DOCTORAL DISSERTATION

"Factors and signaling pathways implicated in the browning of white adipose tissue and in the effects of exercise on adipose tissue"

By:

Eleni Nintou

Supervisor:

Dr. Andreas D. Flouris

A thesis submitted in fulfillment of the requirements
for the degree of Doctor of Philosophy in the

Department of Physical Education and Sport Science, University of Thessaly

31st of May 2023

Members of the examination committee (in alphabetical order).

Full name	University	Information
Konstantinos Dimas	University of Thessaly	External examiner
Ioannis Fatouros	University of Thessaly	UTH committee
Anastasios Filippou	University of Athens	External examiner
Andreas D. Flouris	University of Thessaly	Supervisor
Athanasios Jamurtas	University of Thessaly	UTH committee
Stefania Mitola	University of Brescia	External examiner
Marco Rossato	University of Padova	External examiner

Declaration

I, Eleni Nintou, hereby declare that this thesis has not been previously submitted in this University or any other University for the award of any degree, diploma, associateship, fellowship, or other similar titles of recognition.

Thesis title:	"Factors and signaling pathways implicated in the browning of white adipose tissue and in the effects of exercise on adipose tissue"
Pages:	212
Words:	108.519
Figures:	16
Tables:	5

PhD candidate: Eleni Nintou
Signed by: Eleni Nintou
Date: 31/05/ 2023

Plagiarism Disclaimer

I declare that this thesis is based on a total of three studies/papers all of which have been published. These papers are referred to and cited in the thesis.

	Published articles
Chapter 2 [1] :	Prevalence of uncoupling protein one genetic polymorphisms and their relationship with cardiovascular and metabolic health
Chapter 3 [2]:	Effects of In Vitro Muscle Contraction on Thermogenic Protein Levels in Co-Cultured Adipocytes
Chapter 4 [3]:	Characteristics of the Protocols Used in Electrical Pulse Stimulation of Cultured Cells for Mimicking In Vivo Exercise: A Systematic Review, Meta-Analysis, and Meta-Regression

PhD candidate: Eleni Nintou
Signed by: Eleni Nintou
Date: 31/05/ 2023

Table of Contents

List of Figures

List of Tables

Dedicated to my loving family

Acknowledgements

This thesis has been a long tough, joyful, insightful, interesting journey. Like any journey the people you have around you shape it along the way. I was lucky enough to meet people, visit places, interact with an interdisciplinary team and learn that every experience is a valuable lesson for the future.

Thankfully, throughout these years, Prof. Andreas Flouris, was there for me, supporting me, doubting me and pushing me and finally helping me become a better scientist and have a less edgy personality. Once I came to FAMELab, I knew that FAMEpeople are not just co-workers or future "network", but are friends for life, supporting each other all the way. Having very different personalities, scientific backgrounds and interests, we managed to find a common ground for research, understanding and of course mutual respect. The Lab made me a team player and at the same time I learned to stand alone. I would specifically like to thank Dr. Eleni Karligiotou, because she did not quit when I wanted to quit and was like a lifeguard to me.

The experimental work for my thesis was conducted at Prof. Konstantinos Dimas Lab. I am really thankful that I worked with Prof. Dimas and his colleagues, since they shared with me their knowledge and helped me gain high standard technical and scientific skills.

Most important role though throughout my whole life has played my beloved family, my parents Stella and Thanasis, my brother Michalis and my aunt, Athanasia. They were always by my side, when I send my CV to FAMELab, when I decided to do this phd, when I decided to write a fund against all odds...Their love, acceptance and respect has been always my moving force. Last, I would like to thank Constantine Dallas, who is my biggest fan (and I am his) and believes in me more than I believe in myself.

Abstract

Metabolic diseases and related pathologies have been associated with adiposity and relatively recently genetic traits have been identified to have a specific link between white adipose tissue (WAT), cardiovascular disease, body mass index (BMI)-adjusted type 2 diabetes (T2D) and dyslipidemia. Brown adipose tissue (BAT) and beige adipocytes have been identified as major players in the battle against obesity and metabolic disorders. The main protein expressed in BAT and beige adipocytes is UCP1 and an unanswered question is which is the contribution of *UCP1* single nucleotide polymorphisms (SNPs) to susceptibility for cardiometabolic pathologies (CMP) and how their involvement in specific risk factors for these conditions varies across populations. Therefore I investigated the impact of *UCP1* SNPs A-3826G, A-1766G, Ala64Thr and A-112C across Armenia, Greece, Poland, Russia and United Kingdom. In Armenia, GA genotype and A allele of Ala64Thr displayed ~2-fold higher risk for CMP compared to GG genotype and G allele, respectively ($p<0.05$). In Greece, A allele of Ala64Thr decreased risk of CMP by 39%. Healthy individuals with A-3826G GG genotype and carriers of mutant allele of A-112C and Ala64Thr had higher body mass index compared to those carrying other alleles. Heterozygosity of A-112C and Ala64Thr SNPs was related to lower WHR in CMP individuals compared to wild type homozygotes ($p<0.05$). Concluding, the studied SNPs could be associated with the most common CMP and their risk factors in some populations. Apart from UCP1 genetic profile in different populations and species, UCP1 expression is affected by external factors such as exercise, thus the effect of exercise on the formation of beige adipocytes has produced controversial results in human studies. My aim was to research- via an in vitro model of co-culturing of C2C12 myotubes and 3T3-L1 adipocytes under the stimuli of electrical pulse stimulation (EPS) mimicking muscle contraction- the impact of the the direct crosstalk between adipocytes and stimulated muscle cells. When EPS was applied, the t co-culturing led to increases in UCP1 (p = 0.044; d = 1.29) and IL-6 (p = 0.097; d = 1.13) protein expression in the 3T3-L1 adipocytes. In vitro co-culturing of C2C12 myotubes and 3T3-L1

adipocytes under the stimuli of EPS leads to increased expression of thermogenic proteins. I detected changes in the expression pattern of proteins related to browning of adipose tissue, supporting the use of this in vitro model to study the crosstalk between adipocytes and contracting muscle. Although, exercise benefits a wide spectrum of diseases and affects most tissues and organs, as proven by my previous study, many aspects of its underlying mechanistic effects remain unsolved. In vitro exercise, mimicking neuronal signals leading to muscle contraction, can be a valuable tool to address this issue. I performed a systematic review and metanalysis for relevant studies assessing in vitro exercise using electrical pulse stimulation to mimic exercise. I observed variability among existing protocols of in vitro exercise and heterogeneity among protocols of the same type of exercise. The analyses showed that biological indices in vitro followed the patterns of in vivo exercise, and that these effects were correlated with the duration of stimulation, leading to the conclusion that in vitro exercise follows motifs of exercise in humans, allowing biological parameters, such as the aforementioned, to be valuable tools in defining the types of in vitro exercise.

ΠΕΡΙΛΗΨΗ

Τα μεταβολικά νοσήματα και οι συναφείς παθολογίες έχουν συσχετιστεί με το λιπώδη ιστό και σχετικά πρόσφατα εντοπίστηκαν γενετικά χαρακτηριστικά που έχουν ειδική σχέση μεταξύ του λευκού λιπώδους ιστού (ΛΛΙ), των καρδιαγγειακών νοσημάτων, του διαβήτη τύπου 2 (ΔΤ2) και της δυσλιπιδαιμίας, προσαρμοσμένου στο δείκτη μάζας σώματος (ΔΜΣ). Ο καφέ λιπώδης ιστός (ΚΛΙ) και τα μπεζ λιποκύτταρα έχουν αναγνωριστεί ως σημαντικοί παράγοντες στη μάχη κατά της παχυσαρκίας και των μεταβολικών διαταραχών. Η κύρια πρωτεΐνη που εκφράζεται στο ΚΛΙ και στα μπεζ λιποκύτταρα είναι η UCP1 και ένα αναπάντητο ερώτημα είναι ποια είναι η συμβολή των μονονουκλεοτιδικών πολυμορφισμών (SNPs) της UCP1 στις καρδιομεταβολικές παθολογίες (ΚΜΠ) και πώς η συμβολή τους σε συγκεκριμένους παράγοντες κινδύνου για αυτές ποικίλλει μεταξύ των πληθυσμών. Ως εκ τούτου, διερεύνησα την επίδραση των UCP1 SNPs A-3826G, A-1766G, Ala64Thr και A-112C στην Αρμενία, την Ελλάδα, την Πολωνία, τη Ρωσία και το Ηνωμένο Βασίλειο. Στην Αρμενία, ο γονότυπος GA και το αλληλόμορφο A του Ala64Thr εμφάνισαν ~2 φορές υψηλότερο κίνδυνο για ΚΜΠ σε σύγκριση με τον γονότυπο GG και το αλληλόμορφο G, αντίστοιχα ($p<0,05$). Στην Ελλάδα, το αλληλόμορφο A του Ala64Thr μείωσε τον κίνδυνο εμφάνισης ΚΜΠ κατά 39%. Τα υγιή άτομα με γονότυπο A-3826G GG και οι φορείς των μεταλλαγμένων αλληλόμορφων A-112C και Ala64Thr είχαν υψηλότερο δείκτη μάζας σώματος σε σύγκριση με εκείνους που έφεραν άλλα αλληλόμορφα. Η ετεροζυγωτία των SNPs A-112C και Ala64Thr σχετιζόταν με χαμηλότερο WHR σε άτομα με ΚΜΠ σε σύγκριση με τους ομοζυγώτες φυσιολογικού γονότυπου ($p<0,05$). Συμπερασματικά, τα SNPs που μελετήθηκαν θα μπορούσαν να συσχετιστούν με τις πιο συχνές ΚΜΠ και τους παράγοντες κινδύνου τους σε ορισμένους πληθυσμούς. Εκτός όμως, από το γενετικό προφίλ της UCP1 σε διαφορετικούς πληθυσμούς και είδη, η έκφραση της UCP1 επηρεάζεται από εξωτερικούς παράγοντες, όπως η άσκηση, η επίδραση της οποίας στο σχηματισμό μπεζ λιποκυττάρων είναι αμφιλεγόμενη με βάση μελέτες σε ανθρώπους. Σκοπός μου ήταν

να ερευνήσω- μέσω ενός in vitro μοντέλου συγκαλλιέργειας μυοκυττάρων C2C12 και λιποκυττάρων 3T3-L1 υπό το ερέθίσμα της ηλεκτρικής παλμικής διέγερσης (EPS) που μιμείται τη μυϊκή συστολή- την άμεση αλληλεπίδραση μεταξύ λιποκυττάρων και διεγερμένων μυϊκών κυττάρων. Όταν εφαρμόστηκε EPS, στη συγκαλλιέργεια αυξήθηκε η έκφραση των πρωτεϊνών UCP1 (p = 0,044- d = 1,29) και IL-6 (p = 0,097- d = 1,13) στα λιποκύτταρα 3T3-L1., δηλαδή η in vitro συγκαλλιέργεια μυοκυττάρων C2C12 και λιποκυττάρων 3T3-L1 υπό του EPS οδηγεί σε αυξημένη έκφραση θερμογόνων πρωτεϊνών. Οι αλλαγές στο πρότυπο έκφρασης πρωτεϊνών που σχετίζονται με τη φαιοποίηση του λιπώδους ιστού, υποστηρίζουν τη χρήση αυτού του in vitro μοντέλου. Παρόλο που η άσκηση ωφελεί ένα ευρύ φάσμα ασθενειών και επηρεάζει τους περισσότερους ιστούς και όργανα, όπως αποδείχθηκε από την προηγούμενη μελέτη μου, πολλές πτυχές των υποκείμενων μηχανιστικών επιδράσεών της παραμένουν άλυτες. Η άσκηση in vitro, που μιμείται τα νευρικά σήματα που οδηγούν σε μυϊκή συστολή, μπορεί να αποτελέσει πολύτιμο εργαλείο έρευνας. Διεξήγαγα μια συστηματική ανασκόπηση και μετ-ανάλυση για σχετικές μελέτες που αξιολογούν την άσκηση in vitro με τη χρήση ηλεκτρικών παλμών και παρατήρησα ποικιλομορφία μεταξύ των υφιστάμενων πρωτοκόλλων άσκησης in vitro και ετερογένεια μεταξύ πρωτοκόλλων του ίδιου τύπου άσκησης. Οι αναλύσεις έδειξαν ότι οι βιολογικοί δείκτες in vitro ακολουθούσαν τα μοτίβα της άσκησης in vivo και ότι τα αποτελέσματα αυτά συσχετίζονταν με τη διάρκεια της διέγερσης, οδηγώντας στο συμπέρασμα ότι η άσκηση in vitro ακολουθεί μοτίβα της άσκησης στον άνθρωπο, επιτρέποντας σε βιολογικές παραμέτρους, να αποτελέσουν πολύτιμα εργαλεία για τον καθορισμό των τύπων άσκησης in vitro.

Chapter 1

1.1 The importance of Adipose Tissue

Approximately one third of the global population (2.2 billion people) are overweight, of which about 700 million counting for ten percent of the global population are obese[4]. The burden of obesity has been highlighted the last decade as an epidemic and a menace for public health [5] and world health organization's published data gave a raise in public awareness and a subsequent increase in relevant research (>3700 published papers over the last 15 years). Metabolic diseases and characteristics have been related to adiposity and relatively recently genetic traits have been identified to have a specific link between white adipose tissue (WAT), cardiovascular disease, body mass index (BMI)-adjusted type 2 diabetes (T2D) and dyslipidemia [6-8].

1.2 Adipose tissue

Mammalian adipose tissue traditionally can be found in two major subtypes, white adipose tissue and brown adipose tissue (BAT). Those distinct types of adipocytes have distinct roles, location, morphology and embryonic precursors. Another form of adipocytes, called beige adipocytes, having also different role, morphology and precursors [9] has been characterized recently as a distinctive subtype (Fig. 1.1).

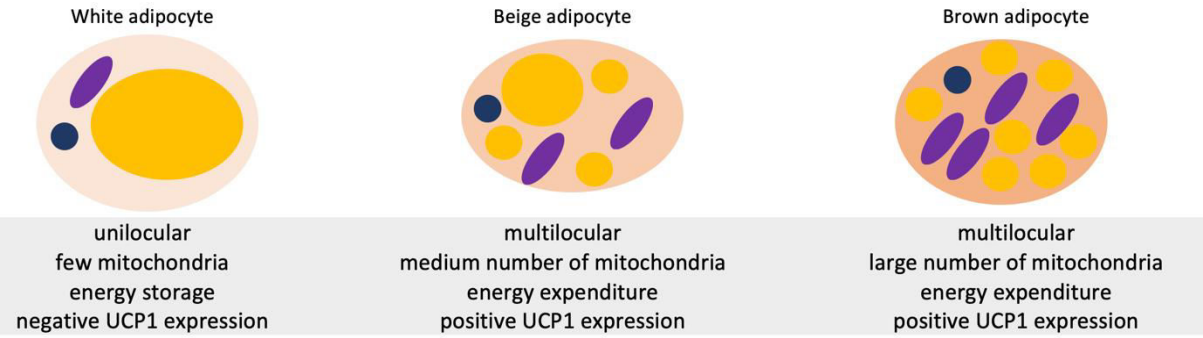


Figure 1.1 The three cell types of adipocytes.
Morphologically, the three cell types share similarities and differences. White adipocytes are unilocular, have few mitochondria and one central lipid droplet. On the other hand, beige and brown adipocytes are multilocular, have more mitochondria, in the BAT are more abundant, and small lipid droplets dispersed in the cytoplasm

1.3 Brown adipose tissue

Brown adipose tissue (BAT) can be detected supraclavicularly, along spinal cord, in the neck and auxiliary area [10], originates from Myf5 positive cells [11] and is responsible for non-shivering thermogenesis and energy expenditure. WAT is present throughout all stages of development, while BAT is mostly prompt in infancy and was thought to be absent in adulthood. However, in 2009, BAT was found in the neck and shoulder regions of adults by ^{18}F-FDG-PET/CT detection [12, 13]. Accumulative evidence, since then, has showed that BAT is present in adults[14, 15], but obese and overweight individuals tend to have lower amounts of active brown adipose tissue compared to lean individuals [16].

The main protein controlling non-shivering thermogenesis is the uncoupling protein 1 (UCP1) and is abundant in BAT [17]. Stimuli such as cold exposure [18], exercise [19] and certain hormones[20] lead to the activation of the PGC-1α transcriptional co-activator, which promotes the expression of several genes involved in mitochondrial biogenesis and oxidative metabolism [21], including UCP1. UCP1 is located in the inner mitochondrial membrane of brown and beige adipocytes and is involved in uncoupling oxidative phosphorylation from ATP synthesis, thereby generating heat instead of ATP. The

electron transport chain generates a proton gradient across the mitochondrial inner membrane, which regulates the activity of UCP1. Upon UCP1 activation the proton gradient dissipates across the membrane, leading to uncoupled respiration and heat production, overpassing the ATP synthase and allowing protons to leak back into the mitochondrial matrix, generating heat [22].

Different mice strains, with different genetic background, also have different levels of expression of UCP1 [23]. Similarly, in humans several Single Nucleotide Polymorphisms (SNPs) in UCP1 (FIg.1.2) or in other related genes such as PRDM16 and PPARg affected the UCP1 expression in adipose tissue. Therefore, the impact of the genetic background on the levels of activity of UCP1 became a new field of research.

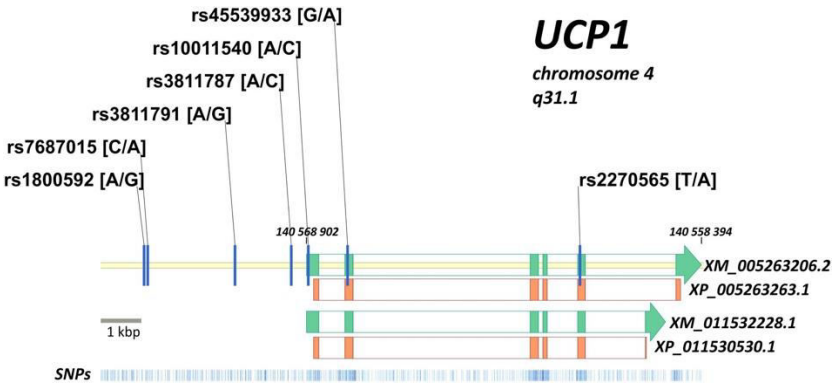


Figure 1.2 UCP1 SNPs

a. A-3826G (rs1800592) located on the upstream region of UCP1, b. A-1766G (rs3811791) a 2kb upstream variant, c. A-112C (rs10011540) on the 5'UTR region, and d. Ala64Thr (rs45539933) a missense variant.

Some SNPs were strongly associated to susceptibility for cardiometabolic pathologies and disease risk [24-26]. For instance, the frequency of AG genotype of A-3826G SNP seemed to vary among different populations with cardiometabolic indices and more specifically, ranges from 24% in Italy [25], to around 50% in Colombia , Japan [27] , and Korea [28], and to 85% in China [8]. Similarly, wide frequency ranges have been reported also for other SNPs across different populations, considering SNPs such as A-1766G,

Ala64Thr and A-112C. However, there was no study describing the genetic effect of UCP1 SNPs in Eastern European countries and the Greek population

Taking into account the existing evidence, I decided to study the effect of polymorphisms of UCP1 from the perspective of population genetics and I tried to identify genetic inheritance effect of UCP1 in healthy and diseased population across different countries/ethnicities.

1.4 White adipose tissue

White adipose tissue is located subcutaneously and viscerally, originates from Myf5 negative cells [11] and is regulating energy homeostasis. Upon energy deficit, WAT promotes lipolysis and upon energy excess moves to lipogenesis, which practically means fatty acid release and fatty acid uptake accordingly[29]. Beyond its role in energy levels regulation, WAT provides insulation and mechanical cushioning and also, acts an endocrine organ [29], presenting intracellular communication with inflammatory (mainly macrophages) and other cell types (muscle), secreting hormones called adipokines (such as adiponectin, leptin, resistin) [30] and adjusting to environmental stimuli.

1.5. White adipose tissue plasticity

White adipose tissue has a surprising ability for plasticity as an adaptive mechanism to environmental changes [31]. During positive energy balance and abundance of feeding, white adipose tissue can either expand (hyperplasia)[24] or increase its cell number (hypertrophy)[32]. However, white adipocytes can also respond to temperature changes, such as cold exposure, by transforming into beige adipocytes[33]. The purpose of this change is to maintain thermogenic capacity and subsequently protect at an organism level from prolonged cold exposure side effects. The plasticity of WAT is reversible over time and after triggering signal ablation.

1.5.1 Beige adipocytes

Along with the discovery of BAT in adults, both healthy and diseased, a new subtype of adipocytes was discovered, namely the beige or brown-like adipocytes. Beige adipocytes share traits with WAT and BAT. They are traced in the neck and supraclavicular area, are Myf5 negative cells and are involved in thermogenesis and energy expenditure.

In the light of these new data and the perspective of a new therapeutic potential against excess adiposity and obesity, a great interest has been aroused.

1.5.2 .Browning of white adipose tissue

Beige adipocytes can be generated within WAT depots via three mechanisms: (1) the differentiation of progenitor cells into new beige adipocytes (i.e., de novo beige adipogenesis), (2) phenotypic conversion of mature white adipocytes into beige adipocytes through the activation (or reactivation) of the thermogenic program, and (3) the proliferation of mature beige adipocytes [34-36].

This adaptation comes along with changes in tissue structure, gene profile (upregulation and downregulation of cell- type specific genes) and metabolic profile [37]. Among these are increased nerve-fiber arborization and angiogenesis and upregulation of UCP1, the characteristic thermogenic protein.

1.5.3. Factors affecting browning of WAT

The browning of WAT is a complex process that involves the interplay of several factors and signaling pathways. Several factors have been identified as promoters of beige adipocytes formation, such as cold

exposure, hormones, transcription factors, inflammatory signaling, nutrient sensors, diet and exercise [38] (Fig. 1.3).

Cold exposure is a potent inducer of browning of WAT, and the process is mediated by the activation of specific signaling pathways and transcription factors, such as the sympathetic nervous system and the transcriptional co-activator PGC-1α. Apart from cold exposure, the most well established factor for browning, several hormones have been shown to play a role in the browning of WAT, such as irisin, which is produced by muscle tissue in response to exercise[39], FGF21 produced by liver [40, 41] and thyroid hormones [41].

Figure 1.3 Factors affecting the browning of WAT
a. Environmental factor such as cold exposure. b. diet, exercise, nutrient sensing and c. biological factors such as transcription factors, inflammatory signaling and hormones.

Another category of activators of browning of WAT are transcription factors, with most well-known to play significant role PPARγ ,PRDM16 and BMP7, that can promote the expression of genes involved in thermogenesis and energy expenditure, and genes involved in fatty acid oxidation and mitochondrial function [42]. Inflammatory signaling pathways, like IL-6 [36] and IL-10 cytokines [43], have been implicated in the browning of WAT. However, nutrient sensing pathways can trigger also, either by their activation (AMPK) [44] or by their inhibition (mTOR)[45], the formation of beige cells.

Exercise may promote browning of WAT through its effects on nutrient-sensing pathways by AMP-activated protein kinase (AMPK), a key regulator of energy metabolism, via the increase in mitochondrial biogenesis and by the inhibition of mTOR signaling. Also, exercise may trigger inflammatory pathways or/and activate sympathetic nervous system and therefore lead to formation of beige cells. The recently identified myokine Irisin, has been shown to be a mediator of browning of adipose tissue due to exercise [39, 46]

However, exercise as a factor promoting browning of WAT has triggered a massive scientific debate, with many controversial results in human studies [47].

1.5.4. Exercise and beige adipose tissue

It has been seen that [48] exercise exerts its effect in many distant organ systems apart from the muscle which is of course the principal organ. It is yet not well defined how these benefits are communicated to the distant target organs like the heart, lungs, adipose tissue and liver. However, there is growing evidence suggesting that exercise along with its other effects can indeed increase the expression of genes involved in the browning of WAT and the activation of beige adipocytes [39].

In rodents voluntary wheel running led to an increase in the expression of genes involved in thermogenesis, mitochondrial biogenesis, and fatty acid oxidation in white adipose tissue, as well as an increase in the number and activity of beige adipocytes [49]. Similarly, in another study in mice endurance exercise training increased the expression of genes involved in thermogenesis and mitochondrial biogenesis in WAT, and also led to an increase in the number and activity of beige adipocytes, as well as both 8- week of aerobic and resistance exercise training led to an induced browning of adipose tissue [50].

The evidence from human studies though has been contradictory and there are studies suggesting that exercise can stimulate the browning of white adipose tissue (WAT) and others found no significant

effect of exercise on beige formation. Independently of weight, exercise intervention in sedentary non-diabetic adults led to brown/beige gene expression changes [51]. Also, acute exercise has been shown to promote the increase of exerkines) that are positively reltaed to BAT volume in young adults.[52]

On the other hand, brown and beige recruitment in endurance athletes was not significantly different when compared to that of untrained sedentary men [53] and neither acute nor repeated bouts of exercise were associated with browning of WAT in sedentary men [54]. Moreover, six weeks of training did not promote browning of adipose tissue in obese men [55] nor 2-week long High intensity interval training (HIIT) seemed to be a stimulus for BAT activation[56].

The different population characteristics (and genetic background), sample sizes and different depots chosen for biopsy [57], detection methods, exercise protocols are the main reasons for this variability among the results in human studies [58].

Upon consideration of these contradictory data, I thought that the most appropriate way to detect the formation of beige cells due to exercise was the creation of an experimental model, where the only direct effect of exercise on WAT could be studied. Therefore, I decided to study the in vitro interaction of muscle cells and adipocytes under the effect of exercise.

1.6. In vitro exercise

There are a few ways to mimic exercise in vitro, such as pharmacological interventions, mechanical streching and electrical pulse stimulation (EPS). EPS has been well established and widely used to mimic in vitro exercise without affecting externally molecular pathways involved in the muscle contraction.

Thus, I chose to built an in vitro model of mimicking exercise based on EPS, in order to study the interaction of contracting muscle cells and adipocytes.

1.6.1. In vitro exercise protocols

A variety of EPS protocols have been used for studying in vitro exercise and muscle contraction. Those exercise protocols were defined as endurance, aerobic, resistance, acute and long-term (chronic) and were performed both in human and mice cell lines. However, the frequency (Hz), pulse duration (ms), applied pulse amplitudes (Vapp), and stimulation duration time of cultured cells in order to achieve exercise-mediating responses are yet to be validated in a systematic way [59].

Therefore, I did a systematic review and meta-analysis to systematically assess the available evidence on the link between the stated type of exercise and the observed biological profile of exercised cells, as well as to present the available EPS-applied protocols mimicking exercise in vitro.

Chapter 2

Prevalence of uncoupling protein one genetic polymorphisms and their relationship with cardiovascular and metabolic health

This work was conducted by Petros C. Dinas, Eleni Nintou, Maria Vliora, Anna E. Pravednikova, Paraskevi Sakellariou, Agata Witkowicz, Zaur M. Kachaev, Victor V. Kerchev, Svetlana N. Larina, James Cotton, Anna Kowalska, Paraskevi Gkiata, Alexandra Bargiota, Zaruhi A. Khachatryan, Anahit A. Hovhannisyan, Mariya A. Antonosyan, Sona Margaryan, Anna Partyka, Pawel Bogdansk, Monika Szulinska, Matylda Kregielska-Narozna , Rafał Czepczyński, Marek Ruchała, Anna Tomkiewicz, Levon Yepiskoposyan, Lidia Karabon, Yulii Shidlovskiiˈ, George S. Metsios, Andreas D Flouris. All authors revised the final draft. Petros C. Dinas, Maria Vliora and myself contributed equally. My contribution to this work included the data collection and experimental data analysis for Greek and UK populations, the statistical analysis for whole sample and the overall data interpretation. Also, I contributed to the original draft preparation and took the responsibility for the integrity of the data and data curation.

As of April of 2022 the present work[1] has been published online by PLoS ONE as follows: Dinas PC, Nintou E, Vliora M, Pravednikova AE, Sakellariou P, Witkowicz A, et al. Prevalence of uncoupling protein one genetic polymorphisms and their relationship with cardiovascular and metabolic health. PLOS ONE. 2022;17(4):e0266386.

Abstract

Contribution of *UCP1* single nucleotide polymorphisms (SNPs) to susceptibility for cardiometabolic pathologies (CMP) and their involvement in specific risk factors for these conditions varies across populations. We tested whether *UCP1* SNPs A-3826G, A-1766G, Ala64Thr and A-112C are associated with common CMP and their risk factors across Armenia, Greece, Poland, Russia and United Kingdom. This case-control study included genotyping of these SNPs, from 2,283 Caucasians. Results were extended via systematic review and meta-analysis. In Armenia, GA genotype and A allele of Ala64Thr displayed ~2-fold higher risk for CMP compared to GG genotype and G allele, respectively ($p<0.05$). In Greece, A allele of Ala64Thr decreased risk of CMP by 39%. Healthy individuals with A-3826G GG genotype and carriers of mutant allele of A-112C and Ala64Thr had higher body mass index compared to those carrying other alleles. In healthy Polish, higher waist-to-hip ratio (WHR) was observed in heterozygotes A-3826G compared to AA homozygotes. Heterozygosity of A-112C and Ala64Thr SNPs was related to lower WHR in CMP individuals compared to wild type homozygotes ($p<0.05$). Meta-analysis showed no statistically significant odds-ratios across our SNPs ($p>0.05$). Concluding, the studied SNPs could be associated with the most common CMP and their risk factors in some ons.

2.1 Introduction

Single nucleotide polymorphisms (SNPs) in a number of candidate genes are highly implicated in energy balance as well as fat and glucose metabolism, modifying disease susceptibility [60-62]. One of these candidate genes codes for uncoupling protein 1 (UCP1), located on chromosome 4 (4q31.1), which is expressed predominantly in brown adipose tissue, holding a critical role in oxidative phosphorylation and overall energy balance [63, 64]. More than 2300 SNPs have been recognized within the *UCP1* gene and its regulatory regions [63], but four have been commonly studied for their impact on metabolism and energy balance [65-69]. These are: (i) A-3826G (rs1800592) located on the upstream region of *UCP1*, (ii) A-1766G (rs3811791) a 2kb upstream variant, (iii) A-112C (rs10011540) on the 5'UTR region, and (iv) Ala64Thr (rs45539933) a missense variant.

The four *UCP1* SNPs have been associated with a number of cardio-metabolic pathologies (CMP) [70]. The G allele of A-3826G, which is associated with reduced mRNA expression of *UCP1* [71], is more common in obese individuals [72, 73] and it is associated with increased body mass index (BMI), percent body fat, blood pressure [74], and lower high-density lipoprotein level [75]. The same allele of this SNP is associated with higher BMI and glucose levels in overweight persons [76] and can increase the risk for proliferative diabetic retinopathy in individuals with type 2 diabetes [77]. The other three SNPs are less prevalent but have been also associated with various risk factors for CMP [[69, 78][79]. The A-112C polymorphism affects *UCP1* gene promoter activity [80] and the C allele is more frequent in individuals with type 2 diabetes than in healthy individuals [81]. The Ala64Thr mutant allele is associated with higher waist-to-hip ratio (WHR) [82], while the A-1766G SNP, which is detected in the genomic region that possibly regulates transcription of *UCP1* [83], is related with obesity [65]. Finally, the GAA haplotype (A-3826G, A-1766G, and Ala64Thr) is associated with decreased abdominal fat tissue, body fat mass, and WHR [84].

The contribution of the four *UCP1* SNPs to the susceptibility for CMP as well as their involvement in specific risk factors for these conditions varies across populations, even within the same race, probably due to environmental impacts. For instance, the frequency of AG genotype of A-3826G in persons with CMP ranges from 24% in Italy [25], to around 50% in Colombia [80], Japan [81], and Korea [75], and to 85% in China [77]. Similarly, wide frequency ranges have been reported also for the other three SNPs across different populations [68, 80, 85, 86]. At the same time, some studies report that *UCP1* SNPs are strongly associated with disease risk [65, 77, 87], while others report no such findings [88-90]. Therefore, it remains unclear if differences in the prevalence of these four *UCP1* SNPs across different populations are associated with the prevalence of CMP.

Our incomplete understanding about the potential involvement of these four *UCP1* SNPs, among others, in disease susceptibility limits the potential for precision medicine to effectively address CMP. An even more direct effect on disease mitigation is that CMP risk factors are currently addressed with equal importance across different populations, ignoring the genotypic/phenotypic complexity of CMP in different countries. Improving our knowledge about the impact of UCP1 variants can contribute to precision medicine, within the context of approaches that consider the polygenicity of cardio-metabolic traits (e.g., polygenic risk scores). This could improve the sustainability of healthcare systems due to increased efficacy of CMP prevention and mitigation guidelines. To address these important knowledge gaps, we investigated if differences in the frequency of A-3826G, A-1766G, Ala64Thr and A-112C SNPs are associated with the most common CMP and their risk factors. This case control study was performed across five countries (Armenia, Greece, Poland, Russia, United Kingdom) since CMP appear to be increased in certain ethnic groups in Eastern Europe and Western Asia [91, 92].To confirm any observed associations between the studied *UCP1* SNPs and cardio-metabolic health, we extended our findings to consider all previously-studied populations by conducting a systematic review and meta-analysis [93]. The literature includes four meta-analyses [24, 88, 94, 95] regarding *UCP1* SNPs and their association

with cardio-metabolic traits. Within these four meta-analyses only A-3826G is examined for its association with metabolic diseases or their risk factors, as the most common variant of *UCP1*, while these meta-analyses do not consider the associations of other *UCP1* SNPs with the risk for disease.

2.2. Materials and Methods

2.2.1 Case-control study

This is a multicenter, multinational study conducted during 2016-2019, across five countries (Armenia, Greece, Poland, Russia, and United Kingdom). The participants were recruited via online and paper advertisements as well as word of mouth. Following approval from the relevant Bioethics Review Board in each country (see Online Supplement section 1.1.1). Written informed consent for participation was signed by the volunteers following detailed explanation of all the procedures and risks involved.

2.2.2. Study design and data collection

The study involved two groups of participants: individuals with CMP as well as healthy controls. We considered the following CMP, as they present with the highest prevalence [96, 97] amongst all health abnormalities related to cardio-metabolic health: cardiovascular disease, hypertension, metabolic syndrome, and type 2 diabetes. The inclusion criteria were: 1) adult; 2) diagnosed presence of CMP for the CMP group and generally healthy (free of CMP based on their medical history) for the control group; 3) non-smokers, or have quit smoking for at least one year; 4) not in a pregnancy or lactation period; 5) no history of eating disorders; 6) no acute illness and/or infection during the last four weeks.

Ethnicity was self-reported by each participant. All participants were assessed for: 1) medical history via a structured interview-based questionnaire; 2) anthropometry (body height, body mass, WHR); 3) percent fat mass via non-invasive bioelectrical impedance analysis; 4) genotypes of the aforementioned four *UCP1* SNPs detected in DNA isolated from blood samples. A detailed description of the adopted

blood handling and genotyping methodologies is provided in the Online Supplement (Section 1.1.2). All participants were instructed, for 12 hours prior to assessments, to avoid the consumption of food, coffee, or alcohol and to refrain from exercise. Also, they were advised to consume two glasses of water about two hours prior to their assessment.

2.2.3. Statistical analysis

The data were analyzed using a general genetic model as previously described [98, 99]. We calculated Hardy-Weinberg equilibrium to ensure unbiased outcomes [100]. Linkage disequilibrium between genetic loci, haplotype analysis, and allele frequencies estimation were performed via the SHEsis platform [101, 102]. We used chi-square tests to determine differences in *UCP1* SNPs between groups, as well as Phi indices to report effect sizes [103]. Also, we calculated odds ratios (OR) to determine associations of genotypes and alleles between groups in the overall sample as well as based on country (Online Supplement, Section 1.1.3). Finally, we used Kruskal Wallis ANOVA with post hoc Mann-Whitney U tests to assess differences in BMI, WHR, and fat percentage between genotype groups for each *UCP1* SNP. The level of statistical significance for the Hardy-Weinberg equilibrium was set at $p<0.05$ and for all other analyses at $p\leq0.05$. We did not adjust for multiple comparisons in our study due to the errors and misplaced emphasis associated with such procedures when applied in actual natural observations [104-107].Unless stated otherwise, the SPSS 26.0 (SPSS Inc., Chicago, IL, USA) software was used to perform the statistical analyses.

2.2.4. Systematic review and meta-analysis

We conducted a systematic review and meta-analysis (PROSPERO review protocol: CRD42019132376) investigating if differences in the frequency of A-3826G, A-1766G, Ala64Thr and A-112C SNPs are associated with the prevalence of the studied CMP. Following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [108], we searched the titles and abstracts in PubMed central, Embase, and Cochrane Library (trials) databases from the date of their inception to

February 23, 2021, for studies that evaluated the prevalence of *UCP1* A-3826G, A-1766G, Ala64Thr and A-112C SNPs and their association with CMP. No date, participants' health status, language, or study design limits were applied. A detailed description of the systematic review methodology and the searching algorithm is provided in the Online Supplement (Section 2.1).

2.3. Results

2.3.1. Case-control study

2.3.1.1. Associations between genotype frequencies and health status.

The study population included 2283 Caucasian individuals (Table 2.1). Our Hardy-Weinberg equilibrium (HWE) analysis for the A-1766G revealed significant deviation in healthy individuals ($\chi 2 = 33.34$, $p<0.001$), indicating that this SNP should be excluded from further analysis [100], for other *UCP1* SNPs no deviation from HWE in healthy individuals was noticed. The frequencies of alleles and genotypes for the studied *UCP1* SNPs in healthy controls and in CMP individuals are shown in Fig 2.1, Table 2.2 and S4-S11 Tables. Odds ratios for the association between genotype and health status (i.e., healthy vs. CMP individuals) for each of the four studied *UCP1* SNPs are shown in Table 2.2 and S10-S11 Tables.

Table 2.1 Characteristics of the studied population.

	Group	(n) / (%)	Males / Females (n)	Age (years)	BMI (kg/m^2)
Entire sample	Healthy	1139 / 50	762 / 528	45 (32,54)	25.5 (23.9,26.9)
	CMP	1144 / 50	397 / 521	59 (50,65)	30.5 (27.4,34.2)
Armenia	Healthy	105 / 32	-	-	-

	Group	(n) / (%)	Males / Females (n)	Age (years)	BMI (kg/m^2)
	CMP	226 / 68	98 / 128	59 (54,64)	29.0 (27.2,31.7)
	Healthy	233 / 47	131 / 102	55 (50,65)	26.8 (24.2,29.9)
Greece					
	CMP	264 / 53	125 / 139	62 (56,68)	31.7 (28.9,34.5)
	Healthy	365 / 59	221 /144	32 (25,44)	23.8 (22.0,25.6)
Poland					
	CMP	252 / 41	89 /163	62 (54.7,67)	31.2 (29.4,33.8)
	Healthy	255 / 45	142 / 113	46 (36.5,54.5)	25.9 (25.3,26.3)
Russia					
	CMP	310 / 55	129 / 181	52 (40,63)	28.9 (26.0,34.6)
	Healthy	181 / 66	140 / 41	43 (30,51)	25.7 (23.2,29.8)
UK					
	CMP	92 / 34	54 / 38	54 (48,57)	30.7 (25.9,38.4)

CMP; gray bars indi

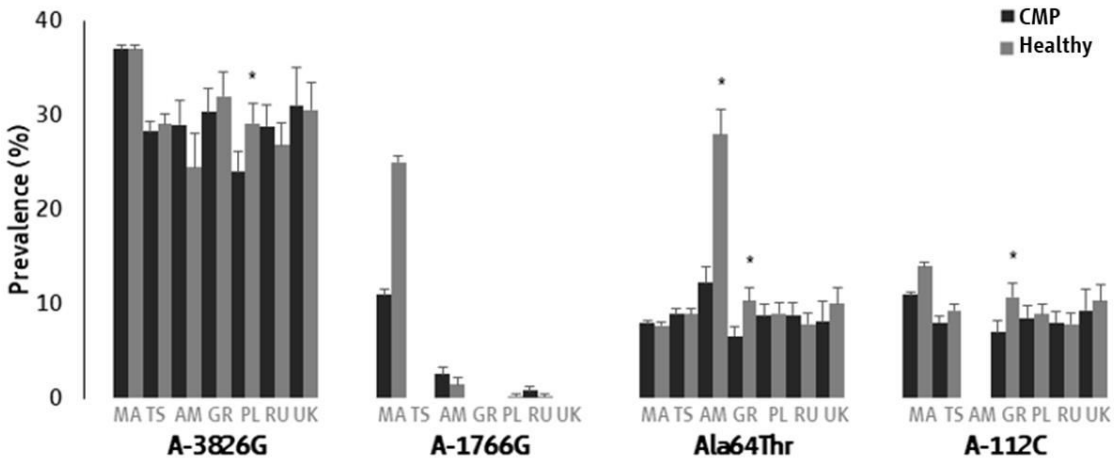


Figure 2.1 Prevalence of the studied UCP1 SNP alleles.

Note: black bars indicate results for individuals with cate results for healthy persons; * indicates differences from CMP persons significant at $p<0.05$. Key: MA= meta-analysis, TS= total sample, AM= Armenia, GR= Greece, PL= Poland, RU= Russia, UK= United Kingdom

Table 2.2 Frequency of genotypes for Ala64Thr in CMP and healthy individuals.

		Healthy		CMP			
						OR (95% CI)	F-test
		(n)	(%)	(n)	(%)		
Total sample	GG	944	83.39	928	82.71		
	GA	175	15.46	188	16.76	1.09 (0.87–1.37)	4.03 p = 0.203
	AA	13	1.15	6	0.53	0.49 (0.19–1.25)	
	HWE	0.134		0.284			
Armenia	GG	90	86.54	164	75.58		
	GA	14	13.46	53	24.42	2.03 (1.08–3.83)	5.70 p = 0.031
	AA	0	0.00	0	0.00	---	
	HWE	0.462		0.040			
Greece	GG	184	80.70	219	87.25		
	GA	41	17.98	31	12.35	0.64 (0.39–1.05)	4.25 p = 0.115
	AA	3	1.32	1	0.40	0.36 (0.05–2.46)	
	HWE	0.679		0.931			

		Healthy		CMP			
						OR (95% CI)	F-test
		(n)	(%)	(n)	(%)		
	GG	304	83.29	211	83.73		
	GA	58	15.89	38	15.08	0.95 (0.61–1.48)	0.39 p = 0.842
Poland							
	AA	3	0.82	3	1.19	1.44 (0.32–6.40)	
	HWE	0.899		0.394			
	GG	218	85.49	257	82.90		
	GA	34	13.33	51	16.45	1.27 (0.79–2.02)	1.52 p = 0.454
Russia							
	AA	3	1.18	2	0.65	0.61 (0.12–3.10)	
	HWE	0.215		0.758			
	GG	148	82.22	77	83.70		
	GA	28	15.56	15	16.30	1.04 (0.53–2.05)	1.65 p = 0.480
UK							
	AA	4	2.22	0	0.00	0.21 (0.01–4.01)	
	HWE	0.069		0.395			

Key: CMP = cardio-metabolic pathologies; OR = odds ratio; HWE = p value for the Hardy-Weinberg equilibrium.

With regard to country-level stratification, allele frequency analysis (S4-S9 Tables) in the Greek population showed that individuals carrying the C allele of the A-112C SNP or the A allele of the Ala64Thr SNP are 37% and 39% less likely to develop CMP, respectively ($p<0.05$; S6 Table). Moreover, the G allele of the A-3826G SNP was associated with 23% lower risk to develop CMP in the Polish population (S7 Table).

In total, we found no associations between genotype and health status in the overall sample for the studied *UCP1* SNPs ($p>0.05$). Though, we observed an association between genotype and health status for Ala64Thr within the Armenian population, where the GA genotype was carried by 24.4% of the CMP individuals but only by 13.5% of healthy individuals. Also, the GA genotype of Ala64Thr showed a 2-fold higher risk ($p=0.03$) for CMP than the GG genotype in the Armenian population (Table 2).

2.3.1.2. Linkage Disequilibrium

Our analysis for all four SNPs in this study in CMP individuals and healthy controls showed that the A-3826G and Ala64Thr were in strong linkage disequilibrium with a D' value of 0.831. Similar results were observed for the combinations of A-3826G and A-112C, as well as for the Ala64Thr and A-112C which were in strong linkage disequilibrium with D' values of 0.917 and 0.924, respectively. However, the r^2 values for the combinations of A-3826G and Ala64Thr ($r^2=0.165$) as well as A-3826G and A-112C ($r^2=0.195$) were relatively low, indicating that their effects are independent of each other. In contrast, the r^2 value for Ala64Thr and A-112C was high ($r^2=0.848$), indicating a direct link between these two SNPs. Country-specific analysis of linkage disequilibrium between investigated SNPs can be found in S1-2 Figs.

2.3.1.3. Haplotype analysis

In the overall sample, the haplotype analysis revealed that CMP individuals were 24% less likely to carry the GAC (A-3826G, Ala64Thr, A-112C) haplotype compared to healthy controls (OR: 0.76 CI95%: 0.60-0.96 $p=0.023$; S1 Table). Country-specific analysis showed lower CMP risk for this haplotype across

countries but this association reached statistical significance only in the Greek population (OR=0.56, CI95%: 0.34-0.91, p=0.017). Additionally, in the Polish population, we found a higher frequency of the AGA haplotype in CMP individuals compared to healthy persons (74.9% vs 70.6%), which indicates the relationship between this haplotype and higher risk of CMP (OR=1.33, CI95%: 1.03-1.73, p=0.032). On the contrary, for GGA haplotype we found a lower frequency in CMP Polish population compared to healthy individuals (15.6% vs 20.3%) indicating a protective effect in healthy individuals (OR=0.74, CI95%: 0.55-0.99, p=0.047). In the Armenian population, the AA haplotype (A-3826G, Ala64Thr) increased the CMP risk more than 4-fold (OR=4.10, CI95%: 1.12-14.98, p=0.02), while the AG haplotype decreased the susceptibility to CMP (OR=0.65, CI95%=0.45-0.95, p=0.025). The AA haplotype differs from the AG in the second position defined by the mutant allele of Ala64Thr confirming the association of A allele of this SNP with CMP risk. Detailed results for haplotype analysis for each country are provided in S1-2 Tables.

2.3.1.4. Association between UCP1 SNPs with specific CMP risk factors

In healthy individuals, we observed significantly higher BMI in the homozygotes GG of A-3826G as compared to AA and AG individuals (p=0.03) as well as in carriers of the mutant allele of A-112C (p=0.015), and Ala64Thr (p=0.004) compared to the wild type homozygotes (Table 3). We also showed that CMP individuals being heterozygotes of A-112C and Ala64Thr had lower WHR than wild type homozygotes (Table 3). Country-specific analysis showed that in the healthy Greek population, heterozygous individuals of A-112C and Ala64Thr displayed higher BMI and fat mass compared to the wild type homozygotes (BMI p=0.005, body fat p=0.008 and BMI p=0.002, body fat p=0.005, respectively; S14 Table). In the Polish healthy population, mutant homozygotes of the A-112C SNP presented higher BMI compared to heterozygotes and wild type homozygotes (Table S12; p<0.05). Due to linkage disequilibrium between A-112C and Ala64Thr, the same effect was observed for mutant

homozygotes of Ala64Thr. Finally, in Polish healthy individuals, higher WHR was observed in GA heterozygotes (p=0.03) in comparison to wild type homozygous subjects (Table S12).

Table 2.3 Body mass index and waist-to-hip ratio [median (Q1, Q3)] across the different UCP1 SNPs for the entire sample as well as across healthy controls and individuals with CMP.

		Body mass index		**Waist-to-hip ratio**	
SNP	**Genotype**	**Healthy**	**CMP**	**Healthy**	**CMP**
	AA	25.6 (23.5,26.6)	30.3 (27.4,34.1)[1]	0.87 (0.81,0.93)	0.97 (0.92,1.04)[1]
A-3826G	AG	25.4 (23.6,27.0)	30.7 (27.5,34.2)[1]	0.88 (0.81,0.93)	1.00 (0.92,1.04)[1]
	GG	26.2 (24.1,28.7)[2,3]	30.8 (27.2,33.8)[1]	0.88 (0.80,0.92)	1.00 (0.92,1.05)[1]
	AA	25.4 (23.5,26.7)	30.6 (27.5,34.2)[1]	0.87 (0.81,0.93)	0.98 (0.93,1.04)[1]
A-112C	AC	25.9 (23.7,28.3)[2]	31.2 (27.3,34.2)[1]	0.88 (0.82,0.94)	0.96 (0.87,1.02)[1,2]
	CC	26.3 (25.5,27.2)	27.9 (27.3,32.5)[1]	0.87 (0.85,0.89)	0.94 (0.84,1.00)
	GG	25.4 (23.4,26.7)[2,3]	30.5 (27.4,34.10[1]	0.87 (0.81,0.93)	0.98 (0.93,1.04)[1,3]
Ala64Thr	GA	26.0 (23.8,28.3)	30.5 (27.3,33.7)[1]	0.88 (0.82,0.93)	0.97 (0.87,1.03)[1]
	AA	26.3 (26.1,27.4)	29.8 (27.2,32.7)	0.90 (0.84,0.98)	0.92 (0.80,1.02)

Note

1 = difference from healthy significant at p≤0.05

2 = difference from AA significant at p≤0.05

3 = difference from AG significant at p≤0.05. Key: CMP = cardio-metabolic pathologies

2.3.2. Systematic review and meta-analysis

2.3.2.1. Searching procedure

The searching procedure retrieved 817 publications of which 109 were duplicates. We excluded 219 publications being reviews, editorials, and conference proceeding as well as 161 publications which referred to animal studies. From the 328 remaining publications, 276 were excluded as they did not meet the inclusion criteria. In total, 52 eligible publications were included in the analysis. Detailed searching procedure results can be found in a PRISMA flowchart (S3 Fig).

2.3.2.2. Characteristics of included studies and risk of bias assessment

The 52 eligible publications included in the analysis were published between 1998 and 2020 and included data from 24 different countries. The extracted data for all 52 included publications can be found in S17 Table. The risk of bias assessment demonstrated low risk for the vast majority of the eligible studies (Online Supplement Section 2.2).

2.3.2.3. Meta-analysis outcomes

Fifty-one out of the 52 eligible publications [25, 26, 43, 65, 66, 70, 71, 74-76, 80, 81, 85-90, 109-141] were used for prevalence meta-analyses, while 22 eligible publications were used for odds ratios meta-analyses. The results from the meta-analyses are summarized in Fig 2.1 and Table 2.4, while the SNP-specific forest and funnel plots for the prevalence (S5-24 and S35-44 Figs) and the odds ratios (S25-34 and S45-S49 Figs) can be found in the Online Supplement (Sections 2.2.1 and 2.2.2). On the whole, for

the different genotypes and alleles we performed 24 prevalence meta-analyses and 12 odds ratios meta-analyses which included a total of 34,313 cases. No statistically significant differences were observed in the prevalence of the mutant alleles of the four different SNPs (p>0.05; Fig 2.1). Also, when we considered only case-control studies, we found no statistically significant odds ratios in different alleles across the four studied SNPs (p>0.05).

Table 2.4 Meta-analysis results for the prevalence and odds ratios of genotypes of the four different SNPs, between healthy and CMP individuals.

			Prevalence meta-analyses		OR meta-analyses	
SNP	**n**	**Genotypes**	**Healthy (%)**	**CMP (%)**	**OR (95%CI)**	**p**
A-3826G	18568	AA	43	42		
		AG	43	43	1.02 (0.96–1.09)	0.46
		GG	14	15	1.06 (0.96–1.17)	0.23
A-112C	6153	AA	77	78		
		AC	21	21	1.07 (0.80–1.44)	0.65
		CC	2	1	0.92 (0.65–1.32)	0.67
Ala64Thr	4984	GG	85	82		
		GA	14	17	1.07 (0.91–1.27)	0.41

SNP	n	Genotypes	Prevalence meta-analyses		OR meta-analyses	
			Healthy (%)	CMP (%)	OR (95%CI)	p
		AA	1	1	0.64 (0.24–1.67)	0.36
		AA	64	66		
A-1766G	4608	AG	30	29	1.12 (0.81–1.55)	0.51
		GG	6	5	1.04 (0.53–2.04)	0.90

n = number of studied individuals; OR = odds ratio with reference to AA; 95%CI = 95% confidence intervals; p = p value for the Z test indicating the overall effect in the meta-analysis.

2.4. Discussion

Our findings confirm an association between the studied *UCP1* SNPs and cardiometabolic health in a multi-country sample of 2,283 persons. Furthermore, we found that differences in the distribution of genotypes and alleles of the studied SNPs between CMP individuals and healthy controls are associated with the prevalence of one or more of the most common CMP and their risk factors, in some (Armenia, Greece, and Poland) but not all (Russia and United Kingdom) countries.

Within our study population, the A-3826G (AG) was the most prevalent of the four SNPs. In persons with CMP, the prevalence was 40%, ranging from 34% in the UK to 42% in Armenia and Russia. This is very similar to the 43% found in our meta-analysis, and mid-way between the 29% reported in Spain [74] and the ~50% reported in Colombia [66], Japan [81], and Korea [46]. Our findings in the case-control study

indicate that the A-3826G is not associated with CMP, but that it leads to increased BMI within the healthy population. Thus, it may promote the development of CMP in the presence of environmental factors [142] as well as other genetic traits [143].

Our results for Ala64Thr and A-112C indicate a strong linkage disequilibrium between the two SNPs. In our study the mutant A allele of Ala64Thr was detected in 9% of both healthy individuals and persons with CMP, and this frequency was not very different across the five studied countries. This was similar to the 7% for healthy and 9% for CMP individuals found in our meta-analysis that included data from 4984 persons across nine countries. Our observed prevalence rates for the C allele of A-112C were 9% in healthy persons and 8% in individuals with CMP. This was somewhat lower than the 12% prevalence found in our meta-analysis that included data from 6,153 persons across eight countries. In terms of health impacts, we showed that the Ala64Thr and A-112C are associated with opposing effects in healthy individuals and persons with CMP. Our results indicate that the A-112C mutant allele demonstrates its effect when present in its heterozygous form and this may be the reason for C allele's association with decreased risk for CMP development. Specifically, we found that healthy individuals carrying the mutant alleles display higher BMI and, in some countries, body fat percent. On the other hand, persons with CMP who carry the mutant variants have lower WHR. These results partly reflect those reported in previous studies [82, 84]. For instance, the presence of mutant alleles Ala64Thr and A-1766G, in combination with A-3826G, can augment the beneficial effects of caloric restriction resulting in greater reductions in WHR [82]. Unfortunately, we were not able to assess potential associations of these SNPs with biochemical indices or with additional clinical features.

It is important to consider the functional impact of A-3826G, A-1766G and Ala64Thr, which is clear since they directly affect the expression of *UCP1*. In the case of A-112C, it is important to also consider the effect of another variant, rs72941746, that is in linkage disequilibrium [144]. The A-112C seems to modify 4 transcription factor binding sites and its region has specific patterns of chromatin accessibility

in several tissues. It appears that the linked variant is responsible for much more alterations in transcription factor binding site motifs and consequently the binding of other proteins. This indicates that the association observed in this study when A-112C is present could possibly be an effect of rs72941746 influence.

Our findings indicate potential limitations of common analysis of different races, ethnicities, and regions when analyzing our data as an entire sample or via meta-analytic methods. For instance, the frequency of A allele of Ala64Thr across all our studied countries was 9%, similar to the 8% found in our meta-analysis, in both cases suggesting no differences between healthy persons and individuals with CMP. However, our country-specific analysis demonstrated that the prevalence of A allele of Ala64Thr was significantly higher in healthy individuals across the Armenian (27.9%) and the Greek (10.3%) populations, as compared to CMP persons. Considering risk factors, we detected a number of associations with the four studied SNPs across Greece, Armenia and Poland, which were not observed in the other countries. Taken together, these findings suggest that the studied SNPs may be important for promoting risk factors and pathophysiological mechanisms involved in CMP, but that this involvement may be stronger in some races, ethnicities, and/or regions. Nevertheless, it is important to also note that the increased CMP prevalence in certain ethnic groups in Eastern Europe and Western Asia [91, 92] may reflect potential ancestral differential effects. While we made every effort to achieve representativeness and increase our sample sizes, we acknowledge that labeling of ancestral populations by self-reported ethnicity does not fully account for genetic variations.

Our results may reflect that ethnicity was self-determined by the participants and potential relationships between them were not investigated. This approach may not always reflect the inter/intra ethnic variation in the frequency distribution of germline variants of the population examined. Also, we were unable to explore additional factors associated with CMPs, including demographic characteristics (socioeconomic status, etc.) and environmental factors (climate conditions, nutritional habits, etc.).

We conclude that, in some populations, the A-3826G, A-1766G, Ala64Thr and A-112C SNPs of *UCP1* gene may be associated with the prevalence of one or more of the most common CMP and their risk factors. Future studies on these SNPs may shed more light on the genetics of CMP and may uncover potential candidates for precision medicine.

Acknowledgments: The authors are grateful to Monika Jasek, Marta Wagner, and Eleftheria Barmpa for their support during the data collection and analysis. We also thank the Center for Precision Genome Editing and Genetic Technologies for Biomedicine, IGB RAS for the provided equipment.

Funding: • European Union 7th Framework Program (FP7-PEOPLE-2013-IRSES Grant No. 319010; U-GENE project • Russian Science Foundation grant 20-14-00201 (case-control study in the Russian population; meta-analysis). • Polish Ministry of Science and Higher Education 2016-2017 international project co-financed W15/7.PR/2016. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Chapter 3

Effects of In Vitro Muscle Contraction on Thermogenic Protein Levels in Co-Cultured Adipocytes

This work was conducted by Eleni Nintou, Eleni Karligiotou, Maria Vliora, Ioannis G. Fatouros, Athanasios Z. Jamurtas, Nikos Sakellaridis, Konstantinos Dimas and Andreas D. Flouris. We all contributed to the final revision of this paper. Resources were provided by Ioannis G. Fatouros, Athanasios Z. Jamurtas, Nikos Sakellaridis, Konstantinos Dimas and Andreas D. Flouris. All authors revised the final draft. I had the leading role in the investigation, data collection, data analysis, statistical analysis and the writing of the original draft. Andreas D.Flouris and I acquired the funding, obtained the original idea and designed the study. Moreover, I took the responsibility for the integrity of the data and the data curation.

As of 12th November of 2021, this paper[2] has been published online by Life (Special Issue: Human Thermophysiology) as follows: Nintou E, Karligiotou E, Vliora M, Fatouros IG, Jamurtas AZ, Sakellaridis N, et al. Effects of In Vitro Muscle Contraction on Thermogenic Protein Levels in Co-Cultured Adipocytes. Life. 2021;11(11):1227.

Abstract

The crosstalk between the exercising muscle and the adipose tissue, mediated by myokines and metabolites, derived from both tissues during exercise has created a controversy between animal and human studies with respect to the impact of exercise on the browning process. The aim of this study was to investigate whether co-culturing of C2C12 myotubes and 3T3-L1 adipocytes under the stimuli of electrical pulse stimulation (EPS) mimicking muscle contraction can impact the expression of UCP1, PGC-1a, and IL-6 in adipocytes, therefore providing evidence on the direct crosstalk between adipocytes and stimulated muscle cells. In the co-cultured C2C12 cells, EPS increased the expression of PGC-1a (p = 0.129; d = 0.73) and IL-6 (p = 0.09; d = 1.13) protein levels. When EPS was applied, we found that co-culturing led to increases in UCP1 (p = 0.044; d = 1.29) and IL-6 (p = 0.097; d = 1.13) protein expression in the 3T3-L1 adipocytes. The expression of PGC-1a increased by EPS but was not significantly elevated after co-culturing (p = 0.448; d = 0.08). In vitro co-culturing of C2C12 myotubes and 3T3-L1 adipocytes under the stimuli of EPS leads to increased expression of thermogenic proteins. These findings indicate changes in the expression pattern of proteins related to browning of adipose tissue, supporting the use of this in vitro model to study the crosstalk between adipocytes and contracting muscle.

3.1. Introduction

The brite or beige adipocytes, discovered during the last decade, play a central role in the energy expenditure and the associated heat release during non-shivering thermogenesis [145, 146]. Cumulating evidence is indicating the beneficial effects of beige adipocyte proliferation to the increase of insulin sensitivity as well as the reduction of circulating triglycerides and body mass index (BMI), making these cells a candidate therapeutic target in battling illnesses related to metabolism such as obesity and cardiometabolic syndrome [146, 147].

White lipid cells trans-differentiate to beige adipocytes (a process known as “browning”) in response to certain types of stimuli including exposure to cold, presence of thyroid hormones and exercise, while the list of factors that can activate this mechanism is actively growing [148]. The expression of beige adipocytes specific genes and protein is commonly used to evaluate the browning capacity of adipocytes, including beige adipocytes marker protein Uncoupling Protein 1 (UCP-1), thermogenic genes Peroxisome proliferator-activated receptor γ (PPARγ), PPARγ coactivator-1 alpha (PGC-1α), PR domain containing protein 16 (PRDM16) and specific marker molecular of beige pre-adipocytes early B-cell factor 2 (EBf2) [149, 150].

Recent studies showed a crosstalk between the exercising muscle and the adipose tissue, which apparently is mediated by myokines and metabolites, derived from the muscle during exercise [39, 151, 152]. In mice, exercise has been linked to increased mitochondrial activity, changes in gene expression, and an increase in beige adipocyte gene expression levels [153]. These studies consistently showed that exercise leads to browning of adipose tissue in mice [154, 155] and that the degree of beige cell proliferation is linked with the intensity and duration of exercise [156]. However, the respective human studies show highly controversial results regarding the effectiveness of exercise/physical activity on the formation of beige adipose tissue [19, 51, 58]. A recent clinical trial did not detect significant browning of adipose tissue after 12 weeks of exercise [157], which is in line with several studies that have failed to

identify a correlation between physical activity and browning [158-160]. In contrast, other studies using similar exercise protocols reported that exercise upregulated the browning process, but without any positive effects on the metabolism of the volunteers [51]. This stark controversy between animal and human studies with respect to the impact of exercise on the browning process has been demonstrated in a recent series of meta-analyses [47] and has been attributed to (i) heterogeneity of white adipose tissue depots [19], (ii) the variability in sample collection in animals and, mainly, humans [19, 161], and (iii) a host of external factors (e.g., nutrition, environmental temperature, other stressors) that may interfere with the effect of exercise on the browning process [38, 47]. Therefore, there is a need for in vitro experiments aiming to identify the factors and the procedures which can induce browning of white lipocytes under the effect of exercise.

