and therefore, the presence of UCP1 in subcutaneous fat cells in untrained individuals remains to be elucidated.

The inverse association of PGC-1α mRNA with BMI, WHR and %fat mass and its positive association with fat-free mass indicate that PGC-1α is mainly expressed in tissues that demand large amounts of energy such as skeletal muscle. Studies show that PGC-1α increases fatty acid oxidation [372, 373] and fat-free mass is positively associated with REE in humans [374], which indicates increased demands of energy. REE is highly dependent on the amount of fat-free mass in both men and women [375, 376]. This is because the fat-free mass is the most proportional tissue in human body compared to fat mass [377] and it includes skeletal muscles and organs, which demand high amounts of energy [378]. PGC-1α is highly expressed in tissues that demand energy, such as the skeletal muscle [226] while increased PGC-1α activation is associated with increased REE in mice [379]. This is because PGC-1α is responsible for mitochondrial biogenesis that are responsible for energy production in the form of ATP [171].

Even though a positive association between PGC-1α mRNA and fat-free mass was detected in the current study, no association of PGC-1α mRNA with REE was found. Previous evidence showed that when a browning process occurs in WAT in response to exercise this lead to increased REE in mice [380]. Also, physical activity levels are positively associated with REE in humans [68]. However, no association of PGC-1α mRNA with physical activity levels was detected in the current study. Regarding the inverse association of PGC-1α mRNA with fat mass, this is probably because PGC-1α is involved in the regulation of genes that control energy homeostasis in metabolic tissues including WAT [381]. In specific, PGC-1α is involved in controlling the development of obesity by regulating oxidative phosphorylation and
fatty acid oxidation that regulate lipid metabolism and lessen insulin resistance in mice [382].

An inverse association between PPARα mRNA with BMI, WHR and %fat mass was also found. PPARα appears to be more active in BAT, liver, intestine, heart, and kidney than in WAT [383]. It is activated under conditions of energy deprivation [246] and its activation promotes the catabolism of fatty acid beta oxidation [247]. Given that WAT is mainly responsible for energy storage [88], the inverse associations of PPARα mRNA with BMI, WHR and fat mass percentage indicate white adipocytes accumulation. PPARα increases ketogenesis and subsequently the uptake of fatty acids, ameliorates beta oxidation in mitochondria and increases triglyceride uptake [247]. Therefore, it triggers lipid catabolism in WAT and this may explain its inverse association with BMI, WHR and fat mass percentage we observed in the current study.

Overall, the inverse associations of PGC-1α and PPARα mRNAs with fat mass, BMI and WHR indicate a possible positive association of these factors with weight loss. However, in order to assume a browning effect of these factors in WAT in response to physical activity, we would expect to observe negative associations of UCP1 mRNA and protein expression with fat mass, BMI and WHR, which were not detected in the current study. Furthermore, a recent study revealed that UCP1 mRNA of subcutaneous WAT in healthy women was negatively associated with weight loss in response to exercise [368], which indicates no relationship of UCP1 with a browning formation of WAT given that increased UCP1 would expect to cause weight loss via an elevation of REE [30]. Given also that PGC-1α and PPARα genes display multiple interactions with other genes other than UCP1, we cannot assume that the inverse associations of PGC-1α and PPARα mRNAs with fat mass, BMI and WHR that were observed in the current study indicate a browning formation of WAT.
It is reasonable to assume that the present results may have been influenced by methodological limitations such as the lack of prior power calculation to determine the sample size. However, this was not possible given the lack of similar design studies that could be utilised to perform sample size calculations. Therefore, a post-measurements power calculation was conducted using an online software (DSS Research) to test >95% statistical power. This revealed 100% of statistical power based on the UCP1 mRNA value (0.19±0.18) we detected in our study and expected UCP1 mRNA value (0.00010±0.0003) from the only previous controlled trial that examined the effect of exercise on UCP1 mRNA of subcutaneous WAT in humans [236]. The study only measured mRNAs of the PGC-1α, PPARα and PPARγ genes. The mRNA of genes usually indicate the protein production sometimes however, they do not predict it [197-199] given that one mRNA transcription may encode the protein more than one time [197-199]. Therefore, protein concentrations may also have needed for PGC-1α, PPARα and PPARγ to determine the physiological function of these genes.

6.5. CONCLUSIONS
The UCP1 protein expression is negatively associated with physical activity levels while UCP1 mRNA and protein expression are not linked with body weight (BMI, WHR, fat mass) as well as REE. These findings indicate no browning effect of WAT in response to physical activity in healthy men. The mRNA of PGC-1α, PPARα and PPARγ genes that along with UCP1 could indicate browning formation of subcutaneous WAT are not associated with physical activity levels in healthy men. PGC-1α and PPARα mRNAs are negatively associated with increased fat mass accumulation parameters (BMI, WHR and fat mass) suggesting a potential association
between PGC-1α and PPARα with human WAT metabolism. The mechanisms of the latter associations remain to be determined.
7.1. INTRODUCTION

BAT produces heat to maintain body temperature as a response to cold exposure via NST, which occurs through the action of UCP1 [25]. The link between exercise and BAT has been investigated because animal studies have shown that increased BAT activity reduces weight gain, up-regulates insulin sensitivity, lowers free fatty acid levels in serum, as well as the risk for T2D and other metabolic disorders [179-183] while exercise seems to increase BAT activity [185, 190, 195, 384, 385]. Evidence from several animal studies shows that exercise may also transform subcutaneous WAT into brown-like adipose tissue through the action of UCP1 and several other genes (i.e. PGC-1α, PPARα and PPARγ) [245, 277-279]. A recent study that examined the latter in both mice and humans [30] found that exercise increases the activation of PGC-1α in skeletal muscle that up-regulates the FNDC5 protein, which is subsequently cleaved and released into the bloodstream as Irisin. Irisin is attached – through an unknown receptor – on the surface of white adipocytes and generates a cascade of events changing the function of white adipocytes into brown-like adipocytes [30]. This transformation was thought to occur in part because of PPARα involvement that enhances UCP1 mRNA within WAT [30]. Indeed, FNDC5 increased by 3-fold the mRNA of PPARα in vitro and a pharmacological inhibition by antagonists of PPARα limited the action of FNDC5 [30]. This indicates that the increased UCP1 mRNA within the white adipocytes was caused, at least in part, by the action of PPARα [30]. Given that UCP1 is the only known contributor in NST that occurs in brown adipocytes, its presence in white adipocytes may indicate brown-like adipocytes properties [223].
The above mechanism triggered a series of research projects in the area. In particular, human Irisin attracted a lot of attention given its inverse correlation with blood glucose, insulin and cholesterol [266] and its role in the transformation of WAT into brown-like adipose tissue via the action of UCP1 [30]. However, other evidence shows that the FNDC5 gene – the precursor of Irisin – is not significantly increased by exercise in human muscle [267] and that the start codon of FNDC5 gene is mutated in humans [274], which raises doubts about the biological activity of Irisin. In line with this, some human studies show that acute exercise increases circulating Irisin [268, 269]; others show no effects [33, 270] and others show that either circulating Irisin is increased in response to chronic exercise [271] or it is not altered [33]. Also, the first study of this PhD (systematic review) revealed that the previous studies in humans used inaccurate methods to identify circulating Irisin. Therefore, there is no evidence to support that Irisin is increased in response to exercise while the evidence regarding the effects of exercise on UCP1 in WAT in humans is very limited. Indeed, the first study of this PhD (systematic review) detected only one study that investigated the effects of exercise on UCP1 mRNA in WAT in humans.

The current study aimed to examine part of the mechanism that seems to change the mode of WAT into brown-like mode. Apart from the exercise intervention, we also included a de-training period that followed the exercise intervention in order to compare its results with the potential effects of exercise on the indices examined. Specifically, the current study aimed to examine the effects of different types of exercise and de-training on the UCP1 mRNA and protein expression of subcutaneous WAT in healthy untrained men in a parallel RCT design. A secondary aim was to examine the effects of different types of exercise and de-training on the mRNA of PGC-1α, PPARα and PPARγ genes. It was hypothesized that different types of chronic
exercise would increase and the following de-training period would decrease the UCP1 mRNA and protein expression in subcutaneous WAT of healthy untrained men. Anthropometry indices, REE (kcal), physical activity levels, energy intake (kcal) and diet – factors known to influence metabolism – were also recorded throughout the trial for their use as confounding factors.

7.2. METHODS

We measured height and weight to calculate BMI, waist and hip circumferences to calculate WHR, blood pressure and body composition via a bio-electrical impedance device. Also, we measured REE, physical activity levels, we retrieved diet recalls and we obtained subcutaneous fat biopsies. Upon inclusion, a participant was randomly assigned to a group using an online computer software (DSS Research) [293]. The initial allocation and enrol of the participants to an experimental group was done by the Researcher in charge (PhD student). The supervision of the exercise interventions was done by three different assistants, one for each exercise group who were blinded to the aim of the study and they were supervised by the Researcher in charge (PhD student) to ensure adherence to the exercise protocols. The methods of this parallel RCT regarding inclusion criteria, anthropometry, assessments of REE, VO_{2\text{peak}}, energy intake, diet elements, physical activity, subcutaneous fat biopsies procedure, gene and protein expression analyses, exercise protocols (Table 8) and allocation of the participants in groups (Figure 7) are described in details in the general methods in Chapter 4, pages 61-71. Figure 8 depicts the methodological design of the present study, which can be found in Chapter 4, page 64.
7.2.1. Participants and procedures

The current study was conducted in Trikala, Thessaly, Greece, and the experimental protocol started in July of 2013 and ended in June of 2014. A priori power calculation was conducted using an online software (DSS Research) to test statistical power based on previous data of UCP1 mRNA [(baseline=0.00010±0.0003), (post-exercise=0.00020±0.00005)] that retrieved from a similar controlled trial that conducted in humans [236]. The power calculation analysis showed a required sample size of at least seven participants per group. This result ensured 100% of statistical power in the current study (alpha error 5%). The participants were recruited through an advertisement in a local newspaper in the area of Trikala, Thessaly, Greece. The inclusion criteria included: adult men who had not participated in a regular exercise programme, non-smokers, with no chronic disease and/or not on medication.

Once the participants confirmed their participation, they were given a pedometer. This was to measure their physical activity levels during a week period prior to baseline measurements as well as at weeks eight and sixteen (please see general methods, page 65). Diet recalls were retrieved during a week period prior to baseline measurements as well as at weeks eight and sixteen (please see general methods, pages 69-70). Following the methodological design (Figure 8, page 64), the participants were given appointments to measure VO₂peak and 1RM at baseline and at the end of weeks four, eight and sixteen. Three days after the first appointment participants were given a second appointment at baseline week and at the end of weeks eight and sixteen to measure the following in the specific order to avoid interaction effects between measurements: 1) anthropometry, 2) body composition, 3) REE and 4) subcutaneous fat biopsy. For the latter measurements the participants visited the laboratory between 07:00 to 09:00 am after been instructed to refrain from
exercise, alcohol, and passive smoking in 72-hour prior the measurements as well as to undertake a 12-hour fast prior the measurements in order to minimize the risk for misleading results [294, 295]. Written consent forms were obtained from 32 healthy non-smokers men (age: 36.06±7.36, BMI: 27.60±4.62). The baseline characteristics of the participants can be found in Table 13.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>36.06±7.36</td>
</tr>
<tr>
<td>BMI</td>
<td>27.60±4.62</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.91±0.08</td>
</tr>
<tr>
<td>VO₂peak (L/min)</td>
<td>2.49±0.52</td>
</tr>
<tr>
<td>Systolic Blood pressure (mm/Hg)</td>
<td>124.28±10.08</td>
</tr>
<tr>
<td>Diastolic Blood pressure (mm/Hg)</td>
<td>84.28±6.91</td>
</tr>
<tr>
<td>IPAQ (METs-min/week)</td>
<td>914±735</td>
</tr>
<tr>
<td>Steps/day</td>
<td>7842±3321</td>
</tr>
<tr>
<td>1RM chest press (kg)</td>
<td>59±17</td>
</tr>
<tr>
<td>1RM leg extension (kg)</td>
<td>77±36</td>
</tr>
<tr>
<td>Fat mass %</td>
<td>28.3±8.9</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>52.9±5.02</td>
</tr>
<tr>
<td>Resting energy expenditure (kcal)</td>
<td>1567±215</td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>1464±456</td>
</tr>
<tr>
<td>Weight of food intake (gr)</td>
<td>1039±330</td>
</tr>
<tr>
<td>Protein (gr)</td>
<td>64±21</td>
</tr>
<tr>
<td>Carbohydrates (gr)</td>
<td>145±43</td>
</tr>
<tr>
<td>Total fat intake (gr)</td>
<td>65±28</td>
</tr>
<tr>
<td>Total sugar intake (gr)</td>
<td>62±26</td>
</tr>
<tr>
<td>Caffeine (gr)</td>
<td>98±175</td>
</tr>
<tr>
<td>UCP1 mRNA</td>
<td>0.27±0.15</td>
</tr>
<tr>
<td>UCP1 protein expression</td>
<td>1.05±0.69</td>
</tr>
<tr>
<td>PGC-1α mRNA</td>
<td>1.07±0.74</td>
</tr>
<tr>
<td>PPARα mRNA</td>
<td>1.74±0.71</td>
</tr>
<tr>
<td>PPARγ mRNA</td>
<td>0.44±0.29</td>
</tr>
</tbody>
</table>

**BMI**: Body mass index; **VO₂peak**: Maximal oxygen uptake; ml/kg · min⁻¹; Millilitre per kilogram, per minute; **1RM**: One repetition maximum; kg: Kilograms; kcal: kilocalories; gr: Grammars; mm/Hg: Millimetre of mercury; **UCP1**: Uncoupling protein one; **PGC1α**: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; **PPARα**: Peroxisome proliferator-activated receptor alpha; **PPARγ**: Peroxisome Proliferator-Activated Receptor Gamma.
7.2.2. Statistical analysis

The normal distribution of the variables was tested using Kolmogorov-Smirnov. Due to the non-normal distribution of some variables, we calculated the delta scores of baseline and post-exercise values (week 8) as well as of post-exercise (week 8) and de-training values (week 16) for UCP1 mRNA and protein expression as well as PGC-1α, PPARα and PPARγ mRNAs as previously described [386, 387]. The delta scores were again tested for normal distribution using Kolmogorov-Smirnov and displayed normal distribution. ANOVA with Bonferroni post-hoc t-tests were used to assess between and within groups differences of delta scores for UCP1 mRNA and protein expression as well as PGC1-α, PPARα and PPARγ mRNAs to test the effects of exercise interventions and de-training on these variables. Given that the analyses did not display any significant effects of exercise interventions and de-training on the delta scores of UCP1 mRNA and protein expression as well as PGC-1α, PPARα and PPARγ mRNAs, we did not perform additional analyses or used any of the studied confounding factors.

Following previous methodology, the 50th percentile was used to define the low and high responders for UCP1 mRNA and protein expression as well as for PGC-1α, PPARα and PPARγ mRNAs in order to test the effects of exercise interventions and de-training period on the high responders [309], based on the hypothesis of the current study. Chi square tests were used to assess differences between the categorical variable of the experimental group (four groups) and the categorical variable of low and high responders for UCP1 mRNA and protein expression as well as for PGC-1α, PPARα and PPARγ mRNAs. Kruskal–Wallis analysis of variance with post-hoc Mann-Witney U tests were used to assess the effects of exercise and de-training of high responders – both to exercise stimuli and de-training period – on UCP1 mRNA and
protein expression as well as on PGC-1α, PPARα and PPARγ mRNAs. Wilcoxon Signed Rank tests were used to assess the effects of exercise and de-training within groups of the high responders – both to exercise stimuli and de-training period – on UCP1 mRNA and protein expression as well as on PGC-1α, PPARα and PPARγ mRNAs.

Pooled Cohen’s d effect size analyses were also used to assess differences between and within groups [310, 362, 388]. Effect size analysis should be included in a parallel RCT, according to the CONSORT guidelines for RCTs [389], because the relative and/or absolute measures alone may not provide a complete understanding of the practical and/or the mechanistic value of the findings and their implications [389]. Pooled Cohen’s d effect size was calculated by dividing the mean difference of the variables examined with the pooled standard deviation of these variables (please see the following formula): 

\[
\frac{(M_1 - M_2)}{\sqrt{\frac{(N_1-1)(SD_1)^2 + (N_2-1)(SD_2)^2}{N_1+N_2-2}}}
\]

(N=group sample size, M=mean, SD=standard deviation) [310, 311]. According to Cohen an effect size 0.20-0.50 represents a small effect, 0.50-0.80 a moderate effect, and >0.80 a large effect [310]. For instance, a value of 0.20 in Cohen’s d effect size means that the two values differ by 0.2 standard deviations and this difference is not trivial even if it is statistically insignificant [310]. Furthermore, according to Cohen a small effect size represents a real effect but it needs careful exploration while a large effect size it is an obvious effect that is very considerable [310]. Pooled Cohen’s d effect size analyses were used to identify effect sizes between baseline and week 8 and between weeks 8 and 16 within groups and to identify effect sizes between each exercise group with the CG for UCP1 mRNA and protein expression as well as PGC-1α, PPARα and PPARγ mRNAs at all time points.
Wilcoxon Signed Rank tests were also used to calculate differences and to test mediating effects of exercise and de-training within groups for REE, WHR, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), VO\textsubscript{2}peak, 1RM upper body, 1RM lower body, fat mass percentage, fat-free mass (kg), weight of food intake (gr), protein (gr), carbohydrates (gr), total fat intake (gr), total sugar intake (gr), caffeine (gr), total energy intake (kcal) and pedometer steps/day at all time points. All analyses were conducted with SPSS Inc., Chicago, IL, USA, version 22 and a $P \leq 0.05$ level of significance.