Numerous studies employ the culturing of either human or mouse myotubes to identify myokines and metabolites and the use electric pulse stimulation (EPS) to mimic exercise (i.e., contraction of the myotubes) [162, 163]. The electric pulse evokes contraction of the myotubes, mimicking the function of a nerve signal reaching the nerve-muscle synapse. Electrical stimulation of muscle cells in culture increases contractile properties and accelerates sarcomere assembly [164, 165]. Moreover, EPS upregulates classical markers of exercise including interleukin 6 (IL-6) [166], PGC-1a [167], as well as glucose uptake [168]. While innervation and interaction with other organs is missing from in vitro exercise models [169] different protocols have managed to induce adaptations similar to resistance [169] and aerobic exercise [170]. Moreover, the in vitro contraction EPS model has proven to be a valuable tool for the identification of new myokines [171, 172] as well as in the study of metabolism [173, 174]. However, EPS has not been employed to date to investigate mechanisms related to tissue crosstalk in the browning process through co-culturing of different cell lines.

The aim of this study was to investigate whether co-culturing of C2C12 myotubes and 3T3-L1 adipocytes under the stimuli of EPS mimicking muscle contraction can impact the expression of UCP1,

PGC-1a, and IL-6 in adipocytes, therefore providing evidence on the direct crosstalk between adipocytes and stimulated muscle cells. Based on previous findings for the use of EPS for the study of metabolic pathways [174-176], we hypothesized that our in vitro model of co-cultured white adipocytes and electrically stimulated myocytes to simulate exercise would increase the expression of UCP1, PGC-1a, and IL-6 in 3T3-L1 adipocytes. To our knowledge, this is the first time that the two cell types were allowed to interact in vitro under the stimuli of EPS mimicking muscle contraction, to unravel possible browning effects of contracting myotubes on adipose cells. Should our hypothesis be confirmed, this model can be used to provide precise and comprehensive mechanistic data that in vivo studies may not be able to tease apart.

3.2. Materials and Methods

3.2.1. Cell Lines and Cell Cultures

The established and well-characterized murine cell lines C2C12 and 3T3-L1 (American Type Culture Collection (ATCC, Manassas,USA) were used as muscle cells and white fat lipocytes, respectively. All cells were cultured in Dulbecco's modified Eagle's medium (DMEM, with L-glutamine, Gibco/BRL, UK), 10% (v/v) fetal bovine serum (Gibco/BRL, UK) and penicillin/streptomycin (100 IU/mL; Biosera) at 37 °C, in an atmosphere of 5% CO_2 in air with 100% humidity.

3.2.2. C2C12 and 3T3-L1 Differentiation Protocols

Differentiation of C2C12 muscle cells to myotubes was achieved when at about 70–80% confluence were cultured in starvation medium of Dulbecco's modified Eagle's medium (DMEM, with L-glutamine) (Gibco/BRL, UK), 2% (v/v) fetal bovine serum (Gibco/BRL, UK) and penicillin/streptomycin (100 IU/mL; Gibco/BRL, UK) at 37 °C in an atmosphere of 5% CO2 in air with extra humidity [177]. Myotubes were formed after 5–7 days. Transformation was observed under light microscope (Figure 3.1a,b). All experiments were performed for passages 7–8.

Differentiation of 3T3-L1 fibroblasts was achieved by adjusting the established protocol previously described [178]. Briefly, 100% confluent cells were cultured in DMEM (Gibco/BRL, UK), 10% (v/v) fetal bovine serum (Gibco/BRL, UK) and penicillin/streptomycin (100 IU/mL; Biosera, UK) supplemented with 10 μg/mL insulin (Sigma, UK), 1 μM dexamethasone (Sigma, UK) and 0.5 mM 3-isobutyl-methyl-xanthine (IBMX) (Sigma, UK) medium and changed daily for 3 consecutive days, followed by a sustainability medium containing 10% FBS-DMEM containing 10 μg/mL insulin. After the course of three to five days differentiated adipocytes (accumulated lipid droplets in the cytoplasm) could be observed under the phase contrast microscope inverted Axionvert 40C) equipped with a ccd camera (Zeiss, AxionVision software, Germany) until day 12 to 15 of differentiation (Figure 3.1c,d). All experiments were performed for passages 8–10.

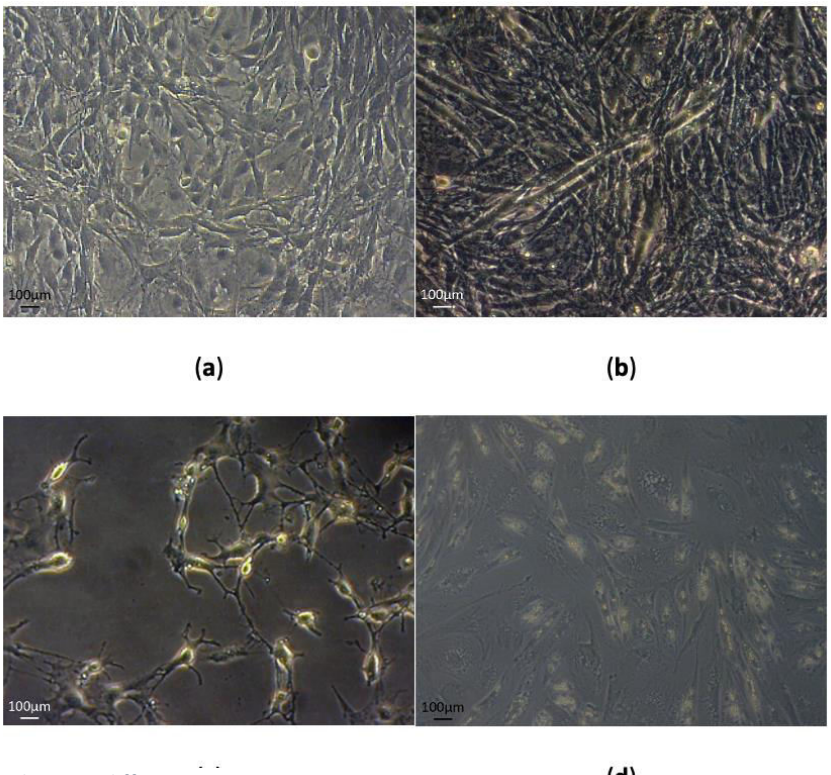


Figure 3.1 C2C12 and 3T3-L1 differentiation process.

The images show undifferentiated (**a**) and differentiated (**b**) C2C12 cells, as well as undifferentiated (**c**) and differentiated (**d**) 3T3-L1 cells. All images were taken at 20× magnification.

3.2.3. Co–Culture Protocol

Muscle myotubes were plated in 6-well plates (SPL Life Sciences), and cells were let grow and differentiate for 6 days, while the 3T3-L1 cells were plated to grow and differentiate for 10 days, in transparent culture inserts, 0.4 μm pore size (cellQart). The inserts were hanged in the wells for the entire duration of the EPS experimental protocol [179].

3.2.4. EPS Protocol

The protocol was performed using a custom-made stimulator device. The EPS protocol used was an adaptation of previous established protocols [165, 180, 181]. Briefly, the fully differentiated C2C12 myotubes were stimulated to contract via carbon electrodes connected to the stimulator. The protocol consisted of 1 h stimulation at 50 mV/1 Hz. Three experimental conditions were established: (a) the co-culture of both cell types with EPS application to the myotubes; (b) the co-culture of both cell types without EPS application; (c) the single culture of the 3T3-L1 cells in the inserts with the EPS application on the well below, filled with medium. All cell types were harvested after 1 h rest from the EPS application (Figure 3.2). Independent experiments were performed at least in duplicates.

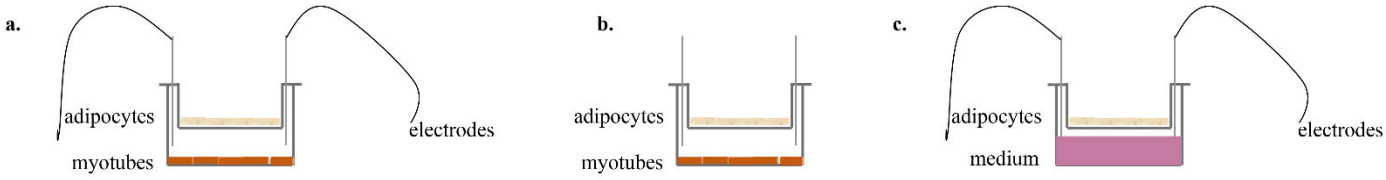


Figure 3.2 Experimental set up.
Schematic representation of the three experimental conditions used: (a) co-culture of both cell types and EPS applied to the myotubes; (b) 3T3-L1 adipocytes were co-cultured with the C2C12 myotubes in absence of EPS; (c) EPS was applied to the well filled with medium, with only the presence of 3T3-L1 cells inserts.

3.2.5. Lactate Dehydrogenase (LDH) Assay

For myotubes, the toxicity of EPS was determined in a colorimetric assay measuring lactate dehydrogenase (LDH) activity in the supernatant of the cell culture at the end of experiment with

Cytotoxicity Detection Kit PLUS (LDH) (Roche Applied Science, Mannheim, Germany) [182] measured in a multimode plate reader (Perkin Elmer-EnSpire).

3.2.6. Western Blot Analysis

Western blot analysis was performed as described previously [183]. In short, cells were treated using lysing buffer (Biorad, Oxfrord, UK), and the lysates were boiled in loading buffer for 10 min. Equal amounts of protein were separated by 8–12% sodium dodecyl sulphate–polyacrylamide gel and transferred onto nitrocellulose membranes (Biorad, Oxford ,UK). The blots were blocked using 5% non-fat dry milk in TBS plus 0.05% Tween 20 and incubated with the primary antibody (Table S1) overnight at 4 °C, followed by 1 h incubation at room temperature with the secondary antibody (Table S1) conjugated to horseradish peroxidase. Detection was carried out using the chemiluminescence (ECL) reaction (Bio-Rad) in Uvitec Alliance imaging system (Uvitec, Cambridge, UK)). All immunoblots were performed in duplicates.

3.2.7. Statistical Analysis

Independent samples t tests and Cohen's d effect size estimates were used to compare relative protein expression levels between co-cultured and non-co-cultured 3T3-L1 adipocytes. The same analyses were used to compare relative protein expression levels between stimulated and non-stimulated C2C12 myotubes. Given the limitations of using statistical tests based on p values for large and, as in this case, small sample sizes [184], the Cohen's d effect size estimates were used to complement the p value comparisons, as a way to provide an additional estimate of the effect that is not based on the number of replicates. We interpreted effect sizes as small (0.2–0.5), moderate (0.5–0.8), and large (>0.80) according to Cohen's recommendations [185]. The level of significance for the t tests was set at $p < 0.05$. All statistical analyses were performed using the statistical software package

SPSS 27 for Windows (SPSS Inc., Chicago, IL, USA). The results are reported as means ± standard deviation, except otherwise indicated.

3.3. Results

3.3.1. Effects of EPS Protocol on C2C12 Myotubes

After 7 days of differentiation, the majority of the C2C12 myoblasts fused together and formed multinucleated myotubes (Figure 1). We applied EPS to differentiated C2C12 myotubes for one hour and observed by optical microscope the contraction. Differentiation and contraction of the myotubes was also confirmed by monitoring the desmin protein expression. During the treatment with EPS, no morphological changes were detected, and cell viability measured via LDH activity was not affected by the contraction protocol (Figure S1).

3.3.2. Effects of EPS Protocol on 3T3-L1 / C2C12 Co-Cultured Cells

Co-culturing of the 3T3-L1 cells with the C2C12 myotubes lasted for the entire contraction protocol and was followed by a resting period of one additional hour. After the resting period, PGC-1a protein levels in C2C12 myotubes showed a 1.1-fold increase and differentiated co-cultured without EPS cells had a 0.8-fold increase compared to differentiated untreated cells (Figure 3.3). The mean difference of PGC-1a protein levels between the two experimental conditions (with and without EPS) was 0.3 ± 0.2. The observed differences did not reach statistical significance (p = 0.129), yet a medium effect size was detected (d = 0.73). Similarly, IL-6 showed the same pattern of expression, presenting 0.86 times higher levels in EPS co-cultured myotubes when compared to differentiated untreated cells and 0.4-fold increase in the case of differentiated co-cultured without EPS cells. This effect of EPS did not reach statistical significance (p = 0.09) but showed a large effect size (d = 1.13).

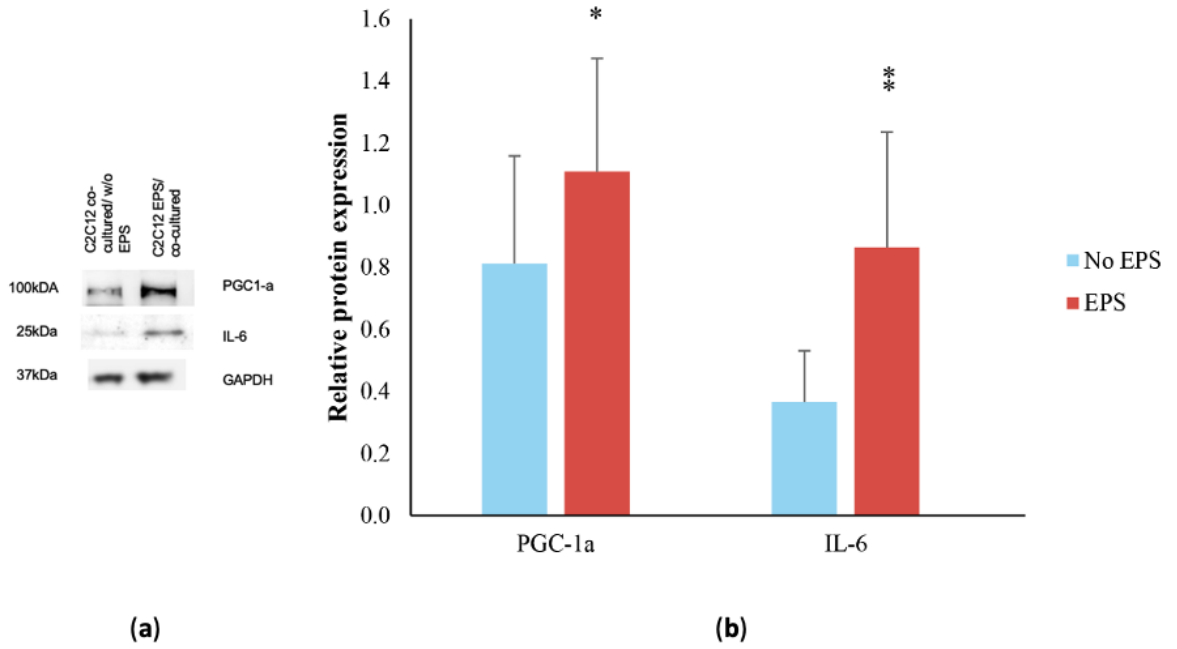


Figure 3.3 Effect of EPS and co-culture on C2C12 myotubes.
(**a**) Representative blots of PGC-1a, IL-6 and GAPDH. GAPDH was used as a loading control (**b**) PGC-1a and IL-6 relative protein expression in C2C12 myotubes co-cultured with 3T3-L1 with and without EPS in relation to C2C12 differentiated and untreated cells. The band intensity was measured by densitometry and was normalized to GAPDH. All immunoblots were performed in duplicates. Graphs represent mean ± SD; asterisks indicate (*) and large (⁑) effect sizes.

The impact of the EPS contracting myotubes on the differentiated 3T3-L1 cells, when in co-culture, was examined through UCP-1, PGC-1a and IL-6 protein expression in relation to 3T3-L1 adipocytes co-cultured with C2C12 without EPS (Figure 3.4). UCP1 protein levels were significantly higher (p = 0.044) in the co-cultured adipocytes with contracted myotubes in comparison to the 3T3-L1 cells when EPS was applied without the presence of the myotubes. Moreover, this difference showed a large effect size (d = 1.29). PGC-1a was similar in EPS co-cultured cells compared to EPS treated cells (without co-culture) (p = 0.448; d = 0.08). Finally, IL-6 expression was higher in EPS co-cultured cells (1.3-fold increase) than EPS treated cells without co-culture (0.98-fold increase). The difference did not reach statistical significance (p = 0.097) but revealed a large effect size (d = 1.13).

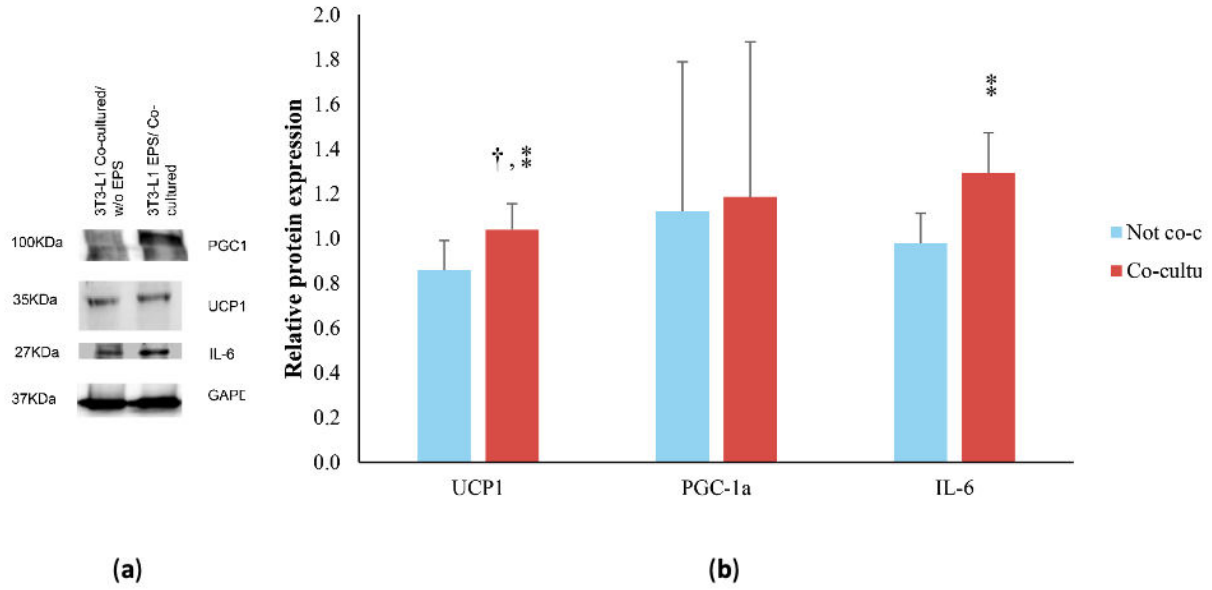


Figure 3.4 Effect of EPS and co-culture on 3T3-L1 adipocytes.

(**a**) Blots of UCP1, PGC-1a, IL-6, and GAPDH. GAPDH was used as a loading control (**b**) UCP1, PGC-1a, and lL-6 relative protein expression in 3T3-L1 adipocytes with EPS co-cultured with and without C2C12 myotubes in relation to 3T3-L1 adipocytes co-cultured with C2C12 without EPS. The band intensity was measured by densitometry and was normalized to GAPDH. All immunoblots were performed in duplicates. Graphs represent mean ± SD; † indicates statistically significant difference ($p < 0.05$); ‡ indicates large effect sizes.

3.4. Discussion

In this study, we let C2C12 myotubes and 3T3-L1 adipocytes interact in vitro under the stimuli of EPS, mimicking muscle contraction. We found that EPS increased the expression of PGC-1a and IL-6 protein levels in the co-cultured C2C12 cells. When EPS was applied, we found that co-culturing led to increases in UCP1 and IL-6 protein expression in the 3T3-L1 adipocytes. These findings suggest the existence of a direct crosstalk between the muscle cells, when contracted, with the adipocytes, resulting in changes in the expression pattern of proteins related to browning of adipose tissue. These findings confirm what has already been shown with in vivo rodent studies, and hence, they confirm that our model can be used in future studies to provide precise and comprehensive mechanistic data that in vivo studies may not be able to tease apart.

During and after exercise, a variety of factors are known to exert and trigger many signaling pathways, while secreted myokines have been described to have an endocrine as well as a paracrine action. Exercise-induced myokines change the profile of both muscle and adipose tissue [186]. This leads to adaptations in white adipose tissue including the reduction of the size of the adipocytes, increased mitochondrial activity, change of the adipokines profile, and changes in gene expression [153, 154].

In vitro studies have investigated myokines, adipokines and metabolites that are considered modulator candidates for the formation of beige cells after exercise [39, 187],[79] but often without including exercise in their experimental design. In the present study, we incorporated a co-culturing of C2C12 and 3T3-L1 cells in vitro with the application of EPS on myotubes. EPS is a well-described exercise proxy that has been documented over the years as a method for inducing contraction in skeletal muscle myotubes, whereas increased levels of PGC-1a expression and other contraction related genes have been recorded [149, 150].

The finding of elevated PGC-1a and IL-6 expression in the C2C12 myotubes after EPS application was expected, as a direct result of the contraction. The increased levels of both PGC-1a and IL-6 after electrical pulse stimulation are indicative of the activation of the metabolic adaptations in myotubes as a response to exercise, since they are known to regulate mitochondrial biogenesis [168, 171, 188, 189]. We found that this process exerted, indirectly, an alteration in the expression of certain proteins derived by the 3T3-L1 adipocytes. Specifically, UCP1 and IL-6 production were increased after EPS induction. Moreover, the expression of PGC-1a increased by EPS but was not significantly elevated after co-culturing. This may be because PGC-1a has a relatively high turnover [190], reaching a peak expression at 15 min post stimulation, whereas our samples were assessed 60 min following EPS. When stimulated by external cues such as exercise and contraction, beige adipocytes express UCP1 protein and exhibit UCP1-dependent thermogenic capacity [161, 191]. Moreover, IL-6 has a dual role: as a major myokine, it activates beige adipocyte development and is required for exercise-induced white adipose tissue

browning in mice [192]; as an adipokine, IL-6 acts as a mediator in non-shivering thermogenesis and is involved in metabolic profile regulation in mice [193, 194]. In line with these findings, we detected a significantly higher expression of UCP1 in 3T3-L1 adipocytes co-cultured with C2C12 myotubes under the effect of muscle contraction, in comparison to the non-co-cultured adipocytes.

A recent study investigated the effect of contracting myotubes on adipocytes where fractionated supernatant was used to culture 3T3-L1 pre-adipocytes, showing that EPS-conditioned medium promoted lipid droplet accumulation in 3T3-L1 pre-adipocytes [195]. Our experimental findings extend this work, demonstrating the existence of a direct crosstalk between the contracting muscle cells and the adipocytes, resulting in increased adipocyte expression of proteins that play key roles in the browning process (UCP-1 and IL-6).

The studied “closed”, controlled, observational in vitro system provides the advantage of having a clearer insight of the interplay between the two cell types, without the intervention of other internal or external factors. As this is the first attempt to depict this crosstalk in a closed in vitro model, through contracting myotubes co-cultured with adipocytes, these findings could ignite further research on the field. Further experimental work should also be performed with different contraction protocols as well as with the use of human myotubes and adipocytes to establish the browning effect of muscle contraction and identify the possible pathway(s) for the suggested crosstalk between cell types. There could be several hypotheses on how this communication occurs and which molecule(s) participate. This has not been elucidated by the present study and requires further investigation. This knowledge will provide a more robust theoretical framework and will, undoubtedly, increase our understanding of metabolic diseases and could be extended to understand potential connections between muscle cells and other cell types, such as cancer cells and osteocytes. Nevertheless, it is important to note that in vitro models of exercise incorporate several limitations, including lack of unanimously accepted exercise protocol characteristics and inability to study systemic effects of exercise. Consequently, results from

such studies may not translate directly to whole body physiology and must be interpreted with caution and combined with in vivo studies.

The present study adopted a previously established EPS protocol [165]. Therefore, our results are limited only to this particular stimulation protocol. Future studies should test a range of muscle stimulation protocols ranging in terms of muscle stimulation characteristics (frequency, intensity, and duration). Moreover, additional browning markers, including Tbx1, Tmem26, and CD137 [196], as well as research methodologies, including gene silencing [197] and gene knock-out [198], should be investigated in future studies to confirm and expand on the present findings. Finally, it is important to note that most of our statistical comparisons based on p values were non-significant. Since the vast majority of our effect size comparisons were moderate or large, the lack of reaching statistical significance in most cases was not caused by increased variability, but instead, it likely reflects the small size of the data pool used in our study. As we aimed to detect biologically meaningful (instead of statistically meaningful) changes in protein expression caused by our EPS protocol, our results and conclusions are based on both p values and effect sizes. In recent years, many experts and scientific societies have called for complementing p value comparisons with other tests, such as Cohen's effect size, to increase robustness and validity in research [184, 199].

Both in vivo and in vitro models are necessary to effectively understand the mechanisms involved in the browning process. This is demonstrated by the controversy observed between in vivo human and animal studies regarding exercise-induced adipose tissue browning, which is likely caused by a variability in the exercise protocols used and different methods of browning detection [47]. Moreover, exercise exerts many whole-body adaptations, which are difficult to study separately through in vivo studies. While this can be addressed by in vitro models, previous in vitro studies did not involve exercise activity in their experimental designs [200]. This is needed to reach to a robust conclusion on the impact of exercise on the browning process. Therefore, there have been recent calls for the development of more

in vitro browning models, particularly involving cell–cell signaling [201]. In the present study, the co-culturing of two different cell types under the stimulus of EPS improved previous in vitro models for studying browning because it considers, for the first time, aspects of in vivo exercise physiology. In this light, the present novel model involving contracting myocytes and adipocytes may play an important role towards the understanding of the browning process. This is particularly true since the proposed model can describe the effects of exercise on other cell types, in this case white adipocytes, which is a vital aspect of in vivo exercise physiology. However, it is important to acknowledge that in vitro models of exercise and browning, such as the present, do not account for inter-organ communication. Moreover, in vitro exercise protocols cannot be easily translated into in vivo situations [202]. Therefore, the simultaneous development of both in vivo and in vitro models that can complement each other should be employed in future studies to generate robust mechanistic data.

3.5. Conclusions

In vitro co-culturing of C2C12 myotubes and 3T3-L1 adipocytes under the stimuli of EPS leads to increased expression of thermogenic proteins. These findings support the use of the present in vitro model to study the direct crosstalk between adipocytes and contracting muscle cells which results in changes in the expression pattern of proteins related to browning of adipose tissue.

Funding: This research was financed by Greece and the European Union (European Social Fund-ESF) through the Operational Program «Human Resources Development, Education and Lifelong Learning 2014–2020» in the context of the project “Effect of the in vitro exercise on browning of white adipose tissue” (grant number MIS: 5048945). This research received no external funding.

Acknowledgments: We are thankful to Applied and Computational Electromagnetics Laboratory, Aristotle University for preparing the electrical pulse stimulator and Alexandros Kalaitzakis for supplying

the C2C12 cell line. We wish to thank Prof. Eirini Dermitzaki and Elina Paflioti for supplying the 3T3-L1 cell line. Moreover, we would like to thank Mrs. Fani Koutsougianni for the technical support during experimental procedure.

Chapter 4

Characteristics of the Protocols Used in Electrical Pulse Stimulation of Cultured Cells for Mimicking In Vivo Exercise: A Systematic Review, Meta-Analysis, and Meta-Regression

This work was conducted by Eleni Nintou, Eleni Karligiotou, Maria Vliora, Leonidas G. Ioannou and Andreas D. Flouris. All authors revised the final draft. The conception of the idea was mine. I contributed to the design of the study, performed the systematic review, drafted the manuscript and took the responsibility for the integrity of the data and the data curation. Each author's contribution can be found in detail at the end of the chapter.

As of November of 2022 this paper[3] has been published online to the International Journal of Molecular Sciences (Special Issue Adipokines, Myokines and Physical Exercise in Health and Disease 2.0) as follows: Nintou E, Karligiotou E, Vliora M, Ioannou LG, Flouris AD. Characteristics of the Protocols Used in Electrical Pulse Stimulation of Cultured Cells for Mimicking In Vivo Exercise: A Systematic Review, Meta-Analysis, and Meta-Regression. International Journal of Molecular Sciences. 2022;23(21):13446.

Abstract

While exercise benefits a wide spectrum of diseases and affects most tissues and organs, many aspects of its underlying mechanistic effects remain unsolved. In vitro exercise, mimicking neuronal signals leading to muscle contraction in vitro, can be a valuable tool to address this issue. Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for this systematic review and meta-analysis, we searched EMBASE and PubMed (from database inception to 4 February 2022) for relevant studies assessing in vitro exercise using electrical pulse stimulation to mimic exercise. Meta-analyses of mean differences and meta-regression analyses were conducted. Of 985 reports identified, 41 were eligible for analysis. We observed variability among existing protocols of in vitro exercise and heterogeneity among protocols of the same type of exercise. Our analyses showed that AMPK, Akt, IL-6, and PGC1a levels and glucose uptake increased in stimulated compared to non-stimulated cells, following the patterns of in vivo exercise, and that these effects correlated with the duration of stimulation. We conclude that in vitro exercise follows motifs of exercise in humans, allowing biological parameters, such as the aforementioned, to be valuable tools in defining the types of in vitro exercise. It might be useful in transferring obtained knowledge to human research.

4. 1. Introduction

Voluminous evidence has strongly linked exercise and physical activity levels with improved health, well-being, and quality of life and has shown that they play important roles in the battle against a wide spectrum of multifactorial diseases, such as cancer [203], diabetes [204], osteoporosis [205], cardiometabolic syndrome, and obesity [206],[207], in addition to many others. As a result, much research has focused on identifying the molecular and biochemical pathways through which exercise benefits muscle as well as other tissues and organs, such as the adipose tissue, heart [208], brain [209], etc. Although many studies have been conducted to unravel the underlying mechanistic effects of exercise and physical activity, there are still many aspects that remain poorly understood [210]. This limits our understanding of important biological and physiological pathways and inhibits the creation of exercise and physical activity regimes that will have a maximized impact on health, wellbeing, and performance. A more-controlled, “closed” system can contribute to addressing these issues, allowing the study of exercise-induced responses in deeper detail [2]. In this light, it has been suggested that electrical pulse stimulation (EPS) can provide the means to mimic muscle contraction both in vitro and ex vivo [211].

Motor neuron activity comprises both mechanical and electrical signals regulating growth and differentiation processes by affecting both cellular-microenvironment modulation and gene-expression pattern [212]. Such signals can be mimicked by EPS of myotubes in cell culture, which leads to increased contraction and accelerates sarcomere assembly [213], while, at the same time, generating changes in the genetic and metabolic profiles [162]. Hence, EPS represents a valuable tool in exercise research, although the limitation of the probability of non-cell-mediated effects should be taken into consideration [214]. Nevertheless, the substitution of the motor neuron activity with the electrical pulse has been shown to cause changes on myokines and muscle proteins in the cultured skeletal muscles [211] and has been used for tissue engineering [213]. However, the frequency (Hz), pulse duration (ms),

applied pulse amplitudes (Vapp), and stimulation duration time of cultured cells in order to achieve exercise-mediating responses are yet to be validated in a systematic way [59].

Published studies have used electrical pulse stimulation to induce acute [167, 169, 215, 216] and chronic [165, 217] exercise; aerobic [218], endurance [169, 219], and resistance training [220]; and high-intensity [221] and moderate activity [222]. The EPS protocols employed and the validation of the efficacy of the stimulation present a noticeable variability [223]. Moreover, the biological footprint of those models of exercise has been partially evaluated, with the main focus on exercise proteins and myokines, such as Akt (protein kinase B) [215],[217, 224], AMPK (5' adenosine monophosphate-activated protein kinase) [219, 225, 226], and IL-6 (Interleucine 6) [215, 221], as well as metabolic indices, mainly glucose metabolism [46, 165, 226]. Therefore, we did a systematic review and meta-analysis to systematically assess the available evidence on the link between the stated type of exercise and the observed biological profile of exercised cells, as well as to present the available EPS-applied protocols mimicking exercise in vitro.

4.2. Methods

4.2.1. Searching Process

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [227] (Table S4), we searched the PubMed and EMBASE databases from their inception to 4 February 2022 for studies that assessed in vitro exercise using EPS as a means to mimic exercise. To increase data availability and method transparency, we uploaded our data to an online repository accessed on 8 October 2022 (https://doi.org/10.6084/m9.figshare.21299523).

The screening of the titles, abstracts, and full texts for eligibility and the selection of studies to be included was performed independently by two investigators (EN and EK). Any conflicts were resolved by a referee investigator (ADF). We included studies where EPS was used to mimic exercise in vitro and the

specific type of exercise achieved was defined by the authors. We considered articles written in English published in peer-reviewed journals. No limits were set for methodological design or sample size. We excluded reviews, conference proceedings, editorials, letters, and magazine articles, but we screened the reference lists of such publications of the retrieved articles for relevant papers. Also, we excluded studies without any information on the characteristics of the stimulation protocol (frequency (Hz), pulse duration (ms), and applied pulse amplitudes (Vapp)) [228], on the duration of the stimulation, on the type of the stimulator, and on the cell type that underwent exercise. Moreover, we excluded studies not providing a definition of the type of mimicked exercise and not clearly stating that pulse stimulation was used in order to mimic exercise (therefore, studies where "muscle contraction" was the term used instead of "exercise"). The search algorithm can be found in Supplement 1.1.

4.2.2. Data Extraction

For all eligible studies, we extracted the first author names, year of publication, country of origin, funding acquisition, and data on the pulse parameters, cell type used, and biological indices measured on the cells under stimuli, and we documented the purpose of each study in relationship to the exercise conducted and any relevant secondary outcome (Table S1–S3). The extracted data are freely available in an online data repository accessed on 8 October 2022 (https://doi.org/10.6084/m9.figshare.21299523). The groups regarding types of exercise studied are based on the definition provided by the authors of each study on the type of exercise achieved, and data was extracted on biological indices.

4.2.3. Meta-Analyses

4.2.3.1 Metanalysis and Meta-Regression

We performed meta-analyses to calculate the differences between control (non-stimulated) and EPS-stimulated cells for the biological indices having enough data for such an analysis. In cases of unreported values, we used WebPlotDigitizer (v4.5, 2021) to extract the information from the given

graphs [229]. Meta-regression analysis was used to evaluate the association between duration of stimulation and levels of expression of the examined biological parameters. In cases where the number of replicates was not identified, we assumed that they were conducted in triplicates, and in cases of a range of number of replicates, we used the mean. Since different methods and scales were utilized in the eligible studies, we used standardized mean differences (SMDs) instead of absolute mean differences to standardize our findings to uniform scale [, 2009 #138]. Missing SDs were imputed using the average coefficient of variation from all complete cases [230]. A random effect model was used to account for heterogeneity due to different cell lines, stimulation protocols, and stimulators. All analyses were performed using the "metafor" package in the R language (Rstudio, version 1.3.1093, PBC, Boston, MA, USA). The "atransf" argument in "metafor" was used for the transformed standardized mean difference as an estimate of the log odds ratio. The level of significance was set at an alpha level of $p < 0.05$.

4.3. Results

4.3.1. General Description of Models

4.3.1.1. Searching and Selection

A total of 985 records were retrieved through our systematic database search. Of these articles, we removed 308, which were duplicates (Figure 4.1). An additional 521 records were classified as non-eligible. 161 were assessed for eligibility. Overall, 41 studies met the inclusion criteria. Of these, 24 studies provided information for meta-analysis. The list of included studies and their main outcomes is provided in the Online Supplement (Table S1–S3).

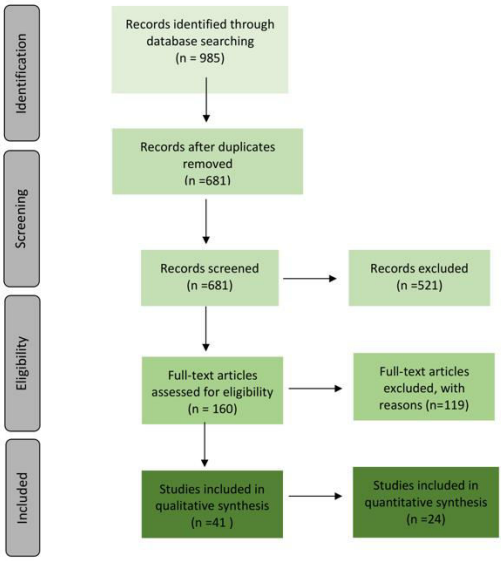


Figure 4.1 Prisma Flow Chart. The selection process of the studies included in the present systematic review.

4.3.1.2. Cell Types and Pulse-Stimulator Types

Two main groups of cell types were used in the included studies: a. cell lines and b. biopsies from humans and mice (Table S2). More specifically, 30 of the eligible studies used cell lines: 24 studies employed the C2C12 cell line [45, 46, 162, 167, 170, 174, 176, 215, 222, 224, 226, 231-243], a mouse myoblast cell line; while one study used the L6 cell line [181], a rat myoblast cell line; one used primary human cells [162]; and one the H-2kb muscle cells (a mouse myoblast cell line) [244]. Of the remaining eligible studies, 12 used human skeletal muscle biopsies [162, 165, 169, 216, 217, 219-221, 225, 245-248] from different sites, such as vastus lateralis, satellite cells, and rectus abdominis, obtained from healthy (*n* = 64), lean (*n* = 32), obese (*n* = 20), and diabetic donors (*n* = 4). One study used rat biopsies from the quadriceps [238], while another study used mouse biopsies from 4–8-week-old mice [218] and one rabbit hindlimbs [249]. Also, we identified two main types of electrical pulse stimulators: custom made stimulators (used by 13 studies) and a commercially available stimulator (used by 28 studies). Also, five commercially available generators and electrodes have been reported (Table S2). The eligible

studies employed a wide range of electric potential (volts), frequency (Hz), and intensity (amps), while a higher homogeneity was observed in the duration of stimulation (Table S3).

4.3.2. In Vitro Types of Exercise

4.3.2.1. Acute and Chronic Exercise

A total of 20 studies [45, 46, 165, 167, 169, 170, 176, 215, 216, 220, 221, 224, 226, 231, 232, 235, 236, 238, 241, 248] reported that their protocol mimicked acute exercise, and we identified an EPS duration time frame of 15 min to 24 h and one case of repeated stimulation for 3 days, 60 min per day. Almost all (95%) of the protocols mimicking acute exercise included an EPS time period of < 100 min. In the case of chronic exercise, the protocols were divided into two major categories. In most studies, chronic exercise was mimicked via a long period of continuous stimulation lasting from 12 to 72 h [165, 217, 220, 221, 238, 239, 243, 246], while in some studies chronic exercise was administered as a brief protocol repeated over several consecutive days (3 to 15 days) [232, 249].

4.3.2.2. Aerobic, Resistance, and Endurance Training

McArdle et al. described their exercise as aerobic activity, where the EPS lasted for 15 min (30 V per well), whilst Nieuwoudt et al. (30 V per well) used a protocol consisting of a 16 h stimulation at 11.5 V per mm. In several studies, the type of exercise was defined in a more qualitative way, describing only the type of training mimicked via the applied protocol. In this case, the authors of seven studies [169, 176, 181, 219, 220, 244, 245] reported that their protocol was comparable to resistance exercise. Further analysis of the stimulation parameters showed that six [169, 181, 219, 220, 244, 245] studies applied the stimulation once (implied as acute) with a range of 15 min to 24 h. Tamura et al. [176], though, used a protocol more similar to that of chronic exercise, applying a 10 min stimulation per day for 3 consecutive days. The protocol used by Breton et al. [220] was the only one where we detected linking both acute (30 min stimulation) and chronic (3 day stimulation) protocols to resistance training in

vitro. Furthermore, three studies [233, 242, 248] identified their EPS model as “endurance training”, either establishing the optimal conditions for EPS to mimic endurance training in vitro (60 min, 11.5 V, 10 Hz) or using an already established protocol (240 min, 20 V, 1 Hz) that was previously proven to mimic endurance exercise in vitro [250].

4.3.2.3. High-Intensity and Moderate Activity

Regarding the intensity of exercise, eight studies characterized their in vitro exercise models as high-intensity [221, 237, 240] or mild/moderate [221, 222, 225, 242, 244] activity. The remaining studies did not provide relevant information. In one study, a 3D-engineered muscle was employed and an EPS protocol consisting of 30 min, 1 V/mm, and 100 Hz was applied. In the 3D-engineered muscle, the high-intensity in vitro protocol mimicked the muscle fatigue of acute high-intensity exercise in humans.

4.3.3. In Vivo vs. In Vitro

Nine studies [162, 167, 170, 218, 226, 233, 238, 242] (Table 4.1) compared their results from exercise mimicking in vitro with their in vivo experiments. A similar pattern of gene expression of MCAD (Medium Chain Acyl CoA Dehydrogenase), Cpt1b (Carnitine Palmitoytransferase-1b), and GLUT4 (Glucose transporter type 4) was observed between EPS-treated muscle cells and chronically exercised mice but not in acutely exercised mice [167]. Similarly, phosphorylated AMPKa1/2 was increased in both exercised mice (chronic exercise of 1 h/day for 3 weeks) and stimulated muscle cells (acute and chronic) [238]. A comparison between mice executing treadmill exercise (75% VO_{2max}) for 60 min and electrically stimulated myotubes (both considered acute exercise) showed a comparable motif of regulation of Rac1, Axin1, and AMPK [226].

Table 4.1 In vitro vs in vivo studies.
The type of exercise as defined by the study authors and the duration of in vitro exercise. These in vitro types of exercise have been compared directly or indirectly to in vivo models of exercise.

Author, Date	Type of Exercise as Defined by the Study Authors	Duration of In Vitro Exercise	In Vivo Protocol	Organism
Burch, 2010[167]	Acute, intermittent, continuous	90 min = acute, 90 min/4 days = intermittent, 24 h = continuous	Treadmill, at 75% of average distance of exhaustion trial (4 days training, 1 day exhaustion, 2 days rest), 6 weeks total	Mice
Fernandez-Verdejo, 2017[233]	Endurance exercise	240 min	Treadmill until exhaustion	Mice
Lee, 2020[238]	Acute and chronic exercise	Acute = 1, 3, 6 h chronic = 12, 24, or 36 h	Treadmill 60 min, 5 d/week, 10 m/min	Mice
McArdle, 2001[218]	Aerobic activity	15 min		
Pattamaprapanont, 2016[170]	Acute exercise	30 min	Cycle ergometer at 80% VO_{2max}, 15 min	Healthy males
Raschke, 2013[162]	Regular exercise	4 to 24 h	Cycle ergometer at 70% VO_{2max}, 60 min	Healthy males
Raschke, 2013[162]	Training model/in humans endurance training	24 h	Treadmill, at 90% of peak heart rate, 3 d/week for 10 weeks	Healthy males
Son, 2019[242]	Mild endurance exercise	60 min	Volunteer wheel running daily for 4 weeks	Mice
Yue, 2020[226]	Acute exercise	60 min	Treadmill, at 75% VO_{2max}, 60 min	Mice

Another approach [242] consisted of comparing the molecular effect of different EPS protocols to that of voluntary wheel running in mice (considered mild endurance exercise), aiming to identify the EPS protocol with the most-similar molecular signature measuring PGC1a (Peroxisome proliferator-activated receptor-gamma coactivator a) levels, AMPK, and p38 phosphorylation. The suggested protocol consisted of 60 min stimulation at 11.5 V and 10 Hz, with a 2 ms pulse stimulus duration.

Pattamapramont and colleagues identified NR4A3 (Nuclear Receptor Subfamily 4 Group A Member 3) as an exercise-induced gene in acutely exercised healthy men, and then they established an EPS model mimicking the effect of exercise on that particular gene expression. An attempt to map the gene activation pattern of FNDC5A (fibronectin type III domain containing 5a) in EP-stimulated human muscle cells and in human biopsies from participants that either underwent 10-week interval endurance

training or 11-week strength training showed no changes in FNDC5 mRNA expression in both exercise models. It should be noted that the EPS protocol was able to enhance PGC1a mRNA expression, which is typical for exercising muscle.

4.3.4. Biological Parameters

Apart from the above-mentioned parameters regarding EPS, the effect of exercise in vitro was evaluated by some authors using exercise-related indicators at biochemical, protein, and translational levels. As previously mentioned, in some studies there was an attempt by authors to correlate biological indices in both in vivo and in vitro experimental setups. These issues are described in the following subsections.

4.3.4.1. AMPK Signalling

AMPK is phosphorylated in skeletal muscle during exercise due to high binding of AMP, whose concentration (and, therefore, availability) depends on the duration and the intensity of exercise [251]. In this perspective, in 10 of the included studies [45, 46, 169, 176, 215, 219, 224-226, 242], AMPK and AMP were measured and were found to be increased after the application of EPS compared to controls in all but one [219] study. The protocol was defined as resistance exercise. However, when two types of EPS contraction (both considered by the study authors as resistance exercise), tetanic vs. twitch, were compared, the phosphorylation of the AMPK a-subunit at post-translational modification site Thr172 (regulating AMPK activity) was found to increase significantly in tetanic but not in twitch contraction [176].

4.3.4.2. Glucose Metabolism

Glucose is the main energy source for exercising skeletal muscle. Glucose availability is determined by the delivery, the transport across the membrane, and the intracellular metabolism; three processes

well-orchestrated and tightly connected [252]. Glucose uptake after EPS was measured in eight of the eligible studies: seven studies [45, 46, 165, 174, 176, 224, 225] reported significant increases in glucose uptake, while one study found a decrease after the stimulation [176]. GLUT4H cell surface receptors, which are responsible for glucose transport into the cell, have also been found higher after applying a 60 min acute exercise protocol in C2C12 cells than in the basal condition. In another study, GLUT4-protein expression remained unchanged after a 16 h aerobic-training protocol in C2C12 cells [174]. A 24 h moderate-exercise protocol applied on human biopsies from lean and obese Caucasians increased GLUT4 only in muscle cells from lean individuals [225].

4.3.4.3. Akt Signalling

Akt signalling pathway is increased by acute bouts of exercise proportionally to the intensity of exercise in human studies, while chronic exercise has minimal effect on Akt activation [253]. In the EPS studies with chronic exercise, Akt levels decreased, while the acute exercise protocols led to an increased phosphorylated Akt [220]. Also, the different timepoints of sample collection seem to play some role, since higher protein levels are detected immediately after the exercise protocol and 180 min later, in contrast to 60 min after the protocol [220].

4.3.4.4. IL-6 as a Myokine

IL-6 is identified as a myokine secreted by skeletal muscle upon exercise [254] and has been measured in eight of the eligible studies [165, 169, 171, 215, 217, 219, 221, 235] at protein and protein-expression levels. Overall, IL-6 secretion increased after the EPS protocol, except for when the muscle cells used were coming from severely obese participants [4]. After a series of measurements over time, Tarum et al. identified a pick at expression levels 4 h after completion of EPS, while, in untreated cells, the IL-6 remained undetected.

4.3.5. Meta-Analyses

4.3.5.1. Mean Differences in Biological Indices between Stimulated and Non-Stimulated Cells

Transformed standardized mean differences between EP-stimulated cells and control (non-stimulated) cells were calculated for the expression levels of Akt, AMPK, IL-6, PGC1-a, and GLUT4, as well as glucose-uptake levels. The analyses showed that EPS cells were much more likely to show higher expression in most of these parameters. Specifically, compared to non-stimulated cells, EPS cells were 2.43 (1.49, 3.95) times (mean (95% CI)) more likely to show higher Akt expression (Figure 4.2); 4.36 (2.09, 9.10) times more likely to show higher AMPK expression (Figure 4.3); 3.73 (2.41, 5.78) times more likely to show higher IL-6 expression (Figure 4.4); 2.01 (1.20, 3.55) times more likely to show higher PGC1a expression (Figure 4.5); and 1.95 (1.02, 3.75) times more likely to show higher glucose-uptake levels (Figure 4.6) (all $p < 0.05$). Compared to non-stimulated cells, EPS cells were 1.42 (0.95, 2.13) times more likely to show higher GLUT4 expression, yet this effect did not reach the level of statistical significance ($p > 0.05$; Figure 4.7).

4.3.5.2. Meta-Regression for the Effect of EPS Depending on Stimulation Duration

The effect of EPS stimulation on AMPK-expression levels was significantly decreased with the duration of stimulation ($p = 0.023$, $R^2 = 0.31$; Figure 4.8). This effect did not reach the level of statistical significance for Akt, IL-6, PGC1a, GLUT4, or glucose uptake ($p > 0.05$; Figure S1–S5). However, when analyzed combined, the overall effect of EPS stimulation on Akt, AMPK, IL-6, and PGC1a also decreased with the duration of stimulation ($p = 0.034$, $R^2 = 0.22$; Figure 8).

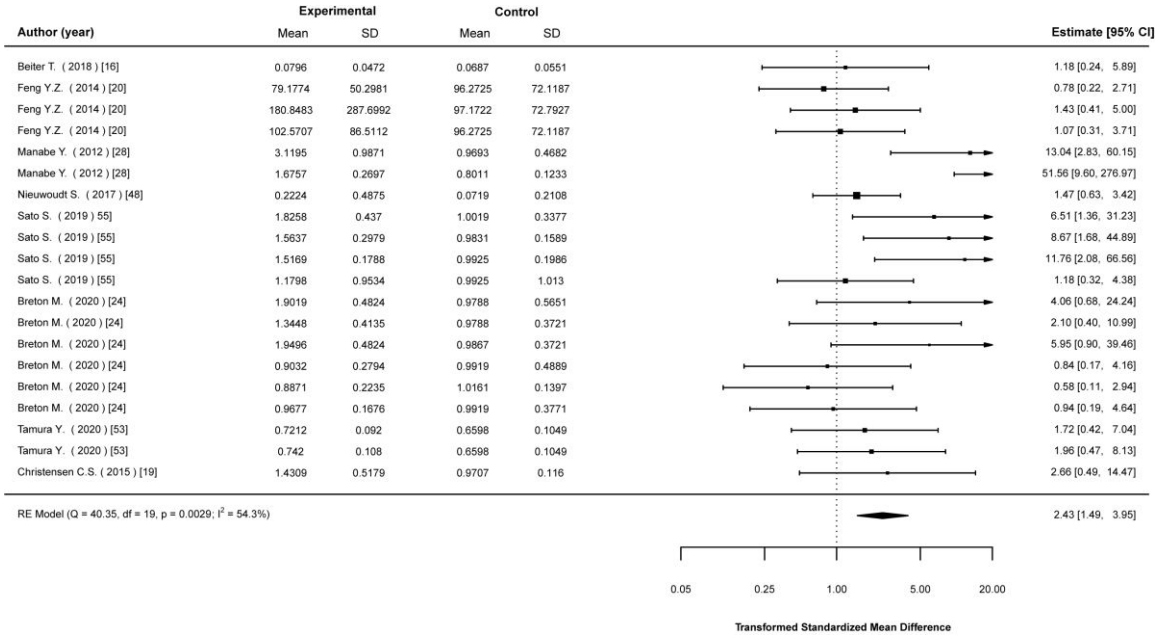


Figure 4.2 Findings of random-effects meta-analysis on the effects of EPS on Akt compared to non-stimulated cells.

Results shown are transformed standardized mean differences and 95% confidence intervals, as an estimate of the log odds ratio. Differences greater than 1.00 favour the EPS cells compared to non-stimulated control cells.

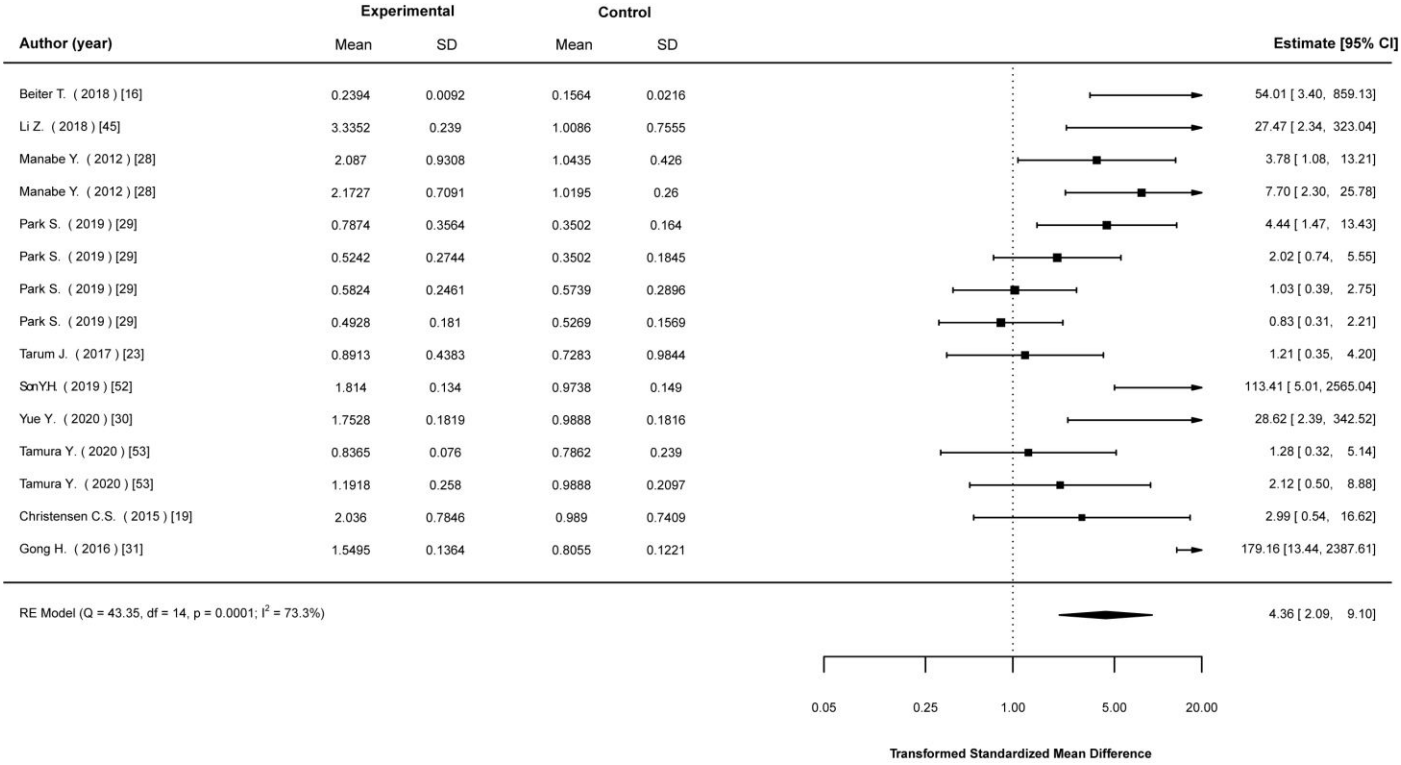


Figure 4.3 Findings of random-effects meta-analysis on the effects of EPS on AMPK compared to non-stimulated cells.

Results shown are transformed standardized mean differences and 95% confidence intervals, as an estimate of the log odds ratio. Differences greater than 1.00 favour the EPS cells compared to non-stimulated control cells.

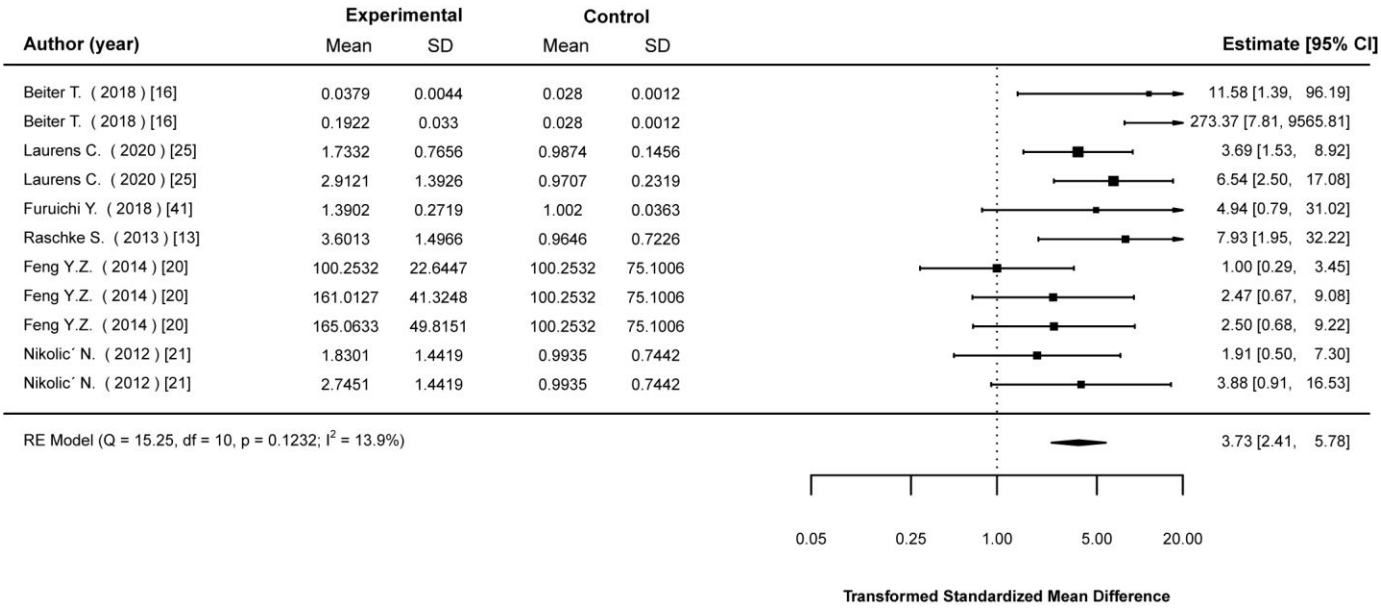


Figure 4.4 Findings of random-effects meta-analysis on the effects of EPS on IL-6 compared to non-stimulated cells.
Results shown are transformed standardized mean differences and 95% confidence intervals, as an estimate of the log odds ratio. Differences greater than 1.00 favour the EPS cells compared to non-stimulated control cells.

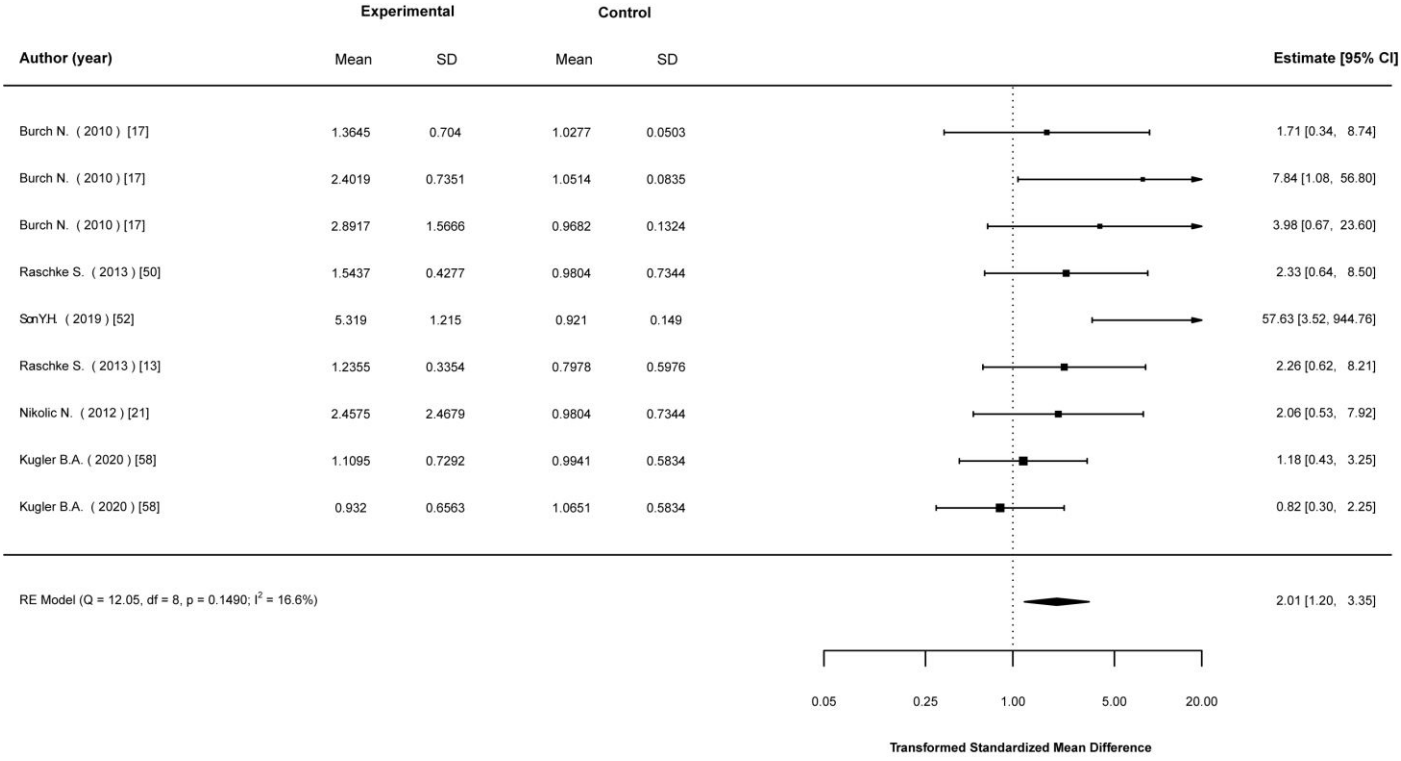


Figure 4.5 Findings of random-effects meta-analysis on the effects of EPS on PGC1a compared to non-stimulated cells.
Results shown are transformed standardized mean differences and 95% confidence intervals, as an estimate of the log odds ratio. Differences greater than 1.00 favour the EPS cells compared to non-stimulated control cells.