The number of the participants randomly assigned in the exercise programmes, the drop outs with reasons and the number of the participants included in the final analysis are displayed in Figure 7, Chapter 4, page 63.

7.3. RESULTS

The average attendance of the participants in the supervised exercise programmes was 31.6 out of 34 sessions (92.9%) for the AEG, 30 out of 31 sessions (96.8%) for the REG and 26.8 out of 29 sessions (92.41%) for the CEG. During the aerobic and combined (aerobic+resistance) exercise programmes heart rate monitors (Polar RS800CX Electro Oy, Kempele, Finland) were used to ensure that the participants follow the appropriate exercise intensity in every exercise session. Also, 1RM tests were used for each resistance exercise at baseline and at week 4 to ensure adherence to the exercise intensities for each participant. No harms (e.g. injuries) were reported from the participants during this experiment.

7.3.1. Primary outcomes

Results from ANOVA analysis
No significant differences between and within groups in delta scores of UCP1 mRNA and protein expression were detected between baseline and post-exercise values (week 8) nor between post-exercise (week 8) and de-training values (week 16). The individual data for UCP1 mRNA are shown in Figure 17A and for UCP1 protein expression are shown in Figure 17B.

**Figure 17**: Individual data for UCP1 mRNA (A) and protein expression (B) at baseline, post exercise (week 8) and de-training (week 16)

Each number in the x axis represents one participant; The participants 1, 5, 11, 13, 18, 19, 20, 24 and 32 belong to the aerobic exercise group; The participants 4, 6, 8, 14, 21, 23, 25, and 29 belong to the resistance exercise group; The participants 2, 10, 12, 15, 26, 28, 30, and 31 belong to the combined (aerobic+resistance) exercise
The participants 3, 7, 9, 16, 17, 22, and 27 belong to the control group; UCP1: Uncoupling protein one.

Results from low responders versus high responders analysis

Regarding UCP1 mRNA and protein expression, the low responders to the exercise stimuli were considered those who displayed values less than the 50th percentile while the high responders to the exercise stimuli were considered those who displayed values equal or more than the 50th percentile. Furthermore, the low responders to the de-training period were considered those who displayed values equal or more than the 50th percentile while the high responders to the de-training period were considered those who displayed values less than the 50th percentile [309]. This is because according to the hypothesis of this study the exercise intervention was expected to increase UCP1 mRNA and protein expression while the de-training period was expected to decrease UCP1 mRNA and protein expression.

Chi square analysis revealed no significant differences between the experimental groups (categorical variable consisted of four groups) and a) UCP1 mRNA (categorical variable of low and high responders to exercise stimuli) \( \chi^2 (3, n=29)=0.60, P>0.05 \) as well as b) UCP1 protein expression (categorical variable of low and high responders to exercise stimuli) \( \chi^2 (3, n=29)=3.92, P>0.05 \). Chi square analysis also showed no significant differences between the experimental groups (categorical variable consisted of four groups) and a) UCP1 mRNA (categorical variable of low and high responders to de-training period) \( \chi^2 (3, n=25)=1.49, P>0.05 \) as well as b) UCP1 protein expression (categorical variable of low and high responders to de-training period) \( \chi^2 (3, n=25)=3.49, P>0.05 \).

Kruskal–Wallis analysis of variance revealed no differences for UCP1 mRNA and protein expression between the experimental groups in high responders to the
exercise stimuli and in high responders to the de-training period at all time points (P>0.05). Wilcoxon Signed Rank tests revealed no differences of UCP1mRNA and protein expression in high responders to the exercise stimuli between baseline and post-exercise as well as in high responders to the de-training period between post-exercise and de-training within each experimental group. The number and the percentage of the low and high responders of UCP1mRNA and protein expression for each experimental group are shown in Table 14.

Table 14: Number and percentage of the low and high responders for UCP1 mRNA and protein expression

<table>
<thead>
<tr>
<th>Group</th>
<th>Low responders</th>
<th></th>
<th>High responders</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UCP1 mRNA</td>
<td>UCP1 protein expression</td>
<td>UCP1 mRNA</td>
<td>UCP1 protein expression</td>
</tr>
<tr>
<td>Exercise stimuli</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEG</td>
<td>3 (37.5%)</td>
<td>4 (50%)</td>
<td>5 (62.5%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>REG</td>
<td>3 (50%)</td>
<td>2 (33.3%)</td>
<td>3 (50%)</td>
<td>4 (66.7%)</td>
</tr>
<tr>
<td>CEG</td>
<td>4 (50%)</td>
<td>6 (75%)</td>
<td>4 (50%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>CG</td>
<td>3 (42.9%)</td>
<td>2 (28.6%)</td>
<td>4 (57.1%)</td>
<td>5 (71.4%)</td>
</tr>
<tr>
<td>De-training period</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEG</td>
<td>2 (33.3%)</td>
<td>2 (33.3%)</td>
<td>4 (66.7%)</td>
<td>4 (66.7%)</td>
</tr>
<tr>
<td>REG</td>
<td>4 (66.7%)</td>
<td>5 (83.3%)</td>
<td>2 (33.3%)</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>CEG</td>
<td>4 (50%)</td>
<td>4 (50%)</td>
<td>4 (50%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>CG</td>
<td>3 (60%)</td>
<td>2 (40%)</td>
<td>2 (40%)</td>
<td>3 (60%)</td>
</tr>
</tbody>
</table>

UCP1: Uncoupling protein one; AEG: Aerobic exercise group; REG: Resistance exercise group; CEG: Combined (aerobic+resistance) exercise group; CG: Control group

Results from Cohen’s d effect size analysis

The UCP1 mRNA was increased in response to aerobic exercise, yet not significantly, while a small effect size (d=0.39) between baseline and post-exercise (Figure 18) was found. The UCP1 mRNA was also increased in response to de-training, yet not significantly, in REC, CEG and CG, while a small effect size in REG (d=0.25) (Figure 19A), in CEG (d=0.49) (Figure 19B) and in CG (d=0.48) (Figure 19C) between post-exercise and de-training was detected.
Figure 18: Cohen’s $d$ effect size in UCP1 mRNA between baseline and post-exercise (week 8) in AEG

*Small effect size ($d=0.39$); Values are depicted as mean and standard deviation; UCP1: Uncoupling protein one
**Figure 19**: Cohen’s $d$ effect size in UCP1 mRNA between post-exercise (week 8) and de-training (week 16) in REG, CEG and CG

![Graphs showing effect size](image)

**Figure 19A**: *Small effect size ($d=0.25$); Figure 19B**: # Small effect size ($d=0.49$); **Figure 19C**: £ Small effect size ($d=0.48$); Values are depicted as mean and standard deviation; UCP1: Uncoupling protein one.

Regarding the baseline values, a small effect size between CG and AEG ($d=0.38$), between CG and REG ($d=0.38$) and between CG and CEG ($d=0.35$) of UCP1 mRNA was identified (Figure 20A). The CG displayed the lowest values compared to the exercise groups. At post-exercise values, a moderate effect size between CG and AEG ($d=0.54$), a small effect size between CG and REG ($d=0.40$) as well as between CG and CEG ($d=0.26$) of UCP1 mRNA was found (Figure 20B).
Similar to the baseline values, the CG displayed the lowest values compared to the exercise groups. Finally, a small effect size between CG and CEG ($d=0.26$) was identified in UCP1 mRNA at de-training (Figure 20C).

**Figure 20**: Cohen’s $d$ effect size in UCP1 mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16)

*Figure 20A*: *Small effect size ($d=0.38$), # Small effect size ($d=0.38$), £ Small effect size ($d=0.35$)*; *Figure 20B*: *Moderate effect size ($d=0.54$), # Small effect size ($d=0.40$), £ Small effect size ($d=0.26$)*; *Figure 20C*: *Small effect size ($d=0.24$)*; Values are depicted as mean and standard deviation; UCP1: Uncoupling protein one.
The UCP1 protein expression was increased in response to exercise, yet not significantly, in AEG, CEG and CG. In this regard a small effect size between baseline and post-exercise was found in AEG ($d=0.26$) (Figure 21A), in CEG ($d=0.30$) (Figure 21C) and in CG ($d=0.31$) (Figure 21D). The UCP1 protein expression was also increased in response to de-training, yet not significantly, in REC and CEG, while a large effect size in REG ($d=1.28$) (Figure 21B) and in CEG ($d=0.88$) (Figure 21C) was detected. Finally, the UCP1 protein expression was decreased in response to de-training in CG while a small effect size between post-exercise and de-training was revealed ($d=0.34$) (Figure 21D).
Figure 21: Cohen’s $d$ effect size in UCP1 protein expression between baseline, post-exercise (week 8) and de-training (week 16)

**Figure 21A:** *Small effect size (d=0.26); Figure 21B: #Large effect size (d=1.28); Figure 21C: *Small effect size (d=0.30), #Large effect size (d=0.88); Figure 21D: *Small effect size (d=0.31), #Small effect size (d=0.34); Values are depicted as mean and standard deviation; UCP1: Uncoupling protein one.
Regarding the baseline values, a small effect size between CG and AEG ($d=0.42$), and a large effect size between CG and CEG ($d=0.90$) of UCP1 protein expression was identified (Figure 22A). In this regard, the CG displayed the highest values compared to the exercise groups. At post-exercise values, a small effect size between CG and AEG ($d=0.38$), a small effect size between CG and REG ($d=0.32$) and a moderate effect size between CG and CEG ($d=0.76$) of UCP1 protein expression was found (Figure 22B). Similar to the baseline values, the CG displayed the highest values compared to the exercise groups. Finally, a large effect size between CG and REG ($d=1.10$) and a small effect size between CG and CEG ($d=0.43$) was identified in UCP1 protein expression at de-training (Figure 22C).
**Figure 22:** Cohen’s $d$ effect size in UCP1 protein expression between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16).

**Figure 22A:** *Small effect size ($d=0.42$), £Large effect size ($d=0.90$); Figure 22B:** *Small effect size ($d=0.38$), #Small effect size ($d=0.32$), £Moderate effect size ($d=0.76$); **Figure 22C:** #Large effect size ($d=1.10$), *Small effect size ($d=0.43$); Values are depicted as mean and standard deviation; UCP1: Uncoupling protein one.
7.3.2. Secondary outcomes

Results from ANOVA analysis

No significant differences between and within groups in delta scores of PGC-1α, PPARα and PPARγ mRNAs were revealed between baseline and post-exercise values (week 8) nor between post-exercise (week 8) and de-training values (week 16). Figure 23 depicts the individual data for PGC-1α (Figure 23A), PPARα (Figure 23B) and PPARγ (Figure 23C) mRNAs.
Figure 23: Individual data for PGC-1α (A), PPARα (B) and PPARγ (C) mRNAs at baseline, post-exercise (week 8) and de-training (week 16)

Each number in the x axis represents one participant; The participants 1, 5, 11, 13, 18, 19, 20, 24 and 32 belong to the aerobic exercise group; The participants 4, 6, 8, 14, 21, 23, 25, and 29 belong to the resistance exercise group; The participants 2, 10, 12, 15, 26, 28, 30, and 31 belong to the combined (aerobic+resistance) exercise
group; The participants 3, 7, 9, 16, 17, 22, and 27 belong to the control group; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα: Peroxisome proliferator-activated receptor alpha; PPARγ: Peroxisome proliferator-activated receptor gamma.

Results from low responders versus high responders analysis

For PGC-1α, PPARα and PPARγ mRNAs the low responders to the exercise stimuli were considered those who displayed values less than the 50th percentile while the high responders to the exercise stimuli were considered those who displayed values equal or more than the 50th percentile. Furthermore, the low responders to the de-training period were considered those who displayed values equal or more than the 50th percentile while the high responders to the de-training period were considered those who displayed values less than the 50th percentile [309]. This is because according to the hypothesis of this study the exercise intervention was expected to increase PGC-1α, PPARα and PPARγ mRNAs while the de-training period was expected to decrease PGC-1α, PPARα and PPARγ mRNAs.

Chi square analyses revealed a significant difference between the experimental groups (categorical variable consisted of four groups) and PGC-1α mRNA $[\chi^2 (3, n=29)=12.64, P=0.005]$ as well as PPARα mRNA $[\chi^2 (3, n=29)=9.47, P=0.02]$ (categorical variables of low and high responders to exercise stimuli). However, no significant difference between the experimental groups (categorical variable consisted of four groups) and PPARγ mRNA (categorical variable of low and high responders to exercise stimuli) $[\chi^2 (3, n=29)=1.110, P>0.05]$ was detected. Similarly, no significant differences between the experimental groups (categorical variable consisted of four groups) and a) PGC-1α mRNA $[\chi^2 (3, n=25)=6.30, P>0.05]$, PPARα mRNA $[\chi^2 (3, n=25)=0.16, P>0.05]$ as well as b) PPARγ mRNA $[\chi^2 (3, n=25)=0.16, P>0.05]$ (categorical variables of low and high responders to de-training period) were found.
Kruskal–Wallis analysis of variance revealed no differences for PGC-1α and PPARγ mRNAs between the experimental groups in high responders to the exercise stimuli and in high responders to the de-training period at all time points (P>0.05). However, Kruskal–Wallis analysis of variance with post-hoc Mann-Witney U tests showed that PPARα mRNA was significantly higher in CG than in REG in high responders to the exercise stimuli at both baseline and post-exercise (P=0.02). Wilcoxon Signed Rank tests revealed no differences of PGC-1α, PPARα and PPARγ mRNAs in high responders to the exercise stimuli between baseline and post-exercise as well as in high responders to the de-training period between post-exercise and de-training within each experimental group. The number and the percentage of the low and high responders of PGC-1α, PPARα and PPARγ mRNAs for each experimental group are shown in Table 15.

<table>
<thead>
<tr>
<th>Group</th>
<th>Low responders</th>
<th>High responders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGC-1α mRNA</td>
<td>PPARα mRNA</td>
</tr>
<tr>
<td></td>
<td>PGC-1α mRNA</td>
<td>PPARα mRNA</td>
</tr>
<tr>
<td>Exercise stimuli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEG</td>
<td>3 (37.5%)</td>
<td>5 (62.5%)</td>
</tr>
<tr>
<td>REG</td>
<td>4 (66.7%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>CEG</td>
<td>7 (87.5%)</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>CG</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>De-training period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEG</td>
<td>2 (33.3%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>REG</td>
<td>2 (33.3%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>CEG</td>
<td>4 (50%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>CG</td>
<td>5 (100%)</td>
<td>3 (60%)</td>
</tr>
</tbody>
</table>

PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα: Peroxisome proliferator-activated receptor alpha; PPARγ: Peroxisome proliferator-activated receptor gamma; AEG: Aerobic exercise group; REG: Resistance exercise group; CEG: Combined (aerobic+resistance) exercise group; CG: Control group.
Results from Cohen’s $d$ effect size analysis

Results for PGC-1α

The PGC-1α mRNA augmented in CEG and CG and reduced in AEG after the exercise protocols while it reduced in response to de-training in all groups. More specifically, in AEG a small effect size ($d=0.21$) between baseline and post-exercise and a large effect size ($d=0.96$) between post-exercise and de-training was found (Figure 24A). In REG a large effect size ($d=0.89$) between post-exercise and de-training was identified (Figure 24B), while in CEG a small effect size ($d=0.26$) between baseline and post-exercise and a small effect size ($d=0.43$) between post-exercise and de-training were detected (Figure 24C). Finally, in CG a moderate effect size ($d=0.50$) between baseline and post-exercise and a small effect size ($d=0.35$) between post-exercise and de-training were found (Figure 24D).
Figure 24: Cohen’s $d$ effect size in PGC-1α mRNA between baseline, post-exercise (week 8) and de-training (week 16)

![Bar charts](image)

Figure 24A: *Small effect size ($d=0.21$), #Large effect size ($d=0.96$); Figure 24B: #Large effect size ($d=0.89$); Figure 24C: *Small effect size ($d=0.26$), #Small effect size ($d=0.43$); Figure 24D: *Moderate effect size ($d=0.50$), #Small effect size ($d=0.35$); Values are depicted as mean and standard deviation; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha.
At baseline values the PGC-1α mRNA was lower in REG than in CG, while a
large effect size \(d=1.01\) between REG and CG was detected (Figure 25A). In CEG
the PGC-1α mRNA was lower than in CG, while a large effect size \(d=1.36\) (Figure
25A) between CEG and CG was found. At post-exercise values the exercise groups
displayed lower PGC-1α mRNA than the CG. A moderate effect size between CG and
AEG \(d=0.73\) as well as a large effect size between CG and REG \(d=1.13\) and
between CG and CEG \(d=1.14\) (Figure 25B) was revealed. Finally, at de-training
values the exercise groups displayed lower PGC-1α mRNA than the CG. A large effect
size between CG and AEG \(d=1.61\), between CG and REG \(d=1.49\) and between
CG and CEG \(d=1.39\) (Figure 25C) was discovered.
Figure 25: Cohen’s $d$ effect size in PGC-1α mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16)

Figure 25A: # Large effect size ($d=1.01$), £ Large effect size ($d=1.36$); Figure 25B: *Moderate effect size ($d=0.73$), # Large effect size ($d=1.13$), £ Large effect size ($d=1.14$); Figure 25C: *Large effect size ($d=1.61$), # Large effect size ($d=1.49$), £ Large effect size ($d=1.39$); Values are depicted as mean and standard deviation; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

Results for PPARα

The PPARα mRNA was decreased, yet not significantly, after the de-training period in all groups. The effect size analyses showed in AEG a small effect size ($d=0.38$) (Figure 26A), in REG a moderate effect size ($d=0.61$) (Figure 26B), in CEG a small effect size ($d=0.47$) (Figure 26C) and in CG also a moderate effect size ($d=0.66$) (Figure 26D)
between post-exercise and de-training. Finally, a small effect size ($d=0.27$) in CEG between baseline and post-exercise was found (Figure 26C).