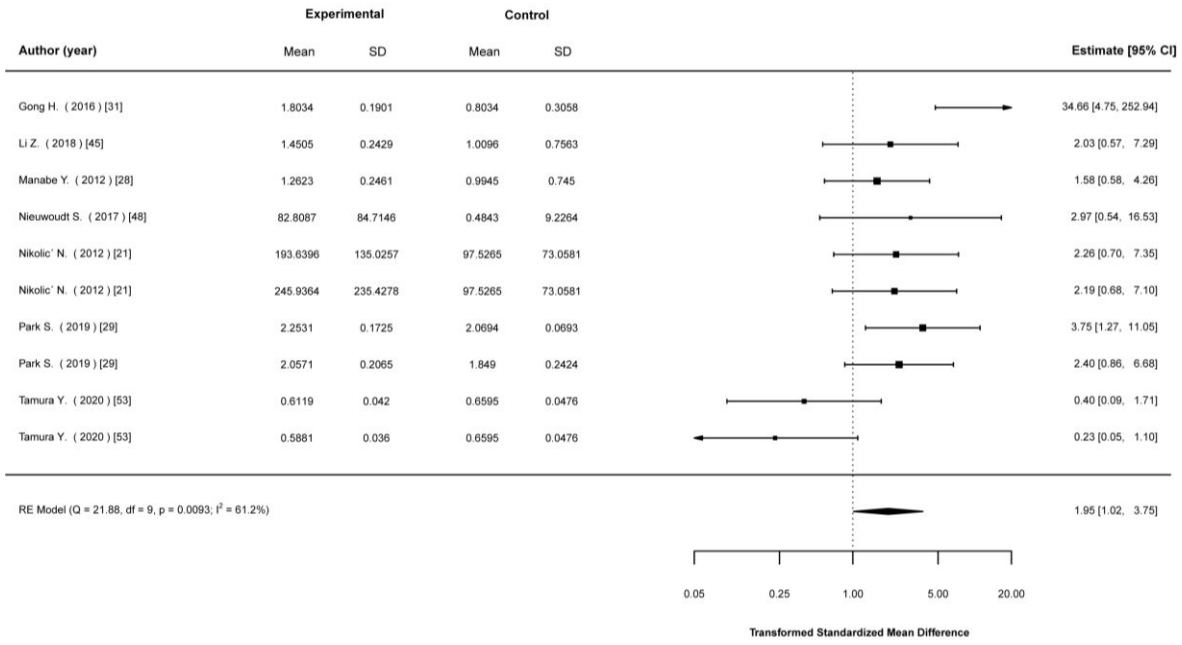


Figure 4.6 Findings of random-effects meta-analysis on the effects of EPS on glucose uptake compared to non-stimulated cells.
Results shown are transformed standardized mean differences and 95% confidence intervals, as an estimate of the log odds ratio. Differences greater than 1.00 favour the EPS cells compared to non-stimulated control cells.

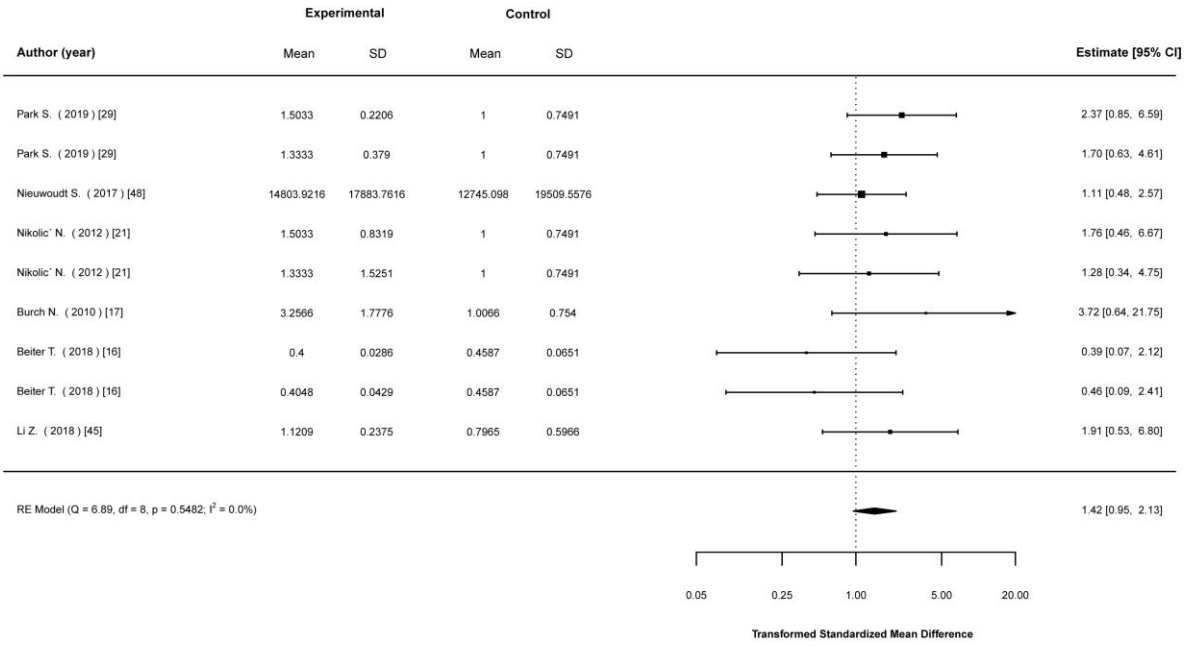


Figure 4.7 Findings of random-effects meta-analysis on the effects of EPS on GLUT4 compared to non-stimulated cells.
Results shown are transformed standardized mean differences and 95% confidence intervals, as an estimate of the log odds ratio. Differences greater than 1.00 favour the EPS cells compared to non-stimulated control cells.

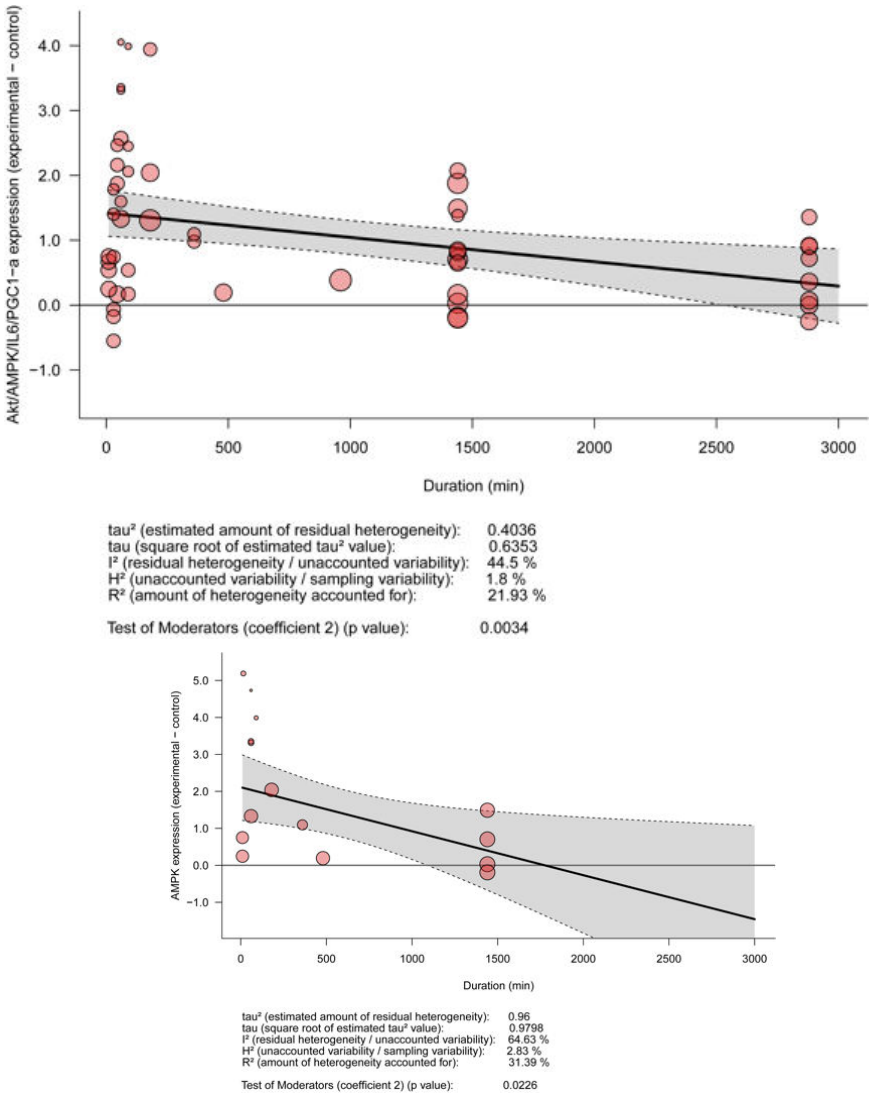


Figure 4.8 Meta-regression for the effect of EPS.

Meta-regression for the effect of EPS depending on stimulation duration in the expression of AMPK (**top**) and combined Akt, AMPK, IL-6, and PGC1a (**bottom**).

4.4. Discussion

In the last decades, exercise has been proposed as a prevention and/or therapeutic strategy for many diseases [203, 204]. Therefore, much research has focused on identifying the molecular and biochemical pathways through which exercise exerts its benefits. A valuable method to study the underlying mechanisms of exercise effect is in vitro mimicking of exercise via EPS [255].

Differences in terms of exercise intensity, duration, and repetitions lead to different (more or less beneficial) effects [255]. Thus, defining the type of exercise in in vitro experiments is essential both for assessing its overall effect and for highlighting the involved pathways. As shown in this systematic review, there is a vast heterogeneity of applied in vitro protocols reflecting different types of exercise. We recorded types of exercise based on duration (chronic and acute), training (endurance, resistance, and aerobic), and intensity (high, mild, and moderate). We observed marked heterogeneity in the protocols used for the same type of mimicked exercise. Furthermore, we observed marked variability in the in vitro studies that conducted and compared their results with in vivo studies. Specifically, for the acute exercise, there were protocols lasting 60 min, while others lasted 360 min and even 24 h. Similarly, chronic exercise protocols ranged from 12 to 36 h. Added to these differences is the important fact that EPS protocols involve many factors, such as pulse duration (ms), applied pulse amplitudes (Vapp), and stimulation duration time, which exert significant impacts on the final outcome.

One could assume that the protocol parameters define the type of exercise; however, the molecular signature of each protocol might be of equal validity. Our meta-analyses showed that EPS protocols exert significant effects in the expression levels of biological parameters that are known to be affected by exercise in in vivo and human studies. Specifically, we found that EPS leads to significant increases in the expression levels of AMPK, Akt, IL-6, and PGC1a and glucose–uptake levels. The above proteins are involved in major biological processes in skeletal muscle triggered by exercise and muscle contraction [252, 256-259]. More specifically, AMPK is acutely activated in response to exercise [256],

and the consequent low-energy status (increased ratio of AMP/ADP: ATP) is involved in metabolic regulation and energy homeostasis by downregulating energy-consuming processes, like fatty acid and cholesterol synthesis, and by upregulating ATP-producing pathways, such as glucose uptake and fatty-acid oxidation [260]. When activated via the Akt/mTORC1 pathway, Akt is key to muscle-mass hypertrophy in the healthy and diseased population [8] and triggered by many extracellular signals, including exercise. IL-6, a pleiotropic myokine, is known to increase in response to exercise exerting both anti- and pro-inflammatory effects [261]. It plays key anti-diabetic roles, enhancing muscular glucose uptake, exerting effects on pancreatic insulin secretion, and promoting fatty-acid oxidation and lipolysis [262]. Upregulation of the p38γ MAPK/PGC-1α pathway and increase of PGC-1α augment mitochondrial biogenesis, fatty-acid oxidation, and insulin sensitivity in healthy and insulin-resistant skeletal muscle, although studies in mice have suggested that PGC1a does not affect insulin sensitivity [188]. Correlating duration of protocol with the mean differences for each of the aforementioned biological indices clearly showed that there was a noteworthy trend for a reduction in the effect of EPS with increasing duration. In particular, the expression of AMPK in stimulated cells significantly decreased with time of stimulation. Likewise, in humans, AMPK has been known to increase in acute exercise and partially in extended chronic exercise [251]. Individually, Akt, IL-6, and PGC1a did not seem to relate with the duration of EPS; although, when analyzed as one group (including AMPK), the effect of stimulation duration became significant. These results may be due to the small number of studies included in our meta-regression but also because the signaling pathways of these molecules are intertwined. For instance, IL-6 has been shown to augment in acute exercise and decrease in plasma of humans both at rest and in response to chronic exercise [263], which is in line with the findings of our meta-regression. Interestingly, glucose uptake and GLUT4 had an opposite trend, increasing with time, meaning that the longer the protocol, the higher the need for glucose uptake and subsequently GLUT4 translocation and expression. Even though AMPK, a regulator of glucose uptake, was found to decrease with time in our meta-regression,

glucose uptake changed in the opposite direction, indicating that in vitro models can mimic contraction-induced glucose uptake involving alternative molecular pathways [264].

The present systematic review, meta-analysis, and meta-regression verified previous statements, that in vitro models of exercise have a massive variability in cell types, protocols, equipment, sample collection time, and measurement methods. In this respect, validating in vitro models by comparing the results to those obtained from in vivo studies is of great value [18]. At present, there are a limited number of studies adopting this research design, inhibiting further data analysis and conclusions.

To our knowledge, this is the first time that key biological parameters for exercise are examined in a meta-analysis and meta-regression in relation to their effect in vitro. It is now evident that in vitro exercise follows motifs of exercise in humans, allowing biological parameters, such as AMPK, Akt, IL-6, PGC1a, and glucose uptake to be valuable tools in defining the types of in vitro exercise. Further research is needed to set the base for a consensus that would provide robustness of results and improved translation of the findings into human studies.

Funding: This research was financed by Greece and the European Union (European Social Fund-ESF) through the Operational Program «Human Resources Development, Education and Lifelong Learning 2014–2020» in the context of the project “Effect of the in vitro exercise on browning of white adipose tissue” (grant number MIS: 5048945). This research received no external funding.

Acknowledgments: We wish to thank Petros Dinas for the guidance and technical support during the searching process.

Chapter 5

The present thesis was an attempt firstly to elucidate the genetic effect of UCP1 polymorphisms on cardiometabolic health and secondly to research the effect of exercise on UCP1 expression levels in adipocytes and to unravel possible exercise models that could be used to mimic exercise in vitro.

The findings in the first study of this Thesis indicate that the A-3826G may promote the development of CMP in the presence of environmental factors [142] as well as other genetic traits [143]. In the case of A-112C, it is important to also consider the effect of another variant, rs72941746, that is in linkage disequilibrium [144]. The A-112C seems to modify transcription factor binding sites and its region has specific patterns of chromatin accessibility in several tissues. It appears that the linked variant is responsible for much more alterations in transcription factor binding site motifs and consequently the binding of other proteins. This indicates that the association I observed in this study when A-112C is present could possibly be an effect of rs72941746 influence. Finally, in some populations, the A-3826G, A-1766G, Ala64Thr and A-112C SNPs of *UCP1* gene may be associated with the prevalence of one or more of the most common CMP and their risk factors. The studied SNPs may be important for promoting risk factors and pathophysiological mechanisms involved in CMP, but this involvement may be stronger in some races, ethnicities, and/or regions. Nevertheless, it is important to note that the increased CMP prevalence in certain ethnic groups in Eastern Europe and Western Asia [91, 92] may reflect potential ancestral differential effects.

The next step, was to investigate, if there is a link in the interaction between adipocytes and myocytes under the effect of exercise. In this study, I let C2C12 myotubes and 3T3-L1 adipocytes interact in vitro under the stimuli of EPS, mimicking muscle contraction. The increased expression of PGC-1a and IL-6 protein levels in the co-cultured C2C12 cells and the increased levels of UCP1 and IL-6 protein expression in the 3T3-L1 adipocytes suggest the existence of a direct crosstalk between the

muscle cells, when contracted, with the adipocytes, resulting in changes in the expression pattern of proteins related to browning of adipose tissue.

It is strongly established that exercise-induced myokines change the profile of both muscle and adipose tissue [186]. This leads to adaptations in white adipose tissue including the reduction of the size of the adipocytes, increased mitochondrial activity, change of the adipokines profile, and changes in gene expression [153, 154].

When stimulated by external cues such as exercise and contraction, beige adipocytes express UCP1 protein and exhibit UCP1-dependent thermogenic capacity [161, 191], In my model I detected a significantly higher expression of UCP1 in 3T3-L1 adipocytes co-cultured with C2C12 myotubes under the effect of muscle contraction, in comparison to the non-co-cultured adipocytes.

My findings demonstrated the existence of a direct crosstalk between the contracting muscle cells and the adipocytes, resulting in increased adipocyte expression of proteins that play key roles in the browning process (UCP-1 and IL-6). However, it is important to acknowledge that in vitro models of exercise and browning, such as the present, do not account for inter-organ communication. Moreover, in vitro exercise protocols cannot be easily translated into in vivo situations [202]. Therefore, the simultaneous development of both in vivo and in vitro models that can complement each other should be employed in future studies to generate robust mechanistic data.

Nevertheless, it is important to note that in vitro models of exercise incorporate several limitations, including lack of unanimously accepted exercise protocol characteristics and inability to study systemic effects of exercise. As shown in my systematic review, there is a vast heterogeneity of applied in vitro protocols reflecting different types of exercise. I observed marked heterogeneity in the protocols used for the same type of mimicked exercise. Furthermore, I observed marked variability in the in vitro studies that conducted and compared their results with in vivo studies. One could assume that the protocol parameters define the type of exercise; however, the molecular signature of each

protocol might be of equal validity. The meta-analyses showed that EPS protocols exert significant effects in the expression levels of biological parameters that are known to be affected by exercise in in vivo and human studies.

The present systematic review, meta-analysis, and meta-regression verified previous statements, that in vitro models of exercise have a massive variability in cell types, protocols, equipment, sample collection time, and measurement methods. In this respect, validating in vitro models by comparing the results to those obtained from in vivo studies is of great value [18]. At present, there are a limited number of studies adopting this research design, inhibiting further data analysis and conclusions.

To my knowledge, this is the first time that key biological parameters for exercise are examined in a meta-analysis and meta-regression in relation to their effect in vitro. It is now evident that in vitro exercise follows motifs of exercise in humans, allowing biological parameters, such as AMPK, Akt, IL-6, PGC1a, and glucose uptake to be valuable tools in defining the types of in vitro exercise.

During this Thesis, although every effort was made, I acknowledge that there are some restrictions. We studied UCP1 variants in certain populations, but we did not account for demographic characteristics (socioeconomic status, etc.) and environmental factors (climate conditions, nutritional habits, etc.), affecting CMP risk factors, such as BMI, WHR a.o.. Additionally, although a power analysis was conducted, the sample size is not that large allowing as safely to perform further analysis by stratifying our sample by gender, age etc.. In the in vitro study of UCP1 expression, the lack of unanimously accepted exercise protocol characteristics, which we also highlighted in our systematic review (chapter 4), and the inability to study systemic effects of exercise and inter-organ communication, is a main restriction in translating those results directly to whole body physiology.

This PhD Thesis answered the scientific questions, verified the hypothesis made at the beginning and created also future perspectives. Our study in UCP1 variants paved the way and highlighted the need for future studies on these SNPs that could possibly unravel potential candidates for precision

medicine. Also, a study that would take into account both genetic background and environmental factors, would be of great importance in uncovering from a more rounded point of view the risk factors and their associations with CMPS. In the in vitro part of this thesis, future studies should test the effect on adipocytes of a range of muscle stimulation protocols ranging in terms of muscle stimulation characteristics (frequency, intensity, and duration) mimicking different types of exercise (anaerobic/aerobic, endurance, acute/ chronic. It would be of great interest to explore the expression of additional browning markers,), as well as research methodologies, including gene silencing and gene knock-out, in order to confirm further and expand the present findings. Moreover, a future application of the in vitro model in human cells might also allow to study how this inter-cellular communication occurs and which molecule(s) participate, providing us a more robust theoretical framework and increasing our knowledge on the interaction of myotubes with other cell types, such as cancer cells.

The present Doctoral Thesis has three major innovative milestones: Firstly. it is the first population genetics study of UCP1 polymorphisms on the Greek population. Secondly, it is the first model of in vitro contracting muscle cells co-cultured with adipocytes and thirdly, it is the first meta-analysis and meta-regression on key biological parameters for exercise and their effect in vitro. The footprint of my research is minor compared to other works, but has one characteristic that makes it complete: it is a spherical approach with both genetic and molecular biology aspect of a topic that has growing interest for scientific community.

The impact of these studies is dual. On the one hand there is a pure research outcome, adding new knowledge to the existing literature, answering research questions and creating new pathways for future research. On the other hand, this process changed also me as a person and as a molecular biologist and geneticist. I gained deeper knowledge, technical skills, soft skills and a work ethic that I will never forget. But above all, I will always remember that one and only moment when I got my first

experimental result. That moment I realized that I knew something that no one else knew, that I was supposed to tell the world and this moment is priceless.

References

1. Afshin, A., M. H. Forouzanfar, M. B. Reitsma, P. Sur, K. Estep, A. Lee, L. Marczak, A. H. Mokdad, M. Moradi-Lakeh, M. Naghavi, J. S. Salama, T. Vos, K. H. Abate, C. Abbafati, M. B. Ahmed, Z. Al-Aly, A. Alkerwi, R. Al-Raddadi, A. T. Amare, A. Amberbir, A. K. Amegah, E. Amini, S. M. Amrock, R. M. Anjana, J. Ärnlöv, H. Asayesh, A. Banerjee, A. Barac, E. Baye, D. A. Bennett, A. S. Beyene, S. Biadgilign, S. Biryukov, E. Bjertness, D. J. Boneya, I. Campos-Nonato, J. J. Carrero, P. Cecilio, K. Cercy, L. G. Ciobanu, L. Cornaby, S. A. Damtew, L. Dandona, R. Dandona, S. D. Dharmaratne, B. B. Duncan, B. Eshrati, A. Esteghamati, V. L. Feigin, J. C. Fernandes, T. Fürst, T. T. Gebrehiwot, A. Gold, P. N. Gona, A. Goto, T. D. Habtewold, K. T. Hadush, N. Hafezi-Nejad, S. I. Hay, M. Horino, F. Islami, R. Kamal, A. Kasaeian, S. V. Katikireddi, A. P. Kengne, C. N. Kesavachandran, Y. S. Khader, Y. H. Khang, J. Khubchandani, D. Kim, Y. J. Kim, Y. Kinfu, S. Kosen, T. Ku, B. K. Defo, G. A. Kumar, H. J. Larson, M. Leinsalu, X. Liang, S. S. Lim, P. Liu, A. D. Lopez, R. Lozano, A. Majeed, R. Malekzadeh, D. C. Malta, M. Mazidi, C. McAlinden, S. T. McGarvey, D. T. Mengistu, G. A. Mensah, G. B. M. Mensink, H. B. Mezgebe, E. M. Mirrakhimov, U. O. Mueller, J. J. Noubiap, C. M. Obermeyer, F. A. Ogbo, M. O. Owolabi, G. C. Patton, F. Pourmalek, M. Qorbani, A. Rafay, R. K. Rai, C. L. Ranabhat, N. Reinig, S. Safiri, J. A. Salomon, J. R. Sanabria, I. S. Santos, B. Sartorius, M. Sawhney, J. Schmidhuber, A. E. Schutte, M. I. Schmidt, S. G. Sepanlou, M. Shamsizadeh, S. Sheikhbahaei, M. J. Shin, R. Shiri, I. Shiue, H. S. Roba, D. A. S. Silva, J. I. Silverberg, J. A. Singh, S. Stranges, S. Swaminathan, R. Tabarés-Seisdedos, F. Tadese, B. A. Tedla, B. S. Tegegne, A. S. Terkawi, J. S. Thakur, M. Tonelli, R. Topor-Madry, S. Tyrovolas, K. N. Ukwaja, O. A. Uthman, M. Vaezghasemi, T. Vasankari, V. V. Vlassov, S. E. Vollset, E. Weiderpass, A. Werdecker, J. Wesana, R. Westerman, Y. Yano, N. Yonemoto, G. Yonga, Z. Zaidi, Z. M. Zenebe, B. Zipkin, and C. J. L. Murray. "Health Effects of Overweight and Obesity in 195 Countries over 25 Years." *N Engl J Med* 377, no. 1 (2017): 13-27.
2. Tremmel, Maximilian, Ulf-G. Gerdtham, Peter M. Nilsson, and Sanjib Saha. "Economic Burden of Obesity: A Systematic Literature Review." *International Journal of Environmental Research and Public Health* 14, no. 4 (2017): 435.
3. Huang, L. O., A. Rauch, E. Mazzaferro, M. Preuss, S. Carobbio, C. S. Bayrak, N. Chami, Z. Wang, U. M. Schick, N. Yang, Y. Itan, A. Vidal-Puig, M. den Hoed, S. Mandrup, T. O. Kilpeläinen, and R. J. F. Loos. "Genome-Wide Discovery of Genetic Loci That Uncouple Excess Adiposity from Its Comorbidities." *Nat Metab* 3, no. 2 (2021): 228-43.
4. Scott, R. A., L. J. Scott, R. Mägi, L. Marullo, K. J. Gaulton, M. Kaakinen, N. Pervjakova, T. H. Pers, A. D. Johnson, J. D. Eicher, A. U. Jackson, T. Ferreira, Y. Lee, C. Ma, V. Steinthorsdottir, G. Thorleifsson, L. Qi, N. R. Van Zuydam, A. Mahajan, H. Chen, P. Almgren, B. F. Voight, H. Grallert, M. Müller-Nurasyid, J. S. Ried, N. W. Rayner, N. Robertson, L. C. Karssen, E. M. van Leeuwen, S. M. Willems, C. Fuchsberger, P. Kwan, T. M. Teslovich, P. Chanda, M. Li, Y. Lu, C. Dina, D. Thuillier, L. Yengo, L. Jiang, T. Sparso, H. A. Kestler, H. Chheda, L. Eisele, S. Gustafsson, M. Frånberg, R. J. Strawbridge, R. Benediktsson, A. B. Hreidarsson, A. Kong, G. Sigurðsson, N. D. Kerrison, J. Luan, L. Liang, T. Meitinger, M. Roden, B. Thorand, T. Esko, E. Mihailov, C. Fox, C. T. Liu, D. Rybin, B. Isomaa, V. Lyssenko, T. Tuomi, D. J. Couper, J. S. Pankow, N. Grarup, C. T. Have, M. E. Jørgensen, T. Jørgensen, A. Linneberg, M. C. Cornelis, R. M. van Dam, D. J. Hunter, P. Kraft, Q. Sun, S. Edkins, K. R. Owen, J. R. B. Perry, A. R. Wood, E. Zeggini, J. Tajes-Fernandes, G. R. Abecasis, L. L. Bonnycastle, P. S. Chines, H. M. Stringham, H. A. Koistinen, L. Kinnunen, B. Sennblad, T. W. Mühleisen, M. M. Nöthen, S. Pechlivanis, D. Baldassarre, K. Gertow, S. E.

Humphries, E. Tremoli, N. Klopp, J. Meyer, G. Steinbach, R. Wennauer, J. G. Eriksson, S. Männistö, L. Peltonen, E. Tikkanen, G. Charpentier, E. Eury, S. Lobbens, B. Gigante, K. Leander, O. McLeod, E. P. Bottinger, O. Gottesman, D. Ruderfer, M. Blüher, P. Kovacs, A. Tonjes, N. M. Maruthur, C. Scapoli, R. Erbel, K. H. Jöckel, S. Moebus, U. de Faire, A. Hamsten, M. Stumvoll, P. Deloukas, P. J. Donnelly, T. M. Frayling, A. T. Hattersley, S. Ripatti, V. Salomaa, N. L. Pedersen, B. O. Boehm, R. N. Bergman, F. S. Collins, K. L. Mohlke, J. Tuomilehto, T. Hansen, O. Pedersen, I. Barroso, L. Lannfelt, E. Ingelsson, L. Lind, C. M. Lindgren, S. Cauchi, P. Froguel, R. J. F. Loos, B. Balkau, H. Boeing, P. W. Franks, A. Barricarte Gurrea, D. Palli, Y. T. van der Schouw, D. Altshuler, L. C. Groop, C. Langenberg, N. J. Wareham, E. Sijbrands, C. M. van Duijn, J. C. Florez, J. B. Meigs, E. Boerwinkle, C. Gieger, K. Strauch, A. Metspalu, A. D. Morris, C. N. A. Palmer, F. B. Hu, U. Thorsteinsdottir, K. Stefansson, J. Dupuis, A. P. Morris, M. Boehnke, M. I. McCarthy, and I. Prokopenko. "An Expanded Genome-Wide Association Study of Type 2 Diabetes in Europeans." *Diabetes* 66, no. 11 (2017): 2888-902.

5. Shungin, D., T. W. Winkler, D. C. Croteau-Chonka, T. Ferreira, A. E. Locke, R. Mägi, R. J. Strawbridge, T. H. Pers, K. Fischer, A. E. Justice, T. Workalemahu, J. M. W. Wu, M. L. Buchkovich, N. L. Heard-Costa, T. S. Roman, A. W. Drong, C. Song, S. Gustafsson, F. R. Day, T. Esko, T. Fall, Z. Kutalik, J. Luan, J. C. Randall, A. Scherag, S. Vedantam, A. R. Wood, J. Chen, R. Fehrmann, J. Karjalainen, B. Kahali, C. T. Liu, E. M. Schmidt, D. Absher, N. Amin, D. Anderson, M. Beekman, J. L. Bragg-Gresham, S. Buyske, A. Demirkan, G. B. Ehret, M. F. Feitosa, A. Goel, A. U. Jackson, T. Johnson, M. E. Kleber, K. Kristiansson, M. Mangino, I. M. Leach, C. Medina-Gomez, C. D. Palmer, D. Pasko, S. Pechlivanis, M. J. Peters, I. Prokopenko, A. Stančáková, Y. J. Sung, T. Tanaka, A. Teumer, J. V. Van Vliet-Ostaptchouk, L. Yengo, W. Zhang, E. Albrecht, J. Ärnlöv, G. M. Arscott, S. Bandinelli, A. Barrett, C. Bellis, A. J. Bennett, C. Berne, M. Blüher, S. Böhringer, F. Bonnet, Y. Böttcher, M. Bruinenberg, D. B. Carba, I. H. Caspersen, R. Clarke, E. W. Daw, J. Deelen, E. Deelman, G. Delgado, A. S. Doney, N. Eklund, M. R. Erdos, K. Estrada, E. Eury, N. Friedrich, M. E. Garcia, V. Giedraitis, B. Gigante, A. S. Go, A. Golay, H. Grallert, T. B. Grammer, J. Gräßler, J. Grewal, C. J. Groves, T. Haller, G. Hallmans, C. A. Hartman, M. Hassinen, C. Hayward, K. Heikkilä, K. H. Herzig, Q. Helmer, H. L. Hillege, O. Holmen, S. C. Hunt, A. Isaacs, T. Ittermann, A. L. James, I. Johansson, T. Juliusdottir, I. P. Kalafati, L. Kinnunen, W. Koenig, I. K. Kooner, W. Kratzer, C. Lamina, K. Leander, N. R. Lee, P. Lichtner, L. Lind, J. Lindström, S. Lobbens, M. Lorentzon, F. Mach, P. K. Magnusson, A. Mahajan, W. L. McArdle, C. Menni, S. Merger, E. Mihailov, L. Milani, R. Mills, A. Moayyeri, K. L. Monda, S. P. Mooijaart, T. W. Mühleisen, A. Mulas, G. Müller, M. Müller-Nurasyid, R. Nagaraja, M. A. Nalls, N. Narisu, N. Glorioso, I. M. Nolte, M. Olden, N. W. Rayner, F. Renstrom, J. S. Ried, N. R. Robertson, L. M. Rose, S. Sanna, H. Scharnagl, S. Scholtens, B. Sennblad, T. Seufferlein, C. M. Sitlani, A. V. Smith, K. Stirrups, H. M. Stringham, J. Sundström, M. A. Swertz, A. J. Swift, A. C. Syvänen, B. O. Tayo, B. Thorand, G. Thorleifsson, A. Tomaschitz, C. Troffa, F. V. van Oort, N. Verweij, J. M. Vonk, L. L. Waite, R. Wennauer, T. Wilsgaard, M. K. Wojczynski, A. Wong, Q. Zhang, J. H. Zhao, E. P. Brennan, M. Choi, P. Eriksson, L. Folkersen, A. Franco-Cereceda, A. G. Gharavi, K. Hedman Å, M. F. Hivert, J. Huang, S. Kanoni, F. Karpe, S. Keildson, K. Kiryluk, L. Liang, R. P. Lifton, B. Ma, A. J. McKnight, R. McPherson, A. Metspalu, J. L. Min, M. F. Moffatt, G. W. Montgomery, J. M. Murabito, G. Nicholson, D. R. Nyholt, C. Olsson, J. R. Perry, E. Reinmaa, R. M. Salem, N. Sandholm, E. E. Schadt, R. A. Scott, L. Stolk, E. E. Vallejo, H. J. Westra, K. T. Zondervan, P. Amouyel, D. Arveiler, S. J. Bakker, J. Beilby, R. N. Bergman, J. Blangero, M. J. Brown, M. Burnier, H. Campbell, A. Chakravarti, P. S. Chines, S. Claudi-Boehm, F. S. Collins, D. C. Crawford, J. Danesh, U. de Faire, E. J. de Geus, M. Dörr, R. Erbel, J. G. Eriksson, M. Farrall, E. Ferrannini, J. Ferrières, N. G. Forouhi, T. Forrester, O. H. Franco, R. T. Gansevoort, C. Gieger, V. Gudnason, C. A. Haiman, T. B. Harris, A. T. Hattersley, M. Heliövaara, A. A. Hicks, A. D. Hingorani, W.

Hoffmann, A. Hofman, G. Homuth, S. E. Humphries, E. Hyppönen, T. Illig, M. R. Jarvelin, B. Johansen, P. Jousilahti, A. M. Jula, J. Kaprio, F. Kee, S. M. Keinanen-Kiukaanniemi, J. S. Kooner, C. Kooperberg, P. Kovacs, A. T. Kraja, M. Kumari, K. Kuulasmaa, J. Kuusisto, T. A. Lakka, C. Langenberg, L. Le Marchand, T. Lehtimäki, V. Lyssenko, S. Männistö, A. Marette, T. C. Matise, C. A. McKenzie, B. McKnight, A. W. Musk, S. Möhlenkamp, A. D. Morris, M. Nelis, C. Ohlsson, A. J. Oldehinkel, K. K. Ong, L. J. Palmer, B. W. Penninx, A. Peters, P. P. Pramstaller, O. T. Raitakari, T. Rankinen, D. C. Rao, T. K. Rice, P. M. Ridker, M. D. Ritchie, I. Rudan, V. Salomaa, N. J. Samani, J. Saramies, M. A. Sarzynski, P. E. Schwarz, A. R. Shuldiner, J. A. Staessen, V. Steinthorsdottir, R. P. Stolk, K. Strauch, A. Tönjes, A. Tremblay, E. Tremoli, M. C. Vohl, U. Völker, P. Vollenweider, J. F. Wilson, J. C. Witteman, L. S. Adair, M. Bochud, B. O. Boehm, S. R. Bornstein, C. Bouchard, S. Cauchi, M. J. Caulfield, J. C. Chambers, D. I. Chasman, R. S. Cooper, G. Dedoussis, L. Ferrucci, P. Froguel, H. J. Grabe, A. Hamsten, J. Hui, K. Hveem, K. H. Jöckel, M. Kivimaki, D. Kuh, M. Laakso, Y. Liu, W. März, P. B. Munroe, I. Njølstad, B. A. Oostra, C. N. Palmer, N. L. Pedersen, M. Perola, L. Pérusse, U. Peters, C. Power, T. Quertermous, R. Rauramaa, F. Rivadeneira, T. E. Saaristo, D. Saleheen, J. Sinisalo, P. E. Slagboom, H. Snieder, T. D. Spector, K. Stefansson, M. Stumvoll, J. Tuomilehto, A. G. Uitterlinden, M. Uusitupa, P. van der Harst, G. Veronesi, M. Walker, N. J. Wareham, H. Watkins, H. E. Wichmann, G. R. Abecasis, T. L. Assimes, S. I. Berndt, M. Boehnke, I. B. Borecki, P. Deloukas, L. Franke, T. M. Frayling, L. C. Groop, D. J. Hunter, R. C. Kaplan, J. R. O'Connell, L. Qi, D. Schlessinger, D. P. Strachan, U. Thorsteinsdottir, C. M. van Duijn, C. J. Willer, P. M. Visscher, J. Yang, J. N. Hirschhorn, M. C. Zillikens, M. I. McCarthy, E. K. Speliotes, K. E. North, C. S. Fox, I. Barroso, P. W. Franks, E. Ingelsson, I. M. Heid, R. J. Loos, L. A. Cupples, A. P. Morris, C. M. Lindgren, and K. L. Mohlke. "New Genetic Loci Link Adipose and Insulin Biology to Body Fat Distribution." *Nature* 518, no. 7538 (2015): 187-96.

6. Cheng, L., J. Wang, H. Dai, Y. Duan, Y. An, L. Shi, Y. Lv, H. Li, C. Wang, Q. Ma, Y. Li, P. Li, H. Du, and B. Zhao. "Brown and Beige Adipose Tissue: A Novel Therapeutic Strategy for Obesity and Type 2 Diabetes Mellitus." *Adipocyte* 10, no. 1 (2021): 48-65.
7. Heaton, J. M. "The Distribution of Brown Adipose Tissue in the Human." *J Anat* 112, no. Pt 1 (1972): 35-9.
8. Sanchez-Gurmaches, J., and D. A. Guertin. "Adipocyte Lineages: Tracing Back the Origins of Fat." *Biochim Biophys Acta* 1842, no. 3 (2014): 340-51.
9. Cypess, A. M., S. Lehman, G. Williams, I. Tal, D. Rodman, A. B. Goldfine, F. C. Kuo, E. L. Palmer, Y. H. Tseng, A. Doria, G. M. Kolodny, and C. R. Kahn. "Identification and Importance of Brown Adipose Tissue in Adult Humans." *N Engl J Med* 360, no. 15 (2009): 1509-17.
10. Virtanen, K. A., M. E. Lidell, J. Orava, M. Heglind, R. Westergren, T. Niemi, M. Taittonen, J. Laine, N. J. Savisto, S. Enerbäck, and P. Nuutila. "Functional Brown Adipose Tissue in Healthy Adults." *N Engl J Med* 360, no. 15 (2009): 1518-25.
11. Hanssen, M. J., A. A. van der Lans, B. Brans, J. Hoeks, K. M. Jardon, G. Schaart, F. M. Mottaghy, P. Schrauwen, and W. D. van Marken Lichtenbelt. "Short-Term Cold Acclimation Recruits Brown Adipose Tissue in Obese Humans." *Diabetes* 65, no. 5 (2016): 1179-89.
12. Blondin, D. P., F. Frisch, S. Phoenix, B. Guérin, E. Turcotte É, F. Haman, D. Richard, and A. C. Carpentier. "Inhibition of Intracellular Triglyceride Lipolysis Suppresses Cold-Induced Brown Adipose Tissue Metabolism and Increases Shivering in Humans." *Cell Metab* 25, no. 2 (2017): 438-47.
13. Ouellet, V., A. Routhier-Labadie, W. Bellemare, L. Lakhal-Chaieb, E. Turcotte, A. C. Carpentier, and D. Richard. "Outdoor Temperature, Age, Sex, Body Mass Index, and Diabetic Status Determine the Prevalence, Mass, and Glucose-Uptake Activity of 18f-Fdg-Detected Bat in Humans." *J Clin Endocrinol Metab* 96, no. 1 (2011): 192-9.

14. Ikeda, K., and T. Yamada. "Ucp1 Dependent and Independent Thermogenesis in Brown and Beige Adipocytes." *Front Endocrinol (Lausanne)* 11 (2020): 498.
15. Coolbaugh, Crystal L., Bruce M. Damon, Emily C. Bush, E. Brian Welch, and Theodore F. Towse. "Cold Exposure Induces Dynamic, Heterogeneous Alterations in Human Brown Adipose Tissue Lipid Content." *Scientific Reports* 9, no. 1 (2019): 13600.
16. Aldiss, P., J. Betts, C. Sale, M. Pope, H. Budge, and M. E. Symonds. "Exercise-Induced 'Browning' of Adipose Tissues." *Metabolism* 81 (2018): 63-70.
17. Zhang, Qiongyue, Qing Miao, Hongying Ye, Zhaoyun Zhang, Chuantao Zuo, Fengchun Hua, Yihui Guan, and Yiming Li. "The Effects of Thyroid Hormones on Brown Adipose Tissue in Humans: A Pet-Ct Study." *Diabetes/Metabolism Research and Reviews* 30, no. 6 (2014): 513-20.
18. Wu, Zhidan, Pere Puigserver, Ulf Andersson, Chenyu Zhang, Guillaume Adelmant, Vamsi Mootha, Amy Troy, Saverio Cinti, Bradford Lowell, Richard C. Scarpulla, and Bruce M. Spiegelman. "Mechanisms Controlling Mitochondrial Biogenesis and Respiration through the Thermogenic Coactivator Pgc-1." *Cell* 98, no. 1 (1999): 115-24.
19. Smith, Robert E., Jane C. Roberts, and Karl J. Hittelman. "Nonphosphorylating Respiration of Mitochondria from Brown Adipose Tissue of Rats." *Science* 154, no. 3749 (1966): 653-54.
20. Kodela, E., M. Moysidou, S. Karaliota, Y. Koutmani, P. Tsakanikas, K. Kodella, E. A. Karavia, K. E. Kypreos, N. Kostomitsopoulos, and K. P. Karalis. "Strain-Specific Differences in the Effects of Lymphocytes on the Development of Insulin Resistance and Obesity in Mice." *Comp Med* 68, no. 1 (2018): 15-24.
21. Brondani, Letícia A., Tais S. Assmann, Bianca M. de Souza, Ana P. Bouças, Luis H. Canani, and Daisy Crispim. "Meta-Analysis Reveals the Association of Common Variants in the Uncoupling Protein (Ucp) 1–3 Genes with Body Mass Index Variability." *PLOS ONE* 9, no. 5 (2014): e96411.
22. Montesanto, A., A. R. Bonfigli, P. Crocco, P. Garagnani, M. De Luca, M. Boemi, E. Marasco, C. Pirazzini, C. Giuliani, C. Franceschi, G. Passarino, R. Testa, F. Olivieri, and G. Rose. "Genes Associated with Type 2 Diabetes and Vascular Complications." *Aging (Albany NY)* 10, no. 2 (2018): 178-96.
23. Labruna, G., F. Pasanisi, C. Nardelli, G. Tarantino, D. F. Vitale, R. Bracale, C. Finelli, M. P. Genua, F. Contaldo, and L. Sacchetti. "Ucp1 -3826 Ag+Gg Genotypes, Adiponectin, and Leptin/Adiponectin Ratio in Severe Obesity." *J Endocrinol Invest* 32, no. 6 (2009): 525-9.
24. Nagai, N., N. Sakane, K. Kotani, T. Hamada, K. Tsuzaki, and T. Moritani. "Uncoupling Protein 1 Gene -3826 a/G Polymorphism Is Associated with Weight Loss on a Short-Term, Controlled-Energy Diet in Young Women." *Nutr Res* 31, no. 4 (2011): 255-61.
25. Cha, M. H., B. K. Kang, D. Suh, K. S. Kim, Y. Yang, and Y. Yoon. "Association of Ucp1 Genetic Polymorphisms with Blood Pressure among Korean Female Subjects." *J Korean Med Sci* 23, no. 5 (2008): 776-80.
26. Sakers, Alexander, Mirian Krystel De Siqueira, Patrick Seale, and Claudio J. Villanueva. "Adipose-Tissue Plasticity in Health and Disease." *Cell* 185, no. 3 (2022): 419-46.
27. Scheja, Ludger, and Joerg Heeren. "The Endocrine Function of Adipose Tissues in Health and Cardiometabolic Disease." *Nature Reviews Endocrinology* 15, no. 9 (2019): 507-24.
28. Reilly, Shannon M., and Alan R. Saltiel. "Adapting to Obesity with Adipose Tissue Inflammation." *Nature Reviews Endocrinology* 13, no. 11 (2017): 633-43.
29. Nguyen, M. T., S. Favelyukis, A. K. Nguyen, D. Reichart, P. A. Scott, A. Jenn, R. Liu-Bryan, C. K. Glass, J. G. Neels, and J. M. Olefsky. "A Subpopulation of Macrophages Infiltrates Hypertrophic Adipose Tissue and Is Activated by Free Fatty Acids Via Toll-Like Receptors 2 and 4 and Jnk-Dependent Pathways." *J Biol Chem* 282, no. 48 (2007): 35279-92.
30. Cuevas-Ramos, D., R. Mehta, and C. A. Aguilar-Salinas. "Fibroblast Growth Factor 21 and Browning of White Adipose Tissue." *Front Physiol* 10 (2019): 37.

31. Wang, Q. A., C. Tao, R. K. Gupta, and P. E. Scherer. "Tracking Adipogenesis During White Adipose Tissue Development, Expansion and Regeneration." *Nat Med* 19, no. 10 (2013): 1338-44.
32. Shao, M., L. Vishvanath, N. C. Busbuso, C. Hepler, B. Shan, A. X. Sharma, S. Chen, X. Yu, Y. A. An, Y. Zhu, W. L. Holland, and R. K. Gupta. "De Novo Adipocyte Differentiation from Pdgfrβ+ Preadipocytes Protects against Pathologic Visceral Adipose Expansion in Obesity." *Nature Communications* 9, no. 1 (2018).
33. Park, J., S. Shin, L. Liu, I. Jahan, S. G. Ong, P. Xu, D. C. Berry, and Y. Jiang. "Progenitor-Like Characteristics in a Subgroup of Ucp1+ Cells within White Adipose Tissue." *Dev Cell* 56, no. 7 (2021): 985-99.e4.
34. Vliora, M., E. Grillo, M. Corsini, C. Ravelli, E. Nintou, E. Karligiotou, A. D. Flouris, and S. Mitola. "Irisin Regulates Thermogenesis and Lipolysis in 3t3-L1 Adipocytes." *Biochim Biophys Acta Gen Subj* 1866, no. 4 (2022): 130085.
35. Valente, Angelica, Athanasios Z. Jamurtas, Yiannis Koutedakis, and Andreas D. Flouris. "Molecular Pathways Linking Non-Shivering Thermogenesis and Obesity: Focusing on Brown Adipose Tissue Development." *Biological Reviews* 90, no. 1 (2015): 77-88.
36. Boström, P., J. Wu, M. P. Jedrychowski, A. Korde, L. Ye, J. C. Lo, K. A. Rasbach, E. A. Boström, J. H. Choi, J. Z. Long, S. Kajimura, M. C. Zingaretti, B. F. Vind, H. Tu, S. Cinti, K. Højlund, S. P. Gygi, and B. M. Spiegelman. "A Pgc1-A-Dependent Myokine That Drives Brown-Fat-Like Development of White Fat and Thermogenesis." *Nature* 481, no. 7382 (2012): 463-8.
37. Fisher, ffolliott M., Sandra Kleiner, Nicholas Douris, Elliott C. Fox, Rina J. Mepani, Francisco Verdeguer, Jun Wu, Alexei Kharitonenkov, Jeffrey S. Flier, Eleftheria Maratos-Flier, and Bruce M. Spiegelman. "Fgf21 Regulates Pgc-1α and Browning of White Adipose Tissues in Adaptive Thermogenesis." *Genes & Development* 26, no. 3 (2012): 271-81.
38. Triandafillou, Joan, Cynthia Gwilliam, and Jean Himms-Hagen. "Role of Thyroid Hormone in Cold-Induced Changes in Rat Brown Adipose Tissue Mitochondria." *Canadian Journal of Biochemistry* 60, no. 5 (1982): 530-37.
39. Seale, P., S. Kajimura, W. Yang, S. Chin, L. M. Rohas, M. Uldry, G. Tavernier, D. Langin, and B. M. Spiegelman. "Transcriptional Control of Brown Fat Determination by Prdm16." *Cell Metab* 6, no. 1 (2007): 38-54.
40. Choi, E. W., M. Lee, J. W. Song, K. Kim, J. Lee, J. Yang, S. H. Lee, I. Y. Kim, J. H. Choi, and J. K. Seong. "Fas Mutation Reduces Obesity by Increasing Il-4 and Il-10 Expression and Promoting White Adipose Tissue Browning." *Sci Rep* 10, no. 1 (2020): 12001.
41. van der Vaart, J. I., M. R. Boon, and R. H. Houtkooper. "The Role of Ampk Signaling in Brown Adipose Tissue Activation." *Cells* 10, no. 5 (2021).
42. Li, Z., Y. Yue, F. Hu, C. Zhang, X. Ma, N. Li, L. Qiu, M. Fu, L. Chen, Z. Yao, P. J. Bilan, A. Klip, and W. Niu. "Electrical Pulse Stimulation Induces Glut4 Translocation in C2c12 Myotubes That Depends on Rab8a, Rab13, and Rab14." *Am J Physiol Endocrinol Metab* 314, no. 5 (2018): E478-E93.
43. Gong, H., L. Liu, C. X. Ni, Y. Zhang, W. J. Su, Y. J. Lian, W. Peng, J. P. Zhang, and C. L. Jiang. "Dexamethasone Rapidly Inhibits Glucose Uptake Via Non-Genomic Mechanisms in Contracting Myotubes." *Arch Biochem Biophys* 603 (2016): 102-9.
44. Dinas, Petros C., Argyro Krase, Eleni Nintou, Alexandros Georgakopoulos, Marnie Granzotto, Marinos Metaxas, Elena Karachaliou, Marco Rossato, Roberto Vettor, Panagiotis Georgoulias, Tiago S. Mayor, John Koutsikos, Konstantinos Athanasiou, Leonidas G. Ioannou, Paraskevi Gkiata, Andres E. Carrillo, Yiannis Koutedakis, George S. Metsios, Athanasios Z. Jamurtas, Sofia Chatziioannou, and Andreas D. Flouris. "Human White-Fat Thermogenesis: Experimental and Meta-Analytic Findings." *Temperature* 8, no. 1 (2021): 39-52.
45. Gielen, Stephan, Gerhard Schuler, and Volker Adams. "Cardiovascular Effects of Exercise Training." *Circulation* 122, no. 12 (2010): 1221-38.

46. Nigro, Pasquale, Maria Vamvini, Jiekun Yang, Tiziana Caputo, Li-Lun Ho, Danae Papadopoulos, Nicholas P. Carbone, Royce Conlin, Jie He, Michael F. Hirshman, Joseph D. White, Jacques Robidoux, Robert C. Hickner, Søren Nielsen, Bente K. Pedersen, Manolis Kellis, Roeland J. W. Middelbeek, and Laurie J. Goodyear. "Exercise Training Remodels Inguinal White Adipose Tissue through Adaptations in Innervation, Vascularization and the Extracellular Matrix." *bioRxiv* (2022): 2022.08.09.503375.
47. Picoli, Caroline de Carvalho, Gustavo Renan Gilio, Felipe Henriques, Luana Garcia Leal, Jean Carlos Besson, Magno Alves Lopes, Solange Marta Franzói de Moraes, Luzmarina Hernandes, Miguel Luiz Batista Junior, and Sidney Barnabé Peres. "Resistance Exercise Training Induces Subcutaneous and Visceral Adipose Tissue Browning in Swiss Mice." *Journal of Applied Physiology* 129, no. 1 (2020): 66-74.
48. Otero-Díaz, B., M. Rodríguez-Flores, V. Sánchez-Muñoz, F. Monraz-Preciado, S. Ordoñez-Ortega, V. Becerril-Elias, G. Baay-Guzmán, R. Obando-Monge, E. García-García, B. Palacios-González, M. T. Villarreal-Molina, M. Sierra-Salazar, and B. Antuna-Puente. "Exercise Induces White Adipose Tissue Browning across the Weight Spectrum in Humans." *Front Physiol* 9 (2018): 1781.
49. Mendez-Gutierrez, A., C. M. Aguilera, F. J. Osuna-Prieto, B. Martinez-Tellez, M. C. Rico Prados, F. M. Acosta, J. M. Llamas-Elvira, J. R. Ruiz, and G. Sanchez-Delgado. "Exercise-Induced Changes on Exerkines That Might Influence Brown Adipose Tissue Metabolism in Young Sedentary Adults." *Eur J Sport Sci* 23, no. 4 (2023): 625-36.
50. Vosselman, M. J., J. Hoeks, B. Brans, H. Pallubinsky, E. B. Nascimento, A. A. van der Lans, E. P. Broeders, F. M. Mottaghy, P. Schrauwen, and W. D. van Marken Lichtenbelt. "Low Brown Adipose Tissue Activity in Endurance-Trained Compared with Lean Sedentary Men." *Int J Obes (Lond)* 39, no. 12 (2015): 1696-702.
51. Dreher, Simon I., Martin Irmler, Olga Pivovarova-Ramich, Katharina Kessler, Karsten Jürchott, Carsten Sticht, Louise Fritsche, Patrick Schneeweiss, Jürgen Machann, Andreas F. H. Pfeiffer, Martin Hrabě de Angelis, Johannes Beckers, Andreas L. Birkenfeld, Andreas Peter, Andreas M. Niess, Cora Weigert, and Anja Moller. "Acute and Long-Term Exercise Adaptation of Adipose Tissue and Skeletal Muscle in Humans: A Matched Transcriptomics Approach after 8-Week Training-Intervention." *International Journal of Obesity* (2023).
52. Tsiloulis, T., A. L. Carey, J. Bayliss, B. Canny, R. C. R. Meex, and M. J. Watt. "No Evidence of White Adipocyte Browning after Endurance Exercise Training in Obese Men." *Int J Obes (Lond)* 42, no. 4 (2018): 721-27.
53. Motiani, P., K. A. Virtanen, K. K. Motiani, J. J. Eskelinen, R. J. Middelbeek, L. J. Goodyear, A. M. Savolainen, J. Kemppainen, J. Jensen, M. U. Din, V. Saunavaara, R. Parkkola, E. Löyttyniemi, J. Knuuti, P. Nuutila, K. K. Kalliokoski, and J. C. Hannukainen. "Decreased Insulin-Stimulated Brown Adipose Tissue Glucose Uptake after Short-Term Exercise Training in Healthy Middle-Aged Men." *Diabetes Obes Metab* 19, no. 10 (2017): 1379-88.
54. de Jong, J. M., O. Larsson, B. Cannon, and J. Nedergaard. "A Stringent Validation of Mouse Adipose Tissue Identity Markers." *Am J Physiol Endocrinol Metab* 308, no. 12 (2015): E1085-105.
55. Dinas, P. C., I. M. Lahart, J. A. Timmons, P. A. Svensson, Y. Koutedakis, A. D. Flouris, and G. S. Metsios. "Effects of Physical Activity on the Link between Pgc-1a and Fndc5 in Muscle, Circulating Irisin and Ucp1 of White Adipocytes in Humans: A Systematic Review." *F1000Res* 6 (2017): 286.
56. Banan Sadeghian, Ramin, Majid Ebrahimi, and Sahar Salehi. "Electrical Stimulation of Microengineered Skeletal Muscle Tissue: Effect of Stimulus Parameters on Myotube Contractility and Maturation." *Journal of Tissue Engineering and Regenerative Medicine* 12, no. 4 (2018): 912-22.
57. Groop, L. "Genetics of the Metabolic Syndrome." *Br J Nutr* 83 Suppl 1 (2000): S39-48.

58. Mirkov, S., J. L. Myers, J. Ramírez, and W. Liu. "Snps Affecting Serum Metabolomic Traits May Regulate Gene Transcription and Lipid Accumulation in the Liver." *Metabolism* 61, no. 11 (2012): 1523-7.
59. Shastry, B. S. "Snp Alleles in Human Disease and Evolution." *J Hum Genet* 47, no. 11 (2002): 561-6.
60. Dinas, P. C., A. Valente, M. Granzotto, M. Rossato, R. Vettor, A. Zacharopoulou, A. E. Carrillo, N. A. Davies, P. Gkiata, A. Z. Jamurtas, Y. Koutedakis, G. S. Metsios, and A. D. Flouris. "Browning Formation Markers of Subcutaneous Adipose Tissue in Relation to Resting Energy Expenditure, Physical Activity and Diet in Humans." *Horm Mol Biol Clin Investig* 31, no. 1 (2017).
61. Valente, A., A. Z. Jamurtas, Y. Koutedakis, and A. D. Flouris. "Molecular Pathways Linking Non-Shivering Thermogenesis and Obesity: Focusing on Brown Adipose Tissue Development." *Biol Rev Camb Philos Soc* 90, no. 1 (2015): 77-88.
62. Chathoth, S., M. H. Ismail, C. Vatte, C. Cyrus, Z. Al Ali, K. A. Ahmed, S. Acharya, A. M. Al Barqi, and A. Al Ali. "Association of Uncoupling Protein 1 (Ucp1) Gene Polymorphism with Obesity: A Case-Control Study." *BMC Med Genet* 19, no. 1 (2018): 203.
63. Franco-Hincapie, L., C. E. Duque, M. V. Parra, N. Gallego, A. Villegas, A. Ruiz-Linares, and G. Bedoya. "[Association between Polymorphism in Uncoupling Proteins and Type 2 Diabetes in a Northwestern Colombian Population]." *Biomedica* 29, no. 1 (2009): 108-18.
64. Jia, J. J., Y. B. Tian, Z. H. Cao, L. L. Tao, X. Zhang, S. Z. Gao, C. R. Ge, Q. Y. Lin, and M. Jois. "The Polymorphisms of Ucp1 Genes Associated with Fat Metabolism, Obesity and Diabetes." *Mol Biol Rep* 37, no. 3 (2010): 1513-22.
65. Lim, J. H., M. M. Ko, T. W. Moon, M. H. Cha, and M. S. Lee. "Association of the Ucp-1 Single Nucleotide Polymorphism a-3826g with the Dampness-Phlegm Pattern among Korean Stroke Patients." *BMC Complement Altern Med* 12 (2012): 180.
66. Pravednikova, Anna E., Sergey Y. Shevchenko, Victor V. Kerchev, Manana R. Skhirtladze, Svetlana N. Larina, Zaur M. Kachaev, Alexander D. Egorov, and Yulii V. Shidlovskii. "Association of Uncoupling Protein (Ucp) Gene Polymorphisms with Cardiometabolic Diseases." *Molecular Medicine* 26, no. 1 (2020): 51.
67. Brondani, L. A., T. S. Assmann, G. C. Duarte, J. L. Gross, L. H. Canani, and D. Crispim. "The Role of the Uncoupling Protein 1 (Ucp1) on the Development of Obesity and Type 2 Diabetes Mellitus." *Arq Bras Endocrinol Metabol* 56, no. 4 (2012): 215-25.
68. Esterbauer, H., H. Oberkofler, Y. M. Liu, D. Breban, E. Hell, F. Krempler, and W. Patsch. "Uncoupling Protein-1 Mrna Expression in Obese Human Subjects: The Role of Sequence Variations at the Uncoupling Protein-1 Gene Locus." *J Lipid Res* 39, no. 4 (1998): 834-44.
69. Hayakawa, T., Y. Nagai, M. Taniguchi, H. Yamashita, T. Takamura, T. Abe, G. Nomura, and K. Kobayashi. "Phenotypic Characterization of the Beta3-Adrenergic Receptor Mutation and the Uncoupling Protein 1 Polymorphism in Japanese Men." *Metabolism* 48, no. 5 (1999): 636-40.
70. Ramis, J. M., J. L. Gonzalez-Sanchez, A. M. Proenza, M. T. Martinez-Larrad, C. Fernandez-Perez, A. Palou, and M. Serrano-Rios. "The Arg64 Allele of the Beta 3-Adrenoceptor Gene but Not the -3826g Allele of the Uncoupling Protein 1 Gene Is Associated with Increased Leptin Levels in the Spanish Population." *Metabolism* 53, no. 11 (2004): 1411-6.
71. Forga, Ll, M. Corbalan, A. Marti, C. Fuentes, M. A. Martinez-Gonzalez, and A. Martinez. "[Influence of the Polymorphism 03826 a --> G in the Ucp1 Gene on the Components of Metabolic Syndrome]." *An Sist Sanit Navar* 26, no. 2 (2003): 231-6.
72. Oh, H. H., K. S. Kim, S. M. Choi, H. S. Yang, and Y. Yoon. "The Effects of Uncoupling Protein-1 Genotype on Lipoprotein Cholesterol Level in Korean Obese Subjects." *Metabolism* 53, no. 8 (2004): 1054-9.