**Figure 26**: Cohen’s $d$ effect size in PPARα mRNA between baseline, post-exercise (week 8) and de-training (week 16)
The PPARα mRNA displayed lower values in the exercise groups than the CG at all time points. At baseline a large effect size between CG and AEG \((d=1.48)\), between CG and REG \((d=1.80)\), as well as between CG and CEG \((d=1.84)\) (Figure 27A) was revealed. At post-exercise a large effect size between CG and AEG \((d=1.47)\), between CG and REG \((d=1.71)\) and between CG and CEG \((d=1.68)\) (Figure 27B) was found. At de-training a large effect size between CG and AEG \((d=1.21)\), between CG and REG \((d=1.41)\) and between CG and CEG \((d=1.30)\) (Figure 27C) was also detected.
**Figure 27:** Cohen’s $d$ effect size in PPARα mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16)

**Figure 27A:** *Large effect size (d=1.48), #Large effect size (d=1.80), £Large effect size (d=1.84); Figure 27B:** *Large effect size (d=1.47), #Large effect size (d=1.71), £Large effect size (d=1.68); **Figure 27C:** *Large effect size (d=1.21), #Large effect size (d=1.41), £Large effect size (d=1.30); Values are depicted as mean and standard deviation; PPARα: Peroxisome proliferator-activated receptor alpha
**Results for PPARγ**

The PPARγ mRNA was increased in response to exercise, yet not significantly in REG, CEG and CG and decreased in response to de-training in AEG, REG and CEG. In AEG a moderate effect size ($d=0.57$) between post-exercise and de-training was found (Figure 28A). In REG a moderate effect size ($d=0.52$) between baseline and post-exercise and a moderate effect size ($d=0.75$) between post-exercise and de-training was detected (Figure 28B). In CEG the PPARγ mRNA was also increased, yet not significantly in response to exercise, while a small effect size ($d=0.30$) between baseline and post-exercise and a small effect size between post-exercise and de-training ($d=0.22$) was noticed (Figure 28C). Finally, in CG the PPARγ mRNA was reduced, yet not significantly in response to exercise, while a small effect size ($d=0.20$) between baseline and post-exercise was identified (Figure 28D).
**Figure 28:** Cohen’s $d$ effect size in PPARγ mRNA between baseline, post-exercise (week 8) and de-training (week 16)

- **A:** Moderate effect size ($d=0.57$); **B:** Moderate effect size ($d=0.52$); **C:** Moderate effect size ($d=0.75$); **D:** Small effect size ($d=0.30$); **D:** Small effect size ($d=0.22$); **D:** Small effect size ($d=0.20$); Values are depicted as mean and standard deviation; PPARγ: Peroxisome proliferator-activated receptor gamma.
The exercise groups displayed lower PPARγ mRNA compared to the CG at all time points. At baseline a small effect size between CG and AEG ($d=0.49$), a moderate effect size between CG and REG ($d=0.76$) and a large effect size between CG and CEG ($d=0.98$) (Figure 29A) was found. At post-exercise a small effect size between CG and AEG ($d=0.41$) and between CG and REG ($d=0.26$) as well as a moderate effect size between CG and CEG ($d=0.66$) (Figure 29B) was shown. At de-training a moderate effect size between CG and AEG ($d=0.71$), between CG and REG ($d=0.59$) and between CG and CEG ($d=0.67$) (Figure 29C) was detected.
Figure 29: Cohen’s $d$ effect size in PPARγ mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16)

Figure 29A: *Small effect size ($d=0.49$), #Large effect size ($d=0.76$), £Large effect size ($d=0.98$); Figure 29B: *Small effect size ($d=0.41$), #Small effect size ($d=0.26$), £Moderate effect size ($d=0.66$); Figure 29C: *Moderate effect size ($d=0.71$), #Moderate effect size ($d=0.59$), £Moderate effect size ($d=0.67$); Values are depicted as mean and standard deviation; PPARγ: Peroxisome proliferator-activated receptor gamma.

7.3.3. Results from analysis within groups

Aerobic exercise group

Wilcoxon Signed Rank tests showed a reduction in 1RM lower body ($z=-1.992, P=0.05$) between baseline and post-exercise, while a reduction between baseline and
de-training in 1RM lower body (z=-2.032, P=0.04) and in steps per day (z=-2.201, P=0.03) were also detected. Finally, WHR (z=-2.041, P=0.04) and SBP (z=-2.226, P=0.03) were significantly decreased within the AEG between post-exercise and de-training. The mean differences between the time points within the AEG can be found in Table 16.

<table>
<thead>
<tr>
<th>Table 16: Mean and standard deviation of outcome variables in AEG (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td><strong>(week 8)</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
</tr>
<tr>
<td><strong>Waist to hip ratio</strong></td>
</tr>
<tr>
<td><strong>VO2peak (L/min)</strong></td>
</tr>
<tr>
<td><strong>Systolic Blood pressure (mm/Hg)</strong></td>
</tr>
<tr>
<td><strong>Diastolic Blood pressure (mm/Hg)</strong></td>
</tr>
<tr>
<td><strong>Steps/day</strong></td>
</tr>
<tr>
<td><strong>1RM upper body (kg)</strong></td>
</tr>
<tr>
<td><strong>1RM lower body (kg)</strong></td>
</tr>
<tr>
<td><strong>Fat mass %</strong></td>
</tr>
<tr>
<td><strong>Fat-free mass (kg)</strong></td>
</tr>
<tr>
<td><strong>REE (kcal)</strong></td>
</tr>
<tr>
<td><strong>Energy intake (kcal)</strong></td>
</tr>
<tr>
<td><strong>Weight of food intake (gr)</strong></td>
</tr>
<tr>
<td><strong>Protein (gr)</strong></td>
</tr>
<tr>
<td><strong>Carbohydrates (gr)</strong></td>
</tr>
<tr>
<td><strong>Total fat intake (gr)</strong></td>
</tr>
<tr>
<td><strong>Total sugar intake (gr)</strong></td>
</tr>
<tr>
<td><strong>Caffeine (gr)</strong></td>
</tr>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>UCP1 mRNA</td>
</tr>
<tr>
<td>UCP1 protein</td>
</tr>
<tr>
<td>expression</td>
</tr>
<tr>
<td>PGC-1α mRNA</td>
</tr>
<tr>
<td>PPARα mRNA</td>
</tr>
<tr>
<td>PPARγ mRNA</td>
</tr>
</tbody>
</table>

*BMI: Body mass index; VO₂peak: Maximal oxygen uptake; ml/kg⁻¹/min⁻¹; Millilitre per kilogram, per minute; 1RM: One repetition maximum; kg: Kilograms; REE: Resting energy expenditure; kcal: Kilocalories; gr: Grammars; mm/Hg: Millimetre of mercury; UCP1: Uncoupling protein one; PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα: Peroxisome proliferator-activated receptor alpha; PPARγ: Peroxisome Proliferator-Activated Receptor Gamma.*

**Significant differences between post-exercise and de-training**

*Significant differences between baseline and post-exercise*

£ Significant differences between baseline and de-training

**Resistance exercise group**

In REG an increase between baseline and week 4 in 1RM upper body (z=-2.226, P=0.03) was found. Additionally, an increase in VO₂peak between baseline and post-exercise (z=-1.992, P=0.05), and between baseline and de-training (z=-1.992, P=0.05) were revealed. Also, an increase in WHR (z=-2.060, P=0.04) between post-exercise and de-training was detected. An increase in 1RM upper body (z=-2.226, P=0.03) and in 1RM lower body (z=-2.201, P=0.03) between baseline and post-exercise was also identified while a reduction in 1RM upper body (z=-2.023, P=0.04) and in 1RM lower body (z=-2.023, P=0.04) between post-exercise and de-training was found. A decrease in SBP (z=-2.041, P=0.04) between baseline and post-exercise was detected while a decrease in SBP (z=-2.023, P=0.04) between baseline and de-training was noticed. Finally, a reduction in DBP (z=-2.000, P=0.05) between baseline and post-exercise was revealed. The mean differences between the time points within the REG can be found in Table 17.
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 4</th>
<th>Post-exercise (week 8)</th>
<th>De-training (week 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34.38±6.41</td>
<td></td>
<td>34.38±6.41</td>
<td>34.38±6.41</td>
</tr>
<tr>
<td>BMI</td>
<td>27.32±2.95</td>
<td></td>
<td>28.05±3.19</td>
<td>27.98±3.17</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.89±0.07</td>
<td></td>
<td>0.92±0.06*</td>
<td>0.94±0.07*</td>
</tr>
<tr>
<td>VO_{2}\text{peak} (L/min)</td>
<td>2.42±0.46*£</td>
<td>2.47±0.42</td>
<td>2.66±0.24*</td>
<td>2.64±0.46£</td>
</tr>
<tr>
<td>Systolic Blood pressure (mm/Hg)</td>
<td>127±15*£</td>
<td></td>
<td>118±10*</td>
<td>116±12£</td>
</tr>
<tr>
<td>Diastolic Blood pressure (mm/Hg)</td>
<td>81±6*</td>
<td></td>
<td>78±7*</td>
<td>82±7</td>
</tr>
<tr>
<td>Steps/day</td>
<td>7086±2728</td>
<td></td>
<td>6553±3755</td>
<td>6779±4143</td>
</tr>
<tr>
<td>1RM upper body (kg)</td>
<td>70±23*#</td>
<td>78±21#</td>
<td>82±24*¥</td>
<td>78±23¥</td>
</tr>
<tr>
<td>1RM lower body (kg)</td>
<td>81±27*</td>
<td>90±32</td>
<td>111±77*¥</td>
<td>95±60¥</td>
</tr>
<tr>
<td>Fat mass %</td>
<td>28.08±4.7</td>
<td></td>
<td>29.3±3.6</td>
<td>30.2±3.8</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>54.6±4.3</td>
<td></td>
<td>54.5±3.9</td>
<td>53.2±3.3</td>
</tr>
<tr>
<td>REE (kcal)</td>
<td>1597±118</td>
<td></td>
<td>1623±260</td>
<td>1520±172</td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>1401±386</td>
<td></td>
<td>1524±140</td>
<td>1781±601</td>
</tr>
<tr>
<td>Weight of food intake (gr)</td>
<td>996±381</td>
<td></td>
<td>1133±250</td>
<td>1220±361</td>
</tr>
<tr>
<td>Protein (gr)</td>
<td>62±23</td>
<td></td>
<td>65±20</td>
<td>83±25</td>
</tr>
<tr>
<td>Carbohydrates (gr)</td>
<td>146±41</td>
<td></td>
<td>180±34</td>
<td>185±61</td>
</tr>
<tr>
<td>Total fat intake (gr)</td>
<td>58±19</td>
<td></td>
<td>58±9</td>
<td>69±34</td>
</tr>
<tr>
<td>Total sugar intake (gr)</td>
<td>66±32</td>
<td></td>
<td>71±9</td>
<td>85±31</td>
</tr>
<tr>
<td>Caffeine (gr)</td>
<td>20±30</td>
<td></td>
<td>35±63</td>
<td>23±38</td>
</tr>
<tr>
<td>UCP1 mRNA</td>
<td>0.29±0.13</td>
<td></td>
<td>0.31±0.16</td>
<td>0.36±0.19</td>
</tr>
<tr>
<td>UCP1 protein expression</td>
<td>1.30±0.84</td>
<td></td>
<td>1.24±0.48</td>
<td>1.98±0.58</td>
</tr>
<tr>
<td>PGC-1α mRNA</td>
<td>0.86±0.39</td>
<td></td>
<td>0.80±0.25</td>
<td>0.59±0.20</td>
</tr>
<tr>
<td>PPARα mRNA</td>
<td>1.53±0.37</td>
<td></td>
<td>1.52±0.28</td>
<td>1.31±0.38</td>
</tr>
<tr>
<td>PPARγ mRNA</td>
<td>0.39±0.17</td>
<td></td>
<td>0.48±0.18</td>
<td>0.34±0.18</td>
</tr>
</tbody>
</table>

*BMI: Body mass index; VO_{2}\text{peak}: Maximal oxygen uptake; ml/kg^-1/min^-1; Millilitre per kilogram, per minute; 1RM: One repetition maximum; kg: Kilograms; REE: Resting energy expenditure; kcal: kilocalories; gr: Grammars; mm/Hg: Millimetre of mercury; UCP1: Uncoupling protein one; PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα: Peroxisome proliferator-activated receptor alpha; PPARγ: Peroxisome Proliferator-Activated Receptor Gamma.
Combined exercise group

In CEG we detected an increase in 1RM lower body (z=-2.023, P=0.04) between baseline and week 4. There was an increase in BMI (z=-2.035, P=0.04), in 1RM upper body (z=-2.366, P=0.02) and in 1RM lower body (z=-2.366, P=0.02) between baseline and post-exercise. Finally, there was a reduction in 1RM upper body (z=-2.371, P=0.02), in 1RM lower body (z=-2.201, P=0.03), in energy intake (z=-2.521, P=0.01) and in carbohydrates intake (z=-2.380, P=0.02) between post-exercise and de-training. The mean differences between the time points within the CEG can be found in Table 18.

Table 18: Mean and standard deviation of outcome variables in CEG (n=8)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 4</th>
<th>Post-exercise (week 8)</th>
<th>De-training (week 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37.75±9.22</td>
<td>37.75±9.22</td>
<td>37.75±9.22</td>
<td>37.75±9.22</td>
</tr>
<tr>
<td>BMI</td>
<td>27.16±5.78¥</td>
<td>27.43±5.62¥</td>
<td>27.36±5.76</td>
<td></td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.93±0.07</td>
<td>0.93±0.06</td>
<td>0.93±0.05</td>
<td></td>
</tr>
<tr>
<td>VO₂peak (L/min)</td>
<td>2.50±0.58</td>
<td>2.54±0.61</td>
<td>2.81±0.67</td>
<td>2.39±0.56</td>
</tr>
<tr>
<td>Systolic Blood</td>
<td>128±7</td>
<td>122±14</td>
<td>126±8</td>
<td></td>
</tr>
<tr>
<td>pressure (mm/Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic Blood</td>
<td>86±8</td>
<td>86±10</td>
<td>85±11</td>
<td></td>
</tr>
<tr>
<td>pressure (mm/Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steps/day</td>
<td>8968±5040</td>
<td>9490±5104</td>
<td>8195±3017</td>
<td></td>
</tr>
<tr>
<td>1RM upper body (kg)</td>
<td>52±11¥</td>
<td>55±9</td>
<td>57±9¥</td>
<td>51±11*</td>
</tr>
<tr>
<td>1RM lower body (kg)</td>
<td>94±60¥#</td>
<td>122±71#</td>
<td>137±99¥</td>
<td>103±73*</td>
</tr>
<tr>
<td>Fat mass %</td>
<td>28.4±12</td>
<td></td>
<td>28.3±12.03</td>
<td>29.7±10.6</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>50.3±6.2</td>
<td></td>
<td>51.2±6.9</td>
<td>49.5±5.5</td>
</tr>
<tr>
<td>REE (kcal)</td>
<td>1516±171</td>
<td></td>
<td>1625±273</td>
<td>1525±200</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 4</td>
<td>Post-exercise (week 8)</td>
<td>De-training (week 16)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>34.86±8.39</td>
<td>34.86±8.39</td>
<td>34.86±8.39</td>
<td>34.86±8.39</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>25.47±4.74*¥</td>
<td>25.21±4.37*</td>
<td>23.55±3.50*¥</td>
<td></td>
</tr>
<tr>
<td><strong>Waist to hip ratio</strong></td>
<td>0.86±0.08</td>
<td>0.85±0.05</td>
<td>0.83±0.02</td>
<td></td>
</tr>
<tr>
<td>VO2peak (L/min)</td>
<td>2.52±0.50</td>
<td>2.53±0.52</td>
<td>2.69±0.38</td>
<td>2.56±0.32</td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>Systolic Blood pressure (mm/Hg)</td>
<td>122±7</td>
<td>118±10</td>
<td>124±7</td>
<td></td>
</tr>
<tr>
<td>Diastolic Blood pressure (mm/Hg)</td>
<td>82±2</td>
<td>78±6</td>
<td>81±2</td>
<td></td>
</tr>
<tr>
<td>Steps/day</td>
<td>8344±3266</td>
<td>8052±4661</td>
<td>6518±4013</td>
<td></td>
</tr>
<tr>
<td>1RM upper body (kg)</td>
<td>59±16</td>
<td>56±14</td>
<td>55±13</td>
<td>56±13</td>
</tr>
<tr>
<td>1RM lower body (kg)</td>
<td>61±13</td>
<td>65±13</td>
<td>61±14</td>
<td>63±12</td>
</tr>
<tr>
<td>Fat mass %</td>
<td>22.6±9.1</td>
<td>21.2±8.3</td>
<td>19.4±7.1</td>
<td></td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>54±4.1</td>
<td>55.4±4.4*</td>
<td>53.4±5.7*</td>
<td></td>
</tr>
<tr>
<td>REE (kcal)</td>
<td>1412±189</td>
<td>1503±202</td>
<td>1454±140</td>
<td></td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>1384±436</td>
<td>1342±417</td>
<td>1611±240</td>
<td></td>
</tr>
<tr>
<td>Weight of food intake (gr)</td>
<td>965±280</td>
<td>1053±170</td>
<td>1200±362</td>
<td></td>
</tr>
<tr>
<td>Protein (gr)</td>
<td>62±20</td>
<td>56±23</td>
<td>63±12</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates (gr)</td>
<td>145±18</td>
<td>148±41</td>
<td>163±48</td>
<td></td>
</tr>
<tr>
<td>Total fat intake (gr)</td>
<td>58±31</td>
<td>49±23</td>
<td>66±12</td>
<td></td>
</tr>
<tr>
<td>Total sugar intake (gr)</td>
<td>51±16</td>
<td>57±20</td>
<td>66±23</td>
<td></td>
</tr>
<tr>
<td>Caffeine (gr)</td>
<td>116±187</td>
<td>154±282</td>
<td>157±253</td>
<td></td>
</tr>
<tr>
<td>UCP1 mRNA</td>
<td>0.21±0.24</td>
<td>0.23±0.22</td>
<td>0.35±0.26</td>
<td></td>
</tr>
<tr>
<td>UCP1 protein expression</td>
<td>1.24±0.55</td>
<td>1.49±0.89</td>
<td>1.18±0.76</td>
<td></td>
</tr>
<tr>
<td>PGC-1α mRNA</td>
<td>1.37±0.61</td>
<td>1.88±1.28</td>
<td>1.49±0.87</td>
<td></td>
</tr>
<tr>
<td>PPARα mRNA</td>
<td>2.55±0.72</td>
<td>2.51±0.74</td>
<td>2.05±0.65</td>
<td></td>
</tr>
<tr>
<td>PPARγ mRNA</td>
<td>0.62±0.41</td>
<td>0.55±0.30</td>
<td>0.49±0.31</td>
<td></td>
</tr>
</tbody>
</table>

**BMI:** Body mass index; **VO2peak:** Maximal oxygen uptake; **ml/kg⁻¹/min⁻¹:** Millilitre per kilogram, per minute; **1RM:** One repetition maximum; **kg:** Kilograms; **REE:** Resting energy expenditure; **kcal:** Kilocalories; **gr:** Grammars; **mm/Hg:** Millimetre of mercury; **UCP1:** Uncoupling protein one; **PGC1α:** Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; **PPARα:** Peroxisome proliferator-activated receptor alpha; **PPARγ:** Peroxisome Proliferator-Activated Receptor Gamma

*Significant differences between post-exercise and de-training

† Significant differences between baseline and de-training
7.4. DISCUSSION

The primary aim of this study was to examine the effects of different types of exercise and de-training on the UCP1 mRNA and protein expression within the subcutaneous WAT in healthy untrained men. The statistical analysis revealed no effects of exercise and de-training on the UCP1 mRNA and protein expression, neither in the entire sample size nor in the high responders not only to the exercise stimuli, but also to the de-training period. This follows the findings of the first study (systematic review) of this thesis, even though the available evidence was limited. The current study’s results are in accordance with a previous controlled trial in humans that examined the effects of exercise on UCP1 mRNA of subcutaneous WAT that showed no effects – even though the UCP1 mRNA was increased in response to a 12-week combined (aerobic+resistance) exercise programme – in both healthy and pre-diabetic individuals [236]. In this regard, UCP1 mRNA and protein expression were also increased, yet not significantly, in response to aerobic exercise and showed a small effect size in the current study. This small effect size in AEG indicates a trend of UCP1 mRNA and protein expression to be increased in response to aerobic exercise that needs further exploration. The results of the current study are also in accordance with a recent single group design study that examined the effects of a 16-week exercise intervention on browning formation markers of WAT including UCP1 mRNA in healthy women. This study showed no differences between baseline and post-exercise in UCP1 mRNA [368]. The later study also showed an inverse association of UCP1 mRNA in subcutaneous WAT with weight loss of the participants in response to exercise [368]. This evidence cannot indicate that the presence of UCP1 in WAT could lead to weight loss and subsequently to lessen obesity, as previously described [30].
In the current study the PPARα mRNA did not alter in response to exercise and de-training neither in the entire sample size nor in the high responders not only to the exercise stimuli, but also to the de-training period. The proposed mechanism that circulating Irisin increases the UCP1 within the white adipocytes may be partly dependent on the action of PPARα. This is because FNDC5 – the precursor of Irisin – has been shown to increase PPARα mRNA in vitro while pharmacological inhibition by antagonists of PPARα limits the action of FNDC5 [30]. Given that PPARα mRNA did not alter in the current study it could therefore, explain, at least in part, why UCP1 mRNA and protein expression did not also alter. Previous evidence from animal studies showed that exercise increased the positive UCP1 white fat cells in exercised high-fat diet mice, but not in sedentary high-fat diet mice [190]. The fat diet (along with the most of the dietary elements) of our participants did not significantly alter throughout the experimental protocol. This may also explain the non-significant alterations of the UCP1 mRNA and protein expression given that previous evidence showed that high-fat diet increased UCP1 in mice [206, 207].

Surprisingly, a moderate effect size was detected – values increased – of UCP1 mRNA after the de-training period in the CG. However, regarding UCP1 protein expression a small effect size was found – values decreased – between post-exercise and de-training, which is completely different trend than the one of the UCP1 mRNA. The CG displayed significantly decreased BMI after the de-training period which may not explain the finding of the UCP1 protein expression, given that BMI is inversely correlated with BAT activity and subsequently a thermogenic effect that the UCP1 represents [29, 183]. Despite the aforementioned alteration of BMI in CG we found no significant changes in nutrition and physical activity levels – that could explain the BMI reduction – of the participants in the CG throughout the experimental protocol (Table
Even though the participants of the CG were instructed not to participate in exercise and to keep their physical activity and behaviour constant, this could not be fully controlled. Furthermore, at post-exercise there was a moderate effect size in UCP1 mRNA between values of the AEG and the REG with the CG. The UCP1 mRNA in the exercise groups was higher than the CG. This may be explained by the fact that the differences between the AEG and the REG with the CG occur at baseline values as well. Indeed, a moderate effect size was found in UCP1 mRNA at baseline values between the exercised groups and the CG. The effect sizes of the UCP1 mRNA are not in accordance with the effect sizes of the UCP1 protein expression. Indeed, the UCP1 protein expression values of the CG were higher than the values of the AEG, REG and CEG at post-exercise. This may be explained by the fact that the differences between the AEG, REG and the CEG with the CG occur at baseline values as well. Actually, at baseline a small and a large effect size were found between AEG and CG and between CEG and CG, respectively. At de-training however, the values of the CG were lower than the values of the REG and the CEG which is in accordance with the UCP1 mRNA values.

The proposed mechanism that exercise may increase UCP1 within the WAT is that exercise produces Irisin in the bloodstream – via the action of the FNDC5 gene within the skeletal muscle – that then attaches to the surface of white adipocytes [30]. However, this biological activity of the Irisin protein has not been well-established. Indeed, the first study (systematic review) of this thesis failed to confirm that Irisin is produced in response to exercise in humans due to earlier studies' use of inaccurate methods to quantify circulating Irisin (the available commercial antibodies attract cross-reacting proteins) [35], that led to misleading and disparate results. The current study did not measure circulating Irisin – even though this was planned – due to the
latter issues. Human Irisin displays a non-canonical start codon (non-ATG), because the FNDC5 gene – the precursor of Irisin – is mutated in humans [274]. This may lead to a short-length Irisin production into the bloodstream, which misses almost 50% of its sequence [274], and therefore, the biological activity of human Irisin is questioned. Nevertheless, more recent mass spectrometry analysis of Irisin in humans showed that Irisin is translated from its non-canonical start codon (non-ATG) but, it circulates and showed to be increased in response to exercise, which implies a biological activity of the protein [276]. A recent animal study showed that FNDC5 produced in subcutaneous white adipocytes may be more responsible than the action of circulating Irisin [380] for the browning process of WAT in response to exercise. However, the current study did not investigate FNDC5.

Similar to the second study of this PhD (cross-sectional study) the current study also detected positive white adipocytes of the UCP1 gene and protein expression at baseline, post-exercise and de-training. The presence of UCP1 in subcutaneous WAT could be dependent on a synergetic interactions between selective genes and variations in recessive and dominants genes [371] as well as in precursor cells in WAT that may promote rise of brown-like adipocytes that express UCP1 [272]. In this light, the presence of UCP1 in subcutaneous adipocytes after an exercise programme and a de-training period that was detected by the current study remains to be elucidated.

A secondary aim of the current study was to examine the effects of different types of exercise and de-training on the mRNA of the PGC-1α, PPARα and PPARγ genes that indicate browning formation of subcutaneous WAT in healthy untrained men. The ANOVA analysis revealed no effects of exercise and de-training on the mRNA of the PGC-1α, PPARα and PPARγ genes neither in the entire sample size nor in the high responders not only to the exercise stimuli, but also to the de-training
period. Also, the effect size analysis did not detect effect sizes in the exercised groups between baseline and post-exercise values. Moderate and large effect sizes, however, were observed in exercise groups – values decreased – between post-exercise and de-training. Recent evidence has shown that PGC-1α increased in subcutaneous WAT of mice in response to chronic exercise [380]. In this study, the thermogenic capacity of the animals was tested and showed increased body heat temperature during the day/night cycle along with increased energy expenditure [380]. Therefore, PGC-1α was proposed to cause a browning formation of WAT that increases energy expenditure [380]. In the current study PGC-1α mRNA did not alter and REE did not significantly change throughout the experimental protocol. We found moderate and large effect sizes – values decreased – between post-exercise and de-training in exercise groups in PGC-1α mRNA. It could be hypothesized therefore, that PGC-1α was increased in response to exercise and subsequently decreased in response to de-training but, we only detected a small effect size between baseline and post-exercise in the CEG while the AEG displayed decreased PGC-1α mRNA in response to exercise. As such, this PGC-1α trend remains to be elucidated.

PPARγ mRNA displayed a trend of increasing in response to resistance and combined (aerobic+resistance) exercise given the small and moderate effect sizes – values increased – that were detected. However, PPARα mRNA of the REG showed significantly lower values than the CG at post-exercise and de-training in the high responders to the exercise stimuli, which cannot indicate an effect of exercise on the high responders. PPARα may control lipid metabolism [246] and fatty acid oxidation in response to fasting [247] while PPARγ is responsible for the regulation of fatty acid storage and glucose metabolism [254]. Neither the fat mass nor the diet of the
participants in the current study were changed in response to exercise and de-training, that may explain the non-alteration of PPARα and PPARγ.

VO₂peak was significantly increased only in the REG after the exercise programme, while it was increased, yet not significantly, in both AEG by 15% and CEG by 10%. Previous evidence showed that an 8-week of resistance training significantly increased VO₂peak in healthy men [347] while chronic resistance exercise increased VO₂peak in healthy older women that was equal to chronic aerobic exercise [390]. This increase occurs due to the significant improvement of the total muscle aerobic capacity and fat oxidation in response to resistance exercise that led to muscle hypertrophy [347]. Indeed, in the current study the 1RM of upper and lower body was significantly increased in response to resistance exercise that may indicate muscle hypertrophy especially in the early stages of resistance training [391]. Notably, the participants of the current study were untrained and therefore, these adaptations seem reasonable. In line with the VO₂peak adaptations the 1RM of upper and lower body were significantly increased in CEG after the exercise programme.

It would be expected that aerobic training would significantly increase VO₂peak of the participants in the current study. Even though VO₂peak was increased by 15% this was not significant. However, previous evidence showed that high intensities of aerobic training are more effective for improving VO₂max than lower intensities [392]. The exercise programmes in the current study were set at 65% of VO₂peak and 1RM throughout and along with the small number of participants (eight per group) may not have allowed a significant alteration. This was because we followed previous exercise programmes that led to increase of PGC-1α – the precursor of the Irisin mechanism – in skeletal muscle [306].
A number of limitations have been identified in the current study. The mRNA measurements were widely used in the past to determine BAT [22] and/or brown-like adipose tissue functions [31, 32, 236, 266, 267]. However, even though the mRNA of genes usually indicate the protein production sometimes they do not predict it [197-199] given that one mRNA transcription may encode the protein more than one time [197-199]. Only mRNAs for PGC-1α, PPARα and PPARγ were measured in the current study and this is a limitation. Furthermore, the acute effects of exercise were not examined given that in humans the acute effects of exercise on PGC-1α, FNDC5 in muscle and Irisin were examined in the past and showed a significant alteration [236]. As such it could be hypothesized that acute alterations could be identified in the genes and protein expressions examined in the current study. However, this would require more than three surgical biopsies for each participant and therefore, due to ethical considerations it was not included in the measurements. Circulating Irisin was not measured to see if it is altered in response to exercise and its associations with the mRNAs and protein expression of the genes that were examined. Blood samples were retrieved from the participants, because the plan was to measure circulating Irisin, but during the study new evidence showed that the ELISA kits used for Irisin identification were neither valid nor accurate [35]. Because alternative methods to measure this protein (e.g. mass spectrometry) were unavailable within the study laboratory, the Irisin identification in blood samples was omitted; however the samples are stored and will be analysed in the future.

Another limitation is that even though the participants of the control group were instructed not to change their physical activity behaviour during the experimental period, this could not be fully controlled. The participants were also instructed to refrain from passive smoking, and alcohol for 72 hours prior to the REE, anthropometry, body
composition measurements and fat biopsies. This could not be fully controlled and may have caused some disturbances in the measurements and therefore, misleading results. The physical activity data and diet elements data were collected by telephone. This may have allowed a greater risk of bias in the reporting data from the participants.

The exercise programmes were based on a specific intensity (65% VO_{2peak}, 1RM). The effects of various exercise intensities on the genes and protein expressions examined could not be tested. Finally, even though a priori power calculation was used to determine sample size, the effect size analyses showed that more participants may have been needed per group. However, we located only one relevant controlled trial on humans to be used for the power calculation analysis.