73. Heilbronn, L. K., K. L. Kind, E. Pancewicz, A. M. Morris, M. Noakes, and P. M. Clifton. "Association of -3826 G Variant in Uncoupling Protein-1 with Increased Bmi in Overweight Australian Women." *Diabetologia* 43, no. 2 (2000): 242-4.
74. Zhang, Y., N. Meng, Z. Lv, H. Li, and Y. Qu. "The Gene Polymorphisms of Ucp1 but Not Ppar Γ and Tcf7l2 Are Associated with Diabetic Retinopathy in Chinese Type 2 Diabetes Mellitus Cases." *Acta Ophthalmol* 93, no. 3 (2015): e223-9.
75. Fukuyama, K., T. Ohara, Y. Hirota, K. Maeda, S. Kuno, M. Zenibayashi, T. Teranishi, K. Kouyama, E. Maeda, N. Sakamoto, and M. Kasuga. "Association of the -112a>C Polymorphism of the Uncoupling Protein 1 Gene with Insulin Resistance in Japanese Individuals with Type 2 Diabetes." *Biochem Biophys Res Commun* 339, no. 4 (2006): 1212-6.
76. Mori, H., H. Okazawa, K. Iwamoto, E. Maeda, M. Hashiramoto, and M. Kasuga. "A Polymorphism in the 5' Untranslated Region and a Met229-->Leu Variant in Exon 5 of the Human Ucp1 Gene Are Associated with Susceptibility to Type Ii Diabetes Mellitus." *Diabetologia* 44, no. 3 (2001): 373-6.
77. Herrmann, Stefan-Martin, Ji-Guang Wang, Jan A. Staessen, Ercan Kertmen, Klaus Schmidt-Petersen, Walter Zidek, Martin Paul, and Eva Brand. "Uncoupling Protein 1 and 3 Polymorphisms Are Associated with Waist-to-Hip Ratio." *Journal of Molecular Medicine* 81, no. 5 (2003): 327-32.
78. Soo Kim, Kil, Dae-Yeon Cho, Young Joo Kim, Sun Mi Choi, Jong Yeol Kim, Seung Uoo Shin, and Yoo Sik Yoon. "The Finding of New Genetic Polymorphism of Ucp-1 a-1766g and Its Effects on Body Fat Accumulation." *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1741, no. 1 (2005): 149-55.
79. Shin, H. D., K. S. Kim, M. H. Cha, and Y. Yoon. "The Effects of Ucp-1 Polymorphisms on Obesity Phenotypes among Korean Female Subjects." *Biochem Biophys Res Commun* 335, no. 2 (2005): 624-30.
80. Pei, X., L. Liu, J. Cai, W. Wei, Y. Shen, Y. Wang, Y. Chen, P. Sun, M. U. Imam, Z. Ping, and X. Fu. "Haplotype-Based Interaction of the Ppargc1a and Ucp1 Genes Is Associated with Impaired Fasting Glucose or Type 2 Diabetes Mellitus." *Medicine (Baltimore)* 96, no. 23 (2017): e6941.
81. Vimaleswaran, K. S., V. Radha, S. Ghosh, P. P. Majumder, M. R. Rao, and V. Mohan. "A Haplotype at the Ucp1 Gene Locus Contributes to Genetic Risk for Type 2 Diabetes in Asian Indians (Cures-72)." *Metab Syndr Relat Disord* 8, no. 1 (2010): 63-8.
82. Cha, Min Ho, Byoung Kab Kang, Dongchul Suh, Kil Soo Kim, Young Yang, and Yoosik Yoon. "Association of Ucp1 Genetic Polymorphisms with Blood Pressure among Korean Female Subjects." *Journal of Korean medical science* 23, no. 5 (2008): 776-80.
83. de Souza, B. M., L. A. Brondani, A. P. Bouças, D. A. Sortica, C. K. Kramer, L. H. Canani, C. B. Leitão, and D. Crispim. "Associations between Ucp1 -3826a/G, Ucp2 -866g/a, Ala55val and Ins/Del, and Ucp3 -55c/T Polymorphisms and Susceptibility to Type 2 Diabetes Mellitus: Case-Control Study and Meta-Analysis." *PLoS One* 8, no. 1 (2013): e54259.
84. Malczewska-Malec, M., I. Wybranska, I. Leszczynska-Golabek, L. Partyka, J. Hartwich, A. Jabrocka, B. Kiec-Wilk, M. Kwasniak, M. Motyka, and A. Dembinska-Kiec. "Analysis of Candidate Genes in Polish Families with Obesity." *Clin Chem Lab Med* 42, no. 5 (2004): 487-93.
85. Schaffler, A., K. D. Palitzsch, E. Watzlawek, W. Drobnik, H. Schwer, J. Scholmerich, and G. Schmitz. "Frequency and Significance of the a-->G (-3826) Polymorphism in the Promoter of the Gene for Uncoupling Protein-1 with Regard to Metabolic Parameters and Adipocyte Transcription Factor Binding in a Large Population-Based Caucasian Cohort." *Eur J Clin Invest* 29, no. 9 (1999): 770-9.
86. Balkau, Beverley, John E. Deanfield, Jean-Pierre Després, Jean-Pierre Bassand, Keith A. A. Fox, Sidney C. Smith, Jr., Philip Barter, Chee-Eng Tan, Luc Van Gaal, Hans-Ulrich Wittchen, Christine

Massien, and Steven M. Haffner. "International Day for the Evaluation of Abdominal Obesity (Idea): A Study of Waist Circumference, Cardiovascular Disease, and Diabetes Mellitus in 168,000 Primary Care Patients in 63 Countries." *Circulation* 116, no. 17 (2007): 1942-51.

87. WHO. "Global Atlas on Cardiovascular Disease Prevention and Control. Geneva: World Health Organization;World Heart Federation; World Stroke Organization." (2011).
88. Higgins JP, and J. Thomas. *Cochrane Handbook for Systematic Reviews of Interventions*, Version 6: Cochrane collaboration 2019.
89. de Almeida Brondani, Letícia, Bianca Marmontel de Souza, Taís Silveira Assmann, Ana Paula Bouças, Andrea Carla Bauer, Luís Henrique Canani, and Daisy Crispim. "Association of the Ucp Polymorphisms with Susceptibility to Obesity: Case–Control Study and Meta-Analysis." *Molecular biology reports* 41, no. 8 (2014): 5053-67.
90. Liu, Xujia, Zehua Jiang, Guihua Zhang, Tsz Kin Ng, and Zhenggen Wu. "Association of Ucp1 and Ucp2 Variants with Diabetic Retinopathy Susceptibility in Type-2 Diabetes Mellitus Patients: A Meta-Analysis." *BMC ophthalmology* 21, no. 1 (2021): 1-12.
91. WHO. "Cardiovascular Diseases (Cvds)." 2017.
92. ———. "Noncommunicable Diseases." 2018.
93. Clarke, Geraldine M., Carl A. Anderson, Fredrik H. Pettersson, Lon R. Cardon, Andrew P. Morris, and Krina T. Zondervan. "Basic Statistical Analysis in Genetic Case-Control Studies." *Nature Protocols* 6, no. 2 (2011): 121-33.
94. Lunetta, K. L. "Genetic Association Studies." *Circulation* 118, no. 1 (2008): 96-101.
95. Namipashaki, A., Z. Razaghi-Moghadam, and N. Ansari-Pour. "The Essentiality of Reporting Hardy-Weinberg Equilibrium Calculations in Population-Based Genetic Association Studies." *Cell J* 17, no. 2 (2015): 187-92.
96. Li, Z., Z. Zhang, Z. He, W. Tang, T. Li, Z. Zeng, L. He, and Y. Shi. "A Partition-Ligation-Combination-Subdivision Em Algorithm for Haplotype Inference with Multiallelic Markers: Update of the Shesis (Http://Analysis.Bio-X.Cn)." *Cell Res* 19, no. 4 (2009): 519-23.
97. Shi, Y. Y., and L. He. "Shesis, a Powerful Software Platform for Analyses of Linkage Disequilibrium, Haplotype Construction, and Genetic Association at Polymorphism Loci." *Cell Res* 15, no. 2 (2005): 97-8.
98. Kim, Hae-Young. "Statistical Notes for Clinical Researchers: Chi-Squared Test and Fisher's Exact Test." *Restorative dentistry & endodontics* 42, no. 2 (2017): 152-55.
99. Feise, R. J. "Do Multiple Outcome Measures Require P-Value Adjustment?" *BMC Med Res Methodol* 2 (2002): 8.
100. Perneger, T. V. "What's Wrong with Bonferroni Adjustments." *BMJ* 316, no. 7139 (1998): 1236-8.
101. Rothman, K. J. "No Adjustments Are Needed for Multiple Comparisons." *Epidemiology* 1, no. 1 (1990): 43-6.
102. ———. "Six Persistent Research Misconceptions." *J Gen Intern Med* 29, no. 7 (2014): 1060-4.
103. Moher, D., A. Liberati, J. Tetzlaff, D. G. Altman, and Prisma Group. "Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The Prisma Statement." *PLoS Med* 6, no. 7 (2009): e1000097.
104. Bracale, Renata, Giuseppe Labruna, Carmine Finelli, Aurora Daniele, Lucia Sacchetti, Giovannangelo Oriani, F. Contaldo, and Fabrizio Pasanisi. "The Absence of Polymorphisms in Adrb3, Ucp1, Pparγ, and Adipoq Genes Protects Morbid Obese Patients toward Insulin Resistance." *Journal of endocrinological investigation* 35 (2012): 2-4.
105. Brondani, Leticia A, Tais S Assmann, Bianca M de Souza, Ana P Boucas, Luis H Canani, and Daisy Crispim. "Meta-Analysis Reveals the Association of Common Variants in the Uncoupling Protein (Ucp) 1–3 Genes with Body Mass Index Variability." *PloS one* 9, no. 5 (2014): e96411.

106. Brondani, L. A., B. M. de Souza, T. S. Assmann, A. P. Bouças, A. C. Bauer, L. H. Canani, and D. Crispim. "Association of the Ucp Polymorphisms with Susceptibility to Obesity: Case-Control Study and Meta-Analysis." *Mol Biol Rep* 41, no. 8 (2014): 5053-67.
107. Chen, Y., X. Wang, Z. Shen, P. Fan, R. Liu, Y. Liu, R. Ren, L. Ma, and H. Bai. "Effect of the Beta-3 Adrenergic Receptor Trp64arg and Uncoupling Protein 1-3826 a>G Genotypes on Lipid and Apolipoprotein Levels in Overweight/Obese and Non-Obese Chinese Subjects." *Lipids Health Dis* 14 (2015): 34.
108. Csernus, K., G. Pauler, É Erhardt, É Lányi, and D. Molnár. "Effects of Energy Expenditure Gene Polymorphisms on Obesity-Related Traits in Obese Children." *Obes Res Clin Pract* 9, no. 2 (2015): 133-40.
109. Dhall, M., M. M. Chaturvedi, U. Rai, and S. Kapoor. "Sex-Dependent Effects of the Ucp1 -3826 a/G Polymorphism on Obesity and Blood Pressure." *Ethn Dis* 22, no. 2 (2012): 181-4.
110. Gagnon, J., F. Lago, Y. C. Chagnon, L. Pérusse, I. Näslund, L. Lissner, L. Sjöström, and C. Bouchard. "DNA Polymorphism in the Uncoupling Protein 1 (Ucp1) Gene Has No Effect on Obesity Related Phenotypes in the Swedish Obese Subjects Cohorts." *Int J Obes Relat Metab Disord* 22, no. 6 (1998): 500-5.
111. Hamada, T., K. Kotani, N. Nagai, K. Tsuzaki, Y. Matsuoka, Y. Sano, M. Fujibayashi, N. Kiyohara, S. Tanaka, M. Yoshimura, K. Egawa, Y. Kitagawa, Y. Kiso, T. Moritani, and N. Sakane. "Low-Calorie Diet-Induced Reduction in Serum Hdl Cholesterol Is Ameliorated in Obese Women with the -3826 G Allele in the Uncoupling Protein-1 Gene." *Tohoku J Exp Med* 219, no. 4 (2009): 337-42.
112. Jin, P., Z. Li, X. Xu, J. He, J. Chen, X. Xu, X. Du, X. Bai, B. Zhang, X. He, L. Lu, J. Zhu, Y. Shi, and H. Zou. "Analysis of Association between Common Variants of Uncoupling Proteins Genes and Diabetic Retinopathy in a Chinese Population." *BMC Med Genet* 21, no. 1 (2020): 25.
113. Kieć-Wilk, B., I. Wybrańska, M. Malczewska-Malec, L. Leszczyńska-Gołabek, L. Partyka, S. Niedbał, A. Jabrocka, and A. Dembińska-Kieć. "Correlation of the -3826a >G Polymorphism in the Promoter of the Uncoupling Protein 1 Gene with Obesity and Metabolic Disorders in Obese Families from Southern Poland." *J Physiol Pharmacol* 53, no. 3 (2002): 477-90.
114. Kotani, K., S. Fujiwara, K. Tsuzaki, Y. Sano, N. Nagai, T. Yamada, and N. Sakane. "The Association between the Uncoupling Protein-1 Gene a-3826g Polymorphism and High-Density Lipoprotein Cholesterol in a General Japanese Population: A Consideration of the Obesity Status." *J Clin Med Res* 3, no. 6 (2011): 319-24.
115. Kotani, K., N. Sakane, K. Saiga, S. Adachi, H. Shimohiro, H. Mu, and Y. Kurozawa. "Relationship between a-3826g Polymorphism in the Promoter of the Uncoupling Protein-1 Gene and High-Density Lipoprotein Cholesterol in Japanese Individuals: A Cross-Sectional Study." *Arch Med Res* 39, no. 1 (2008): 142-6.
116. Lim, Ji Hye, Mi Mi Ko, Tae-Woong Moon, Min Ho Cha, and Myeong Soo Lee. "Association of the Ucp-1 Single Nucleotide Polymorphism a-3826g with the Dampness-Phlegm Pattern among Korean Stroke Patients." *BMC complementary and alternative medicine* 12 (2012): 180-80.
117. Lin, E., D. Pei, Y. J. Huang, C. H. Hsieh, and L. S. Wu. "Gene-Gene Interactions among Genetic Variants from Obesity Candidate Genes for Nonobese and Obese Populations in Type 2 Diabetes." *Genet Test Mol Biomarkers* 13, no. 4 (2009): 485-93.
118. Lindholm, E., M. Klannemark, E. Agardh, L. Groop, and C. D. Agardh. "Putative Role of Polymorphisms in Ucp1-3 Genes for Diabetic Nephropathy." *J Diabetes Complications* 18, no. 2 (2004): 103-7.
119. Mottagui-Tabar, S., J. Hoffstedt, A. J. Brookes, H. Jiao, P. Arner, and I. Dahlman. "Association of Adrb1 and Ucp3 Gene Polymorphisms with Insulin Sensitivity but Not Obesity." *Horm Res* 69, no. 1 (2008): 31-6.

120. Nakatochi, M., Y. Ushida, Y. Yasuda, Y. Yoshida, S. Kawai, R. Kato, T. Nakashima, M. Iwata, Y. Kuwatsuka, M. Ando, N. Hamajima, T. Kondo, H. Oda, M. Hayashi, S. Kato, M. Yamaguchi, S. Maruyama, S. Matsuo, and H. Honda. "Identification of an Interaction between Vwf Rs7965413 and Platelet Count as a Novel Risk Marker for Metabolic Syndrome: An Extensive Search of Candidate Polymorphisms in a Case-Control Study." *PLoS One* 10, no. 2 (2015): e0117591.
121. Nicoletti, C. F., A. P. de Oliveira, M. J. Brochado, B. P. de Oliveira, M. A. Pinhel, J. S. Marchini, J. E. dos Santos, W. Salgado Junior, W. A. Silva Junior, and C. B. Nonino. "Ucp1 -3826 a>G Polymorphism Affects Weight, Fat Mass, and Risk of Type 2 Diabetes Mellitus in Grade Iii Obese Patients." *Nutrition* 32, no. 1 (2016): 83-7.
122. Nieters, A., N. Becker, and J. Linseisen. "Polymorphisms in Candidate Obesity Genes and Their Interaction with Dietary Intake of N-6 Polyunsaturated Fatty Acids Affect Obesity Risk in a Sub-Sample of the Epic-Heidelberg Cohort." *Eur J Nutr* 41, no. 5 (2002): 210-21.
123. Proenza, A. M., C. M. Poissonnet, M. Ozata, S. Ozen, S. Guran, A. Palou, and A. D. Strosberg. "Association of Sets of Alleles of Genes Encoding Beta3-Adrenoreceptor, Uncoupling Protein 1 and Lipoprotein Lipase with Increased Risk of Metabolic Complications in Obesity." *Int J Obes Relat Metab Disord* 24, no. 1 (2000): 93-100.
124. Rudofsky, G., Jr., A. Schrödter, O. E. Voron'ko, A. Schlotterer, P. M. Humpert, J. Tafel, P. P. Nawroth, A. Bierhaus, and A. Hamann. "Promoter Polymorphisms of Ucp1, Ucp2, and Ucp3 Are Not Associated with Diabetic Microvascular Complications in Type 2 Diabetes." *Horm Metab Res* 39, no. 4 (2007): 306-9.
125. Rudofsky, G., Jr., A. Schroedter, A. Schlotterer, O. E. Voron'ko, M. Schlimme, J. Tafel, B. H. Isermann, P. M. Humpert, M. Morcos, A. Bierhaus, P. P. Nawroth, and A. Hamann. "Functional Polymorphisms of Ucp2 and Ucp3 Are Associated with a Reduced Prevalence of Diabetic Neuropathy in Patients with Type 1 Diabetes." *Diabetes Care* 29, no. 1 (2006): 89-94.
126. Sale, M. M., F. C. Hsu, N. D. Palmer, C. J. Gordon, K. L. Keene, H. M. Borgerink, A. J. Sharma, R. N. Bergman, K. D. Taylor, M. F. Saad, and J. M. Norris. "The Uncoupling Protein 1 Gene, Ucp1, Is Expressed in Mammalian Islet Cells and Associated with Acute Insulin Response to Glucose in African American Families from the Iras Family Study." *BMC Endocr Disord* 7 (2007): 1.
127. Sámano, R., C. Huesca-Gómez, R. López-Marure, A. K. Hernández-Cabrera, A. Rodríguez-Ventura, M. Tolentino, R. M. Morales, and R. Gamboa. "Association between Ucp Polymorphisms and Adipokines with Obesity in Mexican Adolescents." *J Pediatr Endocrinol Metab* 31, no. 5 (2018): 561-68.
128. Sivenius, K., R. Valve, V. Lindi, L. Niskanen, M. Laakso, and M. Uusitupa. "Synergistic Effect of Polymorphisms in Uncoupling Protein 1 and Beta3-Adrenergic Receptor Genes on Long-Term Body Weight Change in Finnish Type 2 Diabetic and Non-Diabetic Control Subjects." *Int J Obes Relat Metab Disord* 24, no. 4 (2000): 514-9.
129. Sramkova, D., S. Krejbichova, J. Vcelak, M. Vankova, P. Samalikova, M. Hill, H. Kvasnickova, K. Dvorakova, K. Vondra, V. Hainer, and B. Bendlova. "The Ucp1 Gene Polymorphism a-3826g in Relation to Dm2 and Body Composition in Czech Population." *Exp Clin Endocrinol Diabetes* 115, no. 5 (2007): 303-7.
130. Sun, H., J. T. Zhang, X. R. Xie, T. Li, X. Y. Li, N. N. Wang, J. P. Li, Z. H. Deng, and C. C. Qiu. "Association of Uncoupling Protein Gene Polymorphisms with Essential Hypertension in a Northeastern Han Chinese Population." *J Hum Hypertens* 33, no. 7 (2019): 524-30.
131. Tiwari, A. K., P. Prasad, K. T. B, K. M. Kumar, A. C. Ammini, A. Gupta, and R. Gupta. "Oxidative Stress Pathway Genes and Chronic Renal Insufficiency in Asian Indians with Type 2 Diabetes." *J Diabetes Complications* 23, no. 2 (2009): 102-11.

132. Verdi, H., S. T. Kınık, H. P. Baysan-Çebi, Y. Y. Yalçın, A. C. Yazıcı-Güvercin, B. Aydın, N. B. Tütüncü, and F. B. Ataç. "Uncoupling Protein Gene Ucp1-3826a/G, Ucp2 Ins/Del and Ucp3-55c/T Polymorphisms in Obese Turkish Children." *Turk J Pediatr* 62, no. 6 (2020): 921-29.
133. Vimaleswaran, K. S., V. Radha, R. Deepa, and V. Mohan. "Absence of Association of Metabolic Syndrome with Ppargc1a, Pparg and Ucp1 Gene Polymorphisms in Asian Indians." *Metab Syndr Relat Disord* 5, no. 2 (2007): 153-62.
134. Yiew, S. K., L. Y. Khor, M. L. Tan, C. L. Pang, V. Y. Chai, S. S. Kanachamy, and Y. H. Say. "No Association between Peroxisome Proliferator-Activated Receptor and Uncoupling Protein Gene Polymorphisms and Obesity in Malaysian University Students." *Obes Res Clin Pract* 4, no. 4 (2010): e247-342.
135. Zhang, Y., N. Meng, Z. Lv, H. Li, and Y. Qu. "The Gene Polymorphisms of Ucp1 but Not Ppar Gamma and Tcf7l2 Are Associated with Diabetic Retinopathy in Chinese Type 2 Diabetes Mellitus Cases." *Acta Ophthalmol* 93, no. 3 (2015): e223-9.
136. Zietz, B., E. Watzlawek, K. D. Palitzsch, J. Schölmerich, and A. Schäffler. "Gg-Genotype in the Promotor Region of Uncoupling-Protein-1 Gene Is Associated with Lower Level of Dehydroepiandrosterone in Type 2 Diabetes." *Exp Clin Endocrinol Diabetes* 109, no. 2 (2001): 102-6.
137. Schwartz, D. A. "Environmental Genomics and Human Health." *G Ital Med Lav Ergon* 33, no. 1 (2011): 31-4.
138. Yoneshiro, T., T. Ogawa, N. Okamoto, M. Matsushita, S. Aita, T. Kameya, Y. Kawai, T. Iwanaga, and M. Saito. "Impact of Ucp1 and B3ar Gene Polymorphisms on Age-Related Changes in Brown Adipose Tissue and Adiposity in Humans." *International Journal of Obesity* 37, no. 7 (2013): 993-98.
139. Ward, Lucas D., and Manolis Kellis. "Haploreg V4: Systematic Mining of Putative Causal Variants, Cell Types, Regulators and Target Genes for Human Complex Traits and Disease." *Nucleic acids research* 44, no. D1 (2016): D877-D81.
140. Vegiopoulos, A., M. Rohm, and S. Herzig. "Adipose Tissue: Between the Extremes." *Embo j* 36, no. 14 (2017): 1999-2017.
141. Bartelt, A., and J. Heeren. "Adipose Tissue Browning and Metabolic Health." *Nat Rev Endocrinol* 10, no. 1 (2014): 24-36.
142. Cinti, S. "Adipose Tissues and Obesity." *Ital J Anat Embryol* 104, no. 2 (1999): 37-51.
143. *Meta-Analytic Interval Estimation for Standardized and Unstandardized Mean Differences*.
144. Qiu, Y., L. Sun, X. Hu, X. Zhao, H. Shi, Z. Liu, and X. Yin. "Compromised Browning Plasticity of Primary Subcutaneous Adipocytes Derived from Overweight Chinese Adults." *Diabetol Metab Syndr* 12 (2020): 91.
145. Wang, W., M. Kissig, S. Rajakumari, L. Huang, H. W. Lim, K. J. Won, and P. Seale. "Ebf2 Is a Selective Marker of Brown and Beige Adipogenic Precursor Cells." *Proc Natl Acad Sci U S A* 111, no. 40 (2014): 14466-71.
146. Rao, R. R., J. Z. Long, J. P. White, K. J. Svensson, J. Lou, I. Lokurkar, M. P. Jedrychowski, J. L. Ruas, C. D. Wrann, J. C. Lo, D. M. Camera, J. Lachey, S. Gygi, J. Seehra, J. A. Hawley, and B. M. Spiegelman. "Meteorin-Like Is a Hormone That Regulates Immune-Adipose Interactions to Increase Beige Fat Thermogenesis." *Cell* 157, no. 6 (2014): 1279-91.
147. Heinonen, I., K. K. Kalliokoski, J. C. Hannukainen, D. J. Duncker, P. Nuutila, and J. Knuuti. "Organ-Specific Physiological Responses to Acute Physical Exercise and Long-Term Training in Humans." *Physiology (Bethesda)* 29, no. 6 (2014): 421-36.
148. Stanford, K. I., R. J. Middelbeek, K. L. Townsend, M. Y. Lee, H. Takahashi, K. So, K. M. Hitchcox, K. R. Markan, K. Hellbach, M. F. Hirshman, Y. H. Tseng, and L. J. Goodyear. "A Novel Role for Subcutaneous Adipose Tissue in Exercise-Induced Improvements in Glucose Homeostasis." *Diabetes* 64, no. 6 (2015): 2002-14.

149. Golbidi, S., and I. Laher. "Exercise Induced Adipokine Changes and the Metabolic Syndrome." *J Diabetes Res* 2014 (2014): 726861.
150. Cao, L., E. Y. Choi, X. Liu, A. Martin, C. Wang, X. Xu, and M. J. During. "White to Brown Fat Phenotypic Switch Induced by Genetic and Environmental Activation of a Hypothalamic-Adipocyte Axis." *Cell Metab* 14, no. 3 (2011): 324-38.
151. Dewal, R. S., and K. I. Stanford. "Effects of Exercise on Brown and Beige Adipocytes." *Biochim Biophys Acta Mol Cell Biol Lipids* 1864, no. 1 (2019): 71-78.
152. Flouris, Andreas D., Petros C. Dinas, Angelica Valente, Cláudia Marlise Balbinotti Andrade, Nair Honda Kawashita, and Paraskevi Sakellariou. "Exercise-Induced Effects on Ucp1 Expression in Classical Brown Adipose Tissue: A Systematic Review." 31, no. 2 (2017).
153. Knudsen, J. G., M. Murholm, A. L. Carey, R. S. Biensø, A. L. Basse, T. L. Allen, J. Hidalgo, B. A. Kingwell, M. A. Febbraio, J. B. Hansen, and H. Pilegaard. "Role of Il-6 in Exercise Training- and Cold-Induced Ucp1 Expression in Subcutaneous White Adipose Tissue." *PLoS One* 9, no. 1 (2014): e84910.
154. Daskalopoulou, S. S., A. B. Cooke, Y. H. Gomez, A. F. Mutter, A. Filippaios, E. T. Mesfum, and C. S. Mantzoros. "Plasma Irisin Levels Progressively Increase in Response to Increasing Exercise Workloads in Young, Healthy, Active Subjects." *Eur J Endocrinol* 171, no. 3 (2014): 343-52.
155. Jedrychowski, M. P., C. D. Wrann, J. A. Paulo, K. K. Gerber, J. Szpyt, M. M. Robinson, K. S. Nair, S. P. Gygi, and B. M. Spiegelman. "Detection and Quantitation of Circulating Human Irisin by Tandem Mass Spectrometry." *Cell Metab* 22, no. 4 (2015): 734-40.
156. Wu, J., P. Boström, L. M. Sparks, L. Ye, J. H. Choi, A. H. Giang, M. Khandekar, K. A. Virtanen, P. Nuutila, G. Schaart, K. Huang, H. Tu, W. D. van Marken Lichtenbelt, J. Hoeks, S. Enerbäck, P. Schrauwen, and B. M. Spiegelman. "Beige Adipocytes Are a Distinct Type of Thermogenic Fat Cell in Mouse and Human." *Cell* 150, no. 2 (2012): 366-76.
157. Raschke, S., K. Eckardt, K. Bjørklund Holven, J. Jensen, and J. Eckel. "Identification and Validation of Novel Contraction-Regulated Myokines Released from Primary Human Skeletal Muscle Cells." *PLoS One* 8, no. 4 (2013): e62008.
158. Scheler, M., M. Irmler, S. Lehr, S. Hartwig, H. Staiger, H. Al-Hasani, J. Beckers, M. H. de Angelis, H. U. Häring, and C. Weigert. "Cytokine Response of Primary Human Myotubes in an in Vitro Exercise Model." *Am J Physiol Cell Physiol* 305, no. 8 (2013): C877-86.
159. Grygiel-Górniak, B., and M. Puszczewicz. "A Review on Irisin, a New Protagonist That Mediates Muscle-Adipose-Bone-Neuron Connectivity." *Eur Rev Med Pharmacol Sci* 21, no. 20 (2017): 4687-93.
160. Nikolić, N., S. S. Bakke, E. T. Kase, I. Rudberg, I. Flo Halle, A. C. Rustan, G. H. Thoresen, and V. Aas. "Electrical Pulse Stimulation of Cultured Human Skeletal Muscle Cells as an in Vitro Model of Exercise." *PLoS One* 7, no. 3 (2012): e33203.
161. Chen, W., M. R. Nyasha, M. Koide, M. Tsuchiya, N. Suzuki, Y. Hagiwara, M. Aoki, and M. Kanzaki. "In Vitro Exercise Model Using Contractile Human and Mouse Hybrid Myotubes." *Sci Rep* 9, no. 1 (2019): 11914.
162. Burch, N., A. S. Arnold, F. Item, S. Summermatter, G. Brochmann Santana Santos, M. Christe, U. Boutellier, M. Toigo, and C. Handschin. "Electric Pulse Stimulation of Cultured Murine Muscle Cells Reproduces Gene Expression Changes of Trained Mouse Muscle." *PLoS One* 5, no. 6 (2010): e10970.
163. Brown, A. E., D. E. Jones, M. Walker, and J. L. Newton. "Abnormalities of Ampk Activation and Glucose Uptake in Cultured Skeletal Muscle Cells from Individuals with Chronic Fatigue Syndrome." *PLoS One* 10, no. 4 (2015): e0122982.
164. Christensen, C. S., D. P. Christensen, M. Lundh, M. S. Dahllof, T. N. Haase, J. M. Velasquez, M. J. Laye, T. Mandrup-Poulsen, and T. P. Solomon. "Skeletal Muscle to Pancreatic Beta-Cell Cross-

Talk: The Effect of Humoral Mediators Liberated by Muscle Contraction and Acute Exercise on Beta-Cell Apoptosis." *J Clin Endocrinol Metab* 100, no. 10 (2015): E1289-98.
165. Pattamaprapanont, P., C. Garde, O. Fabre, and R. Barrès. "Muscle Contraction Induces Acute Hydroxymethylation of the Exercise-Responsive Gene Nr4a3." *Front Endocrinol (Lausanne)* 7 (2016): 165.
166. Raschke, S., M. Elsen, H. Gassenhuber, M. Sommerfeld, U. Schwahn, B. Brockmann, R. Jung, U. Wisloff, A. E. Tjonna, T. Raastad, J. Hallen, F. Norheim, C. A. Drevon, T. Romacho, K. Eckardt, and J. Eckel. "Evidence against a Beneficial Effect of Irisin in Humans." *PLoS One* 8, no. 9 (2013): e73680.
167. Ishiuchi, Y., H. Sato, K. Tsujimura, H. Kawaguchi, T. Matsuwaki, K. Yamanouchi, M. Nishihara, and T. Nedachi. "Skeletal Muscle Cell Contraction Reduces a Novel Myokine, Chemokine (C-X-C Motif) Ligand 10 (Cxcl10): Potential Roles in Exercise-Regulated Angiogenesis." *Biosci Biotechnol Biochem* 82, no. 1 (2018): 97-105.
168. Li, Ling-Jie, Jin Ma, Song-Bo Li, Xue-Fei Chen, and Jing Zhang. "Electric Pulse Stimulation Inhibited Lipid Accumulation on C2c12 Myotubes Incubated with Oleic Acid and Palmitic Acid." *Archives of Physiology and Biochemistry* 127, no. 4 (2021): 344-50.
169. Nieuwoudt, S., A. Mulya, C. E. Fealy, E. Martelli, S. Dasarathy, S. V. Naga Prasad, and J. P. Kirwan. "In Vitro Contraction Protects against Palmitate-Induced Insulin Resistance in C2c12 Myotubes." *Am J Physiol Cell Physiol* (2017): ajpcell.00123.2017.
170. Li, H., M. Dong, W. Liu, C. Gao, Y. Jia, X. Zhang, X. Xiao, Q. Liu, and H. Lin. "Peripheral Il-6/Stat3 Signaling Promotes Beiging of White Fat." *Biochim Biophys Acta Mol Cell Res* 1868, no. 10 (2021): 119080.
171. Tamura, Y., K. Kouzaki, T. Kotani, and K. Nakazato. "Electrically Stimulated Contractile Activity-Induced Transcriptomic Responses and Metabolic Remodeling in C2c12 Myotubes: Twitch Vs. Tetanic Contractions." *Am J Physiol Cell Physiol* 319, no. 6 (2020): C1029-C44.
172. Encyclopedia of DNA Elements at UCSC, https://genome.ucsc.edu/ENCODE/.
173. Zebisch, K., V. Voigt, M. Wabitsch, and M. Brandsch. "Protocol for Effective Differentiation of 3t3-L1 Cells to Adipocytes." *Anal Biochem* 425, no. 1 (2012): 88-90.
174. Pandurangan, M., D. Jeong, T. Amna, H. Van Ba, and I. Hwang. "Co-Culture of C2c12 and 3t3-L1 Preadipocyte Cells Alters the Gene Expression of Calpains, Caspases and Heat Shock Proteins." *In Vitro Cell Dev Biol Anim* 48, no. 9 (2012): 577-82.
175. Chan, F. K., K. Moriwaki, and M. J. De Rosa. "Detection of Necrosis by Release of Lactate Dehydrogenase Activity." *Methods Mol Biol* 979 (2013): 65-70.
176. Wood, E. J. "Molecular Cloning. A Laboratory Manual: By T Maniatis, E F Fritsch and J Sambrook. Pp 545. Cold Spring Harbor Laboratory, New York. 1982. ." *Biochemical Education* 11, no. 2 (1983): 82.
177. Karpen, Samuel C. "P Value Problems." *American Journal of Pharmaceutical Education* 81, no. 9 (2017): 6570.
178. Cohen, Jacob. "Statistical Power Analysis for the Behavioral Sciences " (1988).
179. Huh, J. Y. "The Role of Exercise-Induced Myokines in Regulating Metabolism." *Arch Pharm Res* 41, no. 1 (2018): 14-29.
180. Pettersson-Klein, A. T., M. Izadi, D. M. S. Ferreira, I. Cervenka, J. C. Correia, V. Martinez-Redondo, M. Southern, M. Cameron, T. Kamenecka, L. Z. Agudelo, M. Porsmyr-Palmertz, U. Martens, B. Lundgren, M. Otrocka, A. Jenmalm-Jensen, P. R. Griffin, and J. L. Ruas. "Small Molecule Pgc-1alpha1 Protein Stabilizers Induce Adipocyte Ucp1 Expression and Uncoupled Mitochondrial Respiration." *Mol Metab* 9 (2018): 28-42.

181. Shabalina, I. G., N. Petrovic, J. M. de Jong, A. V. Kalinovich, B. Cannon, and J. Nedergaard. "Ucp1 in Brite/Beige Adipose Tissue Mitochondria Is Functionally Thermogenic." *Cell Rep* 5, no. 5 (2013): 1196-203.
182. Rohleder, N., M. Aringer, and M. Boentert. "Role of Interleukin-6 in Stress, Sleep, and Fatigue." *Ann N Y Acad Sci* 1261 (2012): 88-96.
183. Aboouf, M. A., J. Armbruster, M. Thiersch, M. Gassmann, A. Gödecke, E. Gnaiger, G. Kristiansen, A. Bicker, T. Hankeln, H. Zhu, and T. A. Gorr. "Myoglobin, Expressed in Brown Adipose Tissue of Mice, Regulates the Content and Activity of Mitochondria and Lipid Droplets." *Biochim Biophys Acta Mol Cell Biol Lipids* 1866, no. 12 (2021): 159026.
184. Kristóf, E., Á Klusóczki, R. Veress, A. Shaw, Z. S. Combi, K. Varga, F. Győry, Z. Balajthy, P. Bai, Z. Bacso, and L. Fésüs. "Interleukin-6 Released from Differentiating Human Beige Adipocytes Improves Browning." *Exp Cell Res* 377, no. 1-2 (2019): 47-55.
185. Tamura, K., N. Goto-Inoue, K. Miyata, Y. Furuichi, N. L. Fujii, and Y. Manabe. "Effect of Treatment with Conditioned Media Derived from C2c12 Myotube on Adipogenesis and Lipolysis in 3t3-L1 Adipocytes." *PLoS One* 15, no. 8 (2020): e0237095.
186. Pilkington, Anna-Claire, Henry A. Paz, and Umesh D. Wankhade. "Beige Adipose Tissue Identification and Marker Specificity—Overview." *Frontiers in Endocrinology* 12, no. 8 (2021).
187. Pérez-Sotelo, D., A. Roca-Rivada, I. Baamonde, J. Baltar, A. I. Castro, E. Domínguez, M. Collado, F. F. Casanueva, and M. Pardo. "Lack of Adipocyte-Fndc5/Irisin Expression and Secretion Reduces Thermogenesis and Enhances Adipogenesis." *Scientific Reports* 7, no. 1 (2017): 16289.
188. Shan, Tizhong, Xinrong Liang, Pengpeng Bi, and Shihuan Kuang. "Myostatin Knockout Drives Browning of White Adipose Tissue through Activating the Ampk-Pgc1α-Fndc5 Pathway in Muscle." *The FASEB Journal* 27, no. 5 (2013): 1981-89.
189. Glassman, A. R. "Are P Values Enough?" *JAMA Ophthalmol* 138, no. 5 (2020): 567-68.
190. Dufau, J., J. X. Shen, M. Couchet, T. De Castro Barbosa, N. Mejhert, L. Massier, E. Griseti, E. Mouisel, E. Z. Amri, V. M. Lauschke, M. Rydén, and D. Langin. "In Vitro and Ex Vivo Models of Adipocytes." *Am J Physiol Cell Physiol* 320, no. 5 (2021): C822-c41.
191. Samuelson, I., and A. Vidal-Puig. "Studying Brown Adipose Tissue in a Human in Vitro Context." *Front Endocrinol (Lausanne)* 11 (2020): 629.
192. Carter, S., and T. P. J. Solomon. "In Vitro Experimental Models for Examining the Skeletal Muscle Cell Biology of Exercise: The Possibilities, Challenges and Future Developments." *Pflugers Arch* 471, no. 3 (2019): 413-29.
193. Pudkasam, Supa, Kathy Tangalakis, Nanthapan Chinlumprasert, Vasso Apostolopoulos, and Lily Stojanovska. "Breast Cancer and Exercise: The Role of Adiposity and Immune Markers." *Maturitas* 105 (2017): 16-22.
194. Colberg, Sheri R., Ronald J. Sigal, Jane E. Yardley, Michael C. Riddell, David W. Dunstan, Paddy C. Dempsey, Edward S. Horton, Kristin Castorino, and Deborah F. Tate. "Physical Activity/Exercise and Diabetes: A Position Statement of the American Diabetes Association." *Diabetes Care* 39, no. 11 (2016): 2065-79.
195. Harding, Amy T., and Belinda R. Beck. "Exercise, Osteoporosis, and Bone Geometry." *Sports* 5, no. 2 (2017): 29.
196. Wewege, Michael A., Jeanette M. Thom, Kerry-Anne Rye, and Belinda J. Parmenter. "Aerobic, Resistance or Combined Training: A Systematic Review and Meta-Analysis of Exercise to Reduce Cardiovascular Risk in Adults with Metabolic Syndrome." *Atherosclerosis* 274 (2018): 162-71.
197. Flouris, A. D., C. Bouziotas, A. D. Christodoulos, and Y. Koutedakis. "Longitudinal Preventive-Screening Cutoffs for Metabolic Syndrome in Adolescents." *Int J Obes (Lond)* 32, no. 10 (2008): 1506-12.

198. Muscella, Antonella, Erika Stefàno, and Santo Marsigliante. "The Effects of Exercise Training on Lipid Metabolism and Coronary Heart Disease." *American Journal of Physiology-Heart and Circulatory Physiology* 319, no. 1 (2020): H76-H88.
199. da Costa Daniele, Thiago Medeiros, Pedro Felipe Carvalhedo de Bruin, Robson Salviano de Matos, Gabriela Sales de Bruin, Cauby Maia Chaves, and Veralice Meireles Sales de Bruin. "Exercise Effects on Brain and Behavior in Healthy Mice, Alzheimer's Disease and Parkinson's Disease Model—a Systematic Review and Meta-Analysis." *Behavioural Brain Research* 383 (2020): 112488.
200. Wang, Qiaoyun, and Wenli Zhou. "Roles and Molecular Mechanisms of Physical Exercise in Cancer Prevention and Treatment." *Journal of Sport and Health Science* 10, no. 2 (2021): 201-10.
201. Nintou, E., E. Karligiotou, M. Vliora, I. G. Fatouros, A. Z. Jamurtas, N. Sakellaridis, K. Dimas, and A. D. Flouris. "Effects of in Vitro Muscle Contraction on Thermogenic Protein Levels in Co-Cultured Adipocytes." *Life (Basel)* 11, no. 11 (2021).
202. Lambernd, S., A. Taube, A. Schober, B. Platzbecker, S. W. Gorgens, R. Schlich, K. Jeruschke, J. Weiss, K. Eckardt, and J. Eckel. "Contractile Activity of Human Skeletal Muscle Cells Prevents Insulin Resistance by Inhibiting Pro-Inflammatory Signalling Pathways." *Diabetologia* 55, no. 4 (2012): 1128-39.
203. Song, Yang, Jennifer Soto, Binru Chen, Li Yang, and Song Li. "Cell Engineering: Biophysical Regulation of the Nucleus." *Biomaterials* 234 (2020): 119743.
204. Orfanos, Z., M. P. Godderz, E. Soroka, T. Godderz, A. Rumyantseva, P. F. van der Ven, T. J. Hawke, and D. O. Furst. "Breaking Sarcomeres by in Vitro Exercise." *Sci Rep* 6 (2016): 19614.
205. Evers-van Gogh, I. J., S. Alex, R. Stienstra, A. B. Brenkman, S. Kersten, and E. Kalkhoven. "Electric Pulse Stimulation of Myotubes as an in Vitro Exercise Model: Cell-Mediated and Non-Cell-Mediated Effects." *Sci Rep* 5 (2015): 10944.
206. Beiter, T., J. Hudemann, C. Burgstahler, A. M. Niess, and B. Munz. "Effects of Extracellular Orotic Acid on Acute Contraction-Induced Adaptation Patterns in C2c12 Cells." *Mol Cell Biochem* 448, no. 1-2 (2018): 251-63.
207. Chaves, A. B., E. R. Miranda, J. T. Mey, B. K. Blackburn, K. N. Z. Fuller, B. Stearns, A. Ludlow, D. L. th Williamson, J. A. Houmard, and J. M. Haus. "Exercise Reduces the Protein Abundance of Txnip and Its Interacting Partner Redd1 in Skeletal Muscle: Potential Role for a Pka-Mediated Mechanism." *J Appl Physiol (1985)* 132, no. 2 (2022): 357-66.
208. Feng, Y. Z., N. Nikolic, S. S. Bakke, E. T. Kase, K. Guderud, J. Hjelmesaeth, V. Aas, A. C. Rustan, and G. H. Thoresen. "Myotubes from Lean and Severely Obese Subjects with and without Type 2 Diabetes Respond Differently to an in Vitro Model of Exercise." *Am J Physiol Cell Physiol* 308, no. 7 (2015): C548-56.
209. MCARDLE, A. "Contractile Activity-Induced Oxidative Stress: Cellular Origin and Adaptive Responses." *Am J Physiol Cell Physiol* 280 (2001): 621-27.
210. Tarum, J., M. Folkesson, P. J. Atherton, and F. Kadi. "Electrical Pulse Stimulation: An in Vitro Exercise Model for the Induction of Human Skeletal Muscle Cell Hypertrophy. A Proof-of-Concept Study." *Exp Physiol* 102, no. 11 (2017): 1405-13.
211. Valero-Breton, M., G. Warnier, M. Castro-Sepulveda, L. Deldicque, and H. Zbinden-Foncea. "Acute and Chronic Effects of High Frequency Electric Pulse Stimulation on the Akt/Mtor Pathway in Human Primary Myotubes." *Front Bioeng Biotechnol* 8 (2020): 565679.
212. Laurens, Claire, Anisha Parmar, Enda Murphy, Deborah Carper, Benjamin Lair, Pauline Maes, Julie Vion, Nathalie Boulet, Coralie Fontaine, Marie Marquès, Dominique Larrouy, Isabelle Harant, Claire Thalamas, Emilie Montastier, Sylvie Caspar-Bauguil, Virginie Bourlier, Geneviève Tavernier, Jean-Louis Grolleau, Anne Bouloumié, Dominique Langin, Nathalie Viguerie, Fabrice Bertile, Stéphane Blanc, Isabelle de Glisezinski, Donal O'Gorman, and Cedric Moro. "Growth and

Differentiation Factor 15 Is Secreted by Skeletal Muscle During Exercise and Promotes Lipolysis in Humans." *JCI Insight* 5, no. 6 (2020).
213. Lambertucci, R. H., R. Silveira Ldos, S. M. Hirabara, R. Curi, G. Sweeney, and T. C. Pithon-Curi. "Effects of Moderate Electrical Stimulation on Reactive Species Production by Primary Rat Skeletal Muscle Cells: Cross Talk between Superoxide and Nitric Oxide Production." *J Cell Physiol* 227, no. 6 (2012): 2511-8.
214. Nikolić, N., S. W. Görgens, G. H. Thoresen, V. Aas, J. Eckel, and K. Eckardt. "Electrical Pulse Stimulation of Cultured Skeletal Muscle Cells as a Model for In vitro Exercise - Possibilities and Limitations." *Acta Physiol (Oxf)* 220, no. 3 (2017): 310-31.
215. Manabe, Y., S. Miyatake, M. Takagi, M. Nakamura, A. Okeda, T. Nakano, M. F. Hirshman, L. J. Goodyear, and N. L. Fujii. "Characterization of an Acute Muscle Contraction Model Using Cultured C2c12 Myotubes." *PLoS One* 7, no. 12 (2012): e52592.
216. Park, S., K. D. Turner, D. Zheng, J. J. Brault, K. Zou, A. B. Chaves, T. S. Nielsen, C. J. Tanner, J. T. Treebak, and J. A. Houmard. "Electrical Pulse Stimulation Induces Differential Responses in Insulin Action in Myotubes from Severely Obese Individuals." *J Physiol* 597, no. 2 (2019): 449-66.
217. Yue, Yingying, Chang Zhang, Xuejiao Zhang, Shitian Zhang, Qian Liu, Fang Hu, Xiaoting Lv, Hanqi Li, Jianming Yang, Xinli Wang, Liming Chen, Zhi Yao, Hongquan Duan, and Wenyan Niu. "An Ampk/Axin1-Rac1 Signaling Pathway Mediates Contraction-Regulated Glucose Uptake in Skeletal Muscle Cells." *American Journal of Physiology-Endocrinology and Metabolism* 318, no. 3 (2020): E330-E42.
218. Page, Matthew J, Joanne E McKenzie, Patrick M Bossuyt, Isabelle Boutron, Tammy C Hoffmann, Cynthia D Mulrow, Larissa Shamseer, Jennifer M Tetzlaff, Elie A Akl, Sue E Brennan, Roger Chou, Julie Glanville, Jeremy M Grimshaw, Asbjørn Hróbjartsson, Manoj M Lalu, Tianjing Li, Elizabeth W Loder, Evan Mayo-Wilson, Steve McDonald, Luke A McGuinness, Lesley A Stewart, James Thomas, Andrea C Tricco, Vivian A Welch, Penny Whiting, and David Moher. "The Prisma 2020 Statement: An Updated Guideline for Reporting Systematic Reviews." *BMJ* 372 (2021): n71.
219. Pereira, Marcelo G., Vanessa A. Voltarelli, Gabriel C. Tobias, Lara de Souza, Gabriela S. Borges, Ailma O. Paixão, Ney R. de Almeida, Thomas Scott Bowen, Marilene Demasi, Elen H. Miyabara, and Patricia C. Brum. "Aerobic Exercise Training and in Vivo Akt Activation Counteract Cancer Cachexia by Inducing a Hypertrophic Profile through Eif-2Α Modulation." *Cancers* 14, no. 1 (2022): 28.
220. Rohatgi, A. "Webplotdigitizer. 4.5.".
221. Weir, Christopher J., Isabella Butcher, Valentina Assi, Stephanie C. Lewis, Gordon D. Murray, Peter Langhorne, and Marian C. Brady. "Dealing with Missing Standard Deviation and Mean Values in Meta-Analysis of Continuous Outcomes: A Systematic Review." *BMC Medical Research Methodology* 18, no. 1 (2018): 25.
222. Barlow, J., and T. P. J. Solomon. "Conditioned Media from Contracting Skeletal Muscle Potentiates Insulin Secretion and Enhances Mitochondrial Energy Metabolism of Pancreatic Beta-Cells." *Metabolism* 91 (2019): 1-9.
223. Connor, M. K., I. Irrcher, and D. A. Hood. "Contractile Activity-Induced Transcriptional Activation of Cytochrome C Involves Sp1 and Is Proportional to Mitochondrial Atp Synthesis in C2c12 Muscle Cells." *J Biol Chem* 276, no. 19 (2001): 15898-904.
224. Fernandez-Verdejo, R., A. M. Vanwynsberghe, T. Hai, L. Deldicque, and M. Francaux. "Activating Transcription Factor 3 Regulates Chemokine Expression in Contracting C2c12 Myotubes and in Mouse Skeletal Muscle after Eccentric Exercise." *Biochem Biophys Res Commun* 492, no. 2 (2017): 249-54.

225. Fujita, H., K. Shimizu, and E. Nagamori. "Novel Method for Measuring Active Tension Generation by C2c12 Myotube Using Uv-Crosslinked Collagen Film." *Biotechnol Bioeng* 106, no. 3 (2010): 482-9.
226. Furuichi, Y., Y. Manabe, M. Takagi, M. Aoki, and N. L. Fujii. "Evidence for Acute Contraction-Induced Myokine Secretion by C2c12 Myotubes." *PLoS One* 13, no. 10 (2018): e0206146.
227. Guigni, Blas A., Dennis K. Fix, Joseph J. Bivona 3rd, Bradley M. Palmer, James A. Carson, and Michael J. Toth. "Electrical Stimulation Prevents Doxorubicin-Induced Atrophy and Mitochondrial Loss in Cultured Myotubes." *American Journal of Physiology-Cell Physiology* 317, no. 6 (2019): C1213-C28.
228. Horie, M., E. Warabi, S. Komine, S. Oh, and J. Shoda. "Cytoprotective Role of Nrf2 in Electrical Pulse Stimulated C2c12 Myotube." *PLoS One* 10, no. 12 (2015): e0144835.
229. Lee, J. O., W. S. Byun, M. J. Kang, J. A. Han, J. Moon, M. J. Shin, H. J. Lee, J. H. Chung, J. S. Lee, C. G. Son, K. H. Song, T. W. Kim, E. S. Lee, H. M. Kim, C. H. Chung, K. R. W. Ngoei, N. X. Y. Ling, J. S. Oakhill, S. Galic, L. Murray-Segal, B. E. Kemp, K. M. Kim, S. Lim, and H. S. Kim. "The Myokine Meteorin-Like (Metrnl) Improves Glucose Tolerance in Both Skeletal Muscle Cells and Mice by Targeting Ampkalpha2." *FEBS J* 287, no. 10 (2020): 2087-104.
230. Martin, N. R. W., M. C. Turner, R. Farrington, D. J. Player, and M. P. Lewis. "Leucine Elicits Myotube Hypertrophy and Enhances Maximal Contractile Force in Tissue Engineered Skeletal Muscle in Vitro." *J Cell Physiol* 232, no. 10 (2017): 2788-97.
231. Nakamura, T., S. Takagi, D. Okuzaki, S. Matsui, and T. Fujisato. "Hypoxia Transactivates Cholecystokinin Gene Expression in 3d-Engineered Muscle." *J Biosci Bioeng* 132, no. 1 (2021): 64-70.
232. Small, L., A. Altintas, R. C. Laker, A. Ehrlich, P. Pattamaprapanont, J. Villarroel, N. J. Pillon, J. R. Zierath, and R. Barres. "Contraction Influences Per2 Gene Expression in Skeletal Muscle through a Calcium-Dependent Pathway." *J Physiol* 598, no. 24 (2020): 5739-52.
233. Son, Y. H., S. M. Lee, S. H. Lee, J. H. Yoon, J. S. Kang, Y. R. Yang, and K. S. Kwon. "Comparative Molecular Analysis of Endurance Exercise in Vivo with Electrically Stimulated in Vitro Myotube Contraction." *J Appl Physiol (1985)* 127, no. 6 (2019): 1742-53.
234. Thelen, Marc H.M. "Electrical Stimulation of C2c12 Myotubes Induces Contractions and Represses Thyroid-Hormone-Dependent Transcription of the Fast-Type Sarcoplasmic-Reticulum Ca2+-Atpase Gene." *Biochemistry Journal* 321 (1997): 845-48.
235. Sato, S., M. Nomura, I. Yamana, A. Uchiyama, Y. Furuichi, Y. Manabe, and N. L. Fujii. "A New in Vitro Muscle Contraction Model and Its Application for Analysis of Mtorc1 Signaling in Combination with Contraction and Beta-Hydroxy-Beta-Methylbutyrate Administration." *Biosci Biotechnol Biochem* 83, no. 10 (2019): 1851-57.
236. Pattwell, D. M., A. McArdle, J. E. Morgan, T. A. Patridge, and M. J. Jackson. "Release of Reactive Oxygen and Nitrogen Species from Contracting Skeletal Muscle Cells." *Free Radic Biol Med* 37, no. 7 (2004): 1064-72.
237. Broholm, C., M. J. Laye, C. Brandt, R. Vadalasetty, H. Pilegaard, B. K. Pedersen, and C. Scheele. "Lif Is a Contraction-Induced Myokine Stimulating Human Myocyte Proliferation." *J Appl Physiol (1985)* 111, no. 1 (2011): 251-9.
238. Kugler, B. A., W. Deng, B. Francois, M. Anderson, J. M. Hinkley, J. A. Houmard, P. N. Gona, and K. Zou. "Distinct Adaptations of Mitochondrial Dynamics to Electrical Pulse Stimulation in Lean and Severely Obese Primary Myotubes." *Med Sci Sports Exerc* 53, no. 6 (2021): 1151-60.
239. Løvsletten, Nils, Arild Rustan, Claire Laurens, Hege Thoresen, Cedric Moro, and Nataša Nikolić. "Primary Defects in Lipid Handling and Resistance to Exercise in Myotubes from Obese Donors with and without Type 2 Diabetes." *Applied Physiology, Nutrition, and Metabolism* 45 (2019).

240. Scheler, M., M. H. de Angelis, H. Al-Hasani, H. U. Haring, C. Weigert, and S. Lehr. "Methods for Proteomics-Based Analysis of the Human Muscle Secretome Using an in Vitro Exercise Model." *Methods Mol Biol* 1295 (2015): 55-64.
241. Kubis, H. P., R. J. Scheibe, J. D. Meissner, G. Hornung, and G. Gros. "Fast-to-Slow Transformation and Nuclear Import/Export Kinetics of the Transcription Factor Nfatc1 During Electrostimulation of Rabbit Muscle Cells in Culture." *J Physiol* 541, no. Pt 3 (2002): 835-47.
242. Miyatake, Shouta, Philip J. Bilan, Nicolas J. Pillon, and Amira Klip. "Contracting C2c12 Myotubes Release Ccl2 in an Nf-Kb-Dependent Manner to Induce Monocyte Chemoattraction." *American Journal of Physiology-Endocrinology and Metabolism* 310, no. 2 (2016): E160-E70.
243. Richter, E. A., and N. B. Ruderman. "Ampk and the Biochemistry of Exercise: Implications for Human Health and Disease." *Biochem J* 418, no. 2 (2009): 261-75.
244. Sylow, Lykke, Maximilian Kleinert, Erik A. Richter, and Thomas E. Jensen. "Exercise-Stimulated Glucose Uptake — Regulation and Implications for Glycaemic Control." *Nature Reviews Endocrinology* 13, no. 3 (2017): 133-48.
245. Mann, Gagandeep, Michael C. Riddell, and Olasunkanmi A. J. Adegoke. "Effects of Acute Muscle Contraction on the Key Molecules in Insulin and Akt Signaling in Skeletal Muscle in Health and in Insulin Resistant States." *Diabetology* 3, no. 3 (2022): 423-46.
246. Munoz-Canoves, P., C. Scheele, B. K. Pedersen, and A. L. Serrano. "Interleukin-6 Myokine Signaling in Skeletal Muscle: A Double-Edged Sword?" *FEBS J* 280, no. 17 (2013): 4131-48.
247. Neufer, P. Darrell, Marcas M Bamman, Deborah M Muoio, Claude Bouchard, Dan M Cooper, Bret H Goodpaster, Frank W Booth, Wendy M Kohrt, Robert E Gerszten, Mark P Mattson, Russell T Hepple, William E Kraus, Michael B Reid, Sue C Bodine, John M Jakicic, Jerome L Fleg, John P Williams, Lyndon Joseph, Mary Evans, Padma Maruvada, Mary Rodgers, Mary Roary, Amanda T Boyce, Jonelle K Drugan, James I Koenig, Richard H Ingraham, Danuta Krotoski, Mary Garcia-Cazarin, Joan A McGowan, and Maren R Laughlin. "Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits." *Cell Metabolism* 22, no. 1 (2015): 4-11.
248. Muise, Eric S., Hong-Ping Guan, Jinqi Liu, Andrea R. Nawrocki, Xiaodong Yang, Chuanlin Wang, Carlos G. Rodríguez, Dan Zhou, Judith N. Gorski, Marc M. Kurtz, Danqing Feng, Kenneth J. Leavitt, Lan Wei, Robert R. Wilkening, James M. Apgar, Shiyao Xu, Ku Lu, Wen Feng, Ying Li, Huaibing He, Stephen F. Previs, Xiaolan Shen, Margaret van Heek, Sandra C. Souza, Mark J. Rosenbach, Tesfaye Biftu, Mark D. Erion, David E. Kelley, Daniel M. Kemp, Robert W. Myers, and Iyassu K. Sebhat. "Pharmacological Ampk Activation Induces Transcriptional Responses Congruent to Exercise in Skeletal and Cardiac Muscle, Adipose Tissues and Liver." *PLOS ONE* 14, no. 2 (2019): e0211568.
249. Sakamoto, Kei, David E. W. Arnolds, Ingvar Ekberg, Anders Thorell, and Laurie J. Goodyear. "Exercise Regulates Akt and Glycogen Synthase Kinase-3 Activities in Human Skeletal Muscle." *Biochemical and Biophysical Research Communications* 319, no. 2 (2004): 419-25.
250. Pedersen, B. K., A. Steensberg, C. Fischer, C. Keller, P. Keller, P. Plomgaard, E. Wolsk-Petersen, and M. Febbraio. "The Metabolic Role of Il-6 Produced During Exercise: Is Il-6 an Exercise Factor?" *Proceedings of the Nutrition Society* 63, no. 2 (2004): 263-67.
251. Uguccioni, Giulia, Donna D'Souza, and David A. Hood. "Regulation of Ppar<Svg Style="Vertical-Align:-4.698pt;Width:11.35px;" Id="M1" Height="16.012501" Version="1.1" Viewbox="0 0 11.35 16.012501" Width="11.35" Xmlns:Xlink="Http://Www.W3.Org/1999/Xlink" Xmlns="Http://Www.W3.Org/2000/Svg"> <G Transform="Matrix(.022,-0,0,-.022,.062,10.1)"><Path Id="X1d6fe" D="M478 372q0 -39 -31 -97t-61 -98t-78 -98q-45 -55 -73 -102q-13 -79 -13 -197q-11 -11 -43.5 -25.5t-53.5 -15.5l-15 17q5 35 26 101.5t47 123.5q8 72 -1.5 174.5t-37.5 178.5q-14 37 -29 37q-20 0 -67 -65l-25 21q37 60 73 90.5t63 30.5q47 0 72 -112q13 -56 17.5 -141.5 T0.5 -143.5h2q155 193 155 297q0

26 -12 47q-5 8 -5 15q0 16 12.5 27t29.5 11q21 0 34 -21t13 -55z" /></G> </Svg> Coactivator-1<Svg Style="Vertical-Align:-0.216pt;Width:12.8625px;" Id="M2" Height="10.4375" Version="1.1" Viewbox="0 0 12.8625 10.4375" Width="12.8625" Xmlns:Xlink="Http://Www.W3.Org/1999/Xlink" Xmlns="Http://Www.W3.Org/2000/Svg"> <G Transform="Matrix(.022,-0,0,-.022,.062,10.1)"><Path Id="X1d6fc" D="M545 106q-67 -118 -134 -118q-24 0 -40 37.5t-30 129.5h-2q-47 -72 -103 -119.5t-108 -47.5q-47 0 -76 45.5t-29 119.5q0 113 85 204t174 91q47 0 70 -33.5t43 -119.5h3q32 47 80 140l55 13l10 -9q-47 -80 -138 -201q17 -99 27.5 -136t22.5 -37q23 0 69 61zm333 204 Q-14 98 -31 149.5t-50 51.5q-49 0 -94 -70t-45 -164q0 -55 15.5 -86t40.5 -31q70 0 164 150z" /></G> </Svg> Function and Expression in Muscle: Effect of Exercise." *PPAR Research* 2010 (2010): 937123.

252. O'Neill, H. M. "Ampk and Exercise: Glucose Uptake and Insulin Sensitivity." *Diabetes Metab J* 37, no. 1 (2013): 1-21.
253. Chowdhury, Subrata, Logan Schulz, Biagio Palmisano, Parminder Singh, Julian M. Berger, Vijay K. Yadav, Paula Mera, Helga Ellingsgaard, Juan Hidalgo, Jens Brüning, and Gerard Karsenty. "Muscle-Derived Interleukin 6 Increases Exercise Capacity by Signaling in Osteoblasts." *The Journal of Clinical Investigation* 130, no. 6 (2020): 2888-902.
254. Kistner, Timothy M., Bente K. Pedersen, and Daniel E. Lieberman. "Interleukin 6 as an Energy Allocator in Muscle Tissue." *Nature Metabolism* 4, no. 2 (2022): 170-79.
255. Lira, V. A., C. R. Benton, Z. Yan, and A. Bonen. "Pgc-1alpha Regulation by Exercise Training and Its Influences on Muscle Function and Insulin Sensitivity." *Am J Physiol Endocrinol Metab* 299, no. 2 (2010): E145-61.
256. Fischer, Christian P. "Interleukin-6 in Acute Exercise and Training: What Is the Biological Relevance." *Exerc immunol rev* 12, no. 6-33 (2006): 41.
257. Röhling, Martin, Christian Herder, Theodor Stemper, and Karsten Müssig. "Influence of Acute and Chronic Exercise on Glucose Uptake." *Journal of Diabetes Research* 2016 (2016): 2868652.
258. Viswanathan, M., N. D. Berkman, D. M. Dryden, and L. Hartling. "Ahrq Methods for Effective Health Care." In *Assessing Risk of Bias and Confounding in Observational Studies of Interventions or Exposures: Further Development of the Rti Item Bank*. Rockville (MD): Agency for Healthcare Research and Quality (US), 2013.
259. Margulis, A. V., M. Pladevall, N. Riera-Guardia, C. Varas-Lorenzo, L. Hazell, N. D. Berkman, M. Viswanathan, and S. Perez-Gutthann. "Quality Assessment of Observational Studies in a Drug-Safety Systematic Review, Comparison of Two Tools: The Newcastle-Ottawa Scale and the Rti Item Bank." *Clin Epidemiol* 6 (2014): 359-68.
260. Al-Saleh, M. A., S. Armijo-Olivo, N. Thie, H. Seikaly, P. Boulanger, J. Wolfaardt, and P. Major. "Morphologic and Functional Changes in the Temporomandibular Joint and Stomatognathic System after Transmandibular Surgery in Oral and Oropharyngeal Cancers: Systematic Review." *J Otolaryngol Head Neck Surg* 41, no. 5 (2012): 345-60.
261. 2019, RevMan Web. "Review Manager Web (Revman Web)." The Cochrane Collaboration, 2019.
262. Bracale, R., G. Labruna, C. Finelli, A. Daniele, L. Sacchetti, G. Oriani, F. Contaldo, and F. Pasanisi. "The Absence of Polymorphisms in Adrb3, Ucp1, Pparγ, and Adipoq Genes Protects Morbid Obese Patients toward Insulin Resistance." *J Endocrinol Invest* 35, no. 1 (2012): 2-4.
263. Brondani, L. A., G. C. Duarte, L. H. Canani, and D. Crispim. "The Presence of at Least Three Alleles of the Adrb3 Trp64arg (C/T) and Ucp1 -3826a/G Polymorphisms Is Associated with Protection to Overweight/Obesity and with Higher High-Density Lipoprotein Cholesterol Levels in Caucasian-Brazilian Patients with Type 2 Diabetes." *Metab Syndr Relat Disord* 12, no. 1 (2014): 16-24.
264. Dong, C., Y. Lv, L. Xie, R. Yang, L. Chen, L. Zhang, T. Long, H. Yang, X. Mao, Q. Fan, X. Chen, and H. Zhang. "Association of Ucp1 Polymorphisms with Type 2 Diabetes Mellitus and Their Interaction with Physical Activity and Sedentary Behavior." *Gene* 739 (2020): 144497.

Appendix

Supplement to Chapter 2

Prevalence of uncoupling protein one genetic polymorphisms and their relationship with cardiovascular and metabolic health

1. CASE-CONTROL STUDY

1.1 Materials and Methods

1.1.1 Bioethics approval procedures

This multicenter, multinational study conducted across Armenia, Greece, Poland, Russia, and United Kingdom, received approval from the relevant Bioethics Review Board in each country:

1. Armenia: Institute of Molecular Biology, National Academy of Sciences of Republic of Armenia, ref. No IRB/IEC: IRB00004079, (IORG 0003427)/3-7-2012;

2. Greece: Department of Physical Education and Sport Science, University of Thessaly, ref. No 610/9-7-2012.
3. Poland: Local Research Bioethics Committee, University of Medical Sciences, Poznan, ref. No KB 215/13 revised KB 85/16;
4. Russia: Institute of Gene Biology, Russian Academy of Sciences, ref. no 12318-308/ 6-7-2012;
5. United Kingdom: National Health Services/Manchester East Research Ethics Committee, ref. No 15/NW/0874/12-1-2016.

1.1.2. Blood handling and genotyping

For all the analyzed samples, a phenotype-blind genotyping process was adopted. Validation of the genotyping methodology in all the countries has been performed either by direct sequencing of random samples or by PCR-restriction fragment length polymorphism (PCR-RFLP) method as described below.

1.1.2.a Greece and United Kingdom

We collected 4ml of whole blood in EDTA anti-coagulated vacutainers. Genomic DNA was extracted from 4ml whole blood using a NucleoSpin blood QuickPure kit (Macherey-Nagel). Concentration and purity of isolated DNA were evaluated with Qubit 2.0 fluorometer (ThermoFisher Scientific) and Qubit dsDNA BR Assay Kit (ThermoFisher Scientific) and samples were stored at -20 °C until the day of the genotyping analysis. DNA samples (10 ng) were genotyped using the TaqMan SNP genotyping assays (ThermoFisher Scientific) for the *UCP1* A-3826G (rs1800592), A-1766G (rs10011540), A-112C (rs3811791), and Ala64Thr (rs45539933) polymorphisms. Reactions were conducted in 384-well plates in a reaction volume of 10 ul using 1X TaqMan Universal Master Mix II (ThermoFisher Scientific) and 1X TaqMan assay (ThermoFisher Scientific). The plates were then placed in a real-time PCR thermal cycler (ViiA7 Real-Time PCR System; ThermoFisher Scientific), and thermal cycling conditions were as follow:

incubation at 60˚C for 1 min and 95 ˚C for 10 min, followed by 40 cycles of 95˚C for 15 seconds and 60˚C for 1 min. Fluorescence data files from each plate were analysed using automated allele-calling software (QuantStudio 7 Real-Time PCR Software v1.3; ThermoFisher Scientific). To perform the quality control of the genotyping method, we assessed PCR-products of randomly chosen samples from each genotype by direct sequencing.

1.1.2.b. Armenia

We collected 4ml of whole blood in EDTA anti-coagulated vacutainers. 4 ml of blood sample was added in a 15 ml Falcon tube and centrifuged at 3,000 rpm for 10 min. After removal of supernatant 14 ml of RBC lysis buffer was added and centrifuged at 3,000 rpm for 10 min until a clear white pellet was obtained. 25 µl of Proteinase K (20 mkg/ml) and 5 ml of WBC lysis buffer were added and the pellet was disturbed. The tubes were incubated at 56ºC for 2.5-3 hours. 2 ml of 6 M NaCl was added, the tubes were shaking periodically for 20 minutes and centrifuged at 3,000 rpm for 30 min until a clear supernatant and rigid pellet were obtained. To precipitate DNA aqueous phase was added into the 50 ml transparent clear tubes with 35-40 ml 96% ethanol. The tubes were gently shacked until the DNA medusa was generated. Transferred in 1.5 ml sterile Eppendorf tubes, the DNA medusa was washed in 1 ml 70% ethanol. After drying 400 µl TE buffer (pH=8.0) was added and the tubes with DNAs were put at 37ºC overnight to solve DNAs. Concentration and purity of isolated DNA were evaluated with the ratio of absorbance at 260 nm and 280 nm and DNA samples were stored at -20 °C until the day of the genotyping analysis. Four SNPs in the *UCP1* gene, rs1800592, rs3811791, rs45539933, rs10011540 were genotyped using the TaqManSNP Genotyping Assays, respectively: C 8866368 20, C 2052379 10, C 25619416 30, C 25761748 10. Amplification reactions (10µl/well) were carried out in 96-well plate with 20 ng of template DNA, 5 µl TaqMan Genotyping Master Mix (ThermoFisher Scientific), 0.2µl of appropriate, for tested SNP, fluorogenic probe and 3.8 µl MiliQ water. An initial denaturing step of 10 min at 95°C was followed by 40 cycles of 15 seconds at 95°C, 1 min at 60°C. Reaction was performer on Viia 7 System (Applied Biosystems), the results were analysed using QuantStudio™ Software V1.2.4, ThermoFisher Scientific. Moreover, accuracy of genotyping of *UCP1* SNPs: rs1800592,

rs3811791 and rs45539933 was verified by PCR-restriction fragment length polymorphism (PCR-RFLP) method.

1.1.2.c. Poland

Peripheral blood samples were obtained from 252 individuals with CMP (mean age 59.68 ± 11.37, 163 female/89 male) and 365 healthy, unrelated volunteers (35.19 ± 12.64, 144 female/221 male). Individuals were diagnosed at the Department of Internal Diseases, Poznań University of Medical Science, Poland. Blood samples from healthy volunteers were collected in cooperation with the Regional Center for Blood Donation and Blood Treatment in Wrocław. Genomic DNA was extracted from 3 ml whole blood using Invisorb Spin Blood Midi Kit (*Stratec Molecular GmbH*) according to the manufacturer's protocol. The DNA concentration and purity ($A_{260/280}$) was determined spectrophotometrically (*Denovix*). All samples were stored at -20°C. Four SNPs in the *UCP1* gene, rs1800592, rs3811791, rs45539933, rs10011540 were genotyped using the TaqManSNP Genotyping Assays, respectively: C 8866368 20, C 2052379 10, C 25619416 30, C 25761748 10. Amplification reactions (10µl/well) were carried out in 96-well plate with 20 ng of template DNA, 5 µl TaqMan Genotyping Master Mix (ThermoFisher Scientific), 0.2µl of appropriate, for tested SNP, fluorogenic probe and 3.8 µl MiliQ water. An initial denaturing step of 10 min at 95°C was followed by 40 cycles of 15 s at 95°C, 1 min at 60°C. Reaction was performer on Viia 7 System (Applied Biosystems), the results were analysed using QuantStudio™ Software V1.2.4, ThermoFisher Scientific. Moreover, accuracy of genotyping of *UCP1* SNPs: rs1800592, rs3811791 and rs45539933 was verified by PCR-restriction fragment length polymorphism (PCR-RFLP) method.

1.1.2.d. Russia

We collected 4ml of whole blood in EDTA anti-coagulated vacutainers. Genomic DNA was extracted from 0.2 ml whole blood using a GeneJET Genomic DNA Purification Kit (ThermoFisher Scientific). Concentration of isolated DNA was evaluated with Qubit 2.0 fluorometer (ThermoFisher Scientific) and Qubit dsDNA BR Assay Kit (ThermoFisher Scientific). Purity of DNA was assessed based on the ratio of absorbance at 260 nm and 280 nm using an

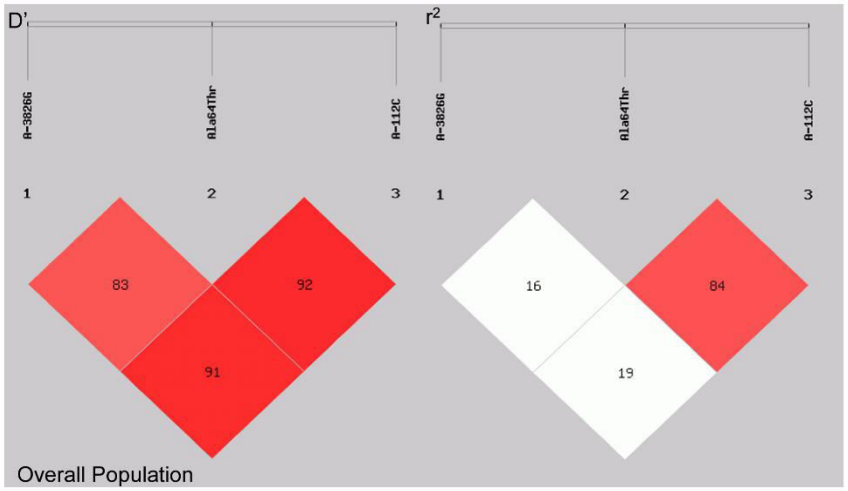
Eppendorf Biospecrometer. Samples were stored at -20 °C until the day of the genotyping analysis. DNA samples (100 ng) were genotyped using polymerase chain reaction with TaqMan probes and primers for the *UCP1* A-3826G (rs1800592), A-1766G (rs3811791), A-112C (rs10011540), and Ala64Thr (rs45539933) polymorphisms. Primers and probes were designed using Primer Express Software (version 3.0; Applied Biosystems). Reactions were conducted in 96-well plates in a reaction volume of 25 ul using 1X Taq Buffer (Evrogen), 1.25 u HS Taq DNA Polymerase (Evrogen), 1 mM dNTPs (ThermoFisher Scientific), 2.5-6 mM (depends on SNP) $MgCl_2$, 0.4 μM of each primer, 0.16 μM of each probe, DNA sample, and mQ water. The plates were then placed in a real-time PCR thermal cycler (CFX96 Touch Real-Time PCR Detection System; BIO-RAD), and thermal cycling conditions were as follow: incubation at 60˚C for 1 min and 95 ˚C for 10 min, followed by 40 cycles of 95˚C for 15 seconds and 58-60˚C (depends on SNP) for 1 min. Fluorescence data files from each plate were analyzed using automated allele-calling software (CFX Maestro Software; BIO-RAD). To perform the quality control of the genotyping method, we assessed PCR-products of randomly chosen samples from each genotype by direct sequencing.

1.1.3. Statistical analysis

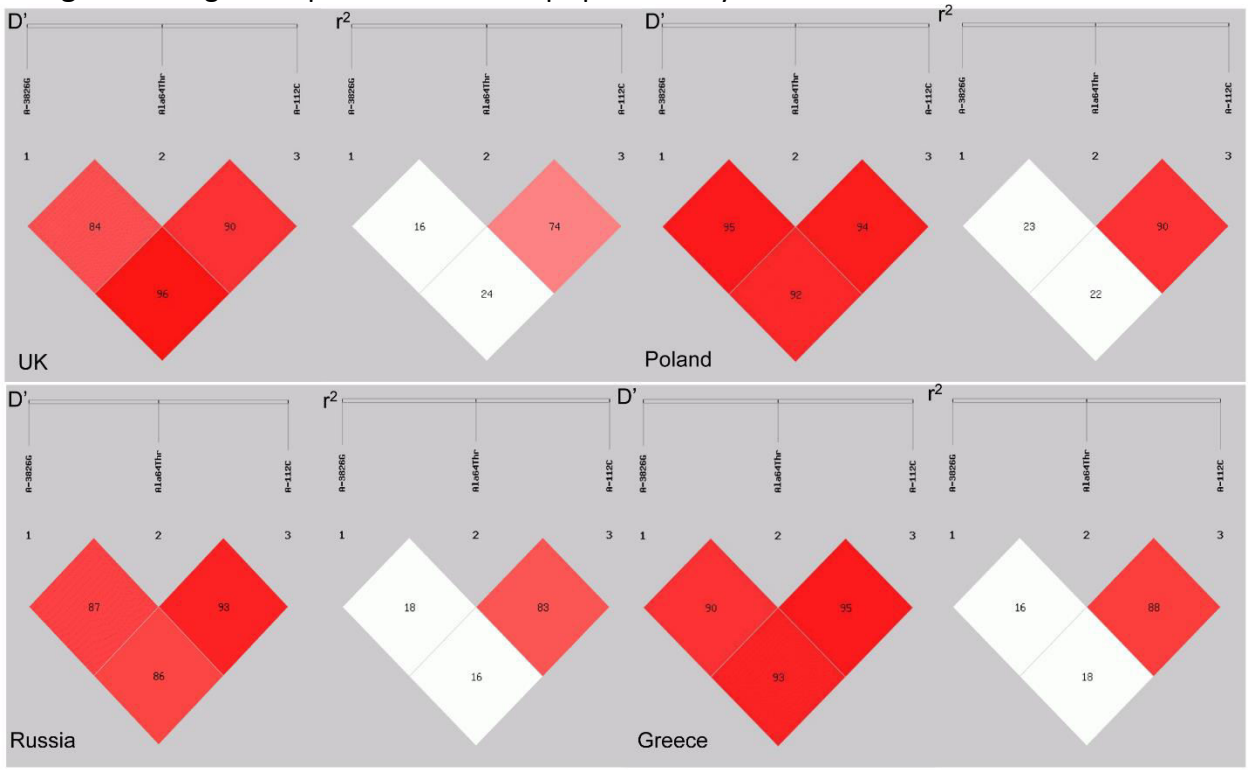
Prevalence rates for each SNP were calculated for: (1) the overall sample size, (2) each country, c) health status (i.e. CMP and healthy). Prevalence was determined by dividing the presence of genotype/allele of each SNP by the overall sample size. Standard error of the prevalence was calculated with the following formula: a (presence of genotype/allele) / [a (presence of genotype/allele) * b (sample size)]^2. The odds ratio (OR) for the analyzed genotype/allele was determined with the following equation: OR= [a (Wild type genotype/allele in healthy participants) * b (Mutated genotype/allele in CMP individuals)]/ c [(Wild type genotype/allele in CMP individuals) * d (Mutated genotype/allele in healthy participants)].

1.2. Results (Tables and Figures)

S1 Figure: Linkage Disequilibrium heat maps for the overall sample size



S2 Figure: Linkage Disequilibrium heat maps per country



S1 Table: Haplotype frequencies [n (prevalence)] for UK, Greece, Poland and Russia as well as for the overall study population for A-3826G, Ala64Thr and A-112C.

Haplotype	Overall		UK		Greece		Poland		Russia	
	CMP	Healthy	CMP	Healthy	CMP	Healthy	CMP	Healthy	CMP	Healthy
A G A	1814.17 (0.712)	1432.11 (0.699)	123.76 (0.691)	244.95 (0.684)	343.10 (0.689)	304.30 (0.673)	370.65 (0.750)	513.99 (0.706)	435.92 (0.703)	369.44 (0.724)
A A	OR: 1.10 (CI95%: 0.96-1.27); x^2=1.845, p=0.174		OR: 1.01 (CI95%: 0.68-1.49); x^2=0.001, p=0.976		OR: 1.10 (CI95%: 0.83-1.45); x^2=0.427, p=0.513		**OR: 1.33 (CI95%: 1.03-1.73); x^2=4.617, p=0.032**		OR: 0,96 (CI95%: 0.74-1.25); x^2=0.09, p=0.764	
A G	278.98 (0.653)		22.02 (0.052)		147.40 (0.744)		2.60 (0.013)			
	OR: 4.10 (CI95%: 1.12-14.98); x^2 = 5.3, p = 0.021									
G A C	119.84 (0.065)	174.03 (0.085)	12.76 (0.070)	30.92 (0.086)	28.60 (0.057)	44.73 (0.099)	37.65 (0.075)	63.00 (0.087)	40.88 (0.066)	35.44 (0.069)
G A	27.98 (0.066)		10.40 (0.033)							
	OR: 0.65 (CI95%: 0.45-0.95); x^2 = 5.011, p = 0.025									
G G	**OR: 0.76 (CI95%: 0.60-0.96); x^2=5.206, p=0.023**		OR: 0.79 (CI95%: 0.40-1.56); x^2=0.463, p=0.496		**OR: 0.56 (CI95%: 0.34-0.91); x^2=5.635, p=0.017**		OR: 0.87 (CI95%: 0.51-1.33); x^2=0.424, p=0.52		OR: 0.94 (CI95%: 0.61-1.55); x^2=0.023, p=0.879	
	97.02 (0.228)		37.60 (0.190)							
	OR: 1.26 (CI95%: 0.83-1.92); x^2 = 1.144, p = 0.285									
G G A	372.72 (0.202)	417.85 (0.204)	39.24 (0.216)	70.97 (0.198)	118.88 (0.239)	98.70 (0.218)	78.33 (0.156)	148.00 (0.203)	125.03 (0.202)	99.55 (0.195)
	OR: 0.999 (CI95%: 0.85-1.17); x^2=0.001, p=0.991		OR: 1.10 (CI95%: 0.71-1.71); x^2=0.184, p=0.668		OR: 1.13 (CI95%: 0.83-1.53); x^2=0.630, p=0.428		**OR: 0.74 (CI95%: 0.55-0.99); x^2=3.948, p=0.047**		OR: 1.07 (CI95%: 0.79-1.43); x^2=0.183, p=0.669	

Note: Haplotypes with frequencies lower than 3 % were omitted.

Key: CMP = individuals with cardio-metabolic pathologies; OR = odds ratio; CI95% = 95% confidence interval.

S2 Table: Haplotype frequencies [n (prevalence)] in Armenia for the A-3826G and Ala64Thr SNPs.

Haplotypes	CMP	Healthy
A G A	123.76 (0.691)	244.95 (0.684)
G A C	12.76 (0.070)	30.92 (0.086)
G G A	39.24 (0.216)	70.97 (0.198)

Key: CMP = individuals with cardio-metabolic pathologies; OR = odds ratio; CI95% = 95% confidence interval.

S3 Table: Prevalence rates for *UCP1* polymorphisms for the overall sample size and per country. SE: standard error; CMP: cardio-metabolic pathologies risk factors; UK: United Kingdom. Data for A-112C *UCP1* polymohism are not available for Armenia.

Polymorphism	Group / Genotype	Events	Sample size	Prevalence	SE
A-1766G	A-1766G overall AA	2216	2283	0.97	0.02
	A-1766G overall AG	40	2283	0.02	0.003
	A-1766G overall GG	3	2283	0.001	0.001
	A-1766G healthy AA	1116	1139	0.98	0.03
	A-1766G healthy AG	17	1139	0.01	0.004
	A-1766G healthy GG	2	1139	0.002	0.001
	A-1766G CMP AA	1079	1144	0.94	0.03
	A-1766G CMP AG	23	1144	0.02	0.004
	A-1766G CMP GG	1	1144	0.001	0.001
	A-1766G UK overall AA	264	273	0.97	0.06
	A-1766G UK overall AG	6	273	0.02	0.01
	A-1766G UK overall GG	2	273	0.01	0.01
	A-1766G UK healthy AA	173	181	0.96	0.07
	A-1766G UK healthy AG	6	181	0.03	0.01
	A-1766G UK healthy GG	1	181	0.01	0.01
	A-1766G UK CMP AA	91	92	0.99	0.10
	A-1766G UK CMP AG	0	92	0.00	0
	A-1766G UK CMP GG	1	92	0.01	0.01
	A-1766G Armenia overall AA	289	331	0.87	0.05
	A-1766G Armenia overall AG	13	331	0.04	0.01
	A-1766G Armenia overall GG	0	331	0.00	0
	A-1766G Armenia healthy AA	102	105	0.97	0.10
	A-1766G Armenia healthy AG	3	105	0.03	0.02
	A-1766G Armenia healthy GG	0	105	0.00	0
	A-1766G Armenia CMP AA	187	226	0.83	0.06
	A-1766G Armenia CMP AG	10	226	0.04	0.01
	A-1766G Armenia CMP GG	0	226	0.00	0
	A-1766G Poland overall AA	615	617	0.997	0.04
	A-1766G Poland overall AG	2	617	0.003	0.002
	A-1766G Poland overall GG	0	617	0.00	0
	A-1766G Poland healthy AA	363	365	0.99	0.05
	A-1766G Poland healthy AG	2	365	0.01	0.004
	A-1766G Poland healthy GG	0	365	0.00	0
	A-1766G Poland CMP AA	0	252	0.00	0
	A-1766G Poland CMP AG	0	252	0.00	0
	A-1766G Poland CMP GG	0	252	0.00	0
	A-1766G Russia overall AA	559	565	0.99	0.04
	A-1766G Russia overall AG	6	565	0.01	0.004
	A-1766G Russia overall GG	0	565	0.00	0
	A-1766G Russia healthy AA	254	255	0.996	0.06
	A-1766G Russia healthy AG	1	255	0.004	0.004
	A-1766G Russia healthy GG	0	255	0.00	0

	A-1766G Russia CMP AA	305	310	0.98	0.06
	A-1766G Russia CMP AG	5	310	0.02	0.01
	A-1766G Russia CMP GG	0	310	0.00	0
	A-1766G Greece overall AA	489	529	0.92	0.04
	A-1766G Greece overall AG	13	529	0.02	0.01
	A-1766G Greece overall GG	1	529	0.002	0.002
	A-1766G Greece healthy AA	224	233	0.96	0.06
	A-1766G Greece healthy AG	5	233	0.02	0.01
	A-1766G Greece healthy GG	1	233	0.004	0.004
	A-1766G Greece CMP AA	244	264	0.92	0.06
	A-1766G Greece CMP AG	8	264	0.03	0.01
	A-1766G Greece CMP GG	0	264	0.00	0
A-3826G	A-3826G Overall AA	1167	2283	0.51	0.01
	A-3826G Overall AG	919	2283	0.40	0.01
	A-3826G Overall GG	193	2283	0.08	0.01
	A-3826G Healthy AA	571	1139	0.50	0.02
	A-3826G Healthy AG	463	1139	0.41	0.02
	A-3826G Healthy GG	97	1139	0.09	0.01
	A-3826G CMP AA	584	1144	0.51	0.02
	A-3826G CMP AG	448	1144	0.39	0.02
	A-3826G CMP GG	95	1144	0.08	0.01
	A-3826G UK overall AA	136	273	0.50	0.04
	A-3826G UK overall AG	105	273	0.38	0.04
	A-3826G UK overall GG	31	273	0.11	0.02
	A-3826G UK healthy AA	88	181	0.49	0.05
	A-3826G UK healthy AG	74	181	0.41	0.05
	A-3826G UK healthy homozygous GG	18	181	0.10	0.02
	A-3826G UK CMP AA	48	92	0.52	0.08
	A-3826G UK CMP AG	31	92	0.34	0.06
	A-3826G UK CMP GG	13	92	0.14	0.04
	A-3826G Armenia overall AA	168	331	0.51	0.04
	A-3826G Armenia overall AG	129	331	0.39	0.03
	A-3826G Armenia overall GG	24	331	0.07	0.01
	A-3826G Armenia healthy AA	57	105	0.54	0.07
	A-3826G Armenia healthy AG	37	105	0.35	0.06
	A-3826G Armenia healthy GG	6	105	0.06	0.02
	A-3826G Armenia CMP AA	111	226	0.49	0.05
	A-3826G Armenia CMP AG	92	226	0.41	0.04
	A-3826G Armenia CMP GG	18	226	0.08	0.02

	A-3826G Poland overall AA	323	617	0.52	0.03
	A-3826G Poland overall AG	254	617	0.41	0.03
	A-3826G Poland overall GG	40	617	0.06	0.01
	A-3826G Poland healthy AA	179	365	0.49	0.04
	A-3826G Poland healthy AG	159	365	0.44	0.03
	A-3826G Poland healthy GG	27	365	0.07	0.01
	A-3826G Poland CMP AA	144	252	0.57	0.05
	A-3826G Poland CMP AG	95	252	0.38	0.04
	A-3826G Poland CMP GG	13	252	0.05	0.01
	A-3826G Russia overall AA	296	565	0.52	0.03
	A-3826G Russia overall AG	222	565	0.39	0.03
	A-3826G Russia overall GG	47	565	0.08	0.01
	A-3826G Russia healthy AA	140	255	0.55	0.05
	A-3826G Russia healthy AG	93	255	0.36	0.04
	A-3826G Russia healthy GG	22	255	0.09	0.02
	A-3826G Russia CMP AA	156	310	0.50	0.04
	A-3826G Russia CMP AG	129	310	0.42	0.04
	A-3826G Russia CMP GG	25	310	0.08	0.02
	A-3826G Greece overall AA	244	529	0.46	0.03
	A-3826G Greece overall AG	209	529	0.40	0.03
	A-3826G Greece overall GG	51	529	0.10	0.01
	A-3826G Greece healthy AA	107	233	0.46	0.04
	A-3826G Greece healthy AG	100	233	0.43	0.04
	A-3826G Greece healthy GG	24	233	0.10	0.02
	A-3826G Greece CMP AA	125	264	0.47	0.04
	A-3826G Greece CMP AG	101	264	0.38	0.04
	A-3826G Greece CMP GG	26	264	0.10	0.02
Ala64Thr	Ala64Thr Overall GG	1893	2283	0.83	0.02
	Ala64Thr Overall GA	363	2283	0.16	0.01
	Ala64Thr Overall AA	19	2283	0.01	0.002
	Ala64Thr Healthy GG	944	1139	0.83	0.03
	Ala64Thr Healthy GA	175	1139	0.15	0.01
	Ala64Thr Healthy AA	13	1139	0.01	0.003
	Ala64Thr CMP GG	928	1144	0.81	0.03
	Ala64Thr CMP GA	188	1144	0.16	0.01
	Ala64Thr CMP AA	6	1144	0.01	0.002
	Ala64Thr UK overall GG	225	273	0.82	0.05
	Ala64Thr UK overall GA	43	273	0.16	0.02
	Ala64Thr UK overall AA	4	273	0.01	0.01

	Ala64Thr UK healthy GG	148	181	0.82	0.07
	Ala64Thr UK healthy GA	28	181	0.15	0.03
	Ala64Thr UK healthy AA	4	181	0.02	0.01
	Ala64Thr UK CMP GG	77	92	0.84	0.10
	Ala64Thr UK CMP GA	15	92	0.16	0.04
	Ala64Thr UK CMP AA	0	92	0.00	0
	Ala64Thr Armenia overall GG	254	331	0.77	0.05
	Ala64Thr Armenia overall GA	67	331	0.20	0.02
	Ala64Thr Armenia overall AA	0	331	0.00	0
	Ala64Thr Armenia healthy GG	90	105	0.86	0.09
	Ala64Thr Armenia healthy GA	14	105	0.13	0.04
	Ala64Thr Armenia healthy AA	0	105	0.00	0
	Ala64Thr Armenia CMP GG	164	226	0.73	0.06
	Ala64Thr Armenia CMP GA	53	226	0.23	0.03
	Ala64Thr Armenia CMP AA	0	226	0.00	0
	Ala64Thr Poland overall GG	515	617	0.83	0.04
	Ala64Thr Poland overall GA	96	617	0.16	0.02
	Ala64Thr Poland overall AA	6	617	0.01	0.004
	Ala64Thr Poland healthy GG	304	365	0.83	0.05
	Ala64Thr Poland healthy GA	58	365	0.16	0.02
	Ala64Thr Poland healthy AA	3	365	0.01	0.005
	Ala64Thr Poland CMP GG	211	252	0.84	0.06
	Ala64Thr Poland CMP GA	38	252	0.15	0.02
	Ala64Thr Poland CMP AA	3	252	0.01	0.01
	Ala64Thr Russia overall GG	475	565	0.84	0.04
	Ala64Thr Russia overall GA	85	565	0.15	0.02
	Ala64Thr Russia overall AA	5	565	0.01	0.004
	Ala64Thr Russia healthy GG	218	255	0.85	0.06
	Ala64Thr Russia healthy GA	34	255	0.13	0.02
	Ala64Thr Russia healthy AA	3	255	0.01	0.01
	Ala64Thr Russia CMP GG	257	310	0.83	0.05
	Ala64Thr Russia CMP GA	51	310	0.16	0.02
	Ala64Thr Russia CMP AA	2	310	0.01	0.005
	Ala64Thr Greece overall GG	424	529	0.80	0.04
	Ala64Thr Greece overall GA	72	529	0.14	0.02
	Ala64Thr Greece overall AA	4	529	0.01	0.004
	Ala64Thr Greece healthy GG	184	233	0.79	0.06
	Ala64Thr Greece healthy GA	41	233	0.18	0.03
	Ala64Thr Greece healthy AA	3	233	0.01	0.01

	Ala64Thr Greece CMP GG	219	264	0.83	0.06
	Ala64Thr Greece CMP GA	31	264	0.12	0.02
	Ala64Thr Greece CMP AA	1	264	0.004	0.004
A-112C	A-112C Overall AA	1634	2283	0.72	0.02
	A-112C Overall AC	300	2283	0.13	0.01
	A-112C Overall CC	18	2283	0.01	0.002
	A-112C Healthy AA	847	1139	0.74	0.03
	A-112C Healthy AC	169	1139	0.15	0.01
	A-112C Healthy CC	11	1139	0.01	0.003
	A-112C CMP AA	766	1144	0.67	0.02
	A-112C CMP AC	131	1144	0.11	0.01
	A-112C CMP CC	7	1144	0.01	0.002
	A-112C UK overall AA	218	273	0.80	0.05
	A-112C UK overall AC	50	273	0.18	0.03
	A-112C UK overall CC	2	273	0.01	0.01
	A-112C UK healthy AA	144	181	0.80	0.07
	A-112C UK healthy AC	33	181	0.18	0.03
	A-112C UK healthy CC	2	181	0.01	0.01
	A-112C UK CMP AA	74	92	0.80	0.09
	A-112C UK CMP AC	17	92	0.18	0.04
	A-112C UK CMP CC	0	92	0.00	0
	A-112C Poland overall AA	515	617	0.83	0.04
	A-112C Poland overall AC	94	617	0.15	0.02
	A-112C Poland overall CC	7	617	0.01	0.004
	A-112C Poland healthy AA	303	365	0.83	0.05
	A-112C Poland healthy AC	57	365	0.16	0.02
	A-112C Poland healthy CC	4	365	0.01	0.01
	A-112C Poland CMP AA	212	252	0.84	0.06
	A-112C Poland CMP AC	37	252	0.15	0.02
	A-112C Poland CMP CC	3	252	0.01	0.01
	A-112C Russia overall AA	480	565	0.85	0.04
	A-112C Russia overall AC	80	565	0.14	0.02
	A-112C Russia overall CC	5	565	0.01	0.004
	A-112C Russia healthy AA	218	255	0.85	0.06
	A-112C Russia healthy AC	34	255	0.13	0.02
	A-112C Russia healthy CC	3	255	0.01	0.01
	A-112C Russia CMP AA	262	310	0.85	0.05
	A-112C Russia CMP AC	46	310	0.15	0.02
	A-112C Russia CMP CC	2	310	0.01	0.005

	A-112C Greece overall AA	421	529	0.80	0.04
	A-112C Greece overall AC	72	529	0.14	0.02
	A-112C Greece overall CC	4	529	0.01	0.004
	A-112C Greece healthy AA	182	233	0.78	0.06
	A-112C Greece healthy AC	45	233	0.19	0.03
	A-112C Greece healthy CC	2	233	0.01	0.01
	A-112C Greece CMP AA	218	264	0.83	0.06
	A-112C Greece CMP AC	31	264	0.12	0.02
	A-112C Greece CMP CC	2	264	0.01	0.01

S4 Table: Allele frequencies in the overall study population

SNP	Allele	CMP individuals		Healthy		OR	%95 CI	p
		no	freq.	no	freq.			
A-1766G	A	2181	0.99	2249	0.99	1.23	0.69-2.20	p=0.49
	G	25	0.01	21	0.01			
A-3826G	A	1616	0.72	1605	0.71	0.96	0.85-1.10	p=0.58
	G	638	0.28	657	0.29			
Ala64Thr	G	2044	0.91	2063	0.91	1.00	0.82-1.23	p=0.97
	A	200	0.09	201	0.09			
A-112C	A	1663	0.92	1863	0.91	0.85	0.68 -1.07	p=0.16
	C	145	0.08	191	0.09			

S5 Table: Allele frequencies in the Armenian population

SNP	Allele	CMP individuals		Healthy		OR	%95 CI	p
		no	freq.	no	freq.			
A-1766G	A	384	0.97	207	0.99	1.80	0.49-6.60	p=0.37
	G	10	0.03	3	0.01			
A-3826G	A	314	0.71	151	0.75	1.26	0.86-1.84	p=0.24
	G	128	0.29	49	0.25			
Ala64Thr	G	381	0.88	294	0.72	0.36	0.25- 0.51	p<0.001
	A	53	0.12	114	0.28			
A-112C	A	-	-	-	-	-	-	
	C	-	-	-	-			

S6 Table: Allele frequencies in the Greek population

SNP	Allele	CMP individuals		Healthy		OR	%95 CI	p
		no	freq.	no	freq.			
A-1766G	A	496	0.99	453	0.99	1.46	0.47 - 4.50	p=0.51
	G	8	0.01	5	0.01			
A-3826G	A	351	0.70	314	0.68	0.92	0.70 - 1.21	p=0.57
	G	153	0.30	148	0.32			
Ala64Thr	G	469	0.93	409	0.9	0.61	0.38 - 0.97	p=0.04
	A	33	0.07	47	0.1			
A-112C	A	467	0.93	409	0.89	0.63	0.40 - 0.98	p=0.04
	C	35	0.07	49	0.11			

S7 Table: Allele frequencies in the Polish population

SNP	Allele	CMP individuals		Healthy		OR	%95 CI	p
		no	freq.	no	freq.			
A-1766G	A	504	100	728	0.99	-	-	
	G	0	0.0	2	0.01			
A-3826G	A	383	0.76	517	0.71	0.77	0.59 - 0.99	p=0.045
	G	121	0.24	213	0.29			
Ala64Thr	G	460	0.91	666	0.91	0.97	0.65 - 1.44	p=0.86
	A	44	0.09	66	0.09			
A-112C	A	461	0.91	663	0.91	0.95	0.64 - 1.42	p=0.81
	C	43	0.01	65	0.09			

S8 Table: Allele frequencies in the UK population

SNP	Allele	CMP individuals		Healthy		OR	%95 CI	p
		no	freq.	no	freq.			
A-1766G	A	182	0.99	352	0.98	0.48	0.10 - 2.30	p=0.35
	G	2	0.01	8	0.02			
A-3826G	A	127	0.69	250	0.69	1.02	0.60 - 1.50	p=0.92
	G	57	0.31	110	0.31			
Ala64Thr	G	169	0.92	324	0.9	0.80	0.43 - 1.50	p=0.48
	A	15	0.08	36	0.1			
A-112C	A	165	0.91	321	0.90	0.89	0.49- 1.64	p=0.72
	C	17	0.09	37	0.10			

S9 Table: Allele frequencies in the Russian population

SNP	Allele	CMP individuals		Healthy		OR	%95 CI	p
		no	freq.	no	freq.			
A-1766G	A	615	0.99	509	0.99	4.14	0.48 - 35.54	p=0.16
	G	5	0.01	1	0.1			
A-3826G	A	441	0.71	373	0.73	1.11	0.85 - 1.44	p=0.45
	G	179	0.29	137	0.27			
Ala64Thr	G	565	0.91	470	0.92	1.14	0.75 - 1.75	p=0.54
	A	55	0.09	40	0.08			
A-112C	A	570	0.92	470	0.92	1.03	0.67 - 1.59	p=0.89
	C	50	0.08	40	0.08			

S10 Table. Frequency of genotypes for A-3826G in CMP and healthy individuals.

		Healthy		CMP		OR (95% CI)	F-test
		(n)	(%)	(n)	(%)		
Total sample	AA	571	50.49	584	51.82		5.29 p=0.153
	AG	463	40.94	448	39.75	0.95 (0.80-1.13)	
	GG	97	8.57	95	8.43	0.96 (0.71-1.30)	
	HWE	0.490		0.819			
Armenia	AA	57	57.00	111	50.23		3.13 p=0.354
	AG	37	37.00	92	41.63	1.27 (0.78-2.09)	
	GG	6	6.00	18	8.14	1.47 (0.57-3.79)	
	HWE	0.998		0.861			
Greece	AA	107	46.32	125	49.60		0.58 p=0.747
	AG	100	43.29	101	40.08	0.87 (0.59-1.26)	
	GG	24	10.39	26	10.32	0.93 (0.51-1.70)	

	HWE	0.929		0.408			
Poland	AA	179	49.04	144	57.14		4.21 p=0.120
	AG	159	43.56	95	37.70	0.74 (0.53-1.04)	
	GG	27	7.40	13	5.16	0.61 (0.31-1.21)	
	HWE	0.302		0.599			
Russia	AA	140	54.90	156	50.32		1.57 p=0.463
	AG	93	36.47	129	41.61	1.24 (0.88-1.76)	
	GG	22	8.63	25	8.07	1.02 (0.55-1.87)	
	HWE	0.251		0.816			
UK	AA	88	48.89	48	52.17		1.96 p=0.396
	AG	74	41.11	31	33.70	0.77 (0.45-1.33)	
	GG	18	10.00	13	14.13	1.33 (0.61-2.92)	
	HWE	0.675		0.042			

Key: CMP = cardio-metabolic pathologies; OR = odds ratio; HWE = p value for the Hardy-Weinberg equilibrium.

S11 Table. Frequency of genotypes for A-112C in CMP and healthy individuals.

		Healthy		CMP		OR (95% CI)	F-test
		(n)	(%)	(n)	(%)		
Total sample	AA	847	82.47	766	84.74		1.93 p=0.367
	AC	169	16.46	131	14.49	0.86 (0.67-1.10)	
	CC	11	1.07	7	0.77	0.72 (0.29-1.82)	
	HWE	0.433		0.593			
Greece	AA	182	79.48	218	86.85		4.92 p=0.73
	AC	45	19.65	31	12.35	0.58 (0.35-0.95)	
	CC	2	0.87	2	0.80	0.58 (0.35-0.95)	
	HWE	0.668		0.448			
Poland	AA	303	83.24	212	84.13		0.20 p=0.947
	AC	57	15.66	37	14.68	0.93 (0.60-1.46)	
	CC	4	1.10	3	1.19	1.11 (0.27-4.54)	
	HWE	0.479		0.347			
Russia	AA	218	85.49	262	84.52		0.75 p=0.717
	AC	34	13.33	46	14.84	1.12 (0.70-1.81)	
	CC	3	1.18	2	0.65	0.59 (0.12-3.04)	
	HWE	0.215		0.990			
UK	AA	144	81.32	74	81.32		0.64

S14 Table. Body mass index, waist-to-hip ratio and body fat percent [median (Q1,Q3)] across the different genotypes of UCP1 SNPs across healthy controls and individuals with CMP in Greece.

	AC	33	18.44	17	18.68	1.01 (0.53-1.93)	p=0.941
	CC	2	1.12	0	0.00	0.39 (0.02-8.18)	
	HWE	0.943		0.326			

Key: CMP = cardio-metabolic pathologies; OR = odds ratio; HWE = p value for the Hardy-Weinberg equilibrium.

S12 Table. Body mass index, waist-to-hip ratio and body fat percent [median (Q1,Q3)] across the different ge
UCP1 SNPs across healthy controls and individuals with CMP in Poland.

		BMI		WHR		Body fa
SNP	Genotype	Healthy	CMP	Healthy	CMP	Healthy
	AA	23.7 (21.7,25.6)	31.3 (29.1,33.8)	0.84 (0.79,0.90)	0.96 (0.87,1.05)	22.6 (18.3,28.
A-3826G	AG	23.9 (22.5,25.6)	31.2 (29.9,33.9)	0.86 (0.81,0.90)[1]	0.95 (0.87,1.04)	23.8 (18.0,27.6
	GG	24.6 (22.3,26.7)	30.1 (28.5,32.2)	0.86 (0.77,0.90)	0.95 (0.84,1.06)	24.8 (22.4,28.
	AA	23.7 (22.0,25.6)	31.2 (29.4,33.8)	0.85 (0.79,0.90)	0.96 (0.88,1.06)	22.9 (18.3,28.
A-112C	AC	23.9 (22.1,25.9)	31.3 (29.5,34.0)	0.85 (0.79,0.90)	0.94 (0.85,1.03)	24.5 (19.4,28.
	CC	26.4 (25.5,27.3)[,2]	27.3 (27.3, 29.8)	0.86 (0.84, 0.88)	0.84 (0.76,0.92)	22.7 (21.9,23.
	GG	23.7 (22.0,25.6)	31.3 (29.4,33.8)	0.85 (0.78,0.89)	0.96 (0.88, 1.06)	22.9 (18.3,28.
Ala64Thr	GA	24.0 (22.2,25.9)[1]	30.9 (29.0,33.8)	0.85 (0.79,0.90)	0.95 (0.85,1.03)	24.3 (19.1,28.
	AA	27.5 (27.3,27.6)	27.3 (27.3,29.8)	0.83 (0.82,0.84)	0.84 (0.76,0.92)	22.1 (21.6,22.

Note: 1 = difference from AA significant at p≤0.05; 2 = difference from AC significant at p≤0.05;

Key: CMP = cardio-metabolic pathologies, BMI= body mass index, WHR= waist-to-hip ratio, Q=quartile.

S13 Table. Body mass index, waist-to-hip ratio and body fat percent [median (Q1,Q3)] across the different ge
UCP1 SNPs across healthy controls and individuals with CMP in Russia.

		BMI		WHR		Body fat %	
SNP	Genotype	Healthy	CMP	Healthy	CMP	Healthy	
	AA	25.9 (25.4,26.3)	28.4 (26.1,34.1)	-	-	29.0 (26.8,32.0)	49.0
A-3826G	AG	25.6 (25.2,26.2)	29.5 (26.0,35.5)	-	-	29.0 (27.0,32.0)	48.3
	GG	25.8 (25.4,26.4)	30.2 (26.9,38.9)	-	-	28.0 (26.0,32.0)	49.9
	AA	25.8 (25.2,26.3)	28.9 (26.1,34.6)	-	-	29.0 (27.0,32.0)	48.6
A-112C	AC	26.0 (25.4,26.5)	30.3 (26.4,36.0)	-	-	30.0 (26.0,33.0)	48.6
	CC	25.9 (25.5,26.1)	29.8 (28.0,31.5)	-	-	31.0 (29.0,33.0)	50.4
	GG	25.8 (25.2,26.3)	28.9 (26.0,34.7)	-	-	29.0 (27.0,32.0)	48.6
Ala64Thr	GA	26.0 (25.5,26.5)	29.9 (26.1,35.9)	-	-	29.5 (26.0,33.0)	48.4
	AA	25.9 (25.5,26.1)	29.8 (28.0,31.5)	-	-	31.0 (29.0,33.0)	50.4

Key: CMP = cardio-metabolic pathologies, BMI= body mass index, WHR= waist-to-hip ratio, Q=quartile.

		BMI		WHR		Body fat %	
SNP	Genotype	Healthy	CMP	Healthy	CMP	Healthy	CMP
A-3826G	AA	26.4 (24.2,29.5)	31.7 (28.9,34.4)	0.94 (0.88,1.00)	1.02 (0.96,1.05)	29.4 (17.3,36.6)	40.4 (34.3,43.4)
	AG	27.2 (23.8,29.2)	31.7 (29.2,33.9)	0.94 (0.88,1.02)	1.02 (0.97,1.04)	29.6 (19.8,36.6)	39.0 (34.5,42.9)
	GG	28.7 (26.2,30.7)	30.9 (28.3,33.5)	0.94 (0.88,1.02)	1.02 (0.97,1.06)	26.5 (19.5,40.5)	37.9 (36.6,44.0)
A-112C	AA	26.5 (23.9,29.2)	31.6 (28.8,34.3)	0.94 (0.88,1.00)	1.03 (0.96,1.05)	28.9 (17.5,36.4)	39.1 (34.5,43.3)
	AC	28.8 (26.0,31.4)[1]	32.3 (29.3,33.6)	0.95 (0.89,1.01)	1.00 (0.96,1.03)	31.4 (25.1,41.7)[1]	39.1 (33.5,44.5)
	CC	26.3	30.4 (29.1,31.6)	1.03	1.00 (0.97,1.03)	19.6	35.7 (30.1, 41.2)
Ala64Thr	GG	26.5 (23.9,29.1)	31.5 (28.8,34.4)	0.94 (0.88,1.00)	1.03 (0.96,1.05)	28.9 (17.6,36.3)	39.1 (34.5,43.4)
	GA	29.0 (26.0,31.8)[2]	32.0 (28.9,33.6)	0.95 (0.90,1.02)	1.00 (0.96,1.04)	34.5 (25.5,41.9)[2]	39.1 (31.4,44.5)
	AA	26.3	32.8	1.00	1.05	19.6	46.7

Note: 1 = difference from AA significant at p≤0.05; 2 = difference from GG significant at p≤0.05; Q1 and Q3 values are reported only where more than one case was detected for a specific genotype.

Key: CMP = cardio-metabolic pathologies, BMI= body mass index, WHR= waist-to-hip ratio, Q=quartile.

S15 Table. Body mass index, waist-to-hip ratio and body fat percent [median (Q1, Q3)] across the different genotypes of UCP1 SNPs across healthy controls and individuals with CMP in UK.

		BMI		WHR		Body fat %	
SNP	Genotype	Healthy	CMP	Healthy	CMP	Healthy	CMP
A-3826G	AA	25.6 (23.0,29.5)	29.2 (26.9,34.5)	0.86 (0.81,0.91)	0.95 (0.92,0.97)	29.6 (24.1,35.1)	33.3 (27.4,38.4)
	AG	25.8 (23.3,29.4)	30.1 (25.7,34.8)	0.83 (0.78,0.90)	0.94 (0.92,0.98)	29.5(24.5,38.0)	33.0 (28.6,38.4)
	GG	27.7 (24.1,31.1)	29.8 (25.9,31.4)	0.84 (0.80,0.89)	0.94 (0.83,1.00)	35.4 (28.2, 40.2)	29.0 (26.9,37.0)
A-112C	AA	25.7 (23.1,29.6)	29.2 (26.5,34.6)	0.85 (0.79,0.91)	0.95 (0.72,0.97)	30.1 (25.1,37.1)	33.0 (27.7,38.2)
	AC	26.0 (23.5,31.1)[1]	30.1 (25.3,33.4)	0.84 (0.80,0.89)	0.93 (0.84,0.97)	29.8 (23.0,39.5)[1]	32.1 (26.9,39.5)
	CC	28.5 (26.6,30.5)	-	0.88 (0.88,0.89)	-	27.8 (19.8,35.8)	-
Ala64Thr	GG	25.7 (22.6,29.6)	29.0 (25.9,34.5)	0.85 (0.79,0.91)	0.95 (0.92,0.97)	30.1 (24.8,37.2)	33.7 (27.1,38.1)
	GA	26.4 (23.7,30.6)[2]	31.4 (28.4,33.6)	0.83 (0.79,0.90)	0.93 (0.87, 0.97)	30.0 (22.6,38.4)[2]	35.7 (28.2,40.3)
	AA	28.6 (24.7,35.3)	-	0.88 (0.86,0.89)	-	39.4 (29.1,46.3)	-

Key: CMP = cardio-metabolic pathologies, BMI= body mass index, WHR= waist-to-hip ratio, Q=quartile.

2. SYSTEMATIC REVIEW

2.1. Materials and Methods

2.1.1. Search strategy, selection criteria and meta-analysis process

The searching procedure, screening of the titles, abstracts and full texts for eligibility as well as the selection of the included studies were conducted independently by two investigators (PS and AEP) and any conflicts were resolved through discussion with a third investigator (PCD). We excluded reviews, conference proceedings and magazine articles and we also searched the reference lists of the included studies to identify potential eligible publications.

Two independent investigators (PS and AEP) evaluated the risk of bias (ROB) of the included studies in the systematic review, via the 13-item of Research Triangle Institute item bank,[265] which is designed for observational studies and has previously shown median interrater agreement of 75%[266] and 93.5%.[267] Conflicts in the risk of bias assessment were resolved by an independent referee investigator (PCD). Data extraction was performed independently by two investigators (PS and AW) and conflicts were resolved through consensus and supervision by a third researcher (PCD). For all studies, we extracted the first author's name, year of publication, methodological design, genotyping method, participants' characteristics and main outcomes (i.e. means±standard deviations/standard error, percentages, confidence intervals, frequencies, etc.).

We conducted prevalence meta-analyses by dividing the incidence of CMP by the overall sample size [a (incidence of genotype)/b (sample size)] of each study for *UCP1* A-3826G, A-1766G, Ala64Thr and A-112C SNPs. These meta-analyses were conducted for each one of the

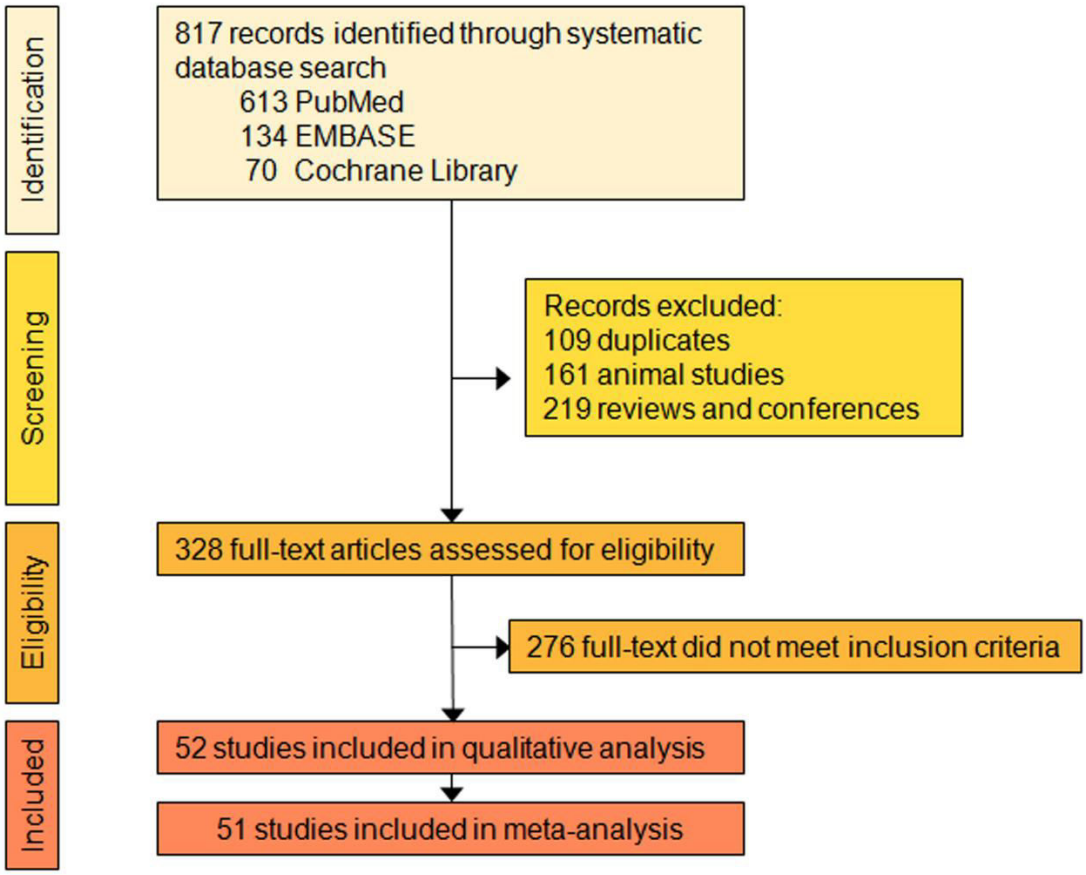
UCP1 homozygous and heterozygous genotypes as well as for the mutant alleles of each studied SNP. Standard errors for these meta-analyses were calculated using the following formula: a (incidence of genotype/allele) /[a (incidence of genotype/allele) *b (sample size)]^2. Standard errors were then used for weighted proportions and the RevMan 5.3 software[268] to generate forest and funnel plots. We also conducted odds ratio meta-analyses, using a dichotomous, inverse variance, random-effect model, via the RevMan 5.3 software. Incidence of each one of the *UCP1* homozygous and heterozygous genotypes and mutant alleles were calculated between a group of CMP individuals and a group of healthy participants, while weighted proportions were calculated based on each study's sample size. For all meta-analyses, we evaluated the 95% confidence interval (CI) and heterogeneity between studies using the I^2 statistic. We considered a statistically significant result for heterogeneity when $p<0.10$, while interpretation of I^2 index was made based on previous guidelines.[93] Where pertinent, standard error (SE) was converted to standard deviation (SD) using the following formula: SD= SE*$\sqrt{n}$.[93]

2.1.1.a. Searching algorithm used in PubMed

(((UCP1 variant*[Title/Abstract]) OR (UCP-1 variant*[Title/Abstract]) OR (uncoupling protein-1 variant*[Title/Abstract]) OR (uncoupling protein 1 variant*[Title/Abstract]) OR (thermogenin variant*[Title/Abstract]) OR (UCP-1 polymorphism*[Title/Abstract]) OR (UCP1 polymorphism*[Title/Abstract]) OR (uncoupling protein-1 polymorphism*[Title/Abstract]) OR (uncoupling protein 1 polymorphism*[Title/Abstract]) OR (thermogenin polymorphism*[Title/Abstract]) OR (UCP-1 gen*[Title/Abstract]) OR (UCP1 gen*[Title/Abstract]) OR (uncoupling protein-1 gen*[Title/Abstract]) OR (uncoupling protein 1 gen*[Title/Abstract]) OR (thermogenin gen*[Title/Abstract]) OR (UCP-1 single nucleotide polymorphism*[Title/Abstract]) OR (UCP1 single nucleotide polymorphism*[Title/Abstract]) OR (uncoupling protein-1 single nucleotide polymorphism*[Title/Abstract]) OR (uncoupling protein 1 single nucleotide polymorphism*[Title/Abstract]) OR (thermogenin single nucleotide polymorphism*[Title/Abstract]) OR (UCP-1 SNP*[Title/Abstract]) OR (UCP1 SNP*[Title/Abstract]) OR (uncoupling protein-1 SNP*[Title/Abstract]) OR (uncoupling protein 1 SNP*[Title/Abstract]) OR (thermogenin SNP*[Title/Abstract]) OR (UCP-1 mut*[Title/Abstract])

OR (UCP1 mut*[Title/Abstract]) OR (uncoupling protein-1 mut*[Title/Abstract]) OR (uncoupling protein 1 mut*[Title/Abstract]) OR (thermogenin mut*[Title/Abstract]) OR (UCP1 haplotype*[Title/Abstract]) OR (UCP-1 haplotype*[Title/Abstract]) OR (A-3826G[Title/Abstract]) OR (A-112C[Title/Abstract]) OR (Ala64Thr[Title/Abstract]) OR (A-3826G[Title/Abstract]) OR (A-1766G[Title/Abstract]) OR (A-112C[Title/Abstract]) OR (+1068G/A[Title/Abstract]) OR (rs10011540[Title/Abstract]) OR (rs45539933[Title/Abstract]) OR (rs3811791[Title/Abstract]) OR (rs1800592[Title/Abstract]) OR (-3826A>G[Title/Abstract]) OR (-112A>C[Title/Abstract]) OR (A-1766G[Title/Abstract]) OR (-1766A>G[Title/Abstract]) OR (uncoupling protein 1[Title/Abstract]) OR (uncoupling protein one[Title/Abstract])) AND ((metabolic syndrome[Title/Abstract]) OR (metabolic dis*[Title/Abstract]) OR (cardiometabolic dis*[Title/Abstract]) OR (CMD*[Title/Abstract]) OR (cardiometabolic disease[MeSH Terms]) OR (obesity[Title/Abstract]) OR (diabetes[Title/Abstract]) OR (T2DM[Title/Abstract]) OR (T2D[Title/Abstract]) OR (type 2 diabetes[Title/Abstract]) OR (type 2 diabetes mellitus[Title/Abstract]) OR (type II diabetes mellitus) OR (cardiovascular dis*[Title/Abstract]) OR (CVD*[Title/Abstract]) OR (cardiovascular disease[MeSH Terms]))) NOT ((animals[MeSH Terms]) NOT (humans[MeSH Terms]))

S3 Figure: PRISMA flowchart



2.2. Results (Tables and Figures)

The risk of bias assessment revealed that 58.97% of the studies displayed low selection bias, 2.54% displayed unclear ROB and 38.49% not applicable ROB. In performance and selective bias all studies displayed low ROB, while in detection bias 2.54% of the studies showed high ROB and 97.44% unclear ROB. In attrition bias, 2.54% of the studies displayed low ROB and 97.44% not applicable ROB. Finally, in confounding bias 89.75% of the studies displayed low ROB, 2.54% displayed high ROB and 7.69% unclear ROB.

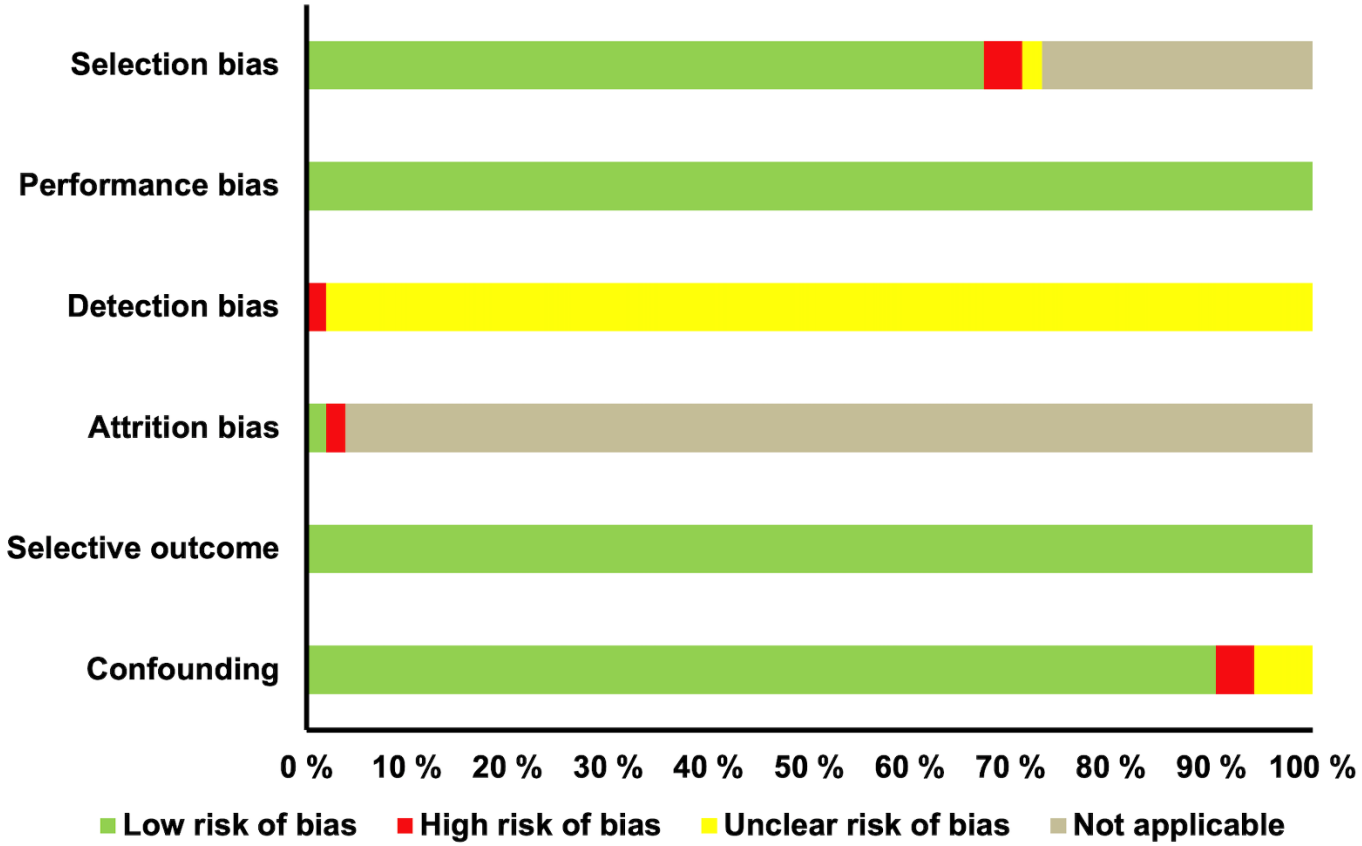
S16 Table: Risk of bias assessment results for the included studies in the systematic review.