7.5. CONCLUSIONS

In conclusion, different types of chronic exercise and de-training do not affect the UCP1 mRNA and protein expression of subcutaneous WAT in healthy untrained men, which may indicate no effect of chronic exercise on browning formation of WAT in humans. In addition, no alterations of body weight and REE in response to exercise were found, which indicate no link between UCP1 and body weight/REE that could designate a browning effect on WAT. Also, no effects of exercise and de-training on the mRNAs of PGC-1α, PPARα and PPARγ genes of subcutaneous WAT in healthy untrained men were found, suggesting no synergetic effect of these genes with UCP1 in order to designate a browning formation of WAT in humans. There is a trend of UCP1 mRNA and protein expression to be increased in response to aerobic exercise, which may indicate a thermogenic function and a browning process of WAT in healthy men. The study found positive UCP1 subcutaneous adipocytes, which cannot be fully attributed to exercise stimuli. There is also a trend of PPARγ mRNA to be increased
in response to both resistance and combined (aerobic+resistance) exercise. This indicates increased lipid metabolism and fatty acid beta oxidation. Finally, there is a trend of PPARγ mRNA to be decreased in response to de-training. This indicates a decline in lipid metabolism and fatty acid beta oxidation. More studies are required to study and understand these phenomena.
8. CHAPTER 8: EFFECTS OF DIFFERENT TYPES OF EXERCISE AND DE-TRAINING ON LEPTIN mRNA EXPRESSION OF SUBCUTANEOUS WHITE ADIPOCYTES IN HUMANS: A RANDOMIZED CONTROL TRIAL (4th study)

8.1. INTRODUCTION

Leptin is an adipokine secreted by the adipose tissue and regulates the appetite levels through its action on the hypothalamus [120, 393]. Increased leptin sensitivity with its receptor stimulates lipolysis in adipose tissue and hepatic fatty acid uptake and oxidation [126]. The leptin resistance condition is an inadequate communication of leptin with its receptor in the hypothalamus and leads to increased circulating leptin concentrations [394]. Increased circulating leptin concentrations may up-regulate the production of TNFα and IL-6 indicating an inflammation state [394], which usually occurs in obese individuals [120]. Leptin production is reduced by the SNS activation [137] and is suppressed in response to cold exposure [25]. When leptin is suppressed in response to cold exposure it may be accompanied by the reduction of fat mass [134]. Also, the reduction in fat mass in response to chronic exercise is usually accompanied by a reduction in circulating leptin concentrations [120, 141, 144, 145].

Adiponectin, an adipokine also secreted by the adipose tissue, controls energy homeostasis and appetite [120]. Low circulating adiponectin concentrations are associated with increased appetite [120] and high circulating adiponectin concentrations may decrease glucose production from the liver, indicating enhanced insulin sensitivity [156, 284]. Chronic exercise has been shown to increase circulating adiponectin concentrations in healthy obese men [13, 395] and postmenopausal women [396]. L/A ratio can be used as an index to identify risk of disease. Increased L/A ratio may indicate increased atherosclerosis and glycated haemoglobin, which is an index of increased insulin resistance [397, 398].

The aim of the present study was to examine the effects of different types of exercise and de-training on the leptin mRNA of subcutaneous WAT in healthy
untrained men in a parallel RCT design. A secondary aim was to examine the effects of different types of exercise and de-training on the adiponectin and L/A ratio mRNAs. It was hypothesized that different types of chronic exercise will decrease (and the following de-training period will increase) the leptin mRNA in subcutaneous WAT of healthy untrained men. Finally, anthropometry indices, REE (kcal), physical activity levels, energy intake (kcal) and diet data were recorded throughout the trial in order to be used as confounding factors.

8.2. METHODS
The methods of this parallel RCT regarding the procedure, the random allocation/blinding procedures and inclusion criteria of the participants, anthropometry measurements, assessments of REE, VO₂peak, energy intake, diet elements, physical activity, subcutaneous fat biopsy procedure, gene expression analysis and exercise protocols (Table 8) are described in detail in the general methods in Chapter 4, pages 61-71. The methodological design can be found in Figure 8 in Chapter 4, page 64.

8.2.1. Participants and procedures
The baseline characteristics of the participants of this study are presented in details in Table 13, page 117. The baseline characteristics of the participants regarding leptin, adiponectin and L/A ratio mRNAs can be found in Table 20.

Table 20: Baseline characteristics of the participants (n=32)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin mRNA</td>
<td>2.85±2.24</td>
</tr>
<tr>
<td>Adiponectin mRNA</td>
<td>1.02±0.44</td>
</tr>
<tr>
<td>Leptin to adiponectin ratio mRNA</td>
<td>2.82±1.77</td>
</tr>
</tbody>
</table>
8.2.2. Statistical analysis

The statistical approach of this study is identical to the statistical approach of the 3\textsuperscript{rd} study and it is described in details on pages 118-120. The number of the participants who were randomly assigned in the exercise programmes (and the drop outs with reasons) as well as the number of the participants included in the final analysis can be found in Figure 7, Chapter 4, page 63.

8.3. RESULTS

Details regarding the exercise attendance and intensity supervision as well as harms of this study are identical to the 3\textsuperscript{rd} study and they are presented on page 120.

8.3.1. Primary outcomes

Results from ANOVA analysis

No significant differences between and within groups in delta scores of mRNA for leptin were detected neither between baseline and post-exercise (week 8) nor between post-exercise (week 8) and de-training (week 16). The individual data for leptin mRNA are shown in Figure 30.


**Figure 30**: Individual data for leptin mRNA at baseline, post exercise (week 8) and de-training (week 16)

Each number in the x axis represents one participant; The participants 1, 5, 11, 13, 18, 19, 20, 24 and 32 belong to the aerobic exercise group; The participants 4, 6, 8, 14, 21, 23, 25, and 29 belong to the resistance exercise group; The participants 2, 10, 12, 15, 26, 28, 30, and 31 belong to the combined (aerobic+resistance) exercise group; The participants 3, 7, 9, 16, 17, 22, and 27 belong to the control group.

**Results from low responders versus high responders analysis**

For leptin mRNA the low responders to the exercise stimuli were considered those who displayed values equal or more than the 50th percentile while the high responders to the exercise stimuli were considered those who displayed values less than the 50th percentile. Furthermore, the low responders to the de-training period were considered those who displayed values less than the 50th percentile while the high responders to the de-training period were considered those who displayed values equal or more than the 50th percentile [309]. This is because according to the hypothesis of this study the exercise intervention was expected to decrease leptin mRNA while the de-training period was expected to increase leptin mRNA.
Chi square analysis revealed no significant differences between the experimental groups (categorical variable consisted of four groups) and a) leptin mRNA (categorical variable of low and high responders to exercise stimuli) \( [\chi^2 (3, n=29)=1.75, P>0.05] \) as well as b) leptin mRNA (categorical variable of low and high responders to de-training period) \( [\chi^2 (3, n=25)=1.32, P>0.05] \).

Kruskal–Wallis analysis of variance revealed no differences for leptin mRNA between the experimental groups in high responders to the exercise stimuli and in high responders to the de-training period at all time points \( (P>0.05) \). Wilcoxon Signed Rank tests revealed no differences of leptin mRNA in high responders to the exercise stimuli between baseline and post-exercise as well as in high responders to the de-training period between post-exercise and de-training within each experimental group. The number and the percentage of the low and high responders of leptin mRNA for each experimental group are shown in Table 21.

<table>
<thead>
<tr>
<th>Group</th>
<th>Low responders</th>
<th>High responders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leptin mRNA</td>
<td>Leptin mRNA</td>
</tr>
<tr>
<td><strong>Exercise stimuli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEG</td>
<td>4 (50%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>REG</td>
<td>3 (50%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>CEG</td>
<td>3 (37.5%)</td>
<td>5 (62.5%)</td>
</tr>
<tr>
<td>CG</td>
<td>5 (71.4%)</td>
<td>2 (28.6%)</td>
</tr>
<tr>
<td><strong>De-training period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEG</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>REG</td>
<td>3 (50%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>CEG</td>
<td>3 (37.5%)</td>
<td>5 (62.5%)</td>
</tr>
<tr>
<td>CG</td>
<td>2 (40%)</td>
<td>3 (60%)</td>
</tr>
</tbody>
</table>

*Table 21:* Number and percentage of the low and high responders for leptin mRNA

AEG: Aerobic exercise group; REG: Resistance exercise group; CEG: Combined (aerobic+resistance) exercise group; CG: Control group.
Results from Cohen’s $d$ effect size analysis

The leptin mRNA was increased in response to aerobic exercise and was decreased in response to de-training. A small effect size ($d=0.33$) between baseline and post-exercise and a large effect size ($d=0.98$) between post-exercise and de-training in AEG (Figure 31A) were found. A small effect size ($d=0.41$) in REG (Figure 31B), in CEG [(d=0.39) (Figure 31C)] and in CG [(d=0.43) (Figure 31D)] between post-exercise and de-training were also detected.
**Figure 31**: Cohen’s $d$ effect size in leptin mRNA between baseline, post-exercise (week 8) and de-training (week 16)

- **A**: Aerobic group
- **B**: Resistance group
- **C**: Combined group
- **D**: Control group

**Figure 31A**: *Small effect size ($d=0.33$); Large effect size ($d=0.98$); Figure 31B: **Small effect size ($d=0.41$); Figure 31C: #Small effect size ($d=0.39$); Figure 31D: #Small effect size ($d=0.43$); Values are depicted as mean and standard deviation.
The leptin mRNA of the CG showed higher values compared to the exercise groups at all time points. At baseline, a moderate effect size between CG and AEG ($d=0.64$), a small effect size between CG and REG ($d=0.35$), and a small effect size between CG and CEG ($d=0.40$) were identified (Figure 32A). At post-exercise, a small effect size between CG and AEG ($d=0.45$), a small effect size between CG and REG ($d=0.35$) and a moderate effect size between CG and CEG ($d=0.50$) were revealed (Figure 32B). At de-training, a large effect size between CG and AEG ($d=0.90$) and a small effect size between CG and CEG ($d=0.37$) were found (Figure 32C).

**Figure 32:** Cohen’s $d$ effect size in leptin mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16)
8.3.2. Secondary outcomes

Results from ANOVA analysis

No significant differences between and within groups in delta scores of adiponectin, and L/A ratio mRNAs were detected between baseline and post-exercise values (week 8) nor between post-exercise (week 8) and de-training values (week 16). The individual data for adiponectin and L/A ratio mRNAs are depicted in Figure 33.
Figure 33: Individual data for adiponectin (A) and leptin to adiponectin ratio (B) mRNAs at baseline, post exercise (week 8) and de-training (week 16)

Each number in the x axis represents one participant; The participants 1, 5, 11, 13, 18, 19, 20, 24 and 32 belong to the aerobic exercise group; The participants 4, 6, 8, 14, 21, 23, 25, and 29 belong to the resistance exercise group; The participants 2, 10, 12, 15, 26, 28, 30, and 31 belong to the combined (aerobic+resistance) exercise group; The participants 3, 7, 9, 16, 17, 22, and 27 belong to the control group; L/A: Leptin to adiponectin.

Results from low responders versus high responders analysis

For adiponectin mRNA the low responders to the exercise stimuli were considered those who displayed values less than the 50th percentile while the high responders to
the exercise stimuli were considered those who displayed values equal or more than the 50th percentile. Furthermore, the low responders to the de-training period were considered those who displayed values equal or more than the 50th percentile while the high responders to the de-training period were considered those who displayed values less than the 50th percentile [309]. This is because according to the hypothesis of this study the exercise intervention was expected to increase adiponectin mRNA while the de-training period was expected to decrease adiponectin mRNA. For L/A ratio mRNA the low responders to the exercise stimuli were considered those who displayed equal or more than the 50th percentile while the high responders to the exercise stimuli were considered those who displayed values less than the 50th percentile. Furthermore, the low responders to the de-training period were considered those who displayed values less than the 50th percentile while the high responders to the de-training period were considered those who displayed values equal or more than the 50th percentile [309]. This is because according to the hypothesis of this study the exercise intervention was expected to decrease L/A ratio mRNA while the de-training period was expected to increase L/A ratio mRNA.

Chi square analyses revealed no significant differences between the experimental groups (categorical variable consisted of four groups) and a) adiponectin mRNA (categorical variable of low and high responders to exercise stimuli) \( \chi^2 (3, n=29)=4.54, P>0.05 \) as well as b) L/A ratio mRNA (categorical variable of low and high responders to exercise stimuli) \( \chi^2 (3, n=29)=1.77, P>0.05 \). Chi square analyses also showed no significant differences between the experimental groups (categorical variable consisted of four groups) and a) adiponectin mRNA (categorical variable of low and high responders to de-training period) \( \chi^2 (3, n=25)=6.13, P>0.05 \) as well as
b) L/A ratio mRNA (categorical variable of low and high responders to de-training period) \[\chi^2 (3, n=25)=1.32, P>0.05\].

Kruskal–Wallis analysis of variance with post-hoc Mann–Witney U tests showed that the REG displayed significantly higher adiponectin mRNA in high responders to the de-training period than the AEG (P=0.03) and the CEG (P=0.03) at post-exercise. Furthermore, Kruskal–Wallis analysis of variance revealed no differences for L/A ratio mRNA between the experimental groups in high responders to the exercise stimuli and in high responders to the de-training period at all time points (P>0.05). Finally, Wilcoxon Signed Rank tests revealed that adiponectin mRNA was significantly higher at post-exercise than after the de-training in high responders to the de-training period in the CEG (z=-2.023, P=0.04). The number and the percentage of the low and high responders of adiponectin and L/A ratio mRNAs for each experimental group are shown in Table 22.

**Table 22:** Number and percentage of the low and high responders for adiponectin and leptin to adiponectin ratio mRNAs

<table>
<thead>
<tr>
<th>Group</th>
<th>Low responders</th>
<th>High responders</th>
<th>Adiponectin mRNA</th>
<th>L/A mRNA</th>
<th>Adiponectin mRNA</th>
<th>L/A mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exercise stimuli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEG</td>
<td>5 (62.5%)</td>
<td>3 (37.5%)</td>
<td>3 (37.5%)</td>
<td>5 (62.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>REG</td>
<td>3 (50%)</td>
<td>4 (66.7%)</td>
<td>3 (50%)</td>
<td>2 (33.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEG</td>
<td>5 (62.5%)</td>
<td>5 (62.5%)</td>
<td>3 (37.5%)</td>
<td>3 (37.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>1 (14.3%)</td>
<td>3 (42.9%)</td>
<td>6 (85.7%)</td>
<td>4 (57.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>De-training period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEG</td>
<td>3 (50%)</td>
<td>4 (66.7%)</td>
<td>3 (50%)</td>
<td>2 (33.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>REG</td>
<td>2 (33.3%)</td>
<td>3 (50%)</td>
<td>4 (66.7%)</td>
<td>3 (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEG</td>
<td>3 (37.5%)</td>
<td>3 (37.5%)</td>
<td>5 (62.5%)</td>
<td>5 (62.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>(100%)</td>
<td>2 (40%)</td>
<td>0 (0%)</td>
<td>3 (60%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

L/A: Leptin to adiponectin ratio; AEG: Aerobic exercise group; REG: Resistance exercise group; CEG: Combined (aerobic+resistance) exercise group; CG: Control group.
Results from Cohen’s $d$ effect size analysis

Results for adiponectin

The adiponectin mRNA was increased in response to exercise in the AEG, REG and CEG and was decreased in response to de-training in all groups. The effect size analyses showed a small effect size ($d=0.20$) in AEG between baseline and post-exercise and a moderate effect size ($d=0.52$) between post-exercise and de-training (Figure 34A). In REG a large effect size ($d=0.81$) between baseline and post-exercise and a large effect size ($d=1.04$) between post-exercise and de-training was detected (Figure 34B). In CEG a small effect size ($d=0.31$) between baseline and post-exercise and a moderate effect size ($d=0.78$) between post-exercise and de-training was identified (Figure 34C). Finally, in CG a large effect size ($d=0.81$) (Figure 34D) between post-exercise and de-training was also noticed.
**Figure 34:** Cohen’s $d$ effect size in adiponectin mRNA between baseline, post-exercise (week 8) and de-training (week 16)

**Figure 34A:** *Small effect size (d=0.20), #Moderate effect size (d=0.52); Figure 34B:** *Large effect size (d=0.81), #Large effect size (d=1.04); **Figure 34C:** *Small effect size (d=0.31), #Moderate effect size (d=0.78); **Figure 34D:** #Large effect size (d=0.81);

*Values are depicted as mean and standard deviation.*
The CG showed higher values in adiponectin mRNA than the exercise groups at all time points. The effect size analysis showed at baseline a large effect size between CG and AEG ($d=0.81$), between CG and REG ($d=1.26$), and between CG and CEG ($d=1.33$) (Figure 35A). At post-exercise, a moderate effect size between CG and AEG ($d=0.73$), a moderate effect size between CG and REG ($d=0.68$) and a large effect size between CG and CEG ($d=1.08$) were identified (Figure 35B). At de-training, a large effect size between CG and AEG ($d=1.03$), between CG and REG ($d=0.91$) and between CG and CEG ($d=1.21$) was revealed (Figure 35C).
Figure 35: Cohen’s $d$ effect size in adiponectin mRNA between control and exercise groups at baseline, post-exercise (week 8) and de-training (week 16)

Figure 35A: *Large effect size ($d=0.81$), #Large effect size ($d=1.26$), £Large effect size ($d=1.33$); Figure 35B: *Moderate effect size ($d=0.73$), #Moderate effect size ($d=0.68$), £Large effect size ($d=1.08$); Figure 35C: *Large effect size ($d=1.03$), #Large effect size ($d=0.91$), £Large effect size ($d=1.21$); Values are depicted as mean and standard deviation.
**Results for leptin to adiponectin ratio**

According to the effect size analysis, the L/A ratio mRNA was decreased after the resistance exercise programme while it was decreased in response to de-training in AEG, REG and CG. Specifically, the L/A ratio mRNA displayed a moderate effect size in AEG ($d=0.77$) between post-exercise and de-training (Figure 36A). In REG the L/A ratio mRNA was decreased and showed a moderate effect size ($d=0.50$) between baseline and post-exercise while it displayed a small effect size ($d=0.20$) between post-exercise and de-training (Figure 36B). In CEG the L/A ratio mRNA showed a small effect size ($d=0.21$) between post-exercise and de-training (Figure 36C) while in CG it also showed a small effect size ($d=0.26$) between post-exercise and de-training (Figure 36D). At baseline and post-exercise, there was any effect size between the CG and the exercise groups. However, at de-training, the AEG displayed lower values than the CG and a moderate effect size was found ($d=0.52$), while the CEG displayed higher values than the CG and a small effect size was detected ($d=0.35$) (Figure 37).
Figure 36: Cohen’s $d$ effect size in leptin to adiponectin ratio mRNA between baseline, post-exercise (week 8) and de-training (week 16)

Figure 36A: *Moderate effect size ($d=0.77$); Figure 36B: *Moderate effect size ($d=0.50$), #Small effect size ($d=0.20$); Figure 36C: #Small effect size ($d=0.21$); Figure 36D: #Small effect size ($d=0.26$); Values are depicted as mean and standard deviation.
**Figure 37:** Cohen’s $d$ effect size in leptin to adiponectin ratio mRNA between control and exercise groups at de-training (week 16)

*Moderate effect size ($d=0.52$), £ Small effect size ($d=0.35$); Values are depicted as mean and standard deviation.

**8.3.3. Results from analysis within groups**

The mean differences of the assessed variables between the time points are identical to the 3rd study of this PhD and can be found in detail in Table 16 for the AEG, Table 17 for the REG, Table 18 for the CEG and Table 19 for the CG on pages 146-151. The mean differences between the time points of all experimental groups regarding leptin, adiponectin and L/A ratio mRNAs can be found in Table 23.

**Table 23:** Mean and standard deviation of leptin, adiponectin and L/A mRNAs

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-exercise (week 8)</th>
<th>De-training (week 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerobic exercise group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin mRNA</td>
<td>2.18±1.27</td>
<td>2.67±1.63</td>
<td>1.36±0.75</td>
</tr>
<tr>
<td>Adiponectin mRNA</td>
<td>0.94±0.58</td>
<td>1.06±0.51</td>
<td>0.83±0.31</td>
</tr>
<tr>
<td>L/A ratio mRNA</td>
<td>2.54±1.35</td>
<td>2.53±1.20</td>
<td>1.71±0.76</td>
</tr>
<tr>
<td><strong>Resistance exercise group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin mRNA</td>
<td>2.92±1.12</td>
<td>2.94±0.97</td>
<td>2.23±2.23</td>
</tr>
<tr>
<td>Adiponectin mRNA</td>
<td>0.94±0.29</td>
<td>1.17±0.26</td>
<td>0.82±0.38</td>
</tr>
<tr>
<td>L/A ratio mRNA</td>
<td>3.16±1.34</td>
<td>2.57±0.89</td>
<td>2.34±1.22</td>
</tr>
<tr>
<td><strong>Combined exercise group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin mRNA</td>
<td>2.64±2.34</td>
<td>2.59±1.58</td>
<td>1.95±1.67</td>
</tr>
</tbody>
</table>
### 8.4. DISCUSSION

The primary aim of the present study was to examine the effects of different types of exercise and de-training on leptin mRNA of subcutaneous adipocytes in healthy untrained men. No effects of exercise and de-training on the mRNA of leptin were found neither in the entire sample size nor in the high responders not only to the exercise stimuli, but also to the de-training period. Previous evidence showed that the reduction of circulating leptin concentrations in response to exercise is usually accompanied by a reduction in fat mass [120, 141, 144]. The fat mass of the participants did not alter in response to exercise and de-training in the current study and thus, this may explain, at least in part, the non-alteration of leptin mRNA. Also, previous data from a human study showed that caloric intake restriction in conjunction with a 2-month exercise programme decreased circulating leptin as opposed to either caloric intake restriction or exercise alone [147]. The participants of the current study did not follow a caloric restriction neither during the exercise programme nor during the de-training period, and this may also explain the non-alteration of leptin mRNA.

Even though statistically insignificant, the leptin mRNA tended to increase in response to exercise in AEG and to decrease in response to de-training in the exercise groups in the current study. This was also depicted by the effect size analyses that showed a small effect size in AEG between baseline and post-exercise (the leptin mRNA was increased) and effects sizes in the exercised groups between post-

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>AEG</th>
<th>HEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin mRNA</td>
<td>0.88±0.35</td>
<td>0.99±0.37</td>
<td>0.68±0.42</td>
</tr>
<tr>
<td>L/A ratio mRNA</td>
<td>2.79±1.92</td>
<td>2.54±0.89</td>
<td>2.81±1.61</td>
</tr>
<tr>
<td>Leptin mRNA</td>
<td>3.86±3.73</td>
<td>3.68±2.75</td>
<td>2.62±1.92</td>
</tr>
<tr>
<td>Adiponectin mRNA</td>
<td>1.35±0.35</td>
<td>1.39±0.36</td>
<td>1.12±0.24</td>
</tr>
<tr>
<td>L/A ratio mRNA</td>
<td>2.81±2.67</td>
<td>2.90±2.83</td>
<td>2.28±1.33</td>
</tr>
</tbody>
</table>

*L/A: Leptin to adiponectin.*
exercise and de-training (the leptin mRNA was decreased). The odd finding of increased leptin mRNA in WAT after a physical activity intervention was previously reported by a RCT. In this study, even though the circulating leptin concentrations were decreased in response to the six month physical activity intervention, the leptin mRNA in WAT was increased in sedentary healthy overweight adults [399]. The latter was also observed in an animal study [400]. A possible explanation is that post transcriptional regulations of the leptin mRNA may not have allowed a natural prediction of circulating leptin [399]. Therefore, both mRNA and circulating protein concentrations measurements would probably have formed a firm conclusion in the current study.

A secondary aim of the current study was to examine the effects of different types of exercise and de-training on the adiponectin and L/A ratio mRNAs of subcutaneous WAT in healthy untrained men. No effects of exercise and de-training on the adiponectin and L/A ratio mRNAs of subcutaneous WAT were found in the entire sample however, the adiponectin mRNA was significantly decreased in response to de-training in the high responders to the de-training in the CEG. Also, even though statistically insignificant, the adiponectin mRNA was increased in response to resistance exercise and decreased in response to de-training in all the exercise groups in the current study. Evidence from a study in rats also showed that a 10-week aerobic exercise increased adiponectin mRNA in adipose tissue [401]. Therefore, the trend of increased adiponectin mRNA in response to exercise and decreased in response to de-training may represent a normal trend in the current study, given that chronic exercise may increase circulating adiponectin concentrations [13, 395, 396]. Low circulating adiponectin concentrations are associated with increased appetite [120] and high circulating adiponectin concentrations may enhance
insulin sensitivity [156, 284]. Furthermore, previous evidence showed that long-term exercise increased circulating adiponectin concentrations in healthy obese men [13, 395] and postmenopausal women [396].

The effect size analyses in the current study showed a decrease of the L/A ratio mRNA in response to resistance exercise and an increase in response to de-training in the AEG. The L/A ratio can be used to identify insulin resistance in T2D patients [397]. Increased L/A ratio may indicate increased atherosclerosis and glycated haemoglobin, which is an index of increased insulin resistance [397, 398]. There is no enough evidence however, to determine a role of L/A ratio in the current study, and instead the trend of the values is reported.

The results regarding the VO2peak alterations as well as the limitations of the current study are identical to the 3rd study of this PhD and they are discussed in details on pages 157-159. The current study did not use a priory power calculation to determine the sample size, given that this RCT is a part of the third RCT study of this PhD. A post measurements power calculation was conducted using an online software (DSS Research) to test statistical power based on the baseline leptin mRNA expression values (2.85±2.24) of the overall sample of the current study that demonstrated at least a sample size of 10 participants per group. Therefore, given the sample size of this study the statistical power was 72%.

8.5. CONCLUSIONS

Based on the statistical analysis, different types of chronic exercise and de-training do not affect mRNA of leptin, adiponectin genes and L/A ratio of subcutaneous WAT in healthy untrained men. There is a trend of adiponectin mRNA to be increased, in response to resistance exercise and to be decreased in response to de-training. There
is also a trend of L/A ratio mRNA to be decreased, in response to resistance exercise and to be increased in response to de-training. Future studies should increase sample sizes and investigate these phenomena.
This PhD study aimed to investigate both the relationship of physical activity to browning formation of WAT as well as the effects of different exercise programmes on browning formation of subcutaneous WAT in humans using mRNA and protein expression measurements of genes involved in this process. It also aimed to examine the effects of exercise on leptin and adiponectin mRNAs of WAT in humans. The approach was to analyse the mRNA and protein expression of several genes through subcutaneous adipose tissue samples obtained via biopsies.

This PhD thesis consists of four studies. The first study is a systematic review conducted to identify evidence regarding the effects of physical activity on the brown-like formation of WAT in humans. In the second study, 46 healthy men were recruited to identify associations of the mRNA and protein expression of genes that indicate brown-like formation of subcutaneous WAT with physical activity levels. From the 46 healthy men, a subset of 32 individuals participated in a RCT. This RCT was split into two studies. The third study, an examination of the effects of different types of exercise and de-training on the UCP1 mRNA and protein expression, and the fourth, an examination of the effects of different types of exercise and de-training on the leptin mRNA of subcutaneous WAT.

*The effects of physical activity on browning formation of white adipocytes: A systematic review (1st study)*

The systematic review examined the effects of physical activity on the link between PGC-1α and FNDC5 in skeletal muscle, circulating Irisin, and UCP1 of WAT in humans. The systematic review found little evidence for the link between PGC-1α and FNDC5 in muscle in response to exercise training or increased physical activity levels. Also, the methods used for Irisin identification were inaccurate in most cases. Only
one study that examined the effects of exercise on UCP1 in WAT was retrieved, which found no effect [236]. Previous evidence showed that the FNDC5 mRNA in muscle is not regularly increased by exercise or differently regulated between those with and without insulin resistance [267]. Also, the antibody used in the initial detection of Irisin was made to correspond against the C-terminal of human FNDC5, which is not the part of the protein that yields the Irisin peptide [360]. The FNDC5 gene – the precursor of Irisin – is mutated in humans due to the non-ATG start codon [274] that questions the biological activity of Irisin [274]. Also, the various generations of antibodies used for Irisin detection in the ELISA kits yield a protein concentration that appears to be ~10-100 times greater than newer mass-spectrometry data [276]. This evidence may explain part or all of the equivocal results of the studies in the systematic review regarding both the effects of exercise on the link between PGC-1α and FNDC5 in muscle and circulating Irisin.

Overall, there is little evidence to determine the link between PGC-1α and FNDC5 in human muscle in response to physical activity. We cannot form any conclusion regarding circulating Irisin in response to physical activity given that the methods used for Irisin identification were inaccurate in most cases. Mass spectrometry detection of Irisin of exercise effects were compromised by the methodological limitations of the existing studies. There was limited evidence on the effects of physical activity on UCP1 in WAT.

*Association of UCP1 with physical activity levels (2nd study)*

The primary aim of the second study was to examine the associations of the UCP1 mRNA and protein expression as well as of the mRNAs of PGC-1α, PPARα and PPARγ genes that indicate browning formation of subcutaneous WAT with physical
activity levels in healthy men. The current study found no associations between the mRNA of UCP1, PGC-1α, PPARα and PPARγ genes of subcutaneous WAT with physical activity levels. There is only one controlled trial that examined the effects of chronic exercise on the UCP1 mRNA of subcutaneous WAT in healthy adults, also confirming no effect of exercise on UCP1 mRNA [236]. The first study of this PhD (systematic review) also failed to confirm a link between physical activity/exercise with a browning formation of WAT in humans. However, the current study revealed an inverse association of UCP1 protein expression with physical activity levels rather than positive. This finding does not support the hypothesis that UCP1 is increased in response to exercise within a white adipocyte that may trigger a browning formation of WAT [30]. Also, this finding may explain a recent discovery that UCP1 mRNA in subcutaneous WAT was negatively correlated with weight loss – indicating no effect of UCP1 on browning formation of WAT – while it was not altered in response to exercise in healthy women [368]. Overall, this evidence suggests that the initial hypothesis of the current study should be rejected.

The current study detected positive white adipocytes of the UCP1 gene which may indicate a browning formation process of white adipocytes; this however, cannot be attributed to physical activity given the lack of relevant associations between physical activity levels and UCP1. It was previously suggested that the presence of the UCP1 in white adipocytes in animals is dependent on a synergetic interactions between selective genes and variations in recessive and dominants genes that affect UCP1 gene even though the mechanisms are unknown [371]. It was also demonstrated that a subset of the precursor cells in white adipocytes may promote rise of brown-like adipocytes that express UCP1 [272]. The mode of the current study
could not highlight the latter issues and therefore, the presence of UCP1 in subcutaneous fat cells in healthy individuals remains to be elucidated.

A secondary aim was to examine the associations of UCP1 mRNA and protein expression as well as of the mRNAs of PGC-1α, PPARα and PPARγ genes with REE, WHR, BMI, age, fat mass, fat-free mass and energy intake. The study found an inverse association of PGC-1α mRNA with BMI, WHR and fat mass percentage and a positive association with fat-free mass. Fat-free mass is positively associated with REE in humans [374], which indicates increased demands of energy, while PGC-1α is highly expressed in tissues that demand energy [226]. Indeed, REE is highly dependent on the amount of fat-free mass in both men and women [375, 376]. This is because the fat-free mass is the most proportional tissue in human body compared to fat mass [377] and it includes skeletal muscles, which demand high amounts of energy [378]. PGC-1α is responsible for mitochondrial biogenesis [171] and it is positively associated with increased REE in mice [379] while it increases fatty acids oxidation [372, 373]. Previous evidence showed that when a browning process occurs in WAT in response to exercise this lead to increased REE in mice [380]. Also, physical activity levels are positively associated with REE in humans [68]. However, we found no association of PGC-1α mRNA with physical activity levels that may explain why PGC-1α and REE have no relationship in the current study. Finally, regarding the inverse association of PGC-1α mRNA with fat mass, this is probably because PGC-1α is involved in oxidative phosphorylation and fatty acid oxidation that regulate lipid metabolism and lessen insulin resistance in mice [382].

An inverse association between PPARα mRNA with BMI, WHR and fat mass percentage was also found. PPARα is responsible for the activation of several genes to regulate lipid metabolism [247]. In specific, it increases ketogenesis and
subsequently the uptake of fatty acids, ameliorates beta oxidation in mitochondria and increases triglyceride uptake [247]. Therefore, it triggers lipid catabolism in WAT and this may explain its inverse association with BMI, WHR and fat mass percentage we found in the current study.

It is concluded that UCP1 protein expression is negatively associated with physical activity levels while UCP1 mRNA and protein expression are not linked with body weight (i.e. BMI, WHR, fat mass) and REE, which indicate no browning effect of WAT in response to physical activity in healthy men. The mRNA of PGC-1α, PPARα and PPARγ genes, that along with UCP1 alterations could indicate a browning formation of subcutaneous WAT, are not associated with physical activity levels in healthy men. PGC-1α and PPARα mRNAs are negatively associated with increased fat mass accumulation parameters (BMI, WHR and fat mass) suggesting a potential association between PGC-1α and PPARα with human WAT metabolism. The mechanisms of the latter associations remain to be determined.