Bracale et al, 2012	N	+	?	N	+	+
Brondan et al i, 2012	+	+	?	N	+	+
Brondani et al, 2014a	N	+	?	N	+	+

Brondani et al, 2014b	+	+	?	N	+	+
Cha et al, 2008	N	+	-	N	+	+
Chathoth et al, 2018	+	+	?	N	+	+
Chen et al, 2015	+	+	?	N	+	+
Csernus et al, 2014	+	+	?	N	+	+
de Souza et al, 2013	+	+	?	N	+	+
Dhall et al, 2012	N	+	?	N	+	-
Dong et al, 2020	+	+	?	N	+	+
Elfasakhany, 2020	-	+	?	N	+	+
Esterbauer, 1998	+	+	?	N	+	+
Forga, 2003	+	+	?	N	+	+
Franco-Hincapie, 2009	+	+	?	N	+	+
Fukuyama, 2006	N	+	?	N	+	+
Gagnon 1998	+	+	?	N	+	+
Hamada, 2009	+	+	?	-	+	+
Heilbronn, 2000	N	+	?	N	+	+
Jin 2020	-	+	?	N	+	+
Kiec-Wilk, 2002	N	+	?	N	+	+
Kotani, 2008	+	+	?	N	+	+
Kotani, 2011	+	+	?	N	+	+
Labruna, 2009	+	+	?	N	+	+
Lim, 2012	+	+	?	N	+	+
Lin, 2009	+	+	?	N	+	+
Lindholm, 2004	+	+	?	N	+	+
Malczewska-Malec, 2004	N	+	?	N	+	+
Montesanto, 2018	+	+	?	N	+	+
Mori, 2001	+	+	?	N	+	?
Mottagui-Tabar, 2008	+	+	?	N	+	?
Nakatochi, 2015	+	+	?	N	+	+
Nicoletti, 2016	N	+	?	N	+	+
Nieters, 2002	+	+	?	N	+	+
Oh, 2004	N	+	?	N	+	+
Pei, 2017	+	+	?	N	+	+
Proenza, 2000	+	+	?	N	+	+
Rudofsky, 2006	+	+	?	N	+	+
Rudofsky, 2007	N	+	?	N	+	+
Sale, 2007	N	+	?	N	+	+
Samano, 2012	+	+	?	N	+	+
Schaffler, 1999	N	+	?	N	+	?
Sivenius, 2000	?	+	?	+	+	+
Sramkova, 2007	+	+	?	N	+	+
Sun, 2018	+	+	?	N	+	+
Tiwari, 2009	+	+	?	N	+	+

Verdi, 2020	+	+	?	N	+	+
Vimaleswaran, 2007	+	+	?	N	+	+
Vimaleswaran, 2010	+	+	?	N	+	+
Yiew, 2010	+	+	?	N	+	-
Zhang, 2015	+	+	?	N	+	+
Zietz, 2001	N	+	?	N	+	+
Key: + = low risk of bias; – = high risk of bias; ? = unclear risk of bias; N = not-applicable.						

S4 Figure: Summary of risk of bias assessment**.**



S17 Table: Data extraction.

First Author-Year	Design	Methods	Participants	Outcomes
1.Bracale et al,2012 [269]	Case-only	Genotyping analysis by TaqMan assay by Real-Time PCR; A-3826G *UCP1* SNP.	Italians (n=112; m=40, f=72; age=32.7±10.5 years; BMI=48.5±7.5 kg/m^2. **Group 1:** severely obese non-diabetic individuals, IR+ (n=50) **Group 2:** severely obese non-diabetic individuals, IR- (n=62).	1. The A-3826G (rs1800592) genotypes were reported more in the IR+ positive (88%) than in IR- (63%) obese individuals (OR= 4.3, 95% CI= 1.6-11.7 p=0.003). 2. Absence of A-3826G UCP1 polymorphism displayed high negative predictive value (100%) for IR.
2.Brondani et al,2012 [70]	Case-control	Genotyping analysis by PCR-RFLPs; A-3826G *UCP1* SNP.	European ancestry, Type I diabetics (n=257) **Group 1:** Patients with Diabetic retinopathy (n=154), age=39.2±12.1 years, BMI= 23.6±4.9kg/m^2 **Group 2:** Patients without Diabetic retinopathy (n=103), age=33.32±13.8 years; BMI= 22.9±5.1 kg/m^2 **Group 3:** Healthy controls (n=29), age=44±7.8 years	1. Genotype frequencies Group1: AA=37%, AG=43.5%, GG=19.5% 2. Genotype frequencies Group 2: AA= 43.7%, AG=49.5%, GG=6.8% 3. G allele frequency Group 3 =33% 4. *UCP1* A-3826G GG genotype is associated with an increased risk of DR in type 1 DM patients.
3.Brondani et al, 2014a [111]	Case-control	Genotyping analysis by TaqMan assay by Real-Time PCR; A-3826G *UCP1* SNP.	Brazilians (n=765) **Group 1:** non-obese+T2DM (n=483); m=53.1%, f=46.9%; age=59.2±10.7 years; BMI= 25.8±2.8 kg/m^2 **Group 2:** obese+T2DM (n=282); m=37.4%, f=62.6%; age=57.3±10.0 years; BMI= 34.4±4.3 kg/m^2	1. A-3826G genotype frequencies in group 1 were for AA 46.7%, for AG 42.7%, and GG 10.6%; in group 2 were 49.4%, 38.6%, and 12%, respectively (p=0.529). 2. -3826G allele frequency in group 1 and group 2 was 0.319 and 0.313, respectively (p=0.839). 3. No difference in the allelic and genotypic distributions between group 1 and group 2 (p>0.05).
4.Brondani et al, 2014b [270]	Case-Control	Genotyping analysis by PCR-fast real system; A-3826G *UCP1* SNP.	Brazilians with Caucasian ancestry (n=1576) **Group 1:** non-diabetic (n=561) **Group 2:** T2DM individuals (n=1015); m=456, f=559; age=59.5±10.5 years; BMI=28.7±5.3 kg/m^2. Obesity was present in 35.9% of participants.	1. Among T2DM individuals, A-3826G genotype frequencies in group 1 were for AA 49.3%, AG 39.5%, and GG 11.2%; in group 2 were 49.8%, 37.7%, and 12.4%, respectively (p= 0.694). 2. -3826G allele frequency in group 1 and group 2 was 0.310 and 0.314, respectively (p = 0.851). 3. No difference in SBP and DBP, BMI, waist circumference, TC,

				HDL-C, LDL-C, HbA1c, FPG, TG levels between A-3826G genotypes (p>0.05).
5.Cha et al, 2008 [28]	Case-only	Genotyping analysis by TaqMan assay by Real-Time PCR; A-1766G, A-3826G and Ala64Thr (+1068G/A) *UCP1* SNPs.	Koreans (n=832) Obese females (n=832); age=27.88±7.80 years; BMI= 25.89±4.27 kg/m^2.	1. A-1766G (rs3811791) genotype distribution were for AA n=458, AG n=307, GG n=63. 2. Ala64Thr (rs45539933) genotype distribution were AA n=706, AG n=111, GG n=5. 3. A-3826G (rs1800592) genotype distribution were AA n= 216, AG n=406, GG=209. 4. -1766G allele frequency was 0.27. 5. Ala64ThrT allele frequency was 0.07. 6. -3826G allele frequency was 0.49 7. AG genotype of A-3826G SNP is associated with SBP in dominant model (p=0.042) and DBP in co-dominant model (p=0.035). 8. No association between A-1766G and Ala64Thr genotypes and SBP and DBP in any of the inheritance models studied (Co-dominant, Dominant and Recessive).
6.Chen et al, 2015 [112]	Case-control	Genotyping analysis by PCR-fast real system; A-3826G *UCP1* SNP.	Chinese (n=418) **Group 1:** non-obese (n=169); m=93, f=76; age=53.35±11.78 years; BMI=20.74±1.6 kg/m^2. **Group 2:** overweight/obese (n=249); m=149, f=100; age=55.04±10 years; BMI= 25.72±2.25 kg/m^2.	1. A-3826G genotype frequencies in group 1 were for AA 0.225, AG 0.479, GG 0.296; in group 2 were: 0.249, 0.454, and 0.297, respectively (p>0.05). 2. -3826G allele frequency in group 1 and group 2 was 0.524 and 0.536, respectively (p = 0.746). 3. Among overweight/obese individuals, -3826AG heterozygotes showed higher serum mean concentration of TG, apo C-II and apo C-III compared with t-3826AA homozygotes (p < 0.05). 4. No association was found of the A-3826G polymorphism and low HDL-cholesterolemia in overweight/obese individuals (p>0.05). 5. No association was found between UCP1 A-3826G polymorphism and

				overweight/obesity.
7.Chathoth et al, 2018 [65]	Case-Control	Genotyping analysis by TaqMan assay by Real-Time PCR; A-3826G, A-1766G, A-112C, *UCP1* SNPs	Saudi Arabians (n=492) **Group 1:** non-obese (n=155); m=76, f=79; age=43.86±14.54 years; BMI=24.09±2.6 kg/m^2. T2DM was present in 36.12% of participants. **Group 2:** obese T2DM (n=235) + obese hypertensive (n=85). The group was subdivided into: a. moderate-obese BMI≥30-39.9 kg/m^2); m=4.96%, f=56.03%; age=50.45±11.17 years; BMI=34.15±2.6 kg/m^2. b. extreme-obese, BMI≥40 kg/m^2; m=36.15%, f=63.84%; age=42.57±13.72 years; BMI= 48.26±11.94 kg/m^2. T2DM was present in 69.73% of participants, and hypertension was present in 25.22% of participants.	1. Among the obese (n=231), the -3826G allele frequency was higher as compared with non-obese (83) (OR=1.52, 95% CI= 1.10-2.08; p=0.009) (adjusted for age, gender and BMI). 2. -1766G allele was associated with the moderate-obesity (OR=2.89, CI=1.33–6.25; p=0.007), but not with extreme obesity (adjusted for age, gender and BMI). 3. -3826G allele frequency was higher in moderate-obese cohort with abnormal HDL, LDL, and hypertriglyceridemia; for hypercholesterolemia, -3826G allele frequency was higher in the extreme-obese cohort. 4. -1766G allele frequency was higher in the moderate-obese with high LDL. 5. -3826G allele frequency was higher in the moderate-obese with T2DM and hypertension. 6. -3826G allele frequency was higher in the extreme-obese males ≤35 years, and higher in the moderate-obese males > 35 years. 7. -1766G allele frequency was higher in females aged ≤35 years with extreme obesity, and in males aged ≤35 years with moderate obesity.
8.Csernus K. et al 2014 [113]	Case only	Genotyping analysis by PCR/PCR-RFLP; A-3826G, *UCP1* SNP.	Obese Hungarian children (n=528), Age:13.2±2.6 years, BMI: 30.6±4.6kg/m^2, m=297 and f=231.	1. No significant differences in measures of obesity adjusted BMR, obesity related metabolic parameters or blood pressure values according to *UCP1* A-3826G. 2. Genotype frequencies: AA=51.1%, AG= 40.5% and GG=8.3%. 3. Minor allele frequency= 0.29
9.de Souza 2013 [88]	Case-Control	Genotyping analysis by TaqMan assay by Real-Time	Brazilians with European ancestry (n=1515) **Group 1:** non-diabetic (n=534); m=55%, f=45%;	1. A-3826G genotype distributions in group 1 were: for AA 49.3%, AG 39.5%, GG 11.2%; in group 2 were: 49.9%, 37.7%, 12.4%, respectively (p=0.694).

		PCR; A-3826G *UCP1* SNP.	age=44.0±7.8 years. **Group 2:** T2DM (n=981); m= 47.4%, f=52.6%; age=59.52±10.63 years; BMI=28.84±5.39 kg/m^2.	2. -3826G allele frequency in group 1 and group 2 was 0.310 and 0.313, respectively (p=0.510). 3. A-3826G allele frequencies did not differ between diabetic and non-diabetic cohorts even when assuming dominant, recessive, additive or co-dominant models of inheritance (p>0.05).
10.Dhall et al, 2012 [114]	Case-only	Genotyping analysis by PCR-fast real system; A-3826G *UCP1* SNP	Indians (n= 96) Individuals with metabolic syndrome; m= 49, age=44±17 years; f=47, age=48±17 years.	1. A-3826G genotype frequencies were: for AA 39.9%, for AG 46.5%, and for GG 13.5%. 2. Among females, the -3826GG homozygotes showed higher BMI, SBP and DBP (p<0.001); among males, homozygotes for -3826A allele showed higher DBP (p<0.001). 3. In female -3826GG homozygotes DBP was correlated with waist circumference and WHtR (p<0.05); in female -3826 AG heterozygotes SBP and DBP were correlated with WC, fat %, BMI, WHtR and WHR (p<0.001). 4. Among males, no association with obesity markers and blood pressure between A-3826G genotypes.
11.Dong et al, 2020 [271]	Case-Control	Mass ARRAY genotyping system, A-1766G *UCP1* SNP	T2D individuals(n=928), 39.2% male and 60.8% female subjects, 60.9±10 years, 25.1±3.6 kg/m^2 Healthy Controls (n=1034), 37.7% male and 62.3% female subjects, 60.0±9.5 years, 24 ±3.2 kg/m^2	1. rs3811791 CC variant genotype conferred a significantly increased risk of T2DM and a higher level of TG 2. rs3811791 of UCP1 may be associated with T2DM and TG. 3. SB interacted with rs3811791 of UCP1 was associated with T2DM, 4. PA interacted with rs3811791 of UCP1 was associated with the level of HOMA-IR, HDL-C, and TG, suggesting that it is of paramount importance for people to take regular physical exercise

12.Elfasakhany et al, 2020	Case control	Genotyping analysis by PCR-RFLP, A-3826G *UCP1* SNP	Saudi Arabians (n=218) **Group 1:** control healthy (n=110), age=39.1 ± 6.07 years, BMI=23.47±1.44 kg/m^2 **Group 2** (n=108) Type 2 diabetes patients, age= 41.2±6.88 years, BMI=23.61± 1.23 kg/m^2	1. Control group→AA=50.9%, AG=40.9%, GG=8.2% 2. Type 2 diabetes group→ AA=47.22%, AG=39.82%, GG=12.96% 3. No significant difference in the genotype frequency between subjects with T2DM and healthy controls (P > 0.05). 4. No significant difference in the allele frequency between both T2DM subjects and healthy controls (P > 0.05) 5. They suggest that UCP1 A/G polymorphism at −3826 promoter region may not contribute to higher susceptibility to the T2DM in the Saudi population of Makkah region.
13.Esterbauer et al, 1998 [71]	Case-only	Genotyping analysis by PCR-RFLPs, A-3826G *UCP1* SNP	Caucasians (n=153)	1. Genotype frequencies: AA=70, AG=66, GG=8 2. -3826G polymorphism is probably a marker for expressional differences, but not the causative mutation. 3. UCP-1 gene locus is identified as a common cause of reduced UCP-1 gene activity in obese subjects.
14.Forga, et al, 2003 [74]	Case-Control	Genotyping analysis by PCR-fast real system; A-3826G *UCP1* SNP.	Spanish (n=313) **Group 1:** non-obese (n=154); BMI=22.3±1.8 kg/m^2 **Group 2:** obese (n=159); BMI=37.6±5.7 kg/m^2 Age=20-60 years.	1. A-3826G genotype distribution in group 1 was 63.6% for AA, 31.2% for AG, 5.2% for GG; in group 2 was 66.7%, 28.9%, and 4.4%, respectively (p=0.574). 2. No differences in UCP1 -3826G allele frequency between group 1 (0.21) and group 2 (0.19) individuals (p=0.574) 3. In obese group, -3826G allele carriers had higher BMI (p<0.05), fat % (p<0.05), SBP (p< 0.01), DBP (p<0.05).
15.Franco-Hincapie et al, 2009 [66]	Case-Control	Genotyping analysis by PCR-fast real system; A-3826G and Ala64Thr UCP1 SNPs.	Colombians (n=994) **Group 1:** non-diabetic (n=449); m=126, f=323; BMI=25.2±3.8 kg/m^2; age>40 years. **Group 2:** T2DM (n=545); m=190, f= 355; BMI=27±4.6 kg/m^2.	1. Association between A-3826G allele and T2DM (OR=0.78; 95% CI: 0.63-0.97; p=0.02).

16.Fukuyama et al, 2006 [80]	Case only	Genotyping analysis by TaqMan assay by Real-Time PCR; A-3826G and A-112C, UCP1 SNPs.	Japanese (n=93) T2DM; m=55, f=38; age=56.6±13.5 years; BMI=25.6±4 kg/m^2.	1. A-3826G genotype frequencies were: for AA 32.3%, for AG 48.4%, and for GG 19.3%. 2. A-112C genotype frequencies were: for AA 88.2%, for AG 10.7%, and for GG1.1%. 3. Carriers of -112C allele showed higher levels of fasting plasma immune-reactive insulin concentration (p=0.0085), HOMA-IR (p= 0.0089), and hepatic lipid content (p= 0.012). 4. No association was found of the A-3826G UCP1 polymorphism and any measured clinical parameters.
17.Gagnon et al, 1998 [115]	Case control	Genotyping analysis by PCR-RFLP, 3826A/G UCP1 SNP	Swedish (n=985) **Group 1:** Obese (n=684) **Group 2:** control subjects (n=311)	**Allele frequencies** 1. Control Group: A allele=473 G allele=149 2. Obese group: A allele= 1013, G allele=335 **Genotype frequencies** 3. Control group: AA=185, AG=103, GG=23 4. Obese group: AA=384, AG=245, GG=45 5. In both genders, there was no difference between carriers and non-carriers for variables pertaining to weight history.
18.Hamada et al. 2009 [116]	Case only	Genotyping analysis by PCR, 3826A/G UCP1 SNP	Japanese obese healthy women (n=32), mean ± S.D.; age 49.9 ± 8.45 years; BMI 28.4 ± 3.3 kg/m^2	1. The distribution of the A/A, A/G, and G/G genotypes was 18%, 49%, and 33%, respectively. 2. No difference in the changes of any physiological and metabolic parameters between the subjects with and without the A allele 3. No difference in the changes of any physiological and metabolic parameters between the subjects with the A/G genotype and those with the G/G genotype 4.
19.Heilbronn et al 2000 [76]	Case-only	Genotyping analysis by PCR-fast real system; A-3826G UCP1 SNP.	Australians (n=526) Female overweight/obese; BMI=34.1 kg/m^2.	1. A-3826G genotype frequencies were: for AA 0.307, for AG 0.190, and for GG 0.29. The -3826G allele frequency was 0.23. 2. -3826G allele was associated

				with higher BMI (p=0.02), insulin (p=0.03), and higher fasting glucose concentrations (p = 0.01). 3. Among T2DM females, -3826G allele carriers were more frequent (p=0.02) and was related with higher fasting glucose concentrations (p = 0.02).
20.Jin P. et al 2020 [117]	Case-control	Genotyping analysis: GWAS, TaqMan assay by Real-Time PCR; A-112C and A-3826G UCP1 SNPs	Han Chinese (n=3107) **Case**= 662 T2D patients with diabetic retinopathy (DR), **Control**=2445 T2D patients without diabetic retinopathy	1. Genotype frequencies: A112C: AA → DR=523, NDR=2046, AC→ DR=127, NDR=342, CC→ DR=4, NDR=10 2. rs10011540 (A-3826G) of the UCP1 gene is marginally significantly associated with DR
21.Kiec-Wilk et al 2002 [118]	Case-only	Genotyping analysis by PCR-fast real system; A-3826G UCP1 SNP.	Polish (n=118) Overweight/obese; m=38, f=80; age=43.4±19.3 years; BMI=33.21±7.73 kg/m^2.	1. A-3826G 1 genotype frequencies were: for AA 51.38%, for AG 33.94%, and for GG 14.68%. 2. -3826G allele frequency was 30.5%. 3. No association was found of -3826G allele with BMI and glucose tolerance. 4. -3526GG homozygotes showed higher fasting levels of TG (p=0.04) and those recorder at 6hrs of OLTT (p=0.058), and the lower HDL levels compared with -3826AA homozygotes (p=0.004). 5. Free fatty acids increased in -3826GG homozygotes, especially at 8 hrs post-OLTT (p=0.031). 6. Carriers of the -3826G allele showed increased LDL levels as compared with -3826AA homozygotes (p=0.027). 7. -3826G allele carriers showed higher beta-tromboglobulin levels compared with -3826AA homozygotes (pAA:A/G= 0.012; pAA:GG=0.055).
22.Kotani et al,2008 [120]	Case only	Genotyping analysis by PCR-RFLPs,	Japanese (n=298), age= 45.2±7.2 years, male=144, female=154	1. Genotype frequencies of UCP-1 genotypes were 26.5, 51.3, and 22.2% for AA, AG, and GG,

		3826A/G UCP1 SNP.		respec tively. 2. Allelic frequency of 0.48 for the G allele. 3. In males, HDL-C levels increased in the order of AA AG GG genotypes, and the levels in GG genotypes (1.75 ± 0.49 mmol/L) were significantly higher than those in the AA genotype (1.45 ± 0.34 mmol/L, p 5 0.015), whereas this trend was non-significantly detected in females. 4. GG genotype may be an independent protective factor associated with low HDL-cholesterolemia in healthy Japanese individuals.
23.Kotani et al,2011 [119]	Case-Control	Genotyping analysis by an intercalater-mediated fluorescent allele-specific PCR method; A-3826G UCP1 SNP.	Japanese (n=294) **Group 1:** non-obese (n=192). **Group 2:** obese (n=102). Age=65±13 years.	5. A-3826G genotype distributions in group 1 were: for AA 31%, AG 46%, and GG 23%; in group 2 were: 31%, 27%, and 27%, respectively (p=0.79). 6. The frequency of the -3826G allele was 0.47. 7. Among obese, -3826 GG homozygotes were more frequent (OR: 6.85, 95% CI: 1.65-28.49; p<0.01). 8. Obese carriers of -3826GG genotype showed higher prevalence of low HDL-cholesterolemia (37%) than those with the AA and AG genotypes (13%) (p<00.1). 9. Obese -3826GG homozygotes showed lower HDLC levels (1.20±0.30) than those carriers of the -3826 AA and AG genotypes (1.39±0.36; p=0.01).
24.Labruna et al,2009 [26]	Case-Control	Genotyping analysis by TaqMan assay by Real-Time PCR; A-3826G UCP1 SNP.	Italians (n= 197) **Group 1:** non-obese (n= 95); m=29, f=66; BMI>20 and <25 kg/m^2, respectively. **Group 2:** obese (n=102); m=41, f=61; age 34.5 and 31 years, respectively; BMI=47.9 and 47.7 kg/m2, respectively. Metabolic syndrome	1. A-3826G frequencies in group 1 were: for AA 54.8%, for AG 34.7%, and for GG 10.5%; in group 2 were: 50%, 41.2%, and 8.8%, respectively. 2. -3826G allele frequency in group 1 and group 2 was 0.28 and 0.29, respectively. 3. In participants with severe liver steatosis -3826 AG and GG genotypes were more frequent than in those with

			was present in 53% males and 66% females, and hypertension was present in 73% males and 31% females.	mild/moderate liver steatosis (21/31; 65% vs 30/70; 43%, p=0.0003). 4. 3826 AG + GG genotypes did not differ among metabolic syndrome+ and metabolic syndrome- obese (46% vs 56%) (p>0.05).
25.Lim et al,2012 [68]	Case-Control	Genotyping analysis by TaqMan assay by Real-Time PCR; A-3826G, A-1766G and Ala64Thr UCP1 SNPs.	Koreans (n= 2180) **Group 1:** heathy (n=587); m=273, f=314; age=64 years; BMI=24.17 kg/m^2. **Group 2:** obese with CIN and DP+ (n= 583); m=314, f=331; age=69 years; BMI=24.6 kg/m^2. **Group 3:** obese with CIN and DP- (n=1010); m=619, f=523; age=70 years; BMI=23.28 kg/m^2.	1. A-3826G minor frequency allele for groups 1, 2 and 3 were 46.73, 48.66, and 50.43, respectively. 2. A-1766G minor frequency allele for groups 1, 2, and 3 were 24.78, 24.4, and 24.09, respectively. 3. Ala64Thr minor frequency allele for groups 1, 2, and 3 were 6.58, 6.45, and 7.84, respectively. 4. Carriers of the -1766AG + GG genotypes were more frequent in DP+ group compared with the normal group in the dominant model (77.76% in DP+ vs.71.77% in normal, OR=1.508, p=0.006, power=85.3%). 5. -1766G allele frequency was lower in the DP- group compared with the normal group in the recessive model (4.77% in DP- vs. 5.10% in normal, OR = 0.606, p =0.0423, power=56.9%). 6. -1766GG homozygotes were less frequent in DP- compared with the normal group in the recessive model (OR=0.606, p=0.042). 7. Carriers of the -3826G allele showed higher serum HLC-C levels in the dominant models (p=0.032). 8. Serum TGs and HDL-C levels were associated with the -1766G allele in the recessive model (p=0.002; p=0.046, respectively).
26.Lin et al,2009 [122]	Case-Control	Genotyping analysis by TaqMan assay by	Taiwanese (n=575) **Group 1:** Non-obese T2DM (n=191); m= 95, f=96; age=57.8±9 years; BMI=22.4±2 kg/m^2.	1. A-3826G genotype frequencies in group 1 were: for AA, 42%, for AG 79%, for GG 57%; in group 2 were 24%, 54%,30%, respectively (p=0.449).

		Real-Time PCR; A-3826G UCP1 SNP.	**Group 2:** Non-obese controls (n=135); m=56, f=79; age=57.1±10.8 years; BMI= 22.1±1.8 kg/m^2. **Group 3:** Obese with T2DM (n=198); m=100, f=98; age=56.9±10.1 years; BMI=28.2±3.1 kg/m^2. **Group 4:** Obese controls (n=51); m=21, f=31; age=57.4±10 years; BMI=27.4±2 kg/m^2.	2. In non-obese - as determined by BMI, the A-3826G polymorphism was not associated with T2DM. 3. A-3826G genotype frequencies in group 3 were for AA 44%, for AG 91%, and for GG 49%; in group 4 were 10%, 15%, and 12%, respectively (p=0.277). 4. In obese- as determined by BMI, no association was found with T2DM for A-3826G polymorphism.
27.Lindholm et al, 2004 [123]	Case-Control	Genotyping analysis by PCR- fast real system; A-3826G UCP1 SNP.	Scandinavians (n=540) **Group 1:** non-diabetic (n=106); m= 61, f=45; age=55.0±14.1 years; BMI=26.2±4.6 kg/m^2. **Group 2:** diabetic + normoalbuminuria (n=218); m=118, f= 100; age=54.3±14.7 years; BMI=25.1±3.9 kg/m^2. **Group 3:** diabetic + micro- or macroalbuminuria (n=216); m=117, f=99; age=55.9±14.6 years; BMI=26.6±4.3 kg/m^2.	1. A-3826G genotype frequencies in group 1 were for AA 64.2% and for AG/GG 35.8%, in group 2 were 55.5% and 44.5%, respectively; in group 3 were 61.1% and 38.9%, respectively. 2. No differences in allele and genotype frequencies in the 3826A/G between the groups were found. 3. Carriers of the -3826G allele showed lower HDL-C levels (p=0.01).
28.Malczewska-Malec et al,2004 [89]	Case-only	Genotyping analysis by PCR- fast real system; A-3826G UCP1 SNP.	Southern Polish (n=122) Members of obese families; m=38, f=84; age=43±19 years. Obese (BMI≥30 kg/m^2), Overweight (BMI≥25 kg/m^2) were 44% and 25%, respectively.	1. No differences in glucose tolerance parameters between the A-3826G genotypes 2. No association between A-3826G polymorphism (i.e. AA and C allele carriers) and BMI and insulin resistance
29.Montesanto et al, 2018 [25]	Case-Control	Genotyping analysis by SEQUENOM MassArray iPLEX technology; Ala64Thr, A-1766G and A-3826G UCP1 SNPs.	Italians (n=940) **Group 1:** non-diabetic (n=505); m=41.2%, f= 58.8%; age=58.59±12.2 years; BMI= 27.1kg/m^2. **Group 2:** T2DM (n=435); m= 56.6%, f=43.4%; age=65.71±7.9 years; BMI=28.7 kg/m^2. The group was subdivided based on the presence and absence of retinopathy	1. No association of Ala64Thr (C/T) polymorphism with T2DM (p=0.969). 2. Ala64ThrT allele was less frequent in individuals with coexisting diabetic retinopathy compared to those without (OR=0.31, 95% CI=0.12-0.82; p=0.010). 3. -3826G allele was less frequent in individuals with nephropathy compared with those without (OR=0.55, 95% CI=0.33-0.98;

			(yes:111/no:324) and nephropathy (yes:54/no:381).	p=0.031).
30.Mori et al, 2001 [81]	Case-Control	Genotyping analysis by PCR- fast real system; A-112C UCP1 SNP.	Japanese (n=570) **Group 1:** healthy controls (n=250); m=145, f=105; age=76.4±7.9 years; BMI=20.9±3.4 kg/m^2. **Group 2:** T2DM (n=320); m=180, f=140; age=62.9±11.8 years; BMI=23.1±3.5 kg/m^2.	1. - 112A/C genotype distributions in group 1 were for AA 220, for AC 29, and for CC 1; in group 2 were 257, 61, and 2, respectively. 2. -112C allele frequency was higher in T2DM (10.2%) than in controls (6.2%) (p= 0.017). 3. A-3826G genotype distributions in Grp1 were for AA 58, for AG 116 and for GG 76; in Grp2 were 83,156, and 81, respectively. 4. The frequency of the -3826G allele did not differ between T2DM (49,7%) and controls (53,6%) (p=0.190).
31.Motaggui-Tabar et al, 2008 [124]	Case-only	Genotyping analysis by Dynamic allele specific hybridization and TaqMan assay by Real-Time PCR; A-3826G UCP1 SNP.	Swedish (n=773) **Group 1:** healthy controls (n=481); Females; BMI=23±3 kg/m^2. **Group 2:** obese (n=292); Females; BMI=39±5 kg/m^2.	1. A-3826G genotype frequencies in group 1 were for AA 59%, for AG 36%, and for GG 5%; in group 2 were 55%, 38%, and 7%, respectively (p=0.53). 2. No allele and genotype differences between the obese and controls. 3. A-3826G polymorphism was not associated with BMI, waist circumference, serum insulin or insulin sensitivity (p>0.05).
32.Nakatochi et al,2015 [125]	Case-Control	Genotyping analysis by DigiTag2 assay; A-3826G *UCP1* SNP.	Japanese (n=2343) **Group 1:** Controls with no metabolic syndrome in 2001 and 2009 (n= 1983); Males; BMI=21.6 kg/m^2. **Group 2:** no metabolic syndrome in 2001, metabolic syndrome in 2009 (n= 360); BMI=24.6 kg/m^2.	1. -3826G allele frequency in groups 1 and 2 were 0.502 and 0.456, respectively (p= 0.022). 2. A-3826G polymorphism was associated with metabolic syndrome (OR=0.83; 95%CI=0.70-0.97; p=0.022).
33.Nicoletti et al 2016 [126]	Case-only	Genotyping analysis by TaqMan assay by Real-Time PCR; A-3826G *UCP1* SNP.	Mixed ethnicity (n=150) Obese; m=20%, f= 80%; age=47.2±10.5 years; BMI ≥35 kg/m^2.	1. A-3826G genotype frequencies were for AA 41.3%, for AG 45.3%, and for GG 13.4%. 2. -3826G allele frequency was 0.36. 3. Carriers of the -3826G allele had lower weight, body fat, and fat free mass for the dominant model (p<0.05). 4. -3826GG homozygotes showed

				lower frequency of T2DM compared with those carriers of -3826AA + GG genotypes. 5. A-3826G polymorphism was associated with weight [r^2:0.417, 95% CI: (-20.020 to -2.259); p=0.015] and with FM [r^2: 0.339, 95% CI: (-15.314 to –2.077); p=0.011] following a multiple regression model adjusted for sex, age, height, physical activity, and energy intake. 6. A-3826G polymorphism was associated with FFM following a simple linear regression model [r^2=0.288, 95% CI: (-8.110 to -1.238); p=0.008]. 7. A-3826G polymorphism was associated with weight [r^2: 0.094, 95%CI: (-25.421 to -4.752); p= 0.005], and FFM [r^2: 0.228, 95%CI: (-8.110 to -1.238); p= 0.008] following a linear regression model.
34.Nieters et al 2002 [127]	Case-control	Genotyping analysis by PCR-RFLPs, 3826A/G *UCP1* SNP.	Germans (n=308) **Group 1**: normal weight (n=154) age=51.3±8.5 years; BMI= 23±1.6 kg/m^2 **Group 2**: grade II and III obese (n=154) age=51.2±8.4 years; BMI= 38.2±2.8 kg/m^2	1. Genotype percent in Group 1: AA=54.9%, AG=41.2%, GG=3.9% 2. Genotype percent in Group 2: AA=54.5%, AG 41.6%, GG=3.9%/
35.Oh et al 2004 [75]		Genotyping analysis by PCR- fast real system; A-3826G *UCP1* SNP.	Koreans (n=190) Obese; m=44, f=146; age=28.38±0.72 years; BMI= 33.88±0.28 kg/m^2.	1. A-3826G UCP1 genotype distribution was for AA 22.1%, for AG 53.7%, and for GG 24.2%. 2. The frequency of the -3826G allele was 0.51. 3. Carriers of the -3826AG+GG genotypes showed higher DBP compared with those carriers of the -3826AA genotype (p=0.023). 4. LDL cholesterol levels were higher in obese carriers of the -3826G allele compared with -3826AA homozygotes type (p=0.011) 5. HDL cholesterol levels were lower in -3826GG homozygotes compared with those carriers of

				the -3826AA+ AG genotypes (p=0.042). 6. The atherogenic index was 22.8% higher in -3826GG homozygotes compared with those carriers of the -3826AA genotype (p=0.027). 7. Obese -3826GG homozygotes showed higher LDL/HDL compared with those carriers of the -3826AA genotype (p=0.001). 8. When obese group was further divided into a normal group and a hyper-LDL cholesterolemia group, the frequency of -3826GG genotype was higher in the hyper 9. LDL cholesterolemia group (71.4%) than in normal group (43.9%) (p=0.05). 10. The frequency of hyper-LDL cholesterolemia was higher in -3826GG genotype carriers (25.6%) compared with those in -3826AA genotype carriers (9.8%) (p=0.05). 11. -3826GG genotype (OR= 4.115; p=0.03) and body fat mass (OR= 1.079; p=0.03), were risk factors of hyper-LDL cholesterolemia.
36.Pei et al 2017 [85]	Case-only	Genotyping analysis by ligase detection reaction; A-3826G and Ala64Thr *UCP1* SNPs.	Chinese (n=528) **Group 1:** normal fasting plasma glucose (n=445); m=174, f=271; age=51.57±13.13 years; BMI=24.00±3.54 kg/m^2. **Group 2:** impaired fasting glucose + T2DM (n=83); m=24, f=59; age=56.71±11.96 years; BMI=26.06±3.56 kg/m^2.	1. Ala64Thr genotype frequencies in group 1 were for CC 87.2%, for CT 12.6, and for TT 0.2%; in group 2 were 84.3%, 15.7%, and 0%, respectively (p>0.05). 2. Ala64Thr T allele frequency for groups 1 and 2 was 6.5% and 7.8%, respectively (p>0.05). 3. A-3826G genotype frequencies in group 1 were for AA 25.8%, for AG 52.6%, and for GG 21.6; in group 2 were 24.1%, 49.4%, and 26.5%, respectively (p>0.05). 4. -3826G allele frequency for groups 1 and 2 was 47.9% and 51.2%, respectively (p>0.05). 5. A-3826G and Ala64Thr genotype distributions and allele frequencies didn't differ between the normal fasting

				plasma glucose group and the impaired fasting glucose + T2DM group using codominant, dominant, and recessive genetic models- even after adjusting for age, sex, drinking status, and BMI (p>0.05).
37.Proenza et al, 2000 [128]	Case-Control	Genotyping analysis by PCR- fast real system; A-3826G *UCP1* SNP was studied.	Turkish (n=240) **Group 1:** lean healthy (n=94); m=77, f=17; age=30±1years; BMI=22.3±0.2 kg/m^2. **Group 2:** obese (n=146); m=83, f=63; age=35±1years; BMI=37.8±0.5 kg/m^2.	1. A-3826G genotype frequencies in group1 were for AA 52.1%, for AG 35.1%, and for GG 12.8%; in group 2 were 47.8%, 43.4%, and 8.8%, respectively (p=0.370). 2. -3826G allele frequency in groups 1 and 2 were 0.30 and 0.31, respectively. 3. -3826GG homozygotes showed higher cholesterol levels associated with BMI compared with carriers of the -3826AA (p=0.027) and -3826AG (p=0.039) genotypes.
38.Rudofsky et al,2007 [129]	Case-only	Genotyping analysis by PCR- fast real system; A-3826G *UCP1* SNP.	Caucasian with T2DM (n=517)	1. A-3826G genotype frequencies were for AA 49.9%, for AG 45.6%, and for GG was, and 4.5%. 2. -3826G allele frequency was 0.27 for G. 3. Genotypic distribution did not differ with respect to the baseline clinical characteristics except of age (p = 0.03). 4. A-3826G genotypes were not associated with diabetes-related microvascular complications: [neuropathy: p = 0.79); (retinopathy: p = 0.48); (nephropathy: p = 0.93)].
39.Rudofsky et al, 2006 [130]	Case only	Genotyping analysis by PCR-RFLPs, A-3826G *UCP1* SNP.	Type 1 diabetes (n=227)	1. 130 patients (57.3%) were (AA), 85 patients (37.4%) were (AG), and 12 (5.3%) were ho-mozygous for the polymorphism (GG) 2. No difference in genotype frequencies was found with respect to diabetes complications 3. No association of the A-3826G polymorphism in the UCP1 gene with diabetic neuropa- thy was observed,
40. Sale et al,	Case-	Genotyping	**Group 1:** African	1. A-3826G polymorphism was

2007 [131]	only	analysis by MassARRAY system; A-3826G and Ala64Thr *UCP1* SNPs.	Americans (n=287); m=43.5%, f= 56.5%; age=43.8±14.8 years; BMI=28.8±6.5 kg/m^2. **Group 2**: Hispanics (n=811), subdivided into: Group 2a: Hispanics from San Antonio (n=493); m= 40%, f=60%, age=43.6±14.8; BMI= 30.1±6.3 kg/m^2. Group 2b Hispanic individuals from San Luis Valley (n= 318); m= 47.8%, f= 52.2%; age=40.3±14 years; BMI=27.5±5.6 kg/m^2. Diabetes prevalence for group 1, group 2a, and group 2b was 11.6%, 17.7%, and 12.8%, respectively.	associated with acute insulin response to glucose in African Americans-adjusted for age, sex, and BMI (p=0.017). 2. A-3826G polymorphism was associated with HDL-C levels in Hispanic families from San Antonio-adjusted for age, sex, BMI- (p=0.001).
41.Samano et al, 2012 [132]	Case control	Genotyping analysis by Taq-Man PCR, A-3826G *UCP1* SNP	Mexican children (n=270) m=173, f=142 **Group 1**= Normal weight n=159, age=16.6±1 year, BMI=21.1±1.9 kg/m^2 **Group 2**= obesity, n=111, age=16.7±1.1 years, BMI=27.8±3.9 kg/m^2	1. The UCP1A-3826G (rs 1800592) polymorphism was associated with high percentage of fat (p = 0.002) and muscle weight (p = 0.019) in a recessive model. 2. Normal weight group: AA=32.1%, AG=50.9%, GG=17% 3. Obesity Group: AA=27.9%, AG=55.9%, GG=16.2%
42.Schaffler et al, 1999 [90]	Case-only	Genotyping analysis by PCR- fast real system; A-3826G *UCP1* SNP.	Germans (n=1020) m=534, f=486; age=51.3±14.6 years; BMI=25.5±4.4 kg/m^2.	1. A-3826G UCP1 genotype frequencies for AA, AG, and GG were 57.0%, 35.4%, and 7.6%, respectively. 2. -3826G allele frequency 0.25. 3. No significant differences between the genotypes and age, gender, BMI, leptin, glucose, fasting insulin, C-peptide, HbA1c, diabetes, TC, and HDL-C (p>0.05).
43.Sivenius et al, 2000 [133]	Case-Control	Genotyping analysis by PCR- fast real system; A-3826G	Finish (n=203) **Group 1:** non-diabetic (n= 123); m=55, f=68; age=58.4±5.3 years; BMI=27.1±4.4 kg/m^2.	1. -3826G allele frequency in groups 1 and 2 was 34.1% and 38.6%, respectively. 2. No difference in the A-3826G polymorphism frequency

		UCP1 SNP.	**Group 2:** T2DM (n=70); m=38, f=32; age=60.1±5.8 years; BMI=30.5±5.2kg/m^2.	between T2DM individuals and healthy controls.
44. Sramkova et al, 2007 [134]	Case-Control	Genotyping analysis by PCR- fast real system; A-3826G *UCP1* SNP.	Czech (n= 415) **Group 1:** healthy controls (n=120); m=42, f=78; age=32.5±11.0 years; BMI=23.3±3.8 kg/ m^2. **Group 2:** T2DM (n=295); m=112, f=183; age=58.8±7.0 years; BMI=30.5±5.5 kg/m^2. **Group 3**: healthy offspring of T2DM (n=113); m=41, f=72; age=38.2±10.4 years; BMI=25.5±4.2 kg/m^2.	1. -3826G allele frequency was 0.26 and not associated with increased risk of T2DM. 2. A-3826G genotype frequencies in group 1 were for AA 50.83%, for AG 40.83%, and for GG 8.33%; in group 2 were 53.22%, 42.03%, and 4.75%, respectively; in group 3 were 53.10%, 45.13%, and 1.77%, respectively. 3. Genotypic distribution did not differ between diabetics and controls (χ^2 = 2.02; p = 0.36). 4. Among diabetic women, -3826AG +GG genotype carriers showed lower WHR (p=0.000) and WHeR (p=0.049) compared with diabetic women carriers of the -3826AA genotype.
45. Sun et al, 2018 [135]	Case-Control	Genotyping analysis by Sequenom MassArray System; A-3826G UCP1 SNP.	Chinese (n=2207) **Group 1:** normotensives (n=1045); m=373, f= 672; age=50.28±8.70 years; BMI=23.73±3.41 kg/m^2 **Group 2:** hypertensives (n=1162); m=573, f=589; age=57.22±11.10; BMI=26.07±5.53 kg/m^2.	1. A-3826G genotype frequencies in group 1 were for AA 25.12%, for AG 50.82%, and for GG 24.06%; in group 2 were 25.2%, 52.01%, and 22.77%, respectively. 2. -3826G allele frequency in groups 1and 2 was 49.47% and 48.78%, respectively. 3. No association between A-3826G genotype and allele distributions and essential hypertension in co-dominant, dominant, and recessive models (p>0.05).
46.Tiwari et al, 2009 [136]	Case-Control	Genotyping analysis by PCR- fast real system; A-3826G, A-112C, and Ala64Thr UCP1 SNPs.	Asian Indians (n=420) **Group 1:** T2DM and no history of kidney disease; <u>Group 1a</u>: from south India T2DM cases (n=149); m=102, f=47; age=60.45±11.5 years; Group 1b: from north India T2DM cases (n=75); m=40, f=35; age=61.03±8.88 years. **Group 2:** T2DM and	1. Among south-Indians A-112C and Ala64Thr polymorphisms were associated with the development of chronic renal insufficiency; In the north-Indians no association for the UCP1 polymorphisms studied was found. 2. Among south-Indians, 112AA homozygotes showed higher percentage of T2DM with chronic renal insufficiency compared with -3826AG and GG

		chronic renal insufficiency. Group 2a: from south India T2DM cases with chronic renal insufficiency (n=106); m=81, f=25; age=55.97±11.5 years; Group 2b: from north India T2DM cases with chronic renal insufficiency (n=90); m=78, f=12; age=53.56±10.99 years.	carriers (OR=2.076, 95%CI=1.1893.625; p=0.0089) 3. Among south-Indians, -112A allele was more frequent in T2DM with chronic renal insufficiency (OR=1.849, 95% CI=1.1422.994; p=0.012) 4. Among south-Indians, Ala64Thr CC homozygotes showed a higher percentage of T2DM with chronic renal insufficiency compared with carriers of Ala64Thr TC and TT genotypes (OR=2.585, 95%CI=1.318–5.072; p=0.0048). 5. Among south-Indians, Ala64Thr C allele was more frequent in T2DM with chronic renal insufficiency individuals (OR=2.099 95%CI=1.146–3.844; p=0.015). 6. A-3826G polymorphism was not associated with the development of chronic renal insufficiency. 7. A-112C genotype in south-Indians and C allele frequencies in group 1 were for AA 59.7%, for AC 35.3%, and for CC 5%, and for C allele 0.227; in group 2 were 75.5%, 21.7%, and 2.8%, and 0.137, respectively. 8. A-112C in north-Indians and C allele frequencies in group 1 were for AA 60%, for AC 36%, and for CC 4%, and for C allele 0.220; in group 2 were 65.5%, 28.9%, and 5.6%, and 0.137, respectively. 9. Ala64Thr T/C in south-Indians and T allele frequencies in group 1 were for TT 1.5%, for TC 29.4%, and for CC 69.1%, and for T allele 0.162; in group 2 were 2.1%, 12.6%, and 85.3%, and 0.084%, respectively. 10. Ala64Thr T/C in north-Indians and T allele frequencies in group 1 were for TT 1.3%, for TC 25.3% and for CC 73.3%, and for T allele 0.140; in group 2 were 2.3%, 21.2%, and 76.5%, and 0.130, respectively.

47. Verdi H et al. 2020 [137]	Case-Control	Genotyping analysis by Real time PCR, melting curve; 3826A/G UCP1 SNP	Turkish (n=189) **Group 1:** obese children n=102 (f=54, f=48), age=12.3±2.8 years, BMI z score=2.6±0.5 **Group 2:** control n=87 (f=48, m=39), age= 11.9±3.2 years, BMI z score=-0.7±0.8	1. A-3826G allele frequencies in obese group G allele=27% and 22% in control group 2. A-3826G genotype frequencies in obese group AA=53%, AG=39%, GG=8%. 3. In control group AA=66%, AG=25% and GG=9%. 4. UCP1 A-3826G genotype is not associated with obesity, metabolic disorders, gender and glucose- insulin responses during oral glucose tolerance test. 11.
48. Vimaleswaran et al, 2010 [86]	Case-Control	Genotyping analysis by PCR- fast real system; A-3826G and A-112C UCP1 SNPs.	Asian Indians (n=1800) **Group 1:** normal glucose tolerant (n=990); m=374, f=616; age=49±12 years; BMI=24±4.7 kg/m^2. **Group 2:** T2DM (n=810); m=353, f=457; age=43±13 years; BMI=26.1±4.2 kg/m^2.	1. A-3826G genotype frequencies in group 1 were for AA 40%, for AG, 45%, and for GG 15%; in group 2 were 36%, 46%, and 18%, respectively (p=0.11). 2. -3826G allele frequency for groups1 and 2 was 0.38 and o.41, respectively (p=0.11). 3. A-112C genotype frequencies in group 1 were for AA 62%, for AC 34%, and for CC 4%; in group 2 were 63%, 33%, and 4%, respectively (p=0.87). 4. -112C allele frequency for groups 1 and 2 was 0.21 (p=0.87). A-3826G and A-112C UCP1 genotype and allele frequencies were not associated with T2DM.
49. Vimaleswaran et al, 2007 [138]		Genotyping analysis by PCR- fast real system; A-3826G and A-112C UCP1 SNPs.	Asian Indians (n=1500) **Group 1:** normal glucose tolerant (n=950); Subdivided into: <u>Group 1a</u>: metabolic syndrome (n=211); m=78, f=133; age=43±11 years; BMI=27.1±4.2 kg/m^2. <u>Group 1b</u>: no metabolic syndrome (n= 739); m=292, f=447; age=37±12 years; BMI=22.4±4.3 kg/m^2. **Group 2:** T2DM (n= 550); Subdivided into: <u>Group 2a</u>: metabolic	1. A-3826G genotype frequencies based in no metabolic syndrome groups (n=887) and in metabolic syndrome groups (n=613) were for AA 58% and 56%, for AG 36% and 39%, and for GG 6% and 5%, respectively. 2. -3826G allele frequency in no metabolic syndrome and metabolic syndrome groups was 0.24, respectively. 3. A-112C genotype frequencies in no metabolic syndrome and metabolic syndrome groups were for AA 74% and 70%, for AC 24% and 28%, and for CC 2%, respectively. 4. -112C allele frequency in no

			syndrome (n=402); m=179, f=223; age=51±11 years; BMI=25.8±4.2 kg/m^2. Group 2b: no metabolic syndrome (n= 148); m=70, f=78; age=51±12 years; BMI=23.6±3 kg/m^2.	metabolic syndrome and metabolic syndrome groups was 0.14 and 0.16, respectively. 5. A-3826G allelic (p=0.89) and genotypic (p=0.26) were not associated with metabolic syndrome. 5. A-112C allelic (p=0.16) and genotypic (p=0.21) distributions were not associated with metabolic syndrome.
50. Yiew et al 2010 [139]	Case only	Genotyping analysis by PCR-RFLPs, A-3826G UCP1 SNP.	Malaysian Chinese (n=256) healthy and unrelated students, age=21.7 ± 1.7 years	1. G allele frequency = 0.58 2. In lean subjects: AA=10%, AG=61, 8%, GG=28,2% 3. In overweight subjects: AA=12.8%, AG=60.5%, GG=26.7% 4. UCP1 −3826A/G SNP is not associated with obesity and its related anthropometric indicators among the Malaysian Chinese university students
51. Zhang et al, 2015 [77]	Case-Control	Genotyping analysis by PCR-ligase detection reactions; A-3826G UCP1 SNP.	Chinese (n=792) **Group 1:** diabetic retinopathy (n=448); m=196, f=252; age=62.35±11.92 years; BMI=25.58±4.18 kg/m^2. Subdivided into: Group 1a**:** diabetic retinopathy proliferative (n= 220); m= 91, f= 119; age=60.36±11.66 years; BMI=27.33±4.06 kg/m^2. Group 1b: diabetic retinopathy non-proliferative (n= 228); m=95, f=133; age=63.03±11.57 years; BMI=25.20±4.13 kg/m^2. **Group 2:** diabetic retinopathy proliferative -no signs of diabetic retinopathy (n=334); m=163, f= 181; age=60.16±11.67 years; BMI=26.16±4.75 kg/m^2.	1. A-3826G genotype frequencies in diabetic retinopathy were for AA 23.6%, AG 48.9%, GG 27.5%; in diabetic non-retinopathy were 28.1%, 48.2%, 3.7%, respectively; in diabetic retinopathy proliferative were 20.7%, 49.3%, 30%, respectively; in diabetic retinopathy non-proliferative 26.4%, 48.5%, 25.1%, respectively. 2. -3826G allele frequency in diabetic retinopathy, diabetic retinopathy proliferative, proliferative diabetic and diabetic retinopathy non-proliferative was 51.9%, 47.8%, 54.6%, and 49.3%, respectively. 3. Frequency of -3826GG genotype was higher in diabetic retinopathy proliferative than in diabetic retinopathy non-proliferative group in the additive model (OR=1.72, 95%CI=1.06–2.79, p=0.03). 4. Frequency of -3826G allele in the additive model was higher in diabetic retinopathy proliferative than in diabetic

				retinopathy non-proliferative (OR=1.32, 95% CI=1.03–1.68; p=0.03). 5. No differences were found for A-3826G allele frequencies and genotype distributions between the diabetic retinopathy and diabetic retinopathy proliferative or diabetic retinopathy non-proliferative and diabetic proliferative (p>0.05). 6. A-3826G is associated with increased risk of diabetic retinopathy proliferative in T2DM.
52. Zietz et al, 2001 [141]	Case-only	Genotyping analysis by PCR- fast real system; A-3826G UCP1 SNP.	Germans (n=549) T2DM; m=312, f=237.	1. A-3826G genotype frequencies were for AA 58.3%, for AG 37.3%, and for GG 4.4%. 2. -3826G allele frequency 0.23. 3. No differences in grade of retinopathy were found among A-3826G genotypes. 4. Serum levels of dehydroepiandrosterone sulfate were lowest in -3826GG homozygotes with no retinopathy compared with those carriers of the -3826AG and AA genotypes (p<0.05). 5. Among female T2DM, dehydroepiandrosterone sulfate was negatively correlated to cholesterol and positively to SBP (p<0.05). 6. No differences in sex, age, BMI known duration of diabetes, cholesterol, glycemic control (HbA1c), SBP, serum levels of C-peptide, cortisol and leptin between A-3826G genotypes were found.

Key: PCR= polymerase chain reaction; UCP1= uncoupling protein one; SNP= single nucleotide polymorphism; m= male; f=female; BMI=body mass index; IR= insulin resistance; OR=odds ratio; T2DM= type 2 diabetes mellitus; SBP= systolic blood pressure; DBP= diastolic blood pressure; TC= total cholesterol; HDL-C= high density lipoprotein-cholesterolemia; LDL-C= low density lipoprotein-cholesterolemia; HbA1c= glycated haemoglobin; FPG= fasting plasma glucose; TG= triglycerides; WHR= waist-to-hip ratio; HOMA-IR= homeostatic Model Assessment of Insulin Resistance; OLTT= oral lipid tolerance test; OGTT= oral glucose tolerance test; CIN= cerebral infarction; DP= Dampness-phlegm; CI= confidence interval; FFM= free fat mass.

2.2.1 Meta-analysis Methodology

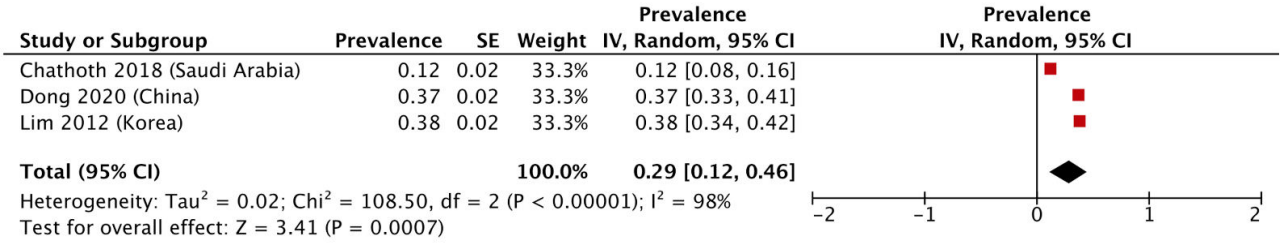
The aim of the systematic review and meta-analysis study was to investigate whether differences in the frequency of A-3826G, A-1766G, Ala64Thr and A-112C SNPs are associated with the most common CMP and their risk factors, in the existing already published literature. An important index to calculate these differences in frequencies, is the prevalence index. Therefore, we conducted prevalence meta-analyses by dividing the incidence of CMP by the overall sample size [a (incidence of genotype)/b (sample size)] of each study for *UCP1* A-3826G, A-1766G, Ala64Thr and A-112C SNPs. These meta-analyses were conducted for each one of the *UCP1* homozygous and heterozygous genotypes as well as for the mutant alleles of each studied SNP. Standard errors for these meta-analyses were calculated using the following formula: a (incidence of genotype/allele) /[a (incidence of genotype/allele) *b (sample size)]^2. Standard errors were then used for weighted proportions and the RevMan 5.3 software[268] to generate forest and funnel plots. Another important index to test whether there is an association between a group of CMP individuals and a group of healthy participants in the *UCP1* homozygous and heterozygous genotypes as well as mutant alleles, is the odds ratio. Therefore, we conducted odds ratio meta-analyses, using a dichotomous, inverse variance, random-effect model, via the RevMan 5.3 software.[4] Incidence of each one of the *UCP1* homozygous and heterozygous genotypes and mutant alleles were calculated between a group of CMP individuals and a group of healthy participants, while weighted proportions were calculated based on each study's sample size. For all meta-analyses, we evaluated the 95% confidence interval (CI) and heterogeneity between studies using the I^2 statistic. Heterogeneity is an index that tests any kind of variability (i.e. effect estimate) among the included studies in a systematic review and meta-

analysis.[93] We considered a statistically significant result for heterogeneity when p<0.10, while interpretation of I^2 index was made based on previous guidelines.[93] A heterogeneity of 0%-40% might not be important, 30%-60% may represent moderate heterogeneity, 50%-90% may represent substantial heterogeneity and 75%-100% represents considerable heterogeneity.[93]

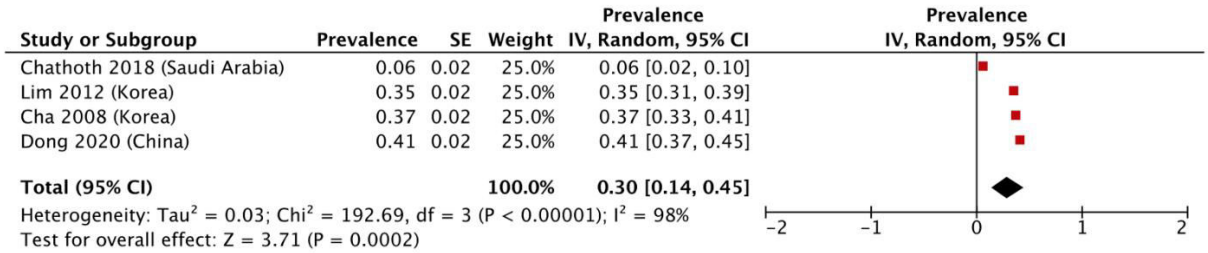
The results from the SNP-specific forest and funnel plots for the prevalence (Figures S7-26) and the odds ratio (Figures S27-36) for different genotypes are shown below. Funnel plots were only produced for those meta-analyses that included >10 studies [93].

From the 52 eligible studies for our Systematic Review, 51 were included in the metanalysis. The publication from Sale et al. [131] was excluded since the population was not stratified according to health status.

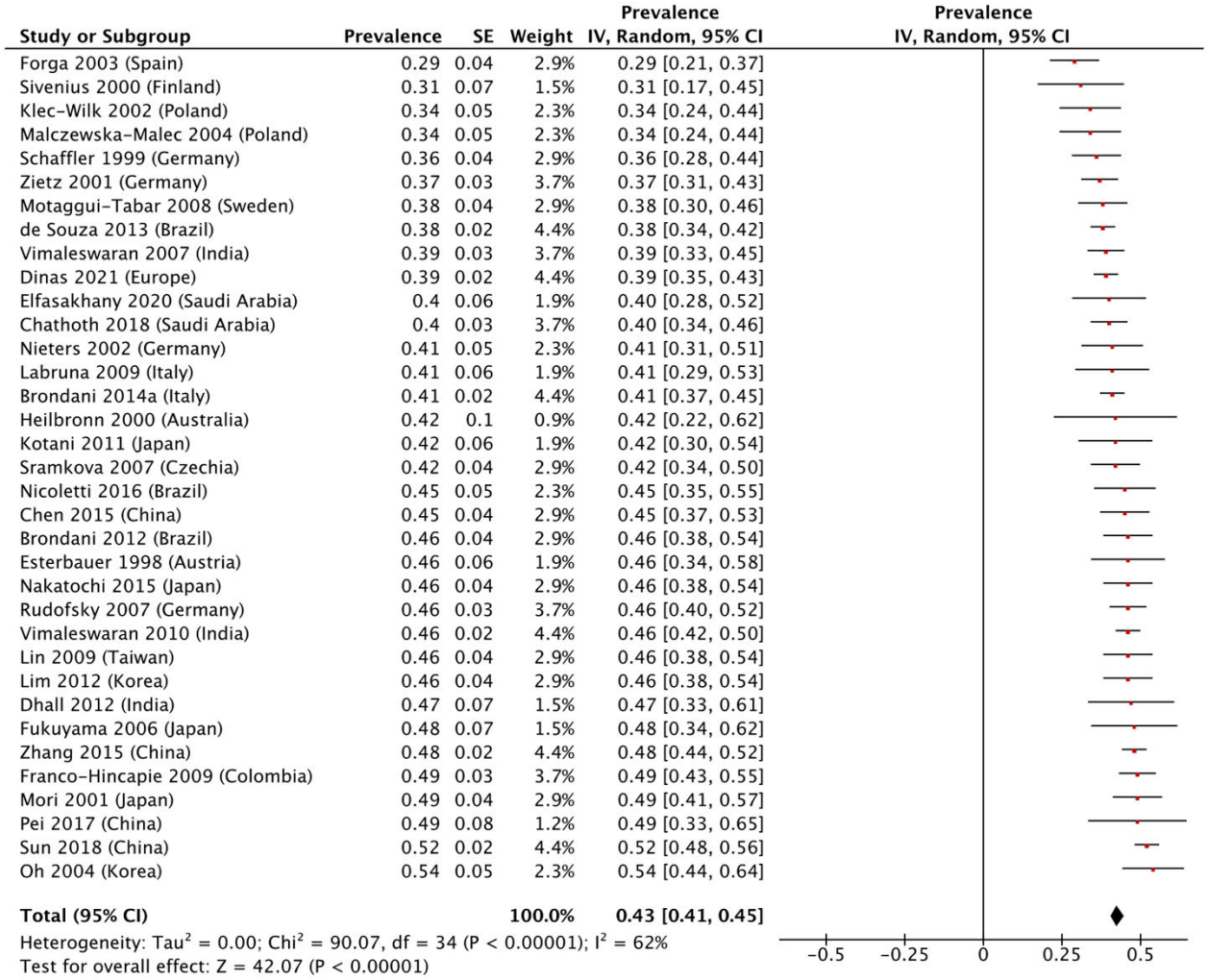
S5 Figure: Forest plot for prevalence of UCP1 A-1766G / AG in the CMP population.



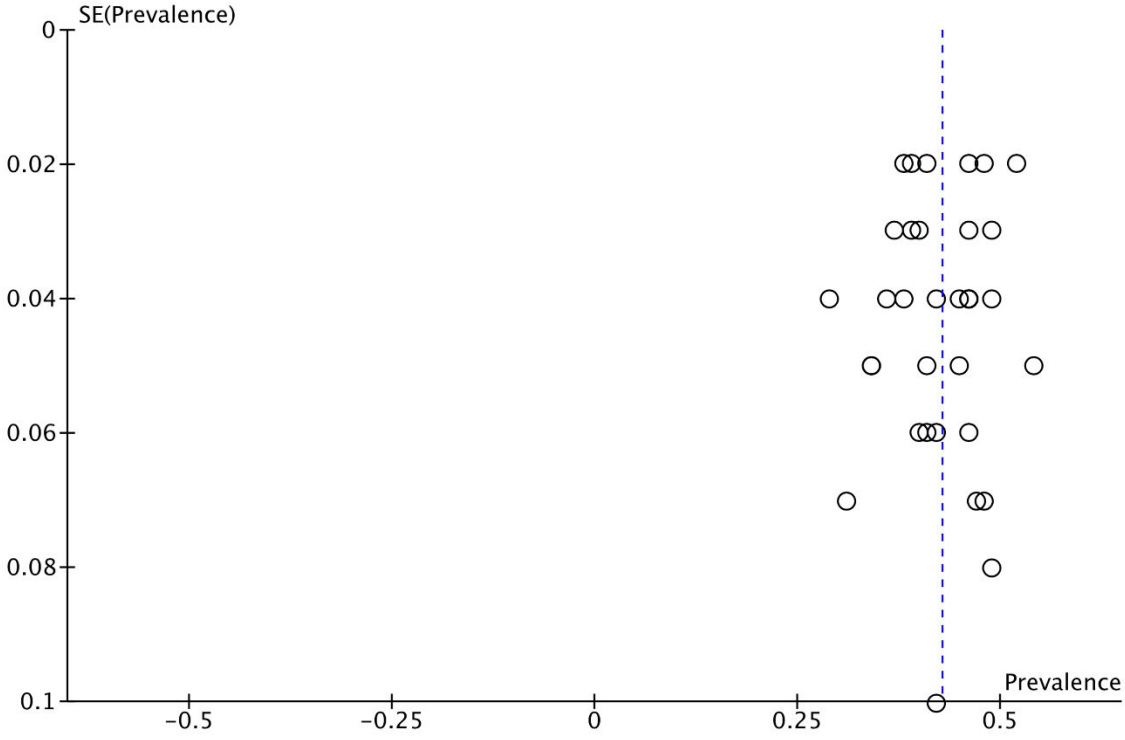
S6 Figure: Forest plot for prevalence of UCP1 A-1766G / AG in healthy individuals.



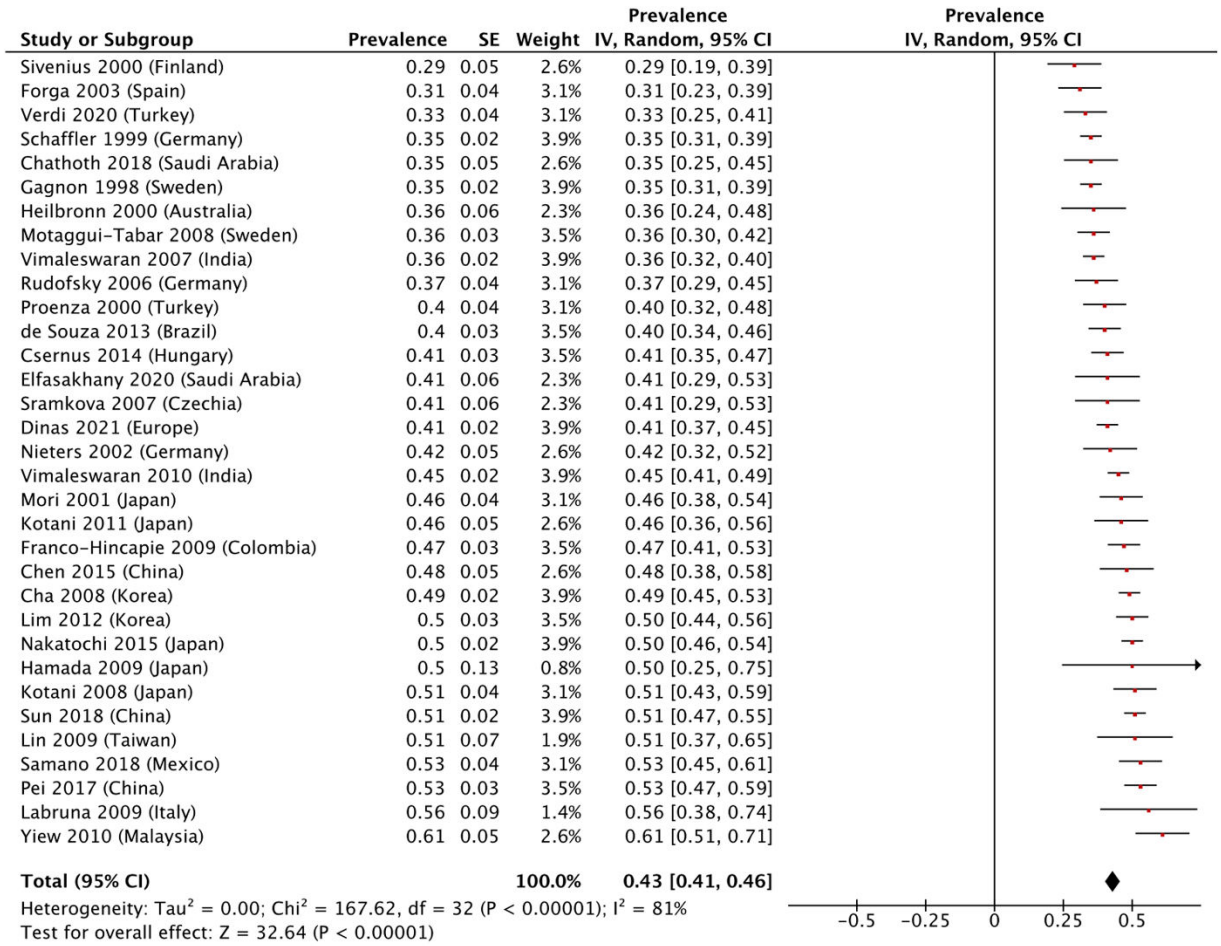
S7 Figure: Forest plot for prevalence of *UCP1* A-3826G / AG in CMP individuals.



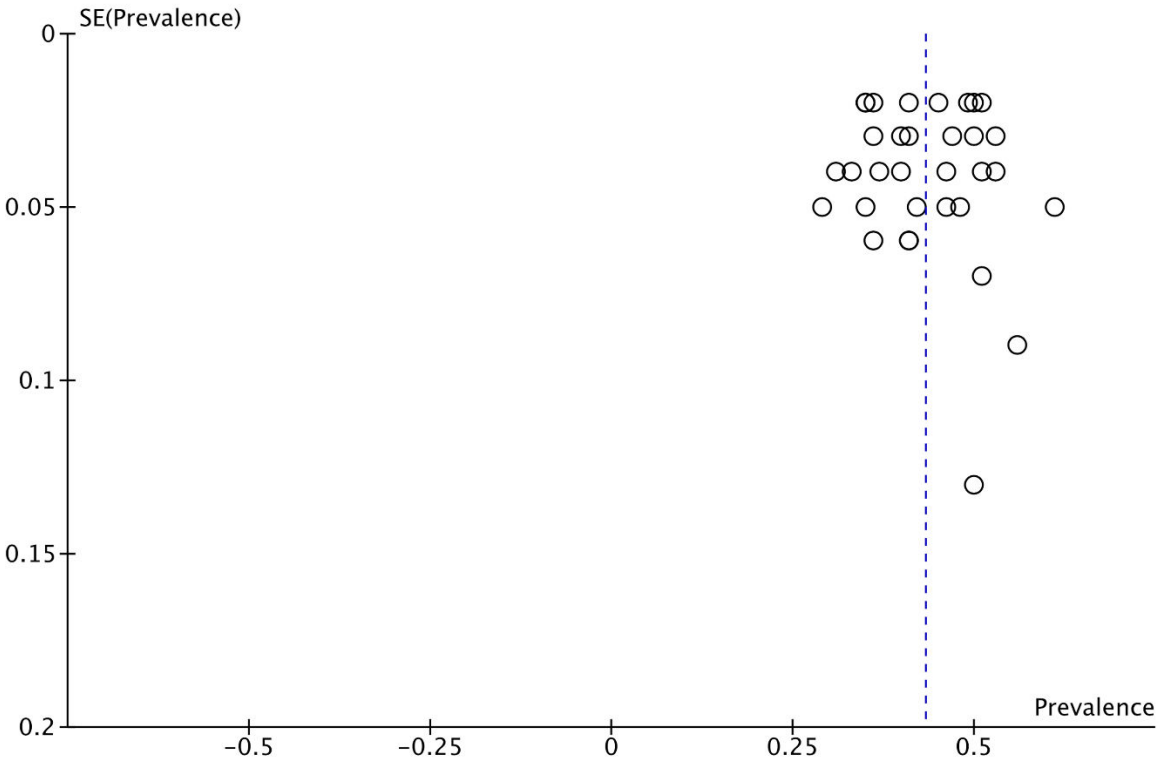
S8 Figure: Funnel plot for prevalence of UCP1 A-3826G / AG in CMP individuals.



S9 Figure: Forest plot for prevalence of UCP1 A-3826G / AG in healthy individuals.



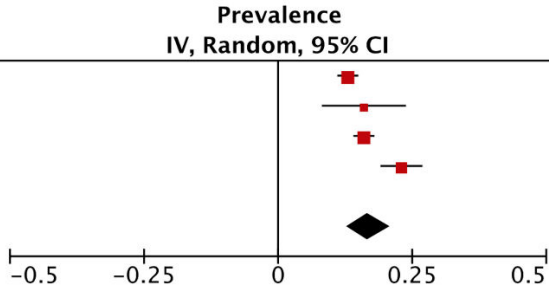
S10 Figure: Funnel plot for prevalence of UCP1 A-3826G / AG in healthy individuals.



S11 Figure: Forest plot for prevalence of UCP1 Ala64Thr / GA in CMP individuals.

Study or Subgroup	Prevalence	SE	Weight	Prevalence IV, Random, 95% CI
Lim 2012 (Korea)	0.13	0.01	30.4%	0.13 [0.11, 0.15]
Pei 2017 (China)	0.16	0.04	14.3%	0.16 [0.08, 0.24]
Dinas 2021 (Europe)	0.16	0.01	30.4%	0.16 [0.14, 0.18]
Tiwari 2009 (India)	0.23	0.02	24.8%	0.23 [0.19, 0.27]
Total (95% CI)			**100.0%**	**0.17 [0.13, 0.21]**

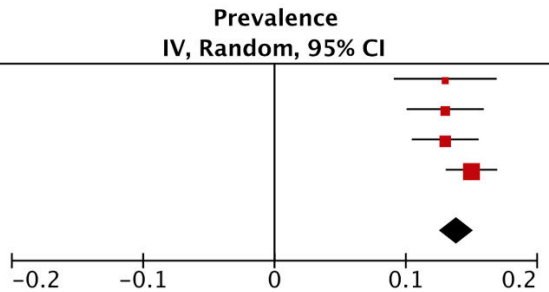
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 20.57$, df = 3 (P = 0.0001); $I^2 = 85\%$
Test for overall effect: Z = 8.34 (P < 0.00001)



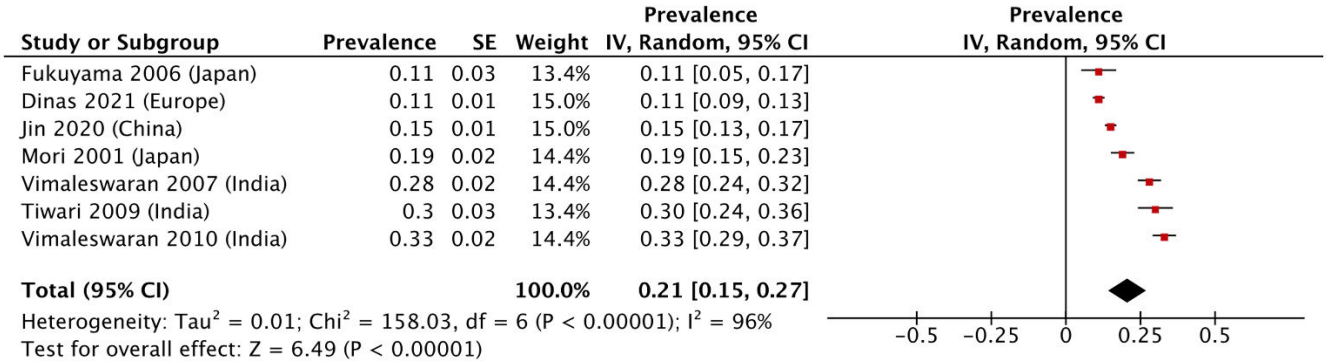
S12 Figure: Forest plot for prevalence of *UCP1* Ala64Thr / GA in healthy individuals.

Study or Subgroup	Prevalence	SE	Weight	Prevalence IV, Random, 95% CI
Pei 2017 (China)	0.13	0.02	10.9%	0.13 [0.09, 0.17]
Lim 2012 (Korea)	0.13	0.015	19.4%	0.13 [0.10, 0.16]
Cha 2008 (Korea)	0.13	0.013	25.9%	0.13 [0.10, 0.16]
Dinas 2021 (Europe)	0.15	0.01	43.7%	0.15 [0.13, 0.17]
Total (95% CI)			**100.0%**	**0.14 [0.13, 0.15]**

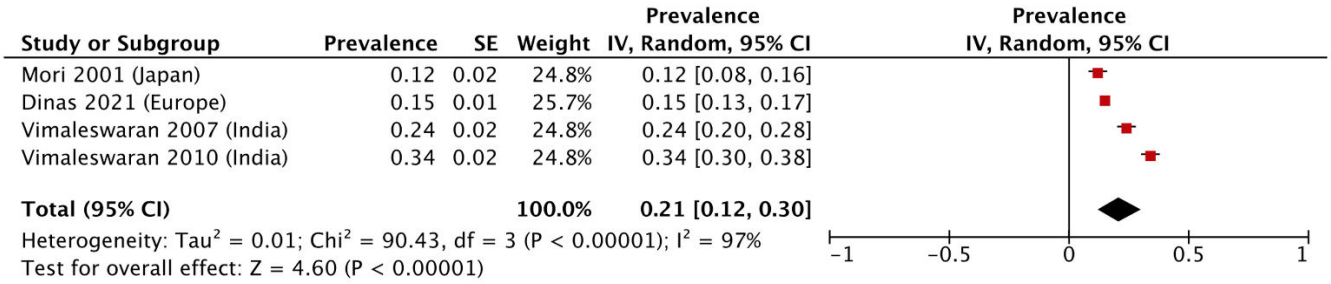
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 2.25$, df = 3 (P = 0.52); $I^2 = 0\%$
Test for overall effect: Z = 20.98 (P < 0.00001)



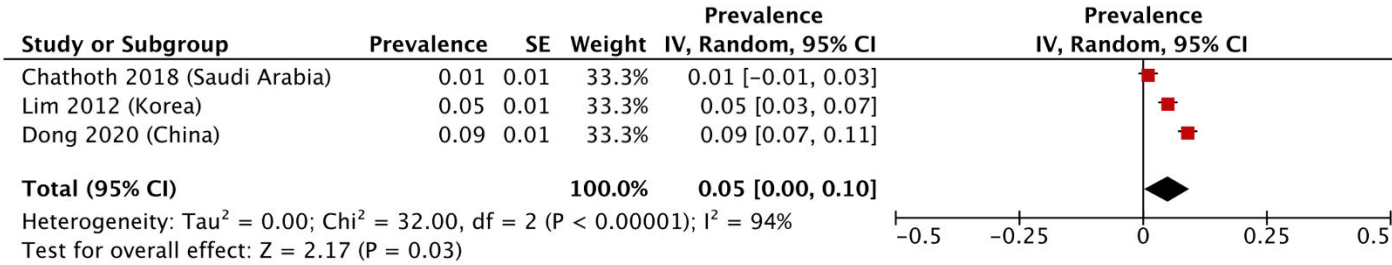
S13 Figure: Forest plot for prevalence of *UCP1* A-112C / AC in CMP individuals.



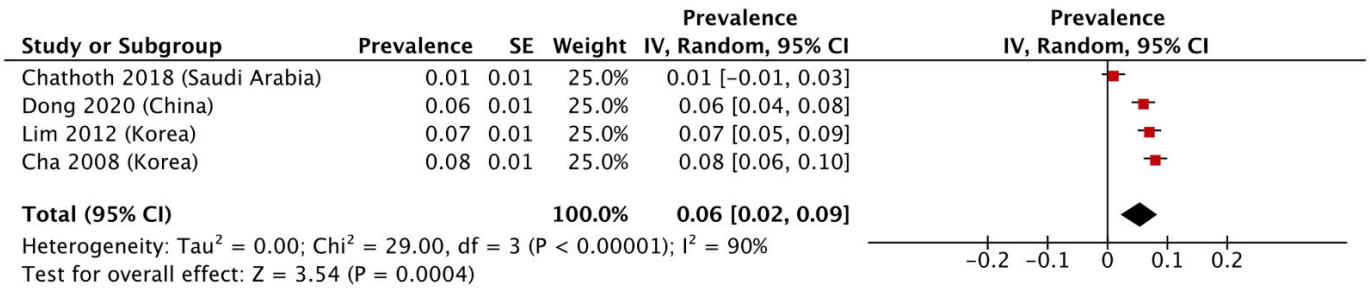
S14 Figure: Forest plot for prevalence of *UCP1* A-112C / AC in healthy individuals.



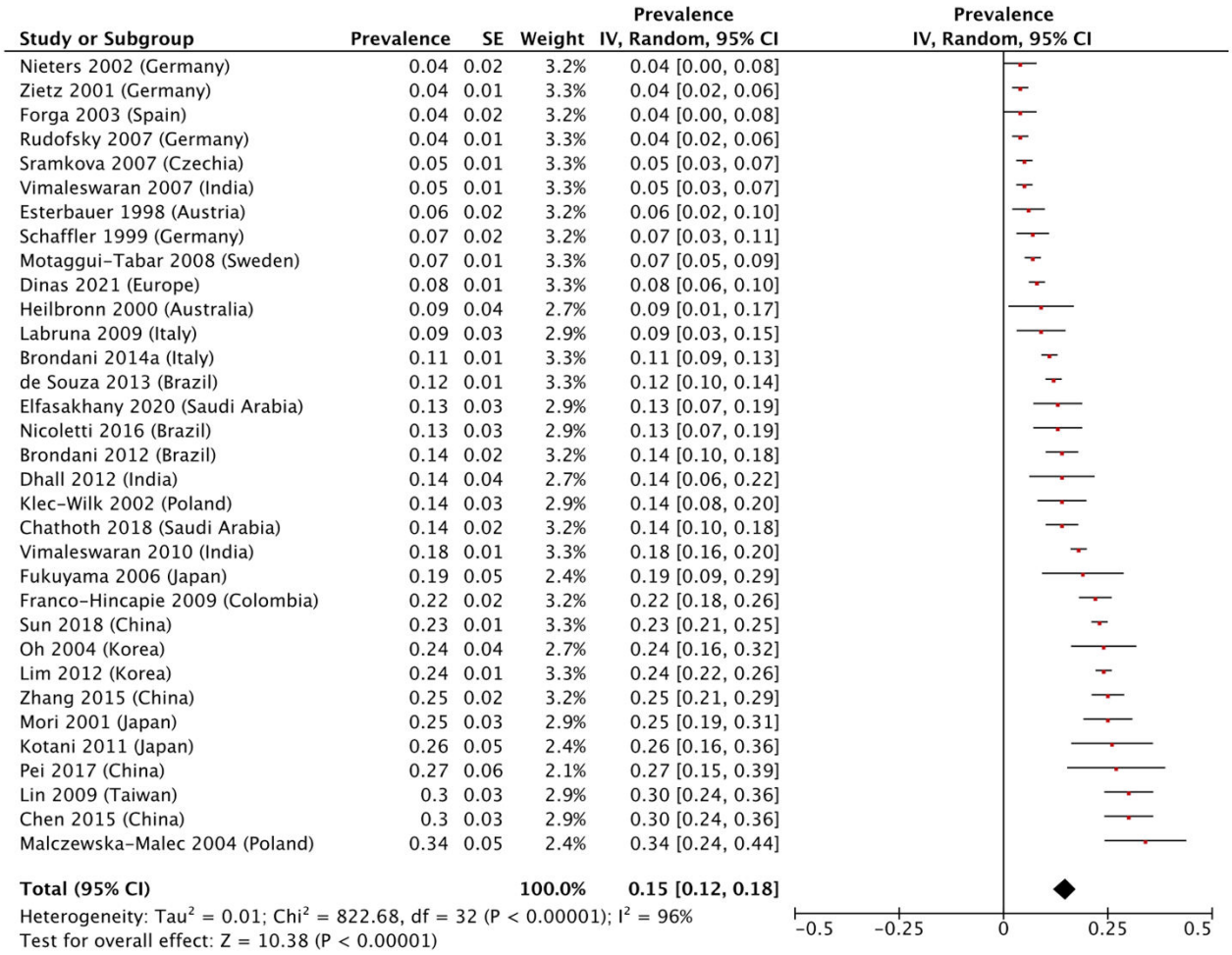
S15 Figure: Forest plot for prevalence of *UCP1* A-1766G / GG in CMP individuals.



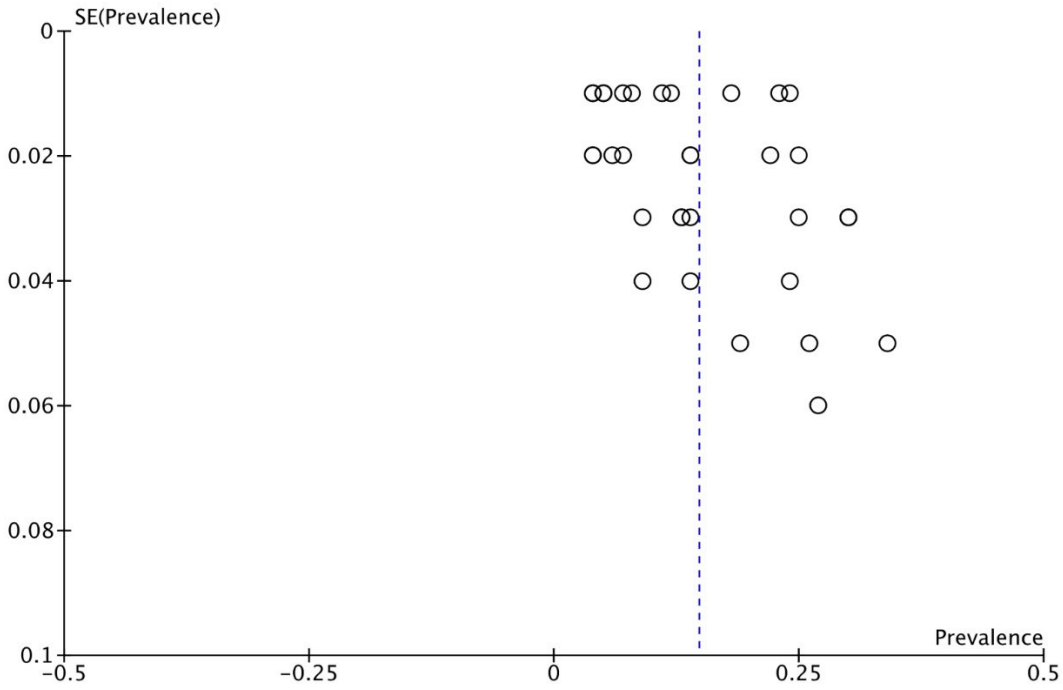
S16 Figure: Forest plot for prevalence of *UCP1* A-1766G / GG in healthy individuals.



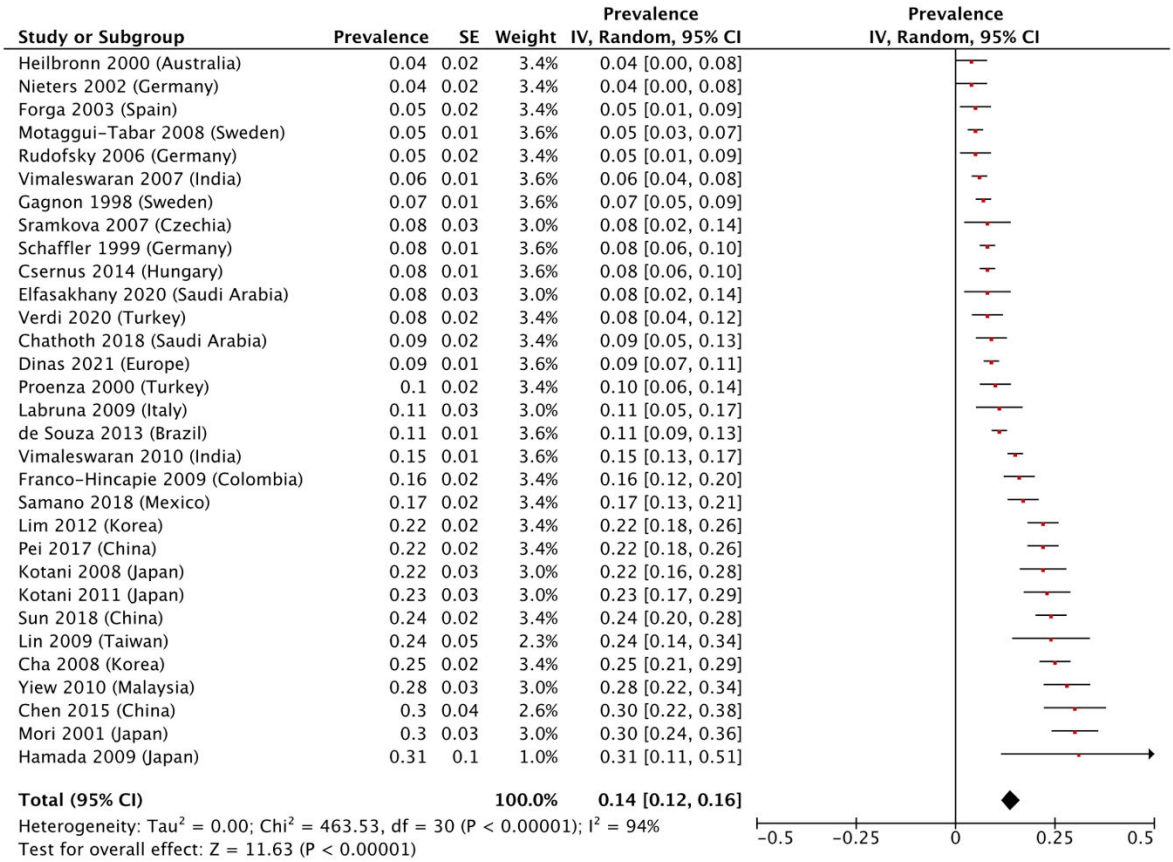
S17 Figure: Forest plot for prevalence of *UCP1* A-3826G / GG in CMP individuals.



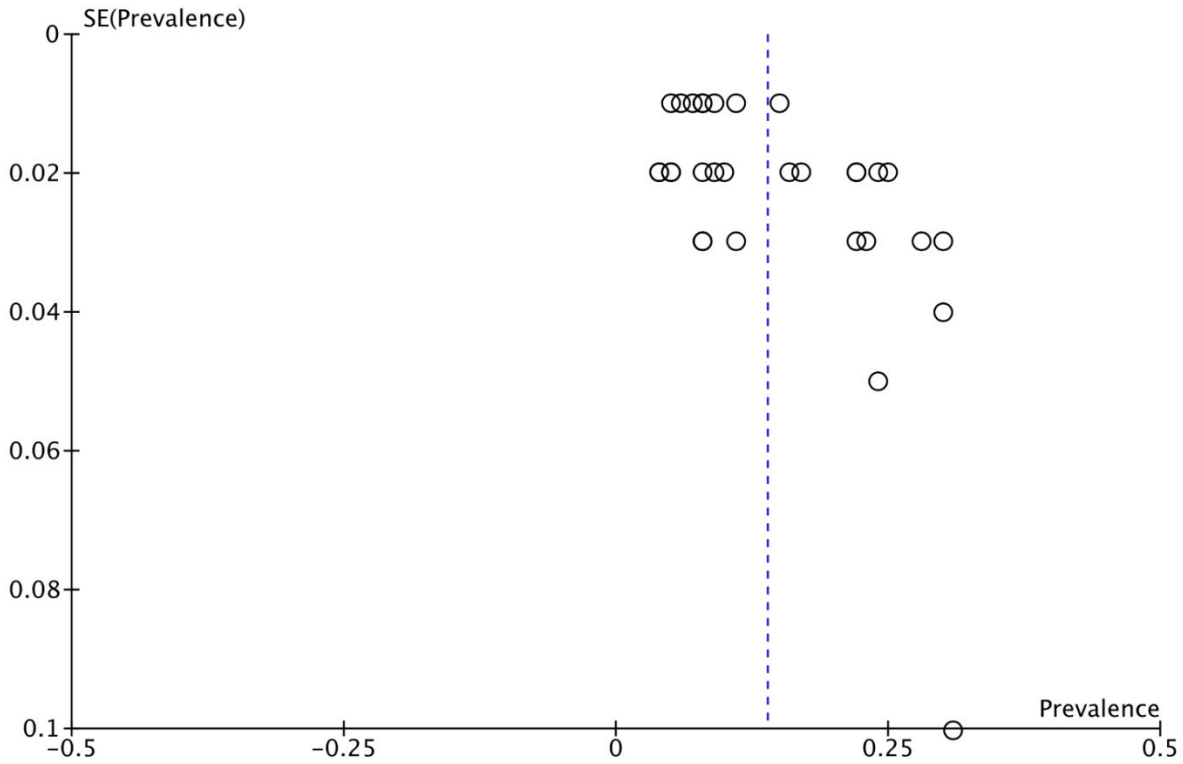
S18 Figure: Funnel plot for prevalence of *UCP1* A-3826G / GG in CMP individuals.



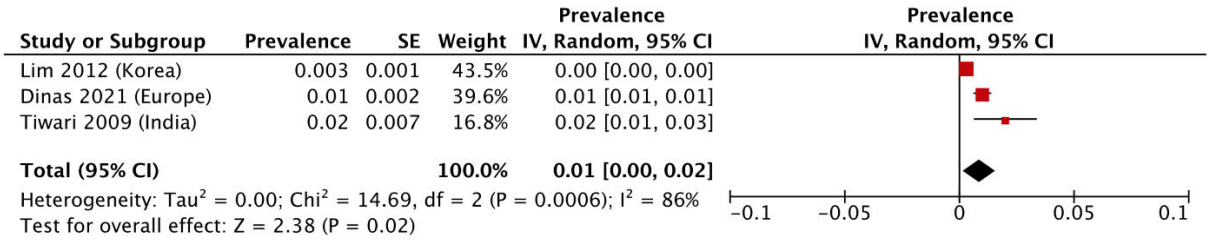
S19 Figure: Forest plot for prevalence of *UCP1* A-3826G / GG in healthy individuals.



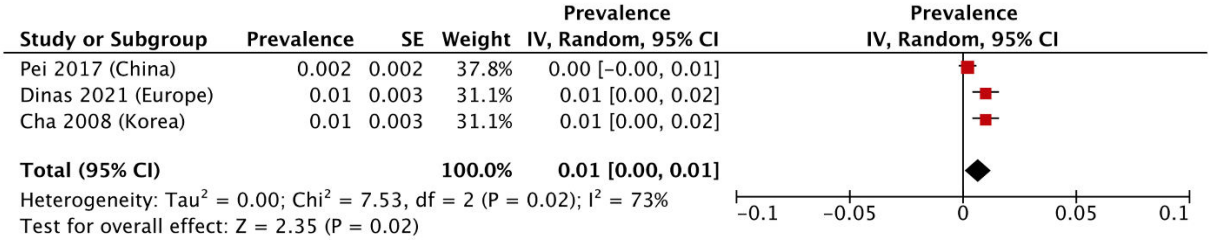
S20 Figure: Funnel plot for prevalence of *UCP1* A-3826G / GG in healthy individuals.



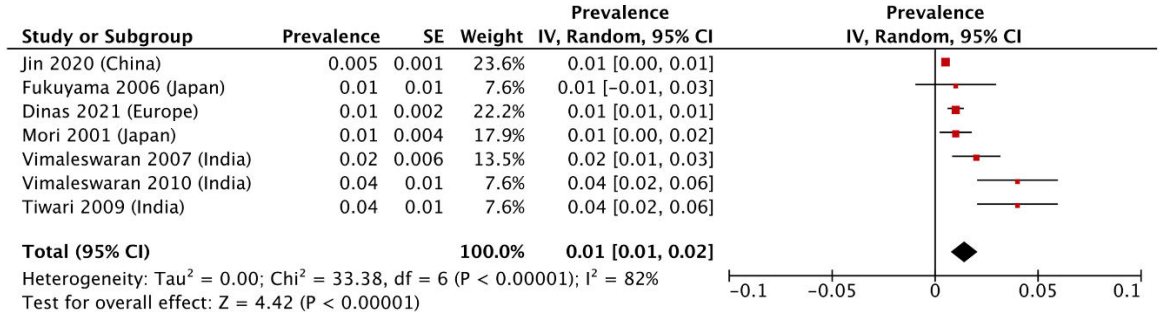
S21 Figure: Forest plot for prevalence of *UCP1* Ala64Thr / AA in CMP individuals.



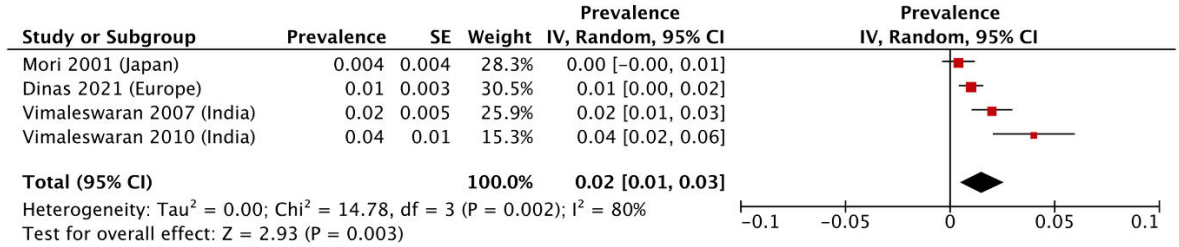
S22 Figure: Forest plot for prevalence of *UCP1* Ala64Thr / AA in healthy individuals.



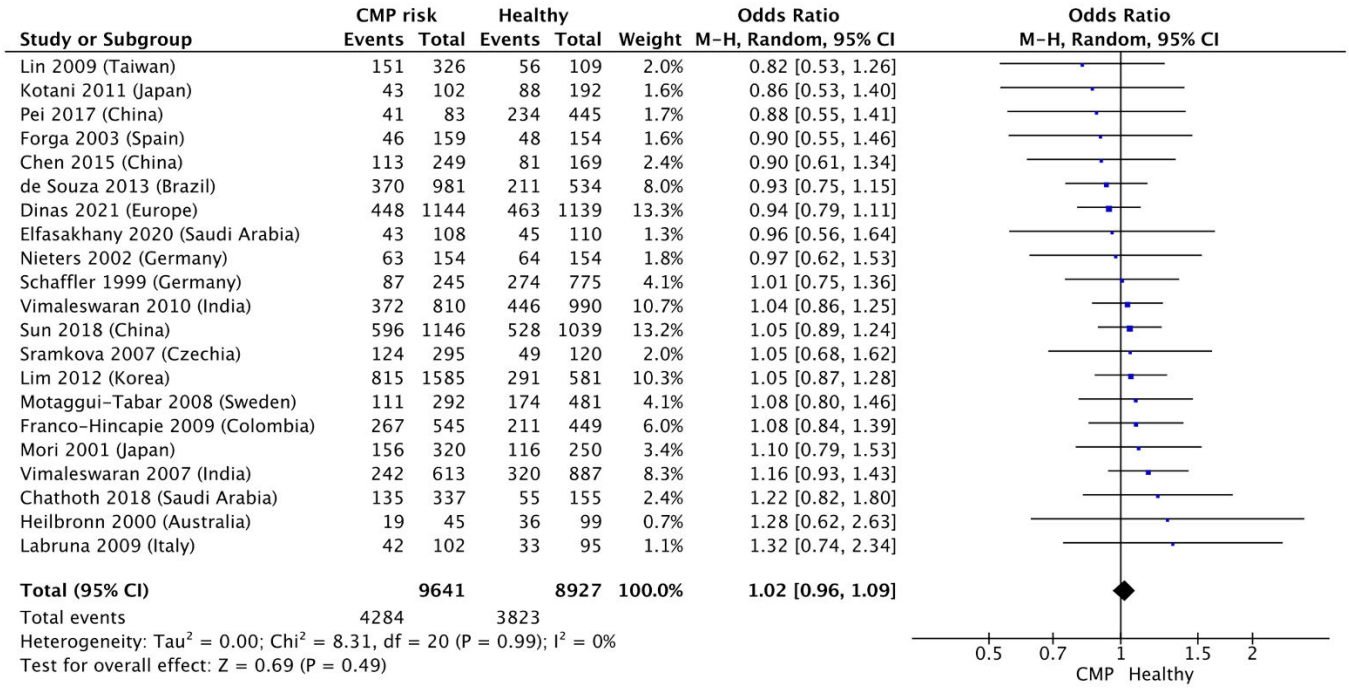
S23 Figure: Forest plot for prevalence of *UCP1* A-112C / CC in CMP individuals.



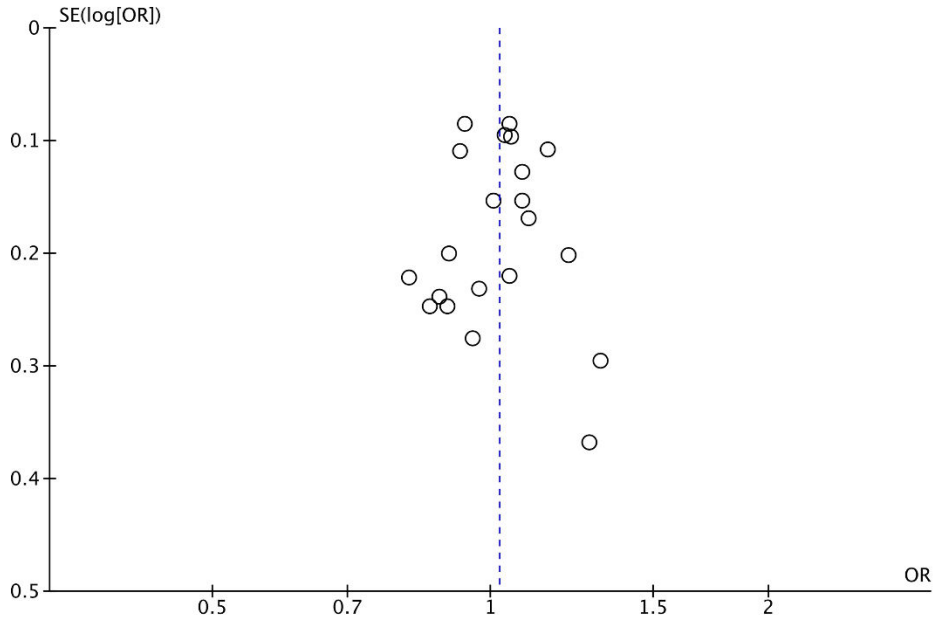
S24 Figure: Forest plot for prevalence of *UCP1* A-112C / CC in healthy individuals.



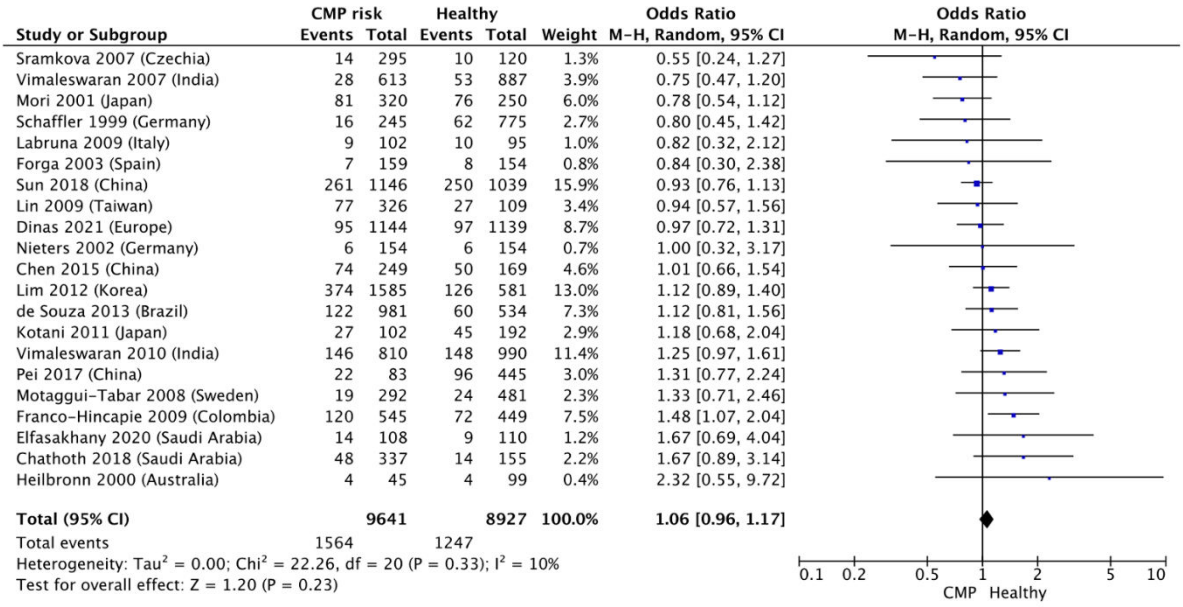
S25 Figure: Forest plot for odds ratio of *UCP1* A-3826G / AG.



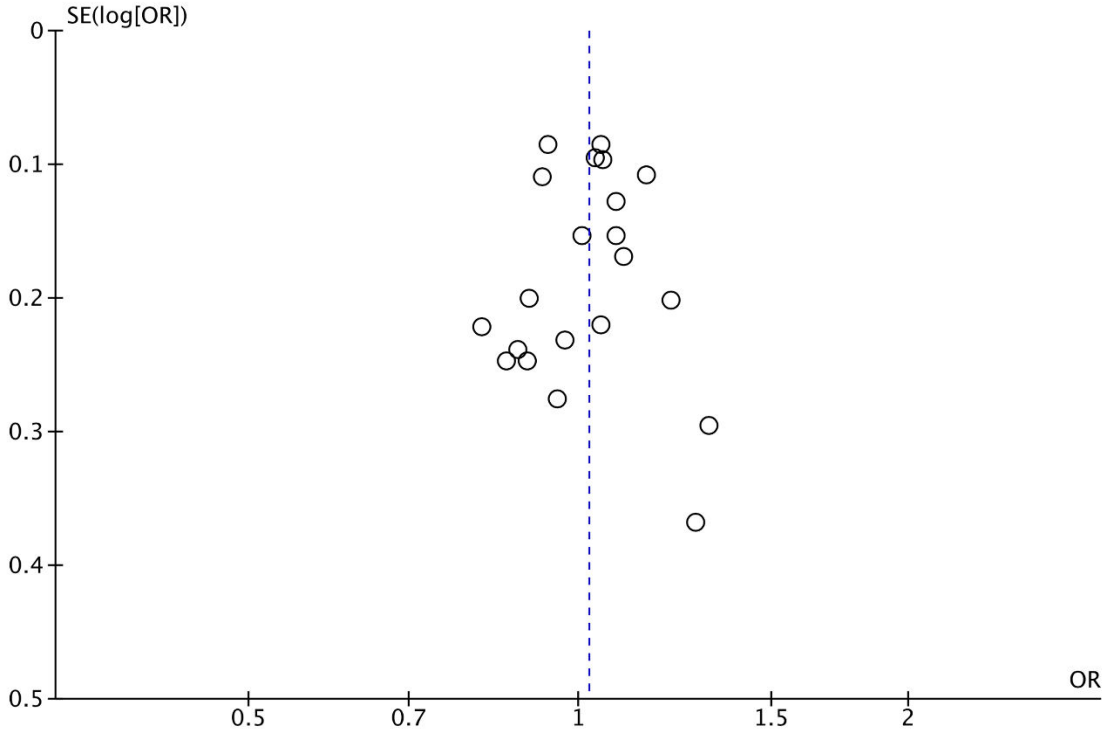
S26 Figure: Funnel plot for odds ratio of *UCP1* A-3826G / AG.



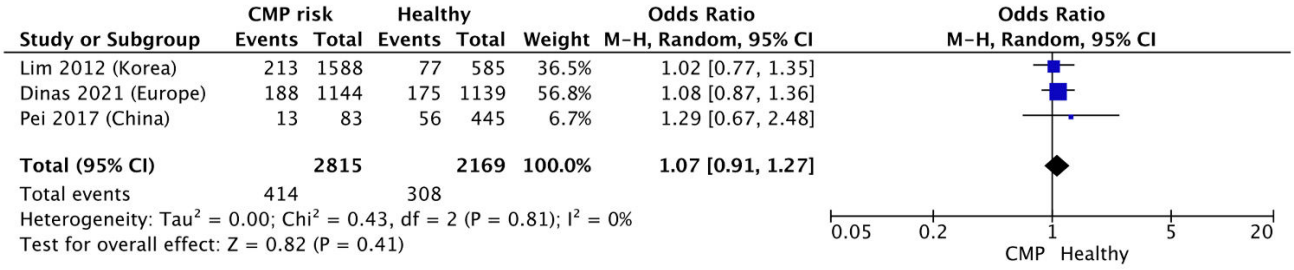
S27 Figure: Forest plot for odds ratio of *UCP1* A-3826G / GG.



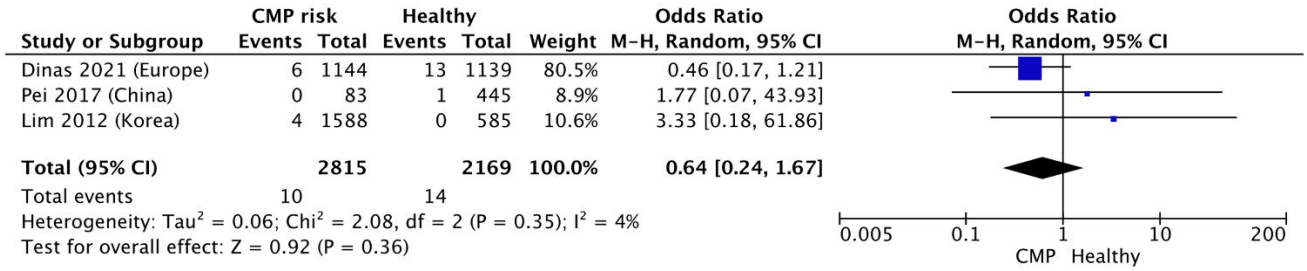
S28 Figure: Funnel plot for odds ratio of *UCP1* A-3826G / GG.



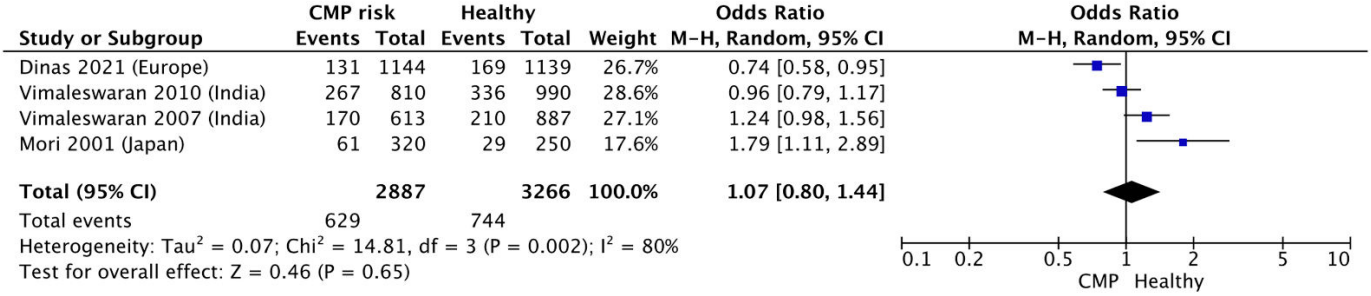
S29 Figure: Forest plot for odds ratio of *UCP1* Ala64Thr / GA.



S30 Figure: Forest plot for odds ratio of *UCP1* Ala64Thr / AA.



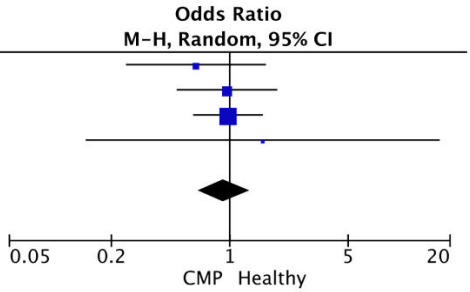
S31 Figure: Forest plot for odds ratio of *UCP1* A-112C / AC.



S32 Figure: Forest plot for odds ratio of *UCP1* A-112C / CC.

Study or Subgroup	CMP risk Events	Total	Healthy Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Dinas 2021 (Europe)	7	1144	11	1139	14.1%	0.63 [0.24, 1.63]
Vimaleswaran 2007 (India)	14	613	21	887	27.2%	0.96 [0.49, 1.91]
Vimaleswaran 2010 (India)	32	810	40	990	56.5%	0.98 [0.61, 1.57]
Mori 2001 (Japan)	2	320	1	250	2.2%	1.57 [0.14, 17.37]
Total (95% CI)		**2887**		**3266**	**100.0%**	**0.92 [0.65, 1.32]**
Total events	55		73			

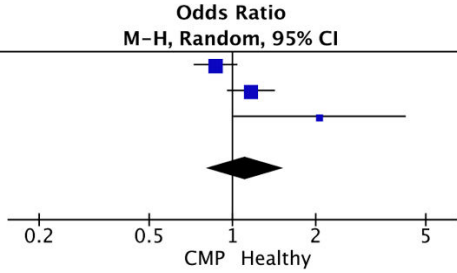
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.87$, df = 3 (P = 0.83); $I^2 = 0\%$
Test for overall effect: Z = 0.43 (P = 0.67)



S33 Figure. Forest plot for the odds ratio of *UCP1* A-1766G /AG.

Study or Subgroup	CMP risk Events	Total	Healthy Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Dong 2020 (China)	346	928	420	1034	43.3%	0.87 [0.72, 1.04]
Lim 2012 (Korea)	603	1575	201	579	42.1%	1.17 [0.96, 1.42]
Chathoth 2018 (Saudi Arabia)	42	337	10	155	14.6%	2.06 [1.01, 4.23]
Total (95% CI)		**2840**		**1768**	**100.0%**	**1.12 [0.81, 1.55]**
Total events	991		631			

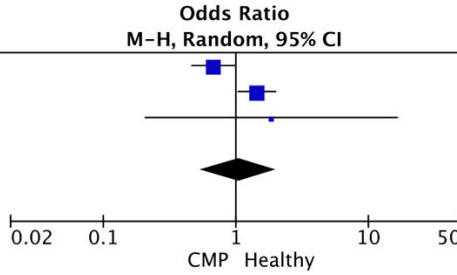
Heterogeneity: $Tau^2 = 0.06$; $Chi^2 = 8.44$, df = 2 (P = 0.01); $I^2 = 76\%$
Test for overall effect: Z = 0.66 (P = 0.51)



S34 Figure. Forest plot for the odds ratio of *UCP1* A-1766G /GG.

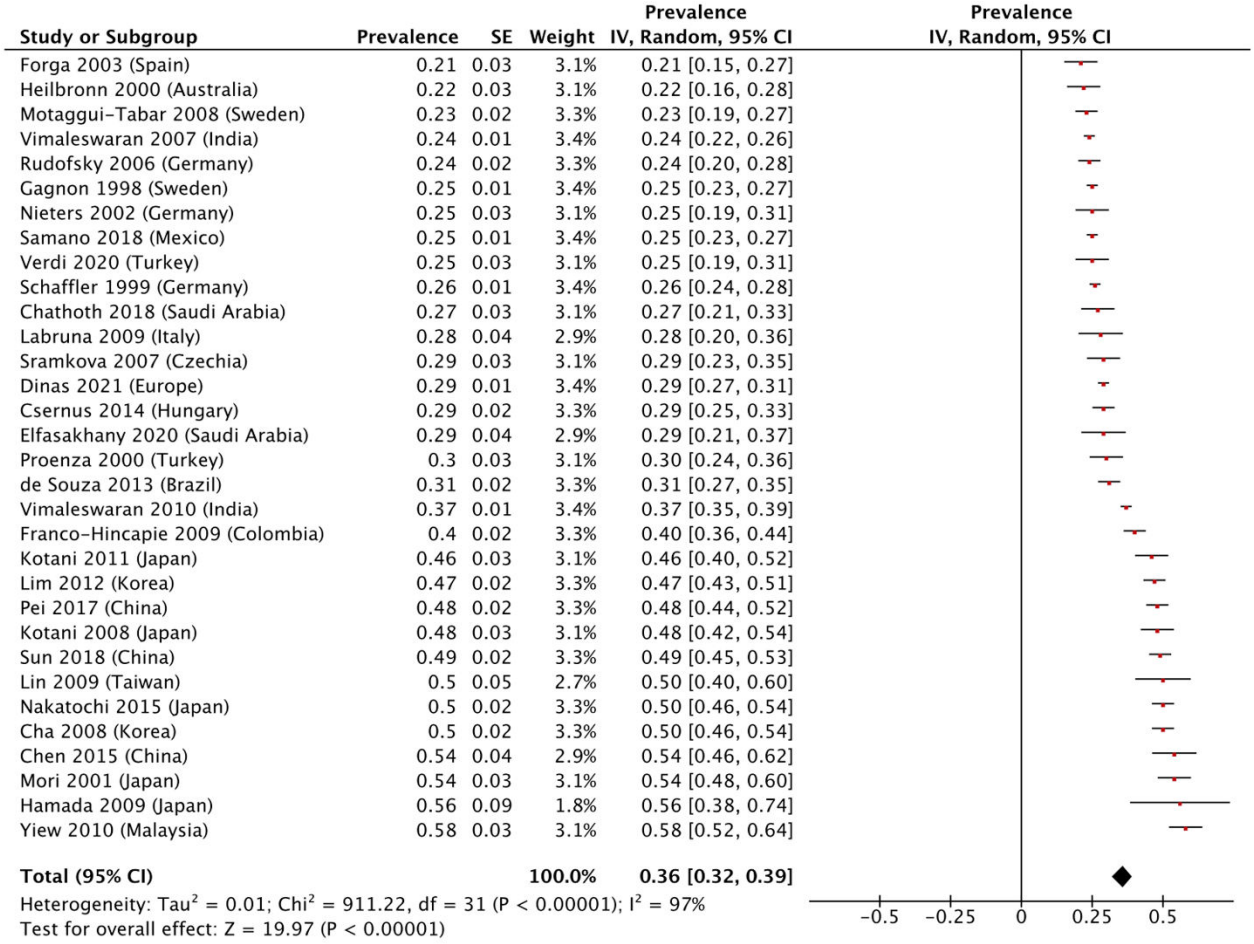
Study or Subgroup	CMP risk Events	Total	Healthy Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Lim 2012 (Korea)	81	1575	43	579	45.3%	0.68 [0.46, 0.99]
Dong 2020 (China)	83	928	66	1034	46.9%	1.44 [1.03, 2.02]
Chathoth 2018 (Saudi Arabia)	4	337	1	155	7.9%	1.85 [0.21, 16.69]
Total (95% CI)		**2840**		**1768**	**100.0%**	**1.04 [0.53, 2.04]**
Total events	168		110			

Heterogeneity: $Tau^2 = 0.22$; $Chi^2 = 8.76$, df = 2 (P = 0.01); $I^2 = 77\%$
Test for overall effect: Z = 0.12 (P = 0.90)

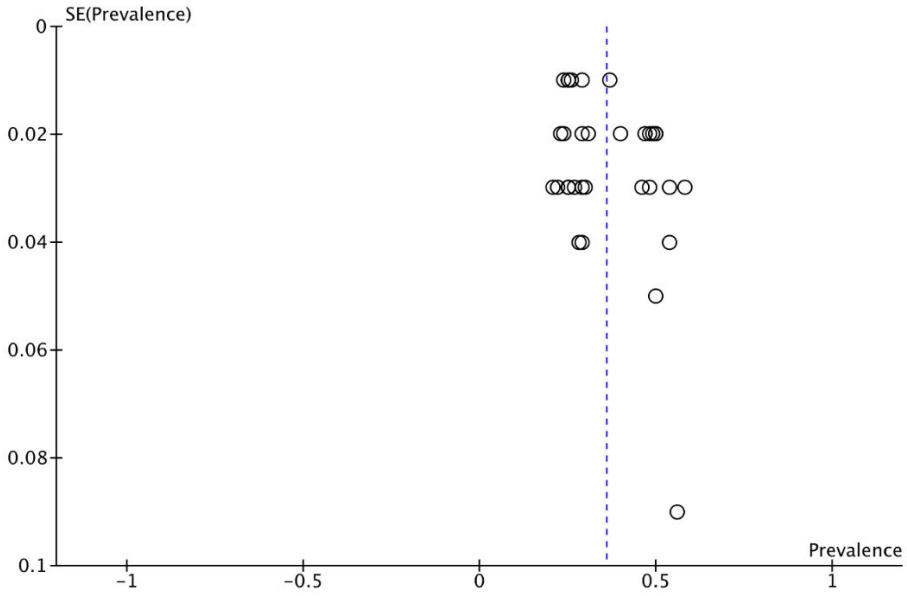


2.2.2 The results from the allele-specific forest and funnel plots for the prevalence (Figures S37-46) and the odds ratio (Figures S47-51) for different alleles are shown below. Funnel plots were only produced for those meta-analyses that included >10 studies [93].

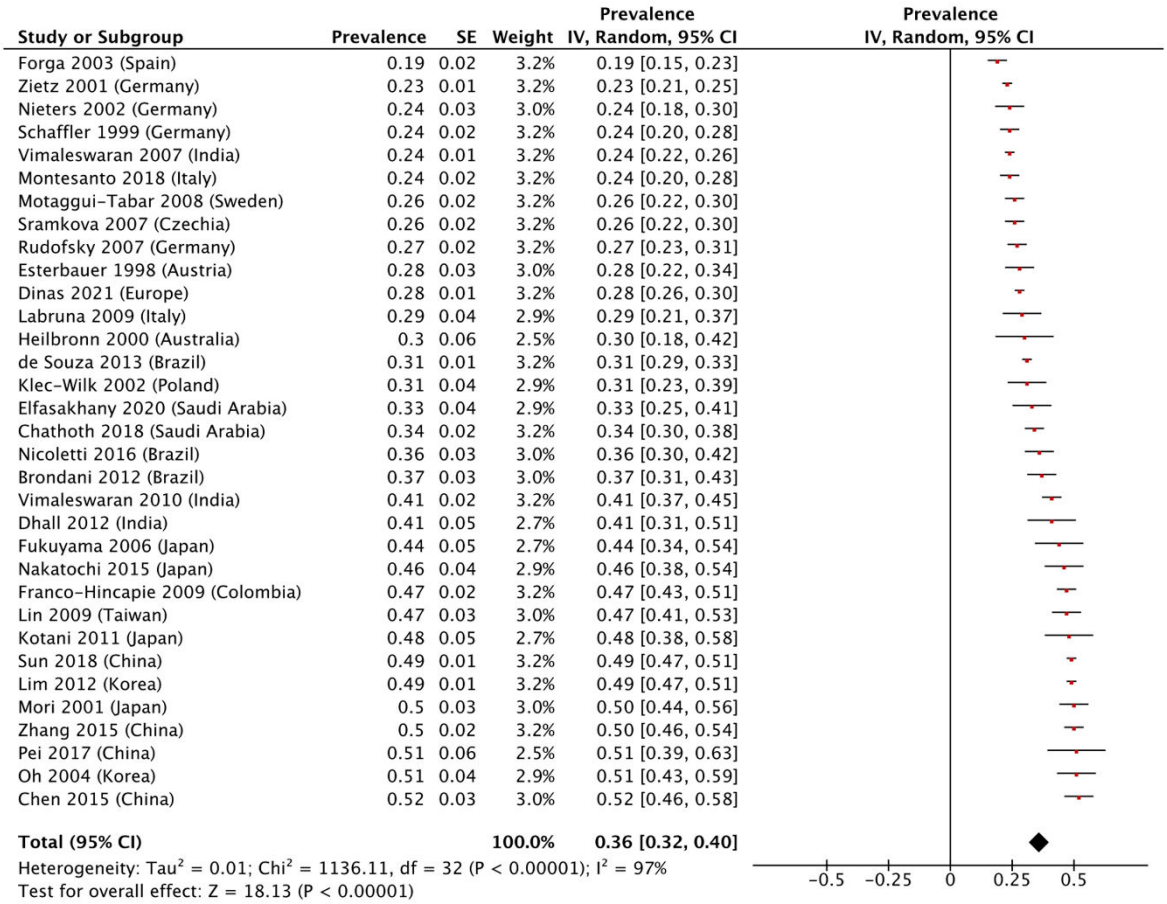
S35 Figure: Forest plot for prevalence of *UCP1* A-3826G / G allele in healthy individuals



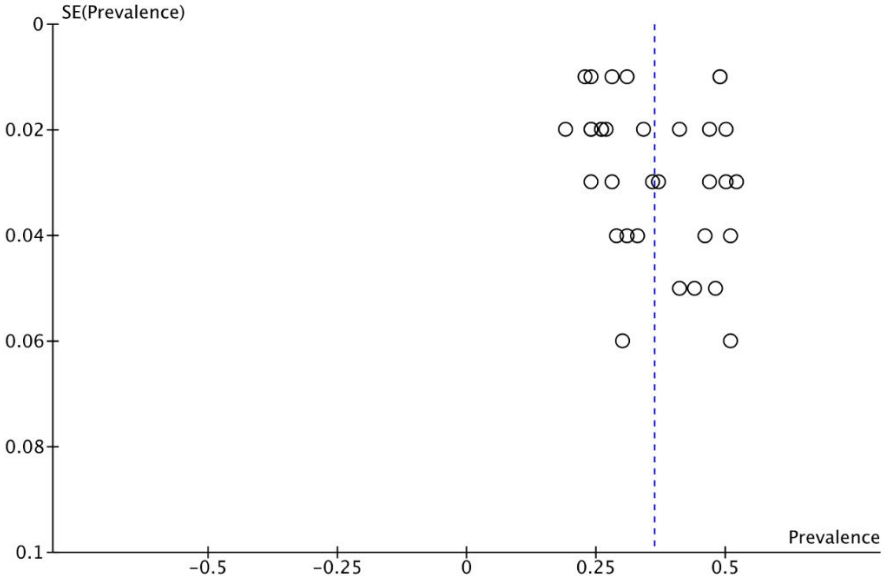
S36 Figure: Funnel plot for prevalence of *UCP1* A-3826G / G allele in healthy individuals



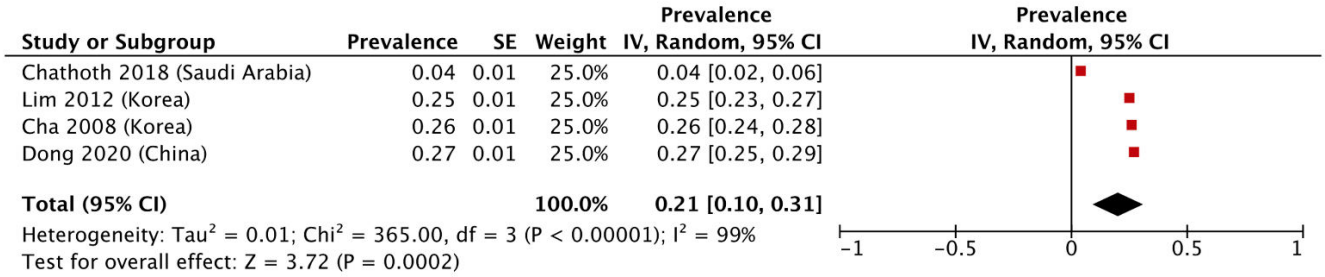
S37 Figure: Forest plot for prevalence of *UCP1* A-3826G / G allele in CMP individuals.



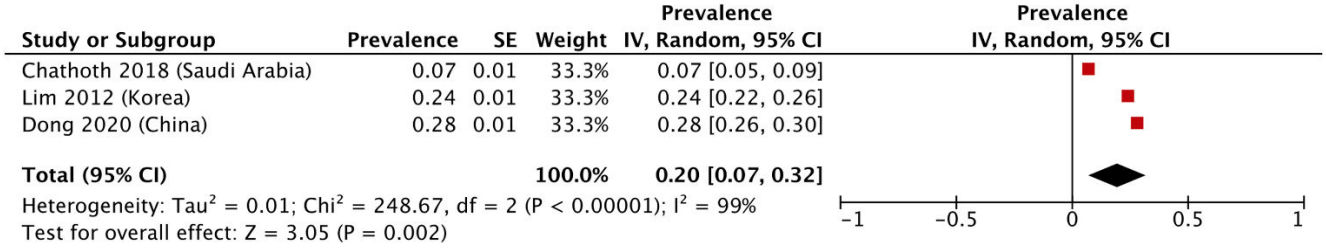
S38 Figure: Funnel plot for prevalence of *UCP1* A-3826G / G allele in CMP individuals.



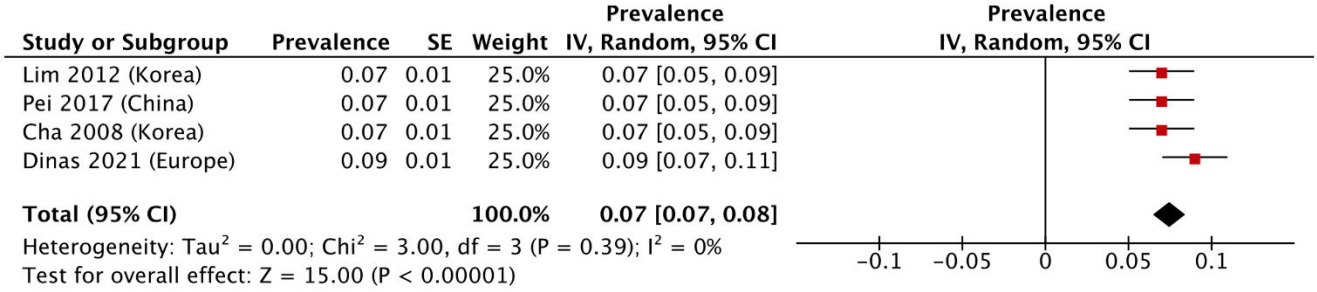
S39 Figure: Forest plot for prevalence of *UCP1* A-1766G / G allele in healthy individuals.



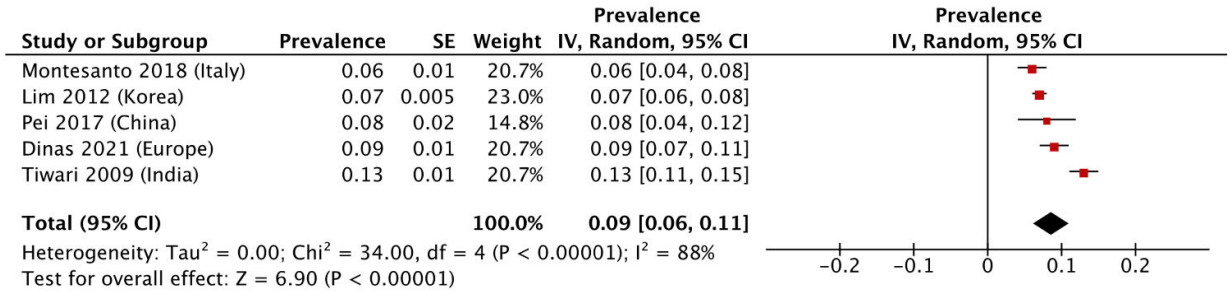
S40 Figure: Forest plot for prevalence of *UCP1* A-1766G / G allele in CMP individuals.



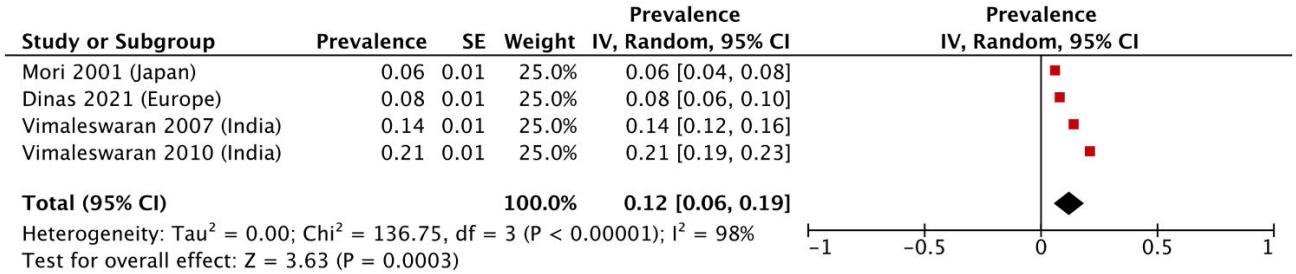
S41 Figure: Forest plot for prevalence of *UCP1* Ala64Thr / A allele in healthy individuals.



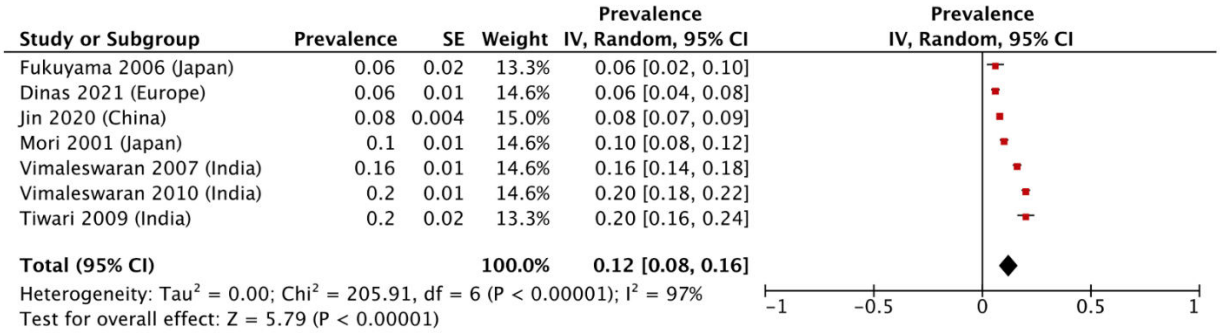
S42 Figure: Forest plot for prevalence of *UCP1* Ala64Thr / A allele in CMP individuals.



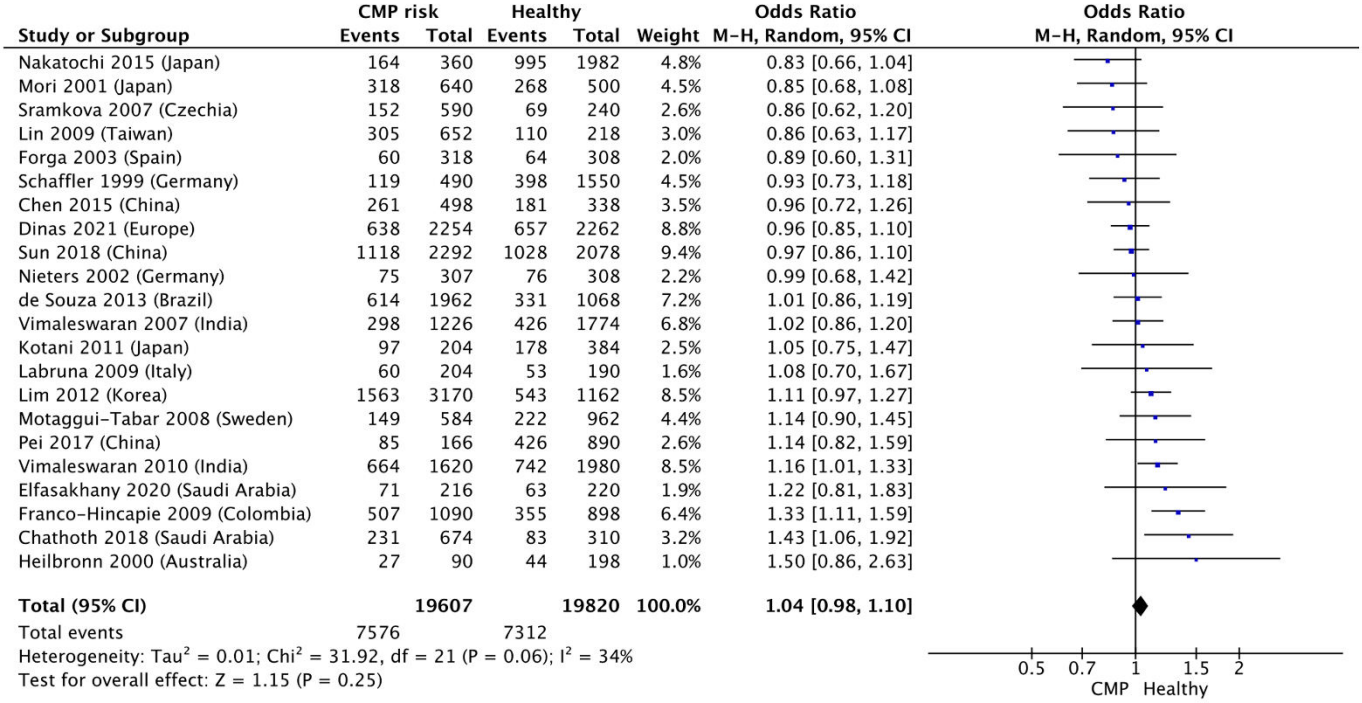
S43 Figure: Forest plot for prevalence of *UCP1* A-112C / C allele in healthy individuals.



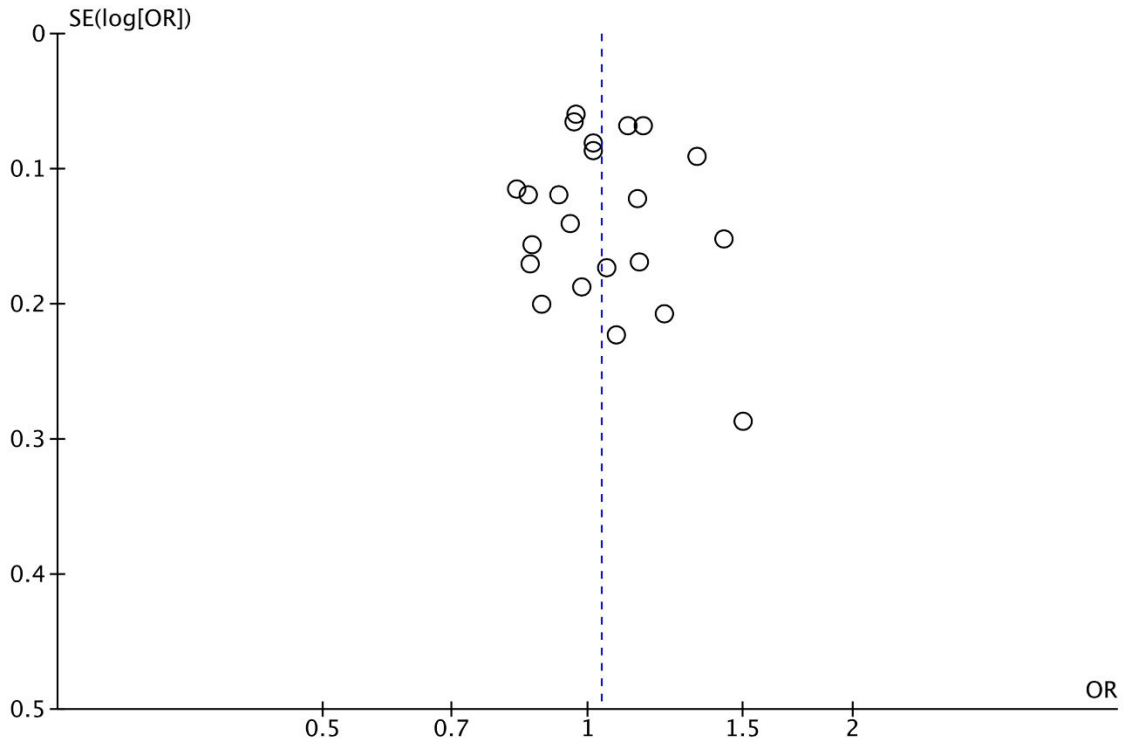
S44 Figure: Forest plot for prevalence of *UCP1* A-112C / C allele in CMP individuals.



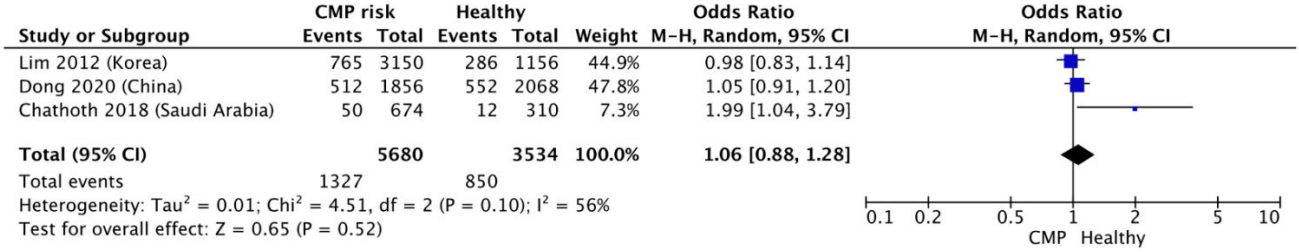
S45 Figure: Forest plot for odds ratio of *UCP1* A-3826G / G allele



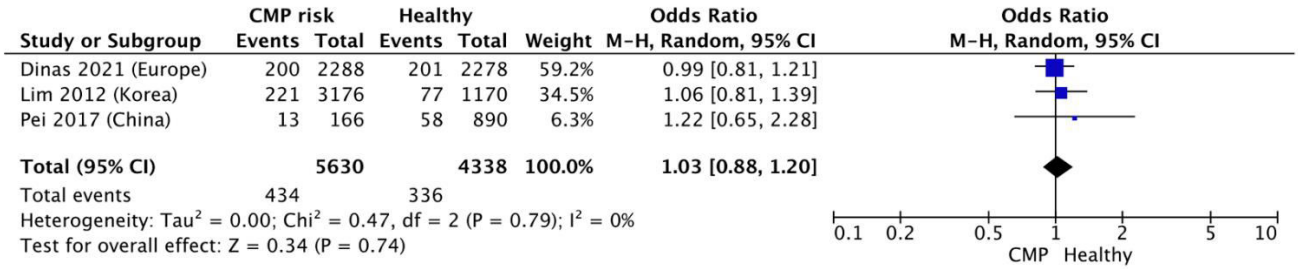
S46 Figure: Funnel plot for odds ratio of *UCP1* A-3826G / G allele



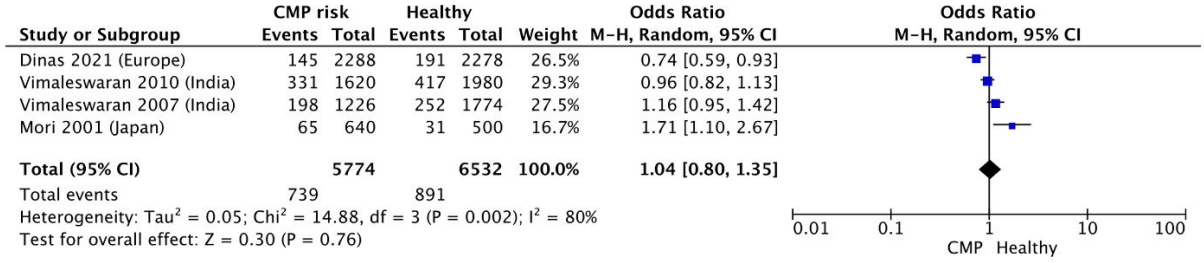
S47 Figure: Forest plot for odds ratio of *UCP1* A-1766G / G allele



S48 Figure: Forest plot for odds ratio of *UCP1* Ala64Thr / A allele



S49 Figure: Forest plot for odds ratio of *UCP1* A-112C / C allele



3.1 REFERENCES CITED IN THIS APPENDIX

1. Viswanathan M, Berkman ND, Dryden DM, Hartling L: **AHRQ Methods for Effective Health Care**. In: *Assessing Risk of Bias and Confounding in Observational Studies of Interventions or Exposures: Further Development of the RTI Item Bank.* Rockville (MD): Agency for Healthcare Research and Quality (US); 2013.
2. Margulis AV, Pladevall M, Riera-Guardia N, Varas-Lorenzo C, Hazell L, Berkman ND, Viswanathan M, Perez-Gutthann S: **Quality assessment of observational studies in a drug-safety systematic review, comparison of two tools: the Newcastle-Ottawa Scale and the RTI item bank**. *Clin Epidemiol* 2014, **6**:359-368.
3. Al-Saleh MA, Armijo-Olivo S, Thie N, Seikaly H, Boulanger P, Wolfaardt J, Major P: **Morphologic and functional changes in the temporomandibular joint and stomatognathic system after transmandibular surgery in oral and oropharyngeal cancers: systematic review**. *J Otolaryngol Head Neck Surg* 2012, **41**(5):345-360.
4. 2019 RW: **Review Manager Web (RevMan Web)**. In.: The Cochrane Collaboration; 2019.
5. Higgins JP, Thomas J: **Cochrane Handbook for Systematic Reviews of Interventions**: Cochrane collaboration 2019.
6. Bracale R, Labruna G, Finelli C, Daniele A, Sacchetti L, Oriani G, Contaldo F, Pasanisi F: **The absence of polymorphisms in ADRB3, UCP1, PPARγ, and ADIPOQ genes protects morbid obese patients toward insulin resistance**. *Journal of endocrinological investigation* 2012, **35**(1):2-4.
7. Brondani LA, Assmann TS, Duarte GC, Gross JL, Canani LH, Crispim D: **The role of the uncoupling protein 1 (UCP1) on the development of obesity and type 2 diabetes mellitus**. *Arquivos brasileiros de endocrinologia e metabologia* 2012, **56**(4):215-225.
8. Brondani LA, de Souza BM, Assmann TS, Bouças AP, Bauer AC, Canani LH, Crispim D: **Association of the UCP polymorphisms with susceptibility to obesity: case-control study and meta-analysis**. *Molecular biology reports* 2014, **41**(8):5053-5067.
9. Brondani LA, Duarte GC, Canani LH, Crispim D: **The presence of at least three alleles of the ADRB3 Trp64Arg (C/T) and UCP1 -3826A/G polymorphisms is associated with protection to overweight/obesity and with higher high-density lipoprotein cholesterol levels in Caucasian-Brazilian patients with type 2 diabetes**. *Metabolic syndrome and related disorders* 2014, **12**(1):16-24.
10. Cha MH, Kang BK, Suh D, Kim KS, Yang Y, Yoon Y: **Association of UCP1 genetic polymorphisms with blood pressure among Korean female subjects**. *Journal of Korean medical science* 2008, **23**(5):776-780.
11. Chen Y, Wang X, Shen Z, Fan P, Liu R, Liu Y, Ren R, Ma L, Bai H: **Effect of the beta-3 adrenergic receptor Trp64Arg and uncoupling protein 1-3826 A>G genotypes on lipid and apolipoprotein levels in overweight/obese and non-obese Chinese subjects**. *Lipids in health and disease* 2015, **14**:34.
12. Chathoth S, Ismail MH, Vatte C, Cyrus C, Al Ali Z, Ahmed KA, Acharya S, Al Barqi AM, Al Ali A: **Association of Uncoupling Protein 1 (UCP1) gene polymorphism with obesity: a case-control study**. *BMC medical genetics* 2018, **19**(1):203.
13. Csernus K, Pauler G, Erhardt É, Lányi É, Molnár D: **Effects of energy expenditure gene polymorphisms on obesity-related traits in obese children**. *Obesity research & clinical practice* 2015, **9**(2):133-140.

14. de Souza BM, Brondani LA, Bouças AP, Sortica DA, Kramer CK, Canani LH, Leitão CB, Crispim D: **Associations between UCP1 -3826A/G, UCP2 -866G/A, Ala55Val and Ins/Del, and UCP3 -55C/T polymorphisms and susceptibility to type 2 diabetes mellitus: case-control study and meta-analysis**. *PloS one* 2013, **8**(1):e54259.
15. Dhall M, Chaturvedi MM, Rai U, Kapoor S: **Sex-dependent effects of the UCP1 -3826 A/G polymorphism on obesity and blood pressure**. *Ethnicity & disease* 2012, **22**(2):181-184.
16. Dong C, Lv Y, Xie L, Yang R, Chen L, Zhang L, Long T, Yang H, Mao X, Fan Q *et al*: **Association of UCP1 polymorphisms with type 2 diabetes mellitus and their interaction with physical activity and sedentary behavior**. *Gene* 2020, **739**:144497.
17. Esterbauer H, Oberkofler H, Liu YM, Breban D, Hell E, Krempler F, Patsch W: **Uncoupling protein-1 mRNA expression in obese human subjects: the role of sequence variations at the uncoupling protein-1 gene locus**. *Journal of lipid research* 1998, **39**(4):834-844.
18. Forga L, Corbalán M, Marti A, Fuentes C, Martínez-González MA, Martínez A: **[Influence of the polymorphism 03826 A --> G in the UCP1 gene on the components of metabolic syndrome]**. *Anales del sistema sanitario de Navarra* 2003, **26**(2):231-236.
19. Franco-Hincapié L, Duque CE, Parra MV, Gallego N, Villegas A, Ruiz-Linares A, Bedoya G: **[Association between polymorphism in uncoupling proteins and type 2 diabetes in a northwestern Colombian population]**. *Biomedica : revista del Instituto Nacional de Salud* 2009, **29**(1):108-118.
20. Fukuyama K, Ohara T, Hirota Y, Maeda K, Kuno S, Zenibayashi M, Teranishi T, Kouyama K, Maeda E, Sakamoto N *et al*: **Association of the -112A>C polymorphism of the uncoupling protein 1 gene with insulin resistance in Japanese individuals with type 2 diabetes**. *Biochemical and biophysical research communications* 2006, **339**(4):1212-1216.
21. Gagnon J, Lago F, Chagnon YC, Pérusse L, Näslund I, Lissner L, Sjöström L, Bouchard C: **DNA polymorphism in the uncoupling protein 1 (UCP1) gene has no effect on obesity related phenotypes in the Swedish Obese Subjects cohorts**. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* 1998, **22**(6):500-505.
22. Hamada T, Kotani K, Nagai N, Tsuzaki K, Matsuoka Y, Sano Y, Fujibayashi M, Kiyohara N, Tanaka S, Yoshimura M *et al*: **Low-calorie diet-induced reduction in serum HDL cholesterol is ameliorated in obese women with the -3826 G allele in the uncoupling protein-1 gene**. *The Tohoku journal of experimental medicine* 2009, **219**(4):337-342.
23. Heilbronn LK, Kind KL, Pancewicz E, Morris AM, Noakes M, Clifton PM: **Association of -3826 G variant in uncoupling protein-1 with increased BMI in overweight Australian women**. *Diabetologia* 2000, **43**(2):242-244.
24. Jin P, Li Z, Xu X, He J, Chen J, Xu X, Du X, Bai X, Zhang B, He X *et al*: **Analysis of association between common variants of uncoupling proteins genes and diabetic retinopathy in a Chinese population**. *BMC medical genetics* 2020, **21**(1):25.
25. Kieć-Wilk B, Wybrańska I, Malczewska-Malec M, Leszczyńska-Gołabek L, Partyka L, Niedbał S, Jabrocka A, Dembińska-Kieć A: **Correlation of the -3826A >G polymorphism in the promoter of the uncoupling protein 1 gene with obesity and metabolic disorders in obese families from southern Poland**. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society* 2002, **53**(3):477-490.
26. Kotani K, Sakane N, Saiga K, Adachi S, Shimohiro H, Mu H, Kurozawa Y: **Relationship between A-3826G polymorphism in the promoter of the uncoupling protein-1 gene and high-density lipoprotein cholesterol in Japanese individuals: a cross-sectional study**. *Archives of medical research* 2008, **39**(1):142-146.
27. Kotani K, Fujiwara S, Tsuzaki K, Sano Y, Nagai N, Yamada T, Sakane N: **The Association Between the Uncoupling Protein-1 Gene A-3826G Polymorphism and High-density Lipoprotein Cholesterol in A General Japanese Population: A Consideration of the Obesity Status**. *Journal of clinical medicine research* 2011, **3**(6):319-324.
28. Labruna G, Pasanisi F, Nardelli C, Tarantino G, Vitale DF, Bracale R, Finelli C, Genua MP, Contaldo F, Sacchetti L: **UCP1 -3826 AG+GG genotypes, adiponectin, and leptin/adiponectin ratio in severe obesity**. *Journal of endocrinological investigation* 2009, **32**(6):525-529.
29. Lim JH, Ko MM, Moon TW, Cha MH, Lee MS: **Association of the UCP-1 single nucleotide polymorphism A-3826G with the dampness-phlegm pattern among Korean stroke patients**. *BMC complementary and alternative medicine* 2012, **12**:180.

30. Lin E, Pei D, Huang YJ, Hsieh CH, Wu LS: **Gene-gene interactions among genetic variants from obesity candidate genes for nonobese and obese populations in type 2 diabetes**. *Genetic testing and molecular biomarkers* 2009, **13**(4):485-493.
31. Lindholm E, Klannemark M, Agardh E, Groop L, Agardh CD: **Putative role of polymorphisms in UCP1-3 genes for diabetic nephropathy**. *Journal of diabetes and its complications* 2004, **18**(2):103-107.
32. Malczewska-Malec M, Wybranska I, Leszczynska-Golabek I, Partyka L, Hartwich J, Jabrocka A, Kiec-Wilk B, Kwasniak M, Motyka M, Dembinska-Kiec A: **Analysis of candidate genes in Polish families with obesity**. *Clinical chemistry and laboratory medicine* 2004, **42**(5):487-493.
33. Montesanto A, Bonfigli AR, Crocco P, Garagnani P, De Luca M, Boemi M, Marasco E, Pirazzini C, Giuliani C, Franceschi C *et al*: **Genes associated with Type 2 Diabetes and vascular complications**. *Aging* 2018, **10**(2):178-196.
34. Mori H, Okazawa H, Iwamoto K, Maeda E, Hashiramoto M, Kasuga M: **A polymorphism in the 5' untranslated region and a Met229-->Leu variant in exon 5 of the human UCP1 gene are associated with susceptibility to type II diabetes mellitus**. *Diabetologia* 2001, **44**(3):373-376.
35. Mottagui-Tabar S, Hoffstedt J, Brookes AJ, Jiao H, Arner P, Dahlman I: **Association of ADRB1 and UCP3 gene polymorphisms with insulin sensitivity but not obesity**. *Hormone research* 2008, **69**(1):31-36.
36. Nakatochi M, Ushida Y, Yasuda Y, Yoshida Y, Kawai S, Kato R, Nakashima T, Iwata M, Kuwatsuka Y, Ando M *et al*: **Identification of an interaction between VWF rs7965413 and platelet count as a novel risk marker for metabolic syndrome: an extensive search of candidate polymorphisms in a case-control study**. *PloS one* 2015, **10**(2):e0117591.
37. Nicoletti CF, de Oliveira AP, Brochado MJ, de Oliveira BP, Pinhel MA, Marchini JS, dos Santos JE, Salgado Junior W, Silva Junior WA, Nonino CB: **UCP1 -3826 A>G polymorphism affects weight, fat mass, and risk of type 2 diabetes mellitus in grade III obese patients**. *Nutrition (Burbank, Los Angeles County, Calif)* 2016, **32**(1):83-87.
38. Nieters A, Becker N, Linseisen J: **Polymorphisms in candidate obesity genes and their interaction with dietary intake of n-6 polyunsaturated fatty acids affect obesity risk in a sub-sample of the EPIC-Heidelberg cohort**. *European journal of nutrition* 2002, **41**(5):210-221.
39. Oh HH, Kim KS, Choi SM, Yang HS, Yoon Y: **The effects of uncoupling protein-1 genotype on lipoprotein cholesterol level in Korean obese subjects**. *Metabolism: clinical and experimental* 2004, **53**(8):1054-1059.
40. Pei X, Liu L, Cai J, Wei W, Shen Y, Wang Y, Chen Y, Sun P, Imam MU, Ping Z *et al*: **Haplotype-based interaction of the PPARGC1A and UCP1 genes is associated with impaired fasting glucose or type 2 diabetes mellitus**. *Medicine* 2017, **96**(23):e6941.
41. Proenza AM, Poissonnet CM, Ozata M, Ozen S, Guran S, Palou A, Strosberg AD: **Association of sets of alleles of genes encoding beta3-adrenoreceptor, uncoupling protein 1 and lipoprotein lipase with increased risk of metabolic complications in obesity**. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* 2000, **24**(1):93-100.
42. Rudofsky G, Jr., Schrödter A, Voron'ko OE, Schlotterer A, Humpert PM, Tafel J, Nawroth PP, Bierhaus A, Hamann A: **Promoter polymorphisms of UCP1, UCP2, and UCP3 are not associated with diabetic microvascular complications in type 2 diabetes**. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme* 2007, **39**(4):306-309.
43. Rudofsky G, Jr., Schroedter A, Schlotterer A, Voron'ko OE, Schlimme M, Tafel J, Isermann BH, Humpert PM, Morcos M, Bierhaus A *et al*: **Functional polymorphisms of UCP2 and UCP3 are associated with a reduced prevalence of diabetic neuropathy in patients with type 1 diabetes**. *Diabetes care* 2006, **29**(1):89-94.
44. Sale MM, Hsu FC, Palmer ND, Gordon CJ, Keene KL, Borgerink HM, Sharma AJ, Bergman RN, Taylor KD, Saad MF *et al*: **The uncoupling protein 1 gene, UCP1, is expressed in mammalian islet cells and associated with acute insulin response to glucose in African American families from the IRAS Family Study**. *BMC endocrine disorders* 2007, **7**:1.
45. Sámano R, Huesca-Gómez C, López-Marure R, Hernández-Cabrera AK, Rodríguez-Ventura A, Tolentino M, Morales RM, Gamboa R: **Association between UCP polymorphisms and adipokines with obesity in Mexican adolescents**. *Journal of pediatric endocrinology & metabolism : JPEM* 2018, **31**(5):561-568.
46. Schäffler A, Palitzsch KD, Watzlawek E, Drobnik W, Schwer H, Schölmerich J, Schmitz G: **Frequency and significance of the A-->G (-3826) polymorphism in the promoter of the gene for uncoupling protein-1 with regard to metabolic parameters and adipocyte transcription factor binding in a large population-based Caucasian cohort**. *European journal of clinical investigation* 1999, **29**(9):770-779.

47. Sivenius K, Valve R, Lindi V, Niskanen L, Laakso M, Uusitupa M: **Synergistic effect of polymorphisms in uncoupling protein 1 and beta3-adrenergic receptor genes on long-term body weight change in Finnish type 2 diabetic and non-diabetic control subjects**. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* 2000, **24**(4):514-519.
48. Sramkova D, Krejbichova S, Vcelak J, Vankova M, Samalikova P, Hill M, Kvasnickova H, Dvorakova K, Vondra K, Hainer V *et al*: **The UCP1 gene polymorphism A-3826G in relation to DM2 and body composition in Czech population**. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association* 2007, **115**(5):303-307.
49. Sun H, Zhang JT, Xie XR, Li T, Li XY, Wang NN, Li JP, Deng ZH, Qiu CC: **Association of uncoupling protein gene polymorphisms with essential hypertension in a northeastern Han Chinese population**. *Journal of human hypertension* 2019, **33**(7):524-530.
50. Tiwari AK, Prasad P, B KT, Kumar KM, Ammini AC, Gupta A, Gupta R: **Oxidative stress pathway genes and chronic renal insufficiency in Asian Indians with Type 2 diabetes**. *Journal of diabetes and its complications* 2009, **23**(2):102-111.
51. Verdi H, Kınık ST, Baysan-Çebi HP, Yalçın YY, Yazıcı-Güvercin AC, Aydın B, Tütüncü NB, Ataç FB: **Uncoupling protein gene UCP1-3826A/G, UCP2 Ins/Del and UCP3-55C/T polymorphisms in obese Turkish children**. *The Turkish journal of pediatrics* 2020, **62**(6):921-929.
52. Vimaleswaran KS, Radha V, Ghosh S, Majumder PP, Rao MR, Mohan V: **A haplotype at the UCP1 gene locus contributes to genetic risk for type 2 diabetes in Asian Indians (CURES-72)**. *Metabolic syndrome and related disorders* 2010, **8**(1):63-68.
53. Vimaleswaran KS, Radha V, Deepa R, Mohan V: **Absence of Association of Metabolic Syndrome with PPARGC1A, PPARG and UCP1 Gene Polymorphisms in Asian Indians**. *Metabolic syndrome and related disorders* 2007, **5**(2):153-162.
54. Yiew SK, Khor LY, Tan ML, Pang CL, Chai VY, Kanachamy SS, Say YH: **No association between peroxisome proliferator-activated receptor and uncoupling protein gene polymorphisms and obesity in Malaysian university students**. *Obesity research & clinical practice* 2010, **4**(4):e247-342.
55. Zhang Y, Meng N, Lv Z, Li H, Qu Y: **The gene polymorphisms of UCP1 but not PPAR γ and TCF7L2 are associated with diabetic retinopathy in Chinese type 2 diabetes mellitus cases**. *Acta ophthalmologica* 2015, **93**(3):e223-229.
56. Zietz B, Watzlawek E, Palitzsch KD, Schölmerich J, Schäffler A: **GG-genotype in the promotor region of uncoupling-protein-1 gene is associated with lower level of dehydroepiandrosterone in type 2 diabetes**. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association* 2001, **109**(2):102-106.

Appendix

Supplement to Chapter 3

Effects of In Vitro Muscle Contraction on Thermogenic Protein Levels in Co-Cultured Adipocytes

C2C12 PGC-1a		

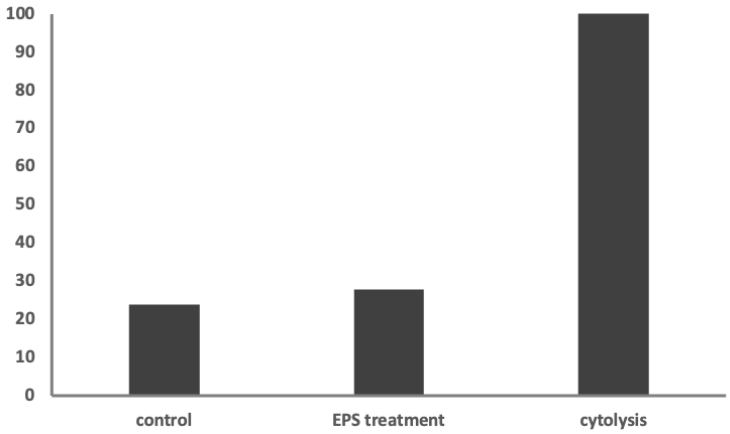


Figure A1. LDH cytotoxicity assay in C2C12 cells. Control represents medium of untreated cells. EPS treatment represents medium from EPS-treated cells and cytolysis describes the control for cell death.

Table A1. List of antibodies used in Western Blot analyses.

Antibody	Dilution	Cat.Numbers	Company
UCP1	1:1000	PA1-24894	Invitrogen
PGC-1a	1:1000	ab77210	Abcam
IL6	1:500	P620	Invitrogen
GAPDH	1:2000	AB2302	Millipore
Anti- Mouse	1:10.000	A9044	Sigma- Aldrich
Anti- Rabbit	1:10.000	7074P2	Cell Signaling

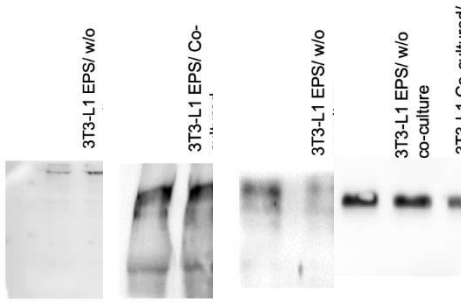


Figure A2. Original Blots for UCP1, PGC1-a, IL-6 and GAPDH in 3T3-L1 cell line.

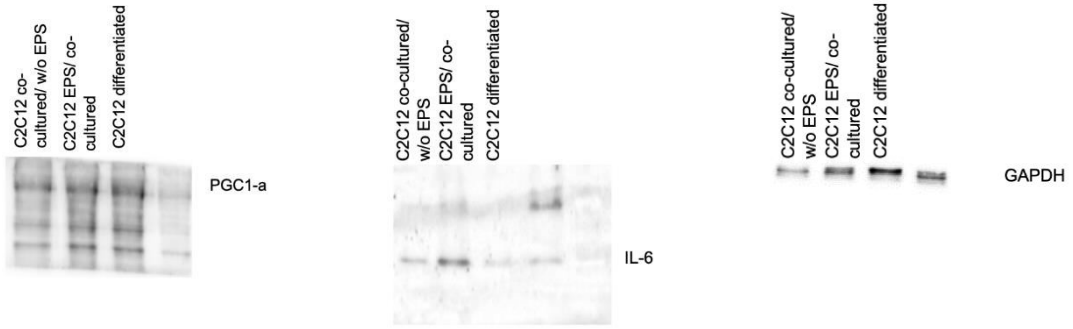
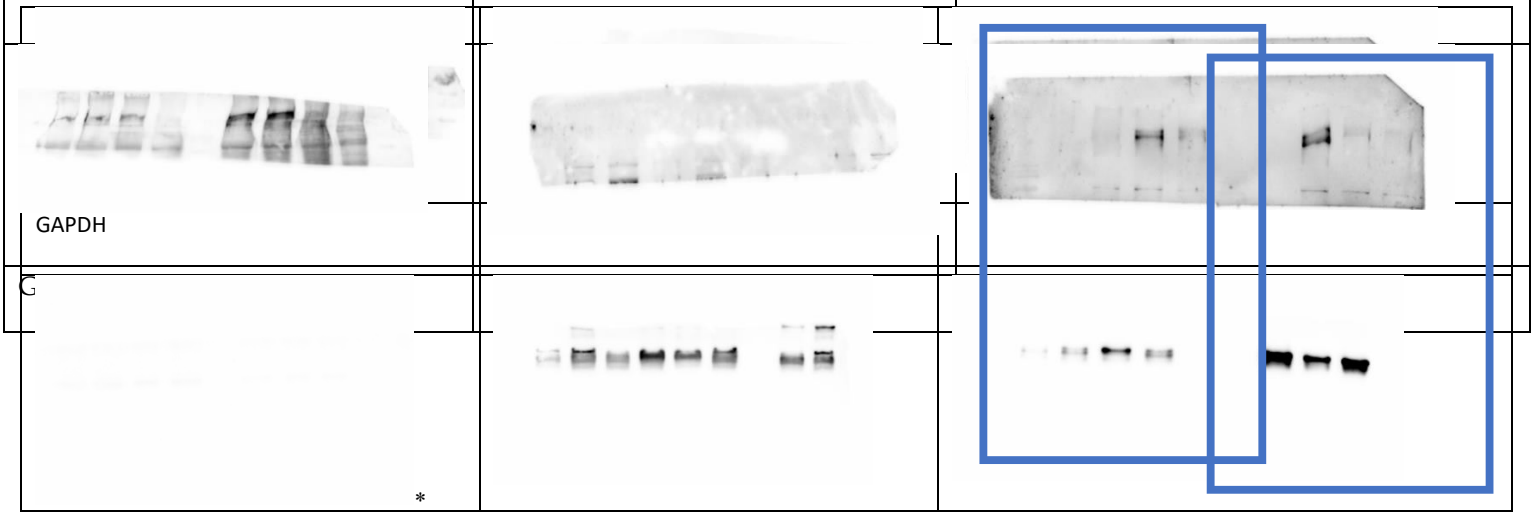


Figure A3. Original Blots for PGC1-a, IL-6 and GAPDH in C2C12 cell line.

∗ This blot's bands can be better visualized once opened with appropriate software (e.g. image j)

3T3-L1 PGC-1a

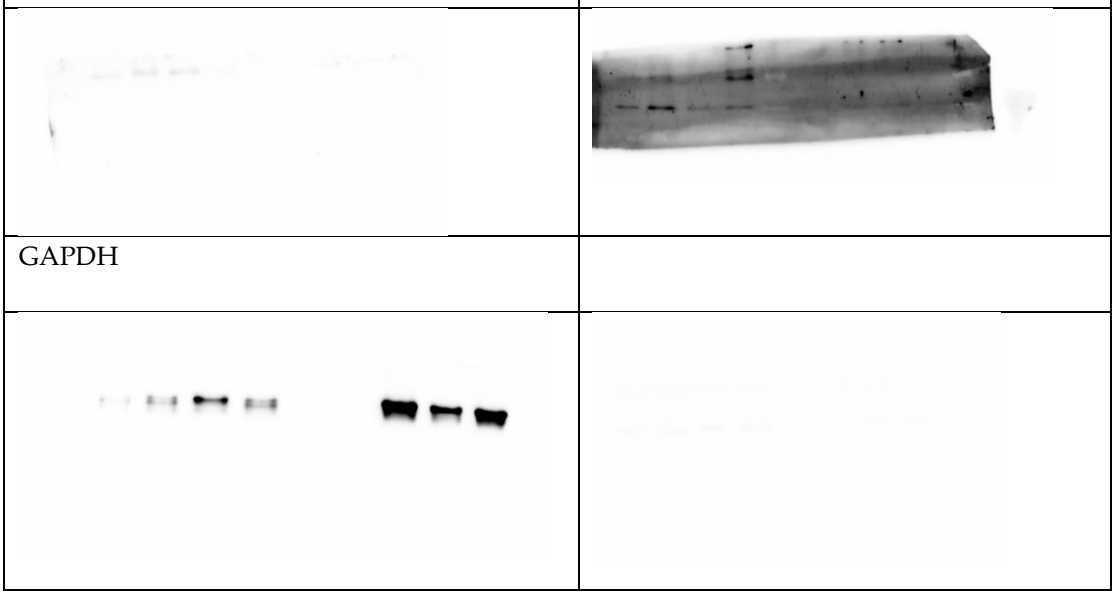


GAPDH

C

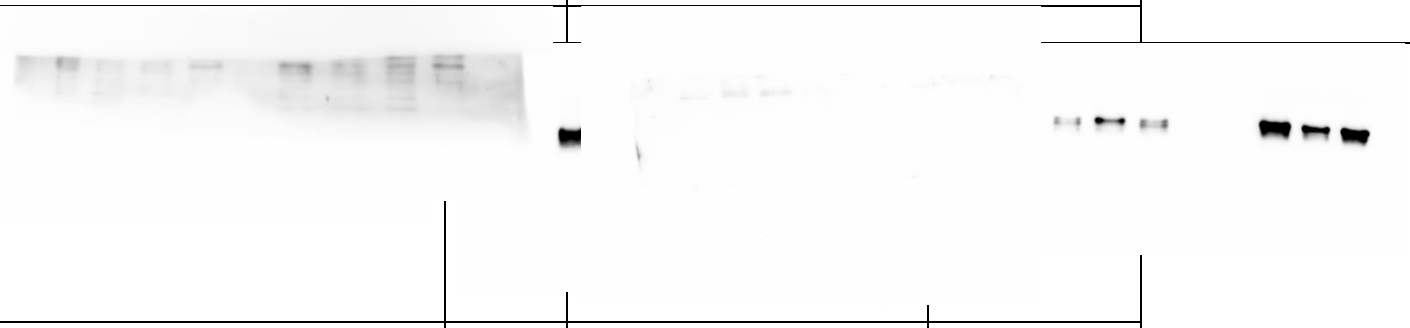
*

C2C12 IL-6

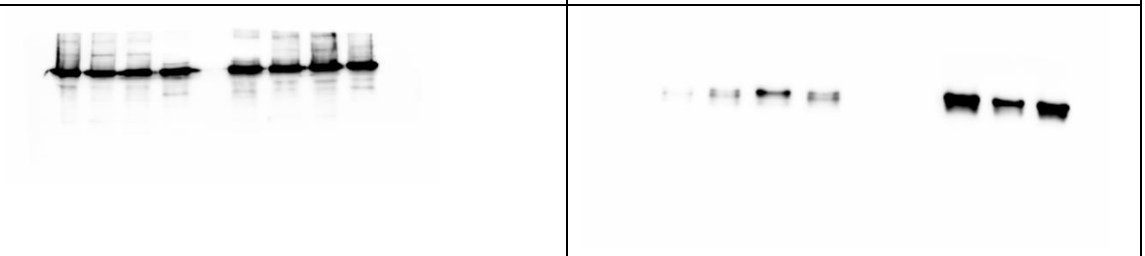


GAPDH

3T3-L1 IL-6

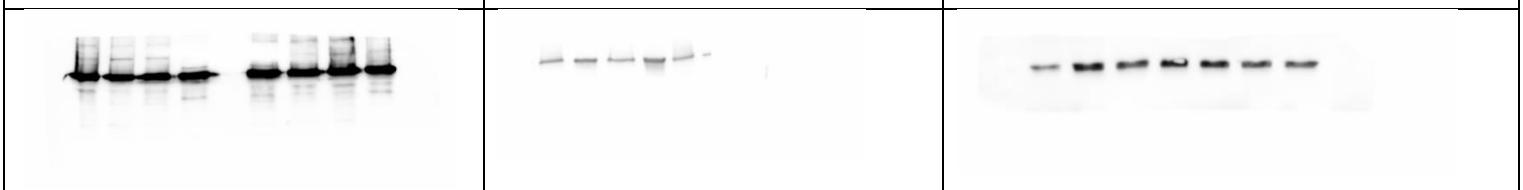


GAPDH



3T3-L1 UCP1

GAPDH



Appendix

Supplement to Chapter 4

Characteristics of the protocols used in electrical pulse stimulation of cultured cells for mimicking in vivo exercise: A systematic review, meta-analysis and meta-regression

1.1 Materials and Methods

1.1.1. Search Strategy

The following algorithm has been used both in EMBASE and PubMed and was accordingly modified, when needed.

The search term combination was the following for both databases:

((((((((((((((pulse*[Title/Abstract])) OR (electric*[Title/Abstract])) OR (stimul*[Title/Abstract])) OR (contract*[Title/Abstract])) OR (frequency[Title/Abstract])) OR (electrode*[Title/Abstract])) OR (field[Title/Abstract])) OR (train[Title/Abstract])) OR (bipolar[Title/Abstract])) OR (pacemaker[Title/Abstract])) OR (c-pace[Title/Abstract]))) AND ((((((((((((cell line*[Title/Abstract])) OR (cell culture*[Title/Abstract])) OR (cell*[Title/Abstract])) OR (musc*[Title/Abstract])) OR (myotube*[Title/Abstract])) OR (myoblast*[Title/Abstract])) OR (muscle cell*[Title/Abstract])) OR (myofiber*[Title/Abstract])) OR (skeletal[Title/Abstract])) OR (myofibril*[Title/Abstract])) OR (contractile activity[Title/Abstract]))) AND ((((physical activity[Title/Abstract])) OR (exercise[Title/Abstract])) OR (training[Title/Abstract]))) AND (((in vitro[Title/Abstract])) NOT (in vivo[Title/Abstract]))

1.1.2. Data extraction

Author, Year	Results- Outcomes
Barlow, 2019 *[231]*	Molecules released as a consequence of acute skeletal muscle contraction, may improve insulin secretion by cells in obese prediabetic and T2D patients who have controlled hyperglycaemia
Beiter T., 2018 *[215]*	The cell culture system does not allow to directly monitor long-term adaptation processes following endurance exercise training, but is quite suitable to analyze acute contraction
Blas A. Guigni, 2019 *[236]*	Results suggest that the beneficial effect of electrical field stimulation derives from the activation of mechanotransductive pathways that downregulate proteolysis and preserve mitochondrial content protect against the atrophic effects of chemotherapeutics
Broholm C., 2011 *[245]*	Leukemia Inhibitory Factor (LIF) mRNA is induced in human skeletal muscle following resistance exercise and LIF protein is secreted from electrically stimulated cultured myotubes
Burch N., 2010 *[167]*	EPS of muscle cells in culture triggers an increase in fatty acid b-oxidation resembling the adaptations of this pathway in chronic endurance exercise. Future combining EPS with mechanical stretch or temporary hypoxia might further help to approximate the environment a fiber a trained muscle's fibre is exposed to
Christensen C.S., 2015 *[169]*	Due to the lack of blood flow, innervation and skeletal connection the EPS HSkM model cannot precisely mimic in vivo exercise. However,

Main outcomes and characteristics of the included studies are presented in the following tables.

TableS1. Main outcomes of the included studies

	the protocol applied elicited molecular adaptations that more likely resemble resistance exercise
Laurens C., 2020 *[221]*	GDF15 is a potentially novel exerkine produced by skeletal muscle contraction and able to target human adipose tissue to promote lipolysis
Connor M.K., 2001 *[232]*	Studies revealed elevated DNA binding in response to contractile activity. This was paralleled by increases in Sp1 protein levels**.** Variations in the rate of mitochondrial ATP synthesis are important in determining cytochrome c gene expression in muscle cells and this is mediated, in part, by Sp1-induced increases in cytochrome c transcription
Feng YZ., 2014 *[217]*	EPS enhanced oxidative capacity of glucose in myotubes from all subjects, in contrast to oleic acid that affected only in lean subjects. Human myotubes display the same phenotype as intact muscle in vivo
Fernández-Verdejo R., 2017 *[233]*	ATF3 is induced by EPS and regulates chemokine mRNA expression in C2C12 myotubes. Part of the ATF3 up-regulation in contracting skeletal muscle occurs in myofibers
Fujita H., 2010 *[234]*	Contractile activity not only enhances myotube maturation in vitro, but additionally induces changes in the sense of a fast-to-slow transition. Successfully managed to have artificially exercised C2C12 myotubes
Furuichi Y., 2018 *[235]*	The secretion of IL-6 increased, following muscle contraction, in the absence of cellular damage, suggesting the presence of a secretory machinery in skeletal muscle cells
Gong H., 2016 *[46]*	EPS stimulation significantly increased intracellular ATP levels
Horie M., 2015 *[237]*	EPS induced excessive ROS production in contracting C2C12 myotubes and metabolism of ROS resulting from Nrf2 activation protected the myotubes from EPS-induced apoptosis
Kubis HP., 2002 *[249]*	A ON period of 5 min in a 45 min stimulation cycle is sufficient to induce MHCI e pression and reduce MHCIId expression (mRNA and protein levels). Shorter ON periods of 1.5 min in a 45 min cycle failed

	to induce a fast-to-slow transition
Lambertucci RH., 2012 *[222]*	Moderate electrical stimulation increases ROS and NO production by primary rat skeletal muscle cells
Lee J.O..,2020 *[272]*	Acute and chronic EPS increased the secretion and expression of metrnl into conditioning media and cell lysates and the phosphorylation of AMPK2a in C2C12 myotubes . Metrnl improves glucose metabolism via AMPK-2 and is a promising therapeutic candidate for glucose-related diseases such as type 2 diabetes
Li Z., 2018 *[45]*	Acute myotube contraction activates signaling of LKB1, AMPK and CaMKII to increase surface GLUT4 levels
Manabe Y., 2012 *[224]*	C2C12 model is suitable for defining the physiological role of intracellular signaling evoked by muscle contraction
Martin N.R.W., 2017 *[239]*	Leucine supplementation may augment skeletal muscle functional capacity, validates the use of engineered skeletal muscle for highly-controlled investigations into nutritional regulation of muscle physiology.
McArdle F., 2001 *[218]*	The short period of contractile activity induced a significant rise in the superoxide level detected in muscle interstitial fluid by microdialysis techniques, but did not induce any significant damage to skeletal muscle fibers
Nieuwoudt S., 2017 *[273]*	EPS protected against palmitate-induced reductions in PI3K activity, despite the reduction in enzyme activity. Also the respond to contraction stimuli happened in a predictable manner. Contraction alone may protect muscle from lipid-induced insulin resistance
Nikolic´ N., 2012 *[165]*	EPS did not induce toxic effects to cultured human skeletal muscle cells and in chronic continuous, low-frequency EPS, improved lipid oxidation and glucose metabolism and a possible fiber-type switch was detected. *In vitro* EPS (Acute, high-frequent as well as chronic, low-frequent) of human myotubes may be used to study effects of

	exercise
Løvsletten N., 2019 *[247]*	Challenging the cells with EPS lead to different responses in myotubes from non-diabetic vs. diabetic subjects
Park S.,2019 *[225]*	Electrical pulse stimulation of primary myotubes from lean and severely obese subjects induced improvements in insulin action, but to a lesser extent in those with severe obesity
Pattamaprapanont P.,2016 *[170]*	This EPS model can mimic some, but not all the features of muscle contraction in vivo
Pattwell DM., 2004 *[244]*	Skeletal muscle cells release multiple ROS during contractile activity and the pattern of release differs depending on the nature of the ROS species and the frequency of stimulation. The reduction of cytochrome c in the supernatant of muscle cells is not related to the number of contractions undertaken or the frequency of stimulation
Raschke S.,2013 *[162]*	DDP4 and PEDF were identified to be also secreted by skeletal muscle cells. The release of 45 myokines is regulated by contraction and among these factors, 18 are described as myokines for the first time.
Raschke S. 2013 *[171]*	This EPS model significantly enhanced PGC1a mRNA expression
Sato S., 2019 *[181]*	The newly established in vitro muscle contraction model is suitable for analyzing the activation of mTORC1 signaling pathway in cultured L6.C11 myotubes
Scheler M., 2013 *[248]*	In vitro exercise model can be used to identify exercise- regulated myokines and can be applied to primary human myotubes to study molecular mechanisms of the individual outcome of exercise intervention
Tarum J., 2017 *[219]*	The study demonstrates that EPS is an *in vitro* exercise model promoting the hypertrophy of human muscle cells, recapitulating a major physiological end-point to resistance exercise in human skeletal muscle
Thelen M.H., 1997 *[243]*	The opposing stimuli of T_3 and (chronic) contractile activity determine the expression of SERCA1, a typical fast isoform, in skeletal muscle

Son Y.H., 2019 *[242]*	High similarity and correlation observed for most parameters between EPS and VWR. Various EPS conditions induce muscle hypertrophy and mitochondrial biogenesis, which are phenotypes displayed in resistance and endurance exercise
Yue Y., 2019 *[226]*	Acute EPS-induced myotube contraction or treadmill exercise regulated Axin1 protein expression in a manner dependent on AMPK activation, while stimulation of Rac1 is AMPK-dependent in both contracted myotubes and exercised skeletal muscle
Valero-Breton M., 2020 *[220]*	The parameters of stimulation developed could be useful for future studies intending to investigate the molecular responses of acute and chronic resistance exercise in an in vitro model in the quest to develop exercise-mimetics.
Chaves A.B., 2021 *[216]*	Acute aerobic exercise was able to significantly reduce T NIP and REDD1 protein expression, which may be mediated by a PKA- or cAMP-related mechanism, as indicated by the in vitro experiments
Kugler B.A., 2021 *[246]*	24 hours of EPS resulted in an improved mitochondrial network structure towards fusion in myotubes derived from lean humans and humans with severe obesity, which was associated with improved skeletal muscle insulin signaling
Nakamura T., 2021 *[240]*	The findings suggested that exposure of 3D-engineered muscle to acute EPS mimicked muscle fatigue during acute high-intensity exercise in humans
Small L., 2020 *[241]*	Results of the study suggest that a proportion of the ability of exercise to entrain the skeletal muscle clock driven directly by muscle contraction. Contraction Interventions may be used to mimic some time of day specific effects of exercise on metabolism and muscle performance
Tamura Y., 2020 *[176]*	From a qualitative perspective, tetanus and twitch were shown to promote metabolic adaptation in the same direction

Table S2. Main extracted data. A general description of the included studies. In the table are shown the cell types, pulse stimulator types, duration of exercise and the type of in vitro exercise.

#	First author, date			C2C12/L6/H2k	Human skeletal muscle biopsies	Primary rat/mouse/rabbit cells	Primary human cells	Custom made stimulator	Commercially available	Duration of stimulation	Acute	Chronic	Aerobic	Resistance	High intensity	Moderate
1	Barlow J., 2019 [231]			☑					☑	64 min	☑					
2	Beiter T., 2018 [215]			☑					☑	90 min	☑					
3	Blas A. Guigni, 2019 [236]			☑					☑	60 min	☑					
4	Broholm C., 2011 [245]				☑				☑	180 min				☑		
5	Burch N., 2010 [167]			☑					☑	90 min, 90 min daily/ 4 consecutive days,24 hrs	☑	☑				
6	Christensen C.S., 2015 [169]				☑				☑	360 min	☑			☑		
7	Laurens C., 2020 [221]				☑				☑	180min and 24h	☑	☑			☑	☑
8	Connor M.K., 2001 [232]			☑				☑		5, 15, 30, 60, or 240 min in one day, 180 min/day	☑	☑				
9	Feng Y.Z., 2014 [217]				☑			☑		48 hrs		☑				
10	Fernández-Verdejo R., 2017 [233]			☑					☑	240min				☑		

11	Fujita H., 2010 [234]			☑					☑	7-11 days							
12	Furuichi Y., 2018 [235]			☑					☑	60min	☑						
13	Gong H., 2016 [46]			☑				☑		1-15 min	☑						
14	Horie M., 2015 [237]			☑					☑	60min, 180min, 360min						☑	
15	Kubis H.P., 2002 [249]					☑		☑		cycles of 45 min		☑					
16	Lambertucci R.H., 2012 [222]					☑		☑		60min							☑
17	Lee J.O..,2020 [272]			☑					☑	60min, 180 min, 360 min, 12hrs, 24hrs, or 36 hrs							
18	Li Z., 2018 [45]			☑					☑	60 min	☑						
19	Manabe Y., 2012 [224]			☑					☑	60 min, 180 min	☑						
20	Martin N.R.W., 2017 [239]			☑				☑		24 hrs		☑					
21	McArdle A., 2001[218]					☑		☑		15 min			☑				
22	Nieuwoudt S., 2017 [273]			☑					☑	16 hrs			☑				
23	Nikolic´ N., 2012 [165]				☑			☑		5–60 min, 12 hrs, 24hrs	☑	☑					
24	Løvsletten N., 2019 [247]				☑				☑	24 hrs							
25	Park S.,2019 [225]				☑				☑	24 hrs							☑
26	Pattamaprapanont P.,2016 [170]			☑					☑	30 min	☑						
27	Pattwell D.M., 2004 [244]			☑				☑		15 min	☑					☑	
28	Raschke S.,2013 [162]						☑		☑	4 to 24 hrs							☑

29	Raschke S. 2013 [171]			☑			☑		☑	24 hrs							
30	Sato S., 2019 [181]			☑					☑	45min				☑			
31	Scheler M., 2013 [248]				☑				☑	2hr, 4hrs, 8hrs, 24 hrs	☑						
32	Tarum J., 2017 [219]				☑				☑	8 hrs				☑			
33	Thelen M.H., 1997 [243]			☑				☑		48 hrs		☑					
34	Son Y.H., 2019 [242]			☑					☑	60 min				☑			☑
35	Yue Y., 2019 [226]			☑					☑	60 min	☑						
36	Breton M., 2020 [220]				☑				☑	30 min, 3 days	☑	☑			☑		
37	Chaves A.B., 2022[216]				☑				☑	24 hrs	☑						
38	Kugler B.A., 2020 [246]				☑				☑	24 hrs		☑					
39	Nakamura T., 2021 [240]			☑					☑	30 min	☑					☑	
40	Small L., 2020 [241]			☑					☑	30 min	☑						
41	Tamura Y., 2020 [176]			☑					☑	10 min					☑		

Table S3. Pulse stimulation characteristics of the included studies

	Pulse duration	Pulse amplitude	Frequency
Barlow, 2019 [231]	2 ms pulses	40 V	1 Hz
Beiter T., 2018 [215]	2 ms pulses	14 V	1 Hz
Blas A. Guigni, 2019 [236]	12 ms pulses	20 V	1 Hz
Broholm C., 2011 [245]	1 ms pulses	40 V	1 Hz
Burch N., 2010 [167]	1 ms pulses	14 V	50 Hz
Christensen C.S., 2015 [169]	2 ms pulses	40 V	1Hz
Laurens C., 2020 [221]	Acute=24-ms pulses / chronic = 2-ms pulses	10 V	Acute=0.5 Hz/ Chronic= 0.1 Hz
Connor M.K., 2001 [232]		65 V	5 Hz
Feng YZ., 2014 [217]	2 ms pulses	30 V	1 Hz
Fernández-Verdejo R., 2017 [233]	2 ms pulses	20 V	1 Hz
Fujita H., 2010 [234]	twitch contraction: 10 ms	1 V/mm	50 Hz
Furuichi Y., 2018 [235]	3ms, 30ms, 50ms pulse duration	various voltages	1-Hz
Gong H., 2016 [46]	11 ms pulses	30 V	1, 4, 10, 30 Hz
Horie M., 2015 [237]	2 ms pulses	14, 20, and 40 V	1 Hz
Kubis HP., 2002 [249]	2.5 ms pulses		II, III & V=1, IV=5 or I=10 Hz
Lambertucci RH., 2012 [222]		5 V	50 Hz
Lee J.O.,2020 [272]	1 ms pulses	25 V	1 Hz
Li