Effects of exercise and de-training on browning formation in white adipose tissue (3rd study)

The primary aim of this study was to examine the effects of different types of exercise and de-training on UCP1 mRNA and protein expression within the subcutaneous WAT in healthy untrained men. The statistical analysis revealed no effects of exercise and de-training on the UCP1 mRNA and protein expression neither in the entire sample size nor in the high responders not only to the exercise stimuli, but also to the de-training period. This is in accordance with the findings of the first study of this PhD thesis (systematic review), even though the available evidence was limited. The results in the current study are in accordance with a previous human study that
examined the effects of exercise on UCP1 mRNA of subcutaneous WAT that showed no effects – even though UCP1 mRNA was increased in response to exercise – in both healthy and pre-diabetic individuals [236]. The results are also in accordance with a single group design study that showed no effect of exercise on UCP1 mRNA of subcutaneous WAT in healthy women [368]. The proposed mechanism that circulating Irisin increases the UCP1 within the white adipocytes may be partly dependent on the action of PPARα. This is because FNDC5 – the precursor of Irisin – increased PPARα mRNA in vitro and pharmacological inhibition by antagonists of PPARα limited the action of FNDC5 [30]. In the current study the PPARα mRNA did not alter in response to exercise and de-training. This may explain, in part, why UCP1 mRNA and protein expression did not also alter. However, UCP1 mRNA and protein expression were increased, yet not significantly, in response to aerobic exercise and showed a small effect size in the current study. This small effect size in AEG indicates a trend of UCP1 mRNA and protein expression to be increased in response to aerobic exercise that needs further exploration.

Similar to the second study of this PhD (cross-sectional study) the current study also detected positive white fat cells of the UCP1 gene at baseline, post-exercise and de-training. The presence of UCP1 in subcutaneous WAT could be dependent on a synergetic interaction between selective genes and variations in recessive and dominants genes [371] as well as in precursor cells in WAT that may promote rise of brown-like adipocytes that express UCP1 [272]. In this light, the presence of UCP1 in subcutaneous fat cells after an exercise programme and a de-training period that was detected by the current study remains to be elucidated.

A secondary aim of the current study was to examine the effects of different types of exercise and de-training on the mRNA of the PGC-1α, PPARα and PPARγ
genes that along with UCP1 alterations could indicate browning formation of subcutaneous WAT in healthy untrained men. The statistical analysis revealed no effects of exercise and de-training on the mRNA of the PGC-1α, PPARα and PPARγ genes neither in the entire sample size nor in the high responders not only to the exercise stimuli, but also to the de-training period. Recent evidence has shown that PGC-1α increased in subcutaneous WAT of mice in response to chronic exercise [380]. In this study the thermogenic capacity of the animals was also tested and showed increased body heat temperature during the day/night cycle, along with increased energy expenditure [380]. Therefore, PGC-1α was proposed to cause a browning formation of WAT that increases energy expenditure [380]. In the current study, PGC-1α mRNA did not alter while REE has not been significantly changed throughout the experimental protocol.

The mRNA of PPARα and PPARγ genes did not change significantly throughout the experimental protocol. Regarding PPARγ mRNA a moderate and a small effect sizes – values increased – were detected between baseline and post-exercise in REG and in CEG, respectively. This indicates a trend of increasing PPARγ mRNA in response to chronic exercise. PPARγ is responsible for the regulation of fatty acid storage and glucose metabolism [254] and it is increased in adipose tissue in response to high fat diet [259]. PPARα increases lipid metabolism [246], is involved in mitochondrial fatty acid beta oxidation [247] and regulates the liver to increase fatty acid oxidation in response to fasting [247]. Neither the fat mass nor the diet of the participants in the current study were changed in response to exercise and de-training, indicating that the action of PPARα and PPARγ was not necessary.

It is concluded that different types of chronic exercise and de-training do not affect the UCP1 mRNA and protein expression of subcutaneous WAT in healthy
untrained men. In addition, no alterations of body weight and REE in response to exercise were found, which potentially means that there is no link between UCP1 and body weight/REE that could designate a browning effect on WAT. There is a trend of UCP1 mRNA and protein expression to be increased in response to aerobic exercise, which may indicate a thermogenic function and a browning process of WAT in healthy men. The study found positive UCP1 subcutaneous fat cells, which cannot be fully attributed to exercise stimuli. There is also a trend of PPARγ mRNA to be increased in response to both resistance and combined (aerobic+resistance) exercise. This indicates increased lipid metabolism and fatty acid beta oxidation. Finally, there is a trend of PPARγ mRNA to be decreased in response to de-training. This indicates a decline lipid metabolism and fatty acid beta oxidation.

**Effects of exercise and de-training on leptin mRNA in white adipose tissue (4th study)**

The primary aim of the fourth study was to examine the effects of different types of exercise and de-training on leptin mRNA of subcutaneous adipocytes in healthy untrained men. No effects of chronic exercise and de-training on the mRNA of leptin were found neither in the entire sample size nor in the high responders not only to the exercise stimuli, but also to the de-training period. Previous evidence showed that the reduction of circulating leptin concentrations in response to exercise is usually accompanied by a reduction in fat mass [120, 141, 144]. The fat mass of the participants did not alter in response to exercise in the current study and thus, this may explain, at least in part, the non-alteration of leptin mRNA. Also, previous data from a human study showed that caloric restriction in conjunction with a 2-month exercise programme decreased circulating leptin as opposed to either caloric restriction or exercise alone [147]. The participants of the current study did not follow a caloric
restriction neither during the exercise programme nor during the de-training period, and this may also explain the non-alteration of leptin mRNA.

A secondary aim of the current study was to examine the effects of different types of exercise and de-training on the adiponectin and L/A ratio mRNAs of subcutaneous WAT in healthy untrained men. No effects of exercise and de-training on the adiponectin and L/A ratio mRNAs of subcutaneous WAT were found in the entire sample size however, the adiponectin mRNA was significantly decreased in response to de-training in the high responders to de-training in the CEG. Also, the adiponectin mRNA was increased in response to resistance exercise, yet not significantly. The effect size analysis showed a large effect size in the REG and a small effect size in the CEG between baseline and post-exercise. This is in accordance with previous evidence showed that chronic exercise increased circulating adiponectin concentrations in healthy obese men [13, 395] and postmenopausal women [396]. The adiponectin mRNA was decreased in response to de-training in the AEG and CEG in the current study, yet not significantly. The effect size analysis showed a large effect size in REG and a moderate effect size in AEG and CEG between post-exercise and de-training. These findings may represent a normal trend, given that chronic exercise may increase circulating adiponectin concentrations [13, 395, 396]. Low circulating adiponectin concentrations are associated with increased appetite [120] and high circulating adiponectin concentrations may enhance insulin sensitivity [156, 284].

The L/A ratio can be used to identify insulin resistance in T2D patients [397]. Increased L/A ratio may indicate increased atherosclerosis and glycated haemoglobin, which is an index of increased insulin resistance [397, 398]. The effect size analyses in this study showed a decrease of the L/A ratio mRNA in response to resistance exercise and an increase in response to de-training in the AEG. However, there is no
enough evidence to determine a role of L/A ratio in the current study, and instead the trend of the values is reported.

It is concluded that different types of chronic exercise and de-training do not affect mRNAs of leptin, adiponectin genes and L/A ratio of subcutaneous WAT in healthy untrained men. There is a trend of adiponectin mRNA to be increased, in response to resistance exercise and to be decreased in response to de-training. There is also a trend of L/A ratio mRNA to be decreased, in response to resistance exercise and to be increased in response to de-training. Future studies should increase sample sizes and investigate these phenomena.

**Overall perspective**

The recent discovery of active BAT in adult humans suggested a potential contribution of this tissue on human REE [22]. It was suggested that 100 gr of a single BAT depot has a rate of 12.2 μmol of glucose uptake per minute, which during a year period could spend energy equal to the energy capacity of 4 kg of WAT [22]. Given also that NST in humans could elevate the daily REE for 25-28% after chronic cold exposure [174, 366], we could hypothesize that in a total of 1500 kcal of daily REE, BAT activity could contribute an additional of 375-420 kcal/day. However, this would be a contribution of BAT to REE under cold exposure, while there is no evidence in humans to support BAT’s contribution to REE under thermoneutral conditions. In this light, the thermogenic capacity of BAT in response to exercise also remains unknown. On the other hand, brown-like adipose tissue seems to not have the same thermogenic capacity and can display only 10% of BAT’s thermogenic capacity based on the hypothesis that the amount of the UCP1 protein expression that is displayed in brown-like adipocytes is significantly lower than the one of brown adipocytes [304].
represents a brown-like adipose tissue contribution to daily REE of 37.5-42 kcal/day in the aforementioned example. However, this would also be a contribution of brown-like adipose tissue to REE under cold exposure, while at the moment there is no evidence to show differences in the thermogenic capacity between brown and brown-like adipocytes in humans. Based on the results of the current PhD study it seems that exercise cannot increase the thermogenic capacity of WAT in humans while there is no link between the thermogenic capacity of white adipocytes and REE. Finally, the current PhD, showed positive UCP1 white adipocytes and a trend of both UCP1 mRNA and protein expression to be increased in response to aerobic exercise. Whether this UCP1 trend could contribute to daily REE in humans should be explored by future studies.
10. CHAPTER 10: LIMITATIONS

The limitations of the current PhD study are:

- In the systematic review only included published literature; grey literature was excluded. There is a potential of publication bias towards peer-reviewed work, but the inclusion of grey literature may itself introduce bias. One reason to include grey literature would be the absence of peer-reviewed sources [365].

- In the systematic review, the secondary outcomes that indicate no positive relationship between Irisin and several health indices were derived from the included studies. These examined the effects of physical activity on Irisin. However, Irisin may have a physiological role in the human body independently of physical activity, therefore, the current systematic review may not be representative enough to determine the relationship between Irisin and health indices.

- Only men participated in all the experimental studies, so gender differences could not be determined.

- There was no priori power calculation for the second (cross-sectional) and the forth (RCT) studies.

- Apart from UCP1, in all the experimental studies, only mRNAs were measured. Protein expressions should have also been required to determine the physiological function of the genes examined.

- In the third study (RCT), Irisin was not assessed in relation to exercise and its association with the genes was not examined. The initial plan was to measure circulating Irisin. That is why, blood samples were retrieved from the participants. However, while this study was conducted, new evidence revealed that the ELISA kits used for Irisin identification gave neither valid nor accurate
data. Given that there was no alternative method within our laboratory, the Irisin identification in blood samples was omitted.

- In the RCT (third, fourth studies), the chronic exercise programmes followed previous research and were set at a specific intensity (65% VO$_2$peak, 1RM). The effects of various intensities on the genes examined could not be tested.
11. CHAPTER 11: SUGGESTIONS FOR FUTURE RESEARCH

The results of the first study, the systematic review, suggest that future RCTs should examine the link between PGC-1α and FNDC5 within the skeletal muscle in humans in response to physical activity. In this regard, the differences between young and older individuals as well as between healthy and patients should be explored given that previous evidence showed that the FNDC5 mRNA in muscle is expressed more in older than in younger individuals and it is differently regulated between those with and without insulin resistance [267]. In addition to that, future studies must re-examine the biological role of Irisin using accurate and valid methods for its analysis. Indeed, the recent evidence via mass spectrometry – the gold standard method for circulating Irisin measurements nowadays – indicates that Irisin is increased in response to exercise [270, 276]. However, these existing studies display several methodological limitations (i.e. post hoc measurements, no control) that do not allow for clear conclusion to be drawn. Therefore, future studies should explore this further in humans, and also need to take into consideration the type and intensities of exercise that may play a role in exercise-induced Irisin changes [266, 337, 339]. In line with this, the role of human Irisin on insulin sensitivity and glucose uptake should also be explored given that the potential of Irisin function is to improve health mainly via an improvement in insulin sensitivity [30]. Furthermore, the link between circulating Irisin and white adipocytes should be investigated, given that the receptor that Irisin was proposed to attach to the surface of white adipocytes is unknown and thus, the biological activity of the protein cannot be fully determined.

The third study of this PhD indicates that UCP1 mRNA and protein expression are not positively linked with physical activity/exercise. Even though one previous single group design study [368], one previous controlled trial [236] and the current PhD/RCT study showed no effect of exercise on UCP1 of WAT in healthy individuals,
two out of the three studies showed a trend of increased UCP1 in response to aerobic exercise. To the best of our knowledge these are the only studies that examined this mechanism in humans and only one of them is a RCT. Therefore, future studies should use the methodological limitations provided within these studies to develop robust methodologies to investigate these phenomena. Also, according to the available evidence the link between Irisin and UCP1 is not well-established and therefore, the possibility of UCP1 to be altered within the human WAT in response to exercise but, not in response to Irisin should be also investigated. This however, implies that important confounding factors (e.g. cold exposure, warm environment) should be taken into consideration given that UCP1 is altered in WAT in response to different environmental conditions [304]. In addition, in the current PhD/RCT the UCP1 mRNA and protein expression were examined only in response to exercise at 65% of VO₂peak and 1 RM. In this light, more studies are needed to explore the effects of different types and intensities of exercise on UCP1 of WAT given the lack of evidence in humans.

Brown-like adipocytes may display a thermogenic capacity lower than the one of brown adipocytes however, this suggestion was based on the hypothesis that the amount of the UCP1 protein expression that is displayed in brown-like adipocytes is significantly lower than the one of brown adipocytes [304]. Therefore, further studies may also be needed to explore the role of UCP1 positive cells in WAT and the thermogenic capacity of these cells compared to the thermogenic capacity of brown adipocytes in humans considering different stimuli (e.g. exercise, cold exposure). This would highlight any potential contribution of brown-like adipocytes on REE and whether this could help towards body weight loss and subsequently to lessen obesity in humans. Regarding the third study of this PhD, future research projects are required
to examine the full mechanism that exercise may cause transformation of white adipocytes into brown-like adipocytes. This would include muscle biopsies to determine any link between PGC-1α and FNDC5, accurate measurements of circulating Irisin to determine any link with FNDC5 and adipose tissue biopsies to determine any link between circulating Irisin and UCP1.

Previous evidence showed that after four weeks of cold exposure, UCP1 was increased in subcutaneous WAT of mice, which subsequently increased hyperlipidemia that could develop atherosclerotic plaques [402]. Given that physical activity/exercise can reduce the risk for atherosclerosis in humans [403], the role of physical activity/exercise on UCP1 in subcutaneous WAT should be explored in combination with any potential effect of UCP1 on atherosclerosis. Furhtermore, specific UCP1 gene polymorphisms (i.e. A-3826G, A-1766G, A-112C, Ala64Thr) may promote the development of cardiovascular disease in humans including T2D [404], while T2D in humans is positively associated with the environmental temperature [405]. In this light, an examination of the associations of the UCP1 gene polymorphisms with cardiovascular disease and physical activity levels could add important information to our existing knowledge around this field.

The second study of this PhD suggests that the role of PGC-1α, PPARα and PPARγ genes in human metabolism should be further explored independently of their potential synergetic action with UCP1 towards the browning formation of WAT. More specifically, the effects of the function of these genes on body weight, fat mass and REE should be explored given the potential of these genes to increase fatty acid oxidation. This however, should be investigated in combination with diet given that PPARα and PPARγ are nuclear receptors for fat vitamins, steroid hormones, and
sterols [247, 248]. Overall, this could highlight potential mechanisms that could help towards body weight loss in humans.

The forth study of this PhD showed no effect of different types of exercise on leptin mRNA. This has been examined to identify potential links of leptin with browning formation of WAT given that leptin is suppressed in response to cold exposure [134], SNS activation and norepinephrine production [122, 123]. Also, Bostrom et al. (2012) showed that leptin mRNA is downregulated by UCP1 in human white adipocytes [30]. This evidence indicates an inverse relationship between leptin and brown/brown-like adipose tissue activity, which needs to be further explored, given that leptin is a key regulator in WAT function. In this PhD/RCT study, only leptin mRNA was examined and the function of circulating leptin has not been determined in response to chronic exercise. Therefore, more experiments are needed to define the role of leptin mRNA and circulating leptin during a potential browning formation of WAT.
1. WHO, Comparative Quantification of Health Risks (Overweight and obesity (high body mass index)), Chapter 8, A.D.L. Majid Ezzati, Anthony Rodgers, and Christopher J.L. Murray, Editor. 2004: Geneva.


282. Hondares, E., et al., Thiazolidinediones and rexinoids induce peroxisome proliferator-activated receptor-coactivator (PGC)-1alpha gene transcription: an autoregulatory loop controls PGC-1alpha expression in adipocytes via


328. Khodadadi, H., et al., The effect of High Intensity Interval Training (HIIT) and pilates on levels of irisin and insulin resistance in overweight women. [Persian]. Iranian Journal of Endocrinology and Metabolism, 2014. 16(3).


13. APPENDICES

13.1. APPENDIX 1: Ethical approval from the University of Wolverhampton

School of Sport, Performing Arts and Leisure

Dean:
John Pye

School of Sport, Performing Arts and Leisure
University of Wolverhampton
Gower Road
Wolverhampton WV1 2SU
United Kingdom

T: +44 (0)1902 329200/329290 (Ext 6)
E: +44 (0)1902 329294
E: s&p@swh.ac.uk

04/09/13

To whom it may concern,

RE: Ethical approval for research projects – Petros Ntinas

This is to confirm that the research projects set out below have been considered by the Ethics Committee of the School of Sport, Performing Arts & Leisure, The University of Wolverhampton. Both projects were considered within the Ethics Committee held on 19th June 2013 and were granted ethical approval.

The effects of acute exercise on brown-like tissue in humans – approved (ethics approval number: 92843)

The effects of prolonged exercise on brown-like tissue formation in humans – approved (ethics approval number: 92844)

Kind regards,

Adam Brown
Administrative assistant
School of Sport, Performing Arts & Leisure
13.2. APPENDIX 2: Ethical approval from the University of Thessaly

University of Thessaly
Department of Physical Education and Sport Science

Internal Ethics Committee

Trikala 13/3/2013
Protocol number: 698

Application for approval of research entitled: The effects of prolonged exercise on brown-like tissue formation in humans.

Scientist responsible – supervisor: Yiannis Koutedakis PhD, Professor in Applied Physiology
Departments: Department of Physical Education and Sport Sciences, School of Sport, Performing Arts and Leisure
Institutions: Thessaly University, Wolverhampton University

Scientific advisor: Andreas D. Flouris PhD, Researcher in Environmental Physiology
Department: FAME Laboratory
Institution: CERETETH

Main researcher – student: Ntinas Petros
Study program: PhD
Department: School of Sport, Performing Arts and Leisure
Institution: Wolverhampton University, UK (collaborating institution)

The proposed research relates to a:
Research grant □  Postgraduate thesis X  Undergraduate thesis □  Independent research □

Contact phones: Yiannis Koutedakis, PhD: +302431047056; Andreas D. Flouris, PhD: +302431500601, Ntinas Petros: +306974010118
Contact emails: Yiannis Koutedakis, PhD: y.koutedakis@pe.uth.gr; Andreas D. Flouris, PhD: andreasflouris@cereteth.gr; Ntinas Petros: petros.cd@gmail.com

The Internal Ethics Committee (IEC) of the Department of PE and Sport Science (DPESS), University of Thessaly, examined the proposal in its 3-4/13-2-2013 meeting and approves the implementation of the proposed research.

The Chair of the IEC – DPESS

Athanasiou Tsiokanos, PhD
13.3. APPENDIX 3: Written consent form

(The form was supplied in Greek due to the participants were Greek citizens)

Written informed consent form

Title of the study: The effects of prolonged exercise on brown-like tissue formation in humans

Scientific responsible-advisor: Yiannis Koutedakis PhD, Professor in Applied Physiology (Tel. 2431047056, email: y.koutedakis@uth.gr)

Scientific Advisor: Andreas Flouris PhD, Researcher in Environmental Physiology (Tel. 2431500601, email: aflouris@cereteth.gr)

Researcher: Petros Ntinas (Tel.6974010118, email: petros.cd@gmail.com)

1. Aim of the study

The aim of the study is to identify the secretion of the protein irisin in the circulation across different types of chronic exercise. A secondary aim is to confirm the presence or absence of transformed white adipocytes to brown-like adipocytes by examining the presence or absence of Uncoupling Protein 1 through subcutaneous white adipose tissue biopsies. Another aim is to assess the relationship between the sympathetic nervous system (SNS) activity and irisin levels by assessing heart rate variability.

2. Procedure

As soon as you sign the present manuscript given your consent to participate in the study, you will be asked to fill via interview a medical history, a short nutrition questionnaire and two short physical activity questionnaires. Then, you will be randomised to be included in one of the following groups: 1) An aerobic exercise group which will perform a 2-month of an aerobic exercise protocol that will start from 20 min at 60% of your maximum oxygen consumption and will lead to 60 min at 70% of your maximum oxygen consumption. The exercise sessions will be performed 3-5 times per week. 2) A resistance exercise group which will perform a 2-month of resistance exercise protocol aiming to muscle hypertrophy, 3-5 times per week at 60-70% of your 1 repetition maximum (your maximum strength). 3) A combined exercise group (aerobic and resistance) which will perform a 2-month of a combined (aerobic and resistance) exercise protocol. Aerobic exercise will start from 20 min at 60% of your maximum oxygen consumption and will lead to 60 min at 70% of your maximum oxygen consumption. Resistance exercise will aim to muscle hypertrophy and will be at 60-70% of your 1 repetition maximum (your maximum strength). The exercise sessions will be performed 3-5 times per week. 4) A control group which will not perform any exercise.

After the exercise period all groups will not follow any exercise programme for 2 more months as a de-training period. During both exercise and de-training period (4-month period) you will be asked to give 20-30 ml of blood 4 times (Baseline, 1st, 2nd, and 4th month). Additionally, you will be asked to apply a recorder (stop watch) Polar RS800CX as to measure heart rate variability 4 times (Baseline, 1st, 2nd, and 4th month). Furthermore, you will be asked to apply a mask of a VO2 analyser to measure your basal metabolic rate 4 times (Baseline, 1st, 2nd, and 4th month). Additionally, you will undergo 4 times (Baseline, 1st, 2nd, and 4th month) a maximal oxygen consumption test and a 1 repetition maximum test (your maximum strength). The latter tests will be done to determine the exercise intensity that you will follow in the exercise programme and to determine your progress in your aerobic capacity and strength. Finally, you will be asked to provide diet recalls, your physical activity levels that you will obtain via a pedometer, anthropometry measurements (height, weight, blood pressure) and body composition measurements during the baseline week and 1st, 2nd, and 4th month.
3. Dangers and Annoyances
There is a risk of skin irritation from the needle injection during the blood sample collection. We inform you that all blood samples will be collected by a certified Phlebotomist to minimize the risk of any discomfort. White adipose tissue biopsies might cause an irritation on the skin by the injection of the scalpel. There is also a small possibility to be remained in the area a small scar. Furthermore, if you do not be aware that you have an allergy in the local anaesthetic (xylocaine) that will be used in the biopsy procedure there is a risk of an allergic reaction. Please note that fat biopsy procedures will be performed by a certified Physician to minimize the risk of any discomfort. There is a risk of injury during the 1 repetition maximum (1RM) test. Before the 1RM test you will perform a 10-min warm up to minimize any injury risk. In this light, before a 1RM test you will be given full instruction by the main researcher and you will perform tests in terms of the correct techniques that you will use in the 1RM test as to minimize any injury risk. Moreover, there is a risk of injury during the exercise period. However, you will be giving full instructions by experts regarding safe exercise techniques as to minimize the risk of any injury.

4. Potential Benefits
By participating in the study you will receive information regarding your fitness level, your physical activity levels, and your nutrition habits. Moreover, you will benefit the positive effects of a chronic exercise programme. This study will help us to better understand the effects of chronic exercise on the action of the protein irisin which may affect brown adipose tissue. This would be a crucial information due to activity of brown adipose tissue may increase human body energy expenditure with a potential effect on weight loss.
13.4. APPENDIX 4: Medical history form (by interview)

**Medical History Form**

Name: ____________________________  Date: ___/___/____  Date of Birth: ___/___/____  
DD  MM  YYYY  DD  MM  YYYY  

**PRESENT STATUS:**

1. Are you in good health at the present time to the best of your knowledge?  Yes ☐  No ☐  
If you answered “No”, please explain:

________________________________________________________________________  
________________________________________________________________________  
________________________________________________________________________  

2. Are you under a doctor's care at the present time?  Yes ☐  No ☐  
If you answered “Yes”, please explain:

________________________________________________________________________  
________________________________________________________________________  
________________________________________________________________________  

3. Are you taking any medications at the present time?  Yes ☐  No ☐  
If you answered “Yes”, please explain:  

**Prescription Drugs (list all):**  

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Are you taking any over the counter medications, vitamins, supplements?  Yes ☐  No ☐  
If you answered “Yes”, please explain:  

**Name (list all):**  

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Do you have a history of high blood pressure?  Yes ☐  No ☐  
6. Do you have a history of diabetes?  Yes ☐  No ☐  
7. Do you have a history of heart attack, chest pain, or other heart condition?  Yes ☐  No ☐  
8. Do you have a history of swelling of feet?  Yes ☐  No ☐  
9. Do you have a history of vascular disease?  Yes ☐  No ☐  
10. Do you have a history of frequent headaches?  Yes ☐  No ☐  
11. Do you have a history of frequent migraines?  Yes ☐  No ☐  
12. Do you have a history of constipation?  Yes ☐  No ☐  
13. Do you have a history of glaucoma?  Yes ☐  No ☐  
14. Do you have a history of sleep apnoea?  Yes ☐  No ☐  
15. Do you have a history of dyslipidemia?  Yes ☐  No ☐  
16. Do you have a history of elevated triglycerides?  Yes ☐  No ☐  
17. Do you have a history of elevated fasting glucose?  Yes ☐  No ☐  
18. Do you have a history of metabolic syndrome?  Yes ☐  No ☐  
19. Do you have a history of kidney disease?  Yes ☐  No ☐  
20. Do you have a history of joint pain/weakness?  Yes ☐  No ☐  
21. Do you have a history of muscle pain/weakness?  Yes ☐  No ☐
22. Do you have a history of thyroid disease?  
Yes ☐  No ☐

23. Do you have a history of immunological disease?  
Yes ☐  No ☐

24. Do you have a history of cancer?  
Yes ☐  No ☐

25. Do you have a history of alcohol abuse?  
Yes ☐  No ☐

26. Do you have a history of drug abuse?  
Yes ☐  No ☐

26A. Do you have a history of Blood Coagulation Disorders?  
Yes ☐  No ☐

26B. Do you have a history of hepatitis?  
Yes ☐  No ☐

26C. Do you have a history of immunosuppression?  
Yes ☐  No ☐

26D. Do you have a history of malignant hyperthermia?  
Yes ☐  No ☐

27. Do you have a history of smoking?  
Yes ☐  No ☐

If you answered “Yes”, please state the average number of packs you smoke per day: _______

If you answered “Yes”, please state the number of years that you smoke or used to smoke: _______

28. Are you a current smoker?  
Yes ☐  No ☐

29. Do you use any other form of tobacco?  
Yes ☐  No ☐

If you answered “Yes”, please explain:
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________

30. Do you have a history of serious injuries?  
Yes ☐  No ☐

If you answered “Yes”, please list all with dates:
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________

31. Do you have a history of surgeries?  
Yes ☐  No ☐

If you answered “Yes”, please list all with dates:
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________

WEIGHT INFORMATION:
32. What is your desired weight? _________ (kg)
33. What is your maximum lifetime weight (non-pregnant)? _________ (kg)
34. What was your weight one year ago? _________ (kg)
35. What was your weight at age 20? _________ (kg)
36. What was your birth weight? _________ (kg)

EARLY LIFE INFORMATION
37. Where were you born?  
Village/Town: __________________ Closer City: ________________ Country: ________________

38. How old was your mother when you were born?  
__________

39. How old was your father when you were born?  
__________

40. How many months did you breastfeed for?  
__________

<table>
<thead>
<tr>
<th>FAMILY HISTORY</th>
<th>Age</th>
<th>Disease</th>
<th>If deceased, state cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brothers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sisters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relationship</td>
<td>Yes</td>
<td>No</td>
<td>Don’t know</td>
</tr>
<tr>
<td>------------------</td>
<td>-----</td>
<td>----</td>
<td>------------</td>
</tr>
<tr>
<td>Glaucoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated triglycerides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated fasting glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
13.5. APPENDIX 5: International Physical Activity Questionnaire

(The questionnarie was supplied in a valid Greek version due to the participants were Greek citizens)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in a usual week. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in a usual week. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During a usual week, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?
   _____ days per week

   [ ] No vigorous physical activities ➔ Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?
   _____ hours per day
   _____ minutes per day

   [ ] Don’t know/Not sure

Think about all the moderate activities that you did in a usual week. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During a usual week, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.
   _____ days per week

   [ ] No moderate physical activities ➔ Skip to question 5

4. How much time did you usually spend doing moderate physical activities on one of those days?
   _____ hours per day
   _____ minutes per day

   [ ] Don’t know/Not sure

Think about the time you spent walking in a usual week. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During a usual week, on how many days did you walk for at least 10 minutes at a time?
   _____ days per week

   [ ] No walking ➔ Skip to question 7
6. How much time did you usually spend walking on one of those days?
   _____ hours per day
   _____ minutes per day
   □ Don’t know/Not sure

The last question is about the time you spent sitting on weekdays during a usual week. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During a usual week, how much time did you spend sitting on a week day?
   _____ hours per day
   _____ minutes per day
   □ Don’t know/Not sure

This is the end of the questionnaire, thank you for participating.
13.6. APPENDIX 6: Physical activity readiness questionnaire

This PAR-Q “Physical Activity Readiness Questionnaire” is for your personal use only. It does not need to be signed and returned to SBCC. Please read the questions carefully, answer them honestly, and consider the recommendations for your personal safety. Thank You.

PAR-Q & YOU
(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before starting to become much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES  NO

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by your doctor?

2. Do you feel pain in your chest when you do physical activity?

3. In the past month, have you had chest pain when you were not doing physical activity?

4. Do you lose your balance because of dizziness or do you ever lose consciousness?

5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?

6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

7. Do you know of any other reason why you should not do physical activity?

YES to one or more questions

Talk to your doctor by phone or in person before you start becoming much more physically active or before you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES:

- You may be able to do any activity you want—just as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.

- Find out which community programs are safe and helpful to you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- Start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.

- Take part in a fitness appraisal - this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or

- If you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

“I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.”

NAME: ___________________________ DATE: ___________________________

SIGNATURE: ___________________________ WITNESS: ___________________________

SIGNATURE OF PARENT: ___________________________

Or GUARDIAN (for participants under the age of majority): ___________________________

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.
13.7. APPENDIX 7: Representative photographic film images of UCP1 protein expression quantification
13.8. APPENDIX 8: Publications

Review Article

Exercise-Induced Biological and Psychological Changes in Overweight and Obese Individuals: A Review of Recent Evidence

Petros C. Dinas,1,2 Aleksandra S. Markati,3 and Andres E. Carrillo4

1 School of Sport, Performing Arts and Leisure, University of Wolverhampton, Walsall WS1 4RD, UK
2 FAME Laboratory, Institute of Research and Technology Thessaly, Centre for Research and Technology Hellas, 42200 Trikala, Greece
3 Department of Physical Education and Sport Science, Kapedasistrimi University of Athens, 17237 Athens, Greece
4 Department of Exercise Science, Chatham University, Pittsburgh, PA 15232, USA

Correspondence should be addressed to Petros C. Dinas; p.ntinas@wlv.ac.uk

Received 10 November 2013; Accepted 23 December 2013; Published 6 February 2014

Academic Editors: A. Bielli, A. N. Kavazis, and A. Tse

Copyright © 2014 Petros C. Dinas et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

On a global scale, the most recent evidence indicates that over 400 million adults are obese while ~20 million children and 1.6 billion adults are overweight. The World Health Organization reveals that, by the year 2025, ~2.3 billion adults will be overweight and more than 700 million will be obese. In this review paper we summarized the current evidence to elucidate the impact of exercise training on biological and psychological health indices in overweight and obese individuals. Endocrine function indices that are discussed herein include leptin, adiponectin, growth hormone, and ghrelin levels. Psychological factors include anxiety and depression, body image, and motivation for exercise. Overall, exercise promotes physical and psychological health in overweight and obese individuals particularly because exercise-induced adaptations occur across a multitude of systems within the active human. The impact of exercise on specific biological and psychological health indices contributes to overall health in overweight and obese individuals.

1. Introduction

Increased adiposity and physical inactivity have been identified as harmful health indices [1]. It has been widely acknowledged that obesity leads to health problems such as cardiovascular disease, type 2 diabetes, and hypertension [2, 3]. On a global scale, the most recent evidence indicated that over 400 million adults are obese while ~20 million children and 1.6 billion adults are overweight [2]. An unpleasant estimation by the World Health Organization revealed that by the year 2025 ~2.3 billion adults will be overweight and more than 700 million will be obese [2]. Thus, it is necessary to establish practical/cost effective strategies as well as update and renew current health initiatives, such as physical activity participation, that will lessen the risk of weight gain and disease in overweight and obese populations.

It is well known that physical activity contributes, at least in part, to a healthy quality of life [4]. Indeed, compelling evidence supports that cardiorespiratory fitness (an objective measure of habitual physical activity) is an independent predictor of all-cause and cardiovascular disease mortality [5]. Specifically, recent evidence indicates that individuals with the highest level of physical activity had a greater total life expectancy compared to individuals with low physical activity participation [6, 7]. Conversely, physical inactivity has been linked to a plethora of health conditions such as type 2 diabetes [2] and has been identified as a greater risk for morbidity and mortality than a large adipose tissue mass [8]. In this light, it is essential to constantly monitor relevant research, which continues to provide nascent exercise-induced adaptations that are invaluable for weight management, disease prevention, and to establish a high quality of life.

The aim of this paper was to review and summarize the current biological and psychological evidence to further understand the relationship between exercise and health in overweight and obese individuals.

http://www.hindawi.com/journals/isrn/2014/964627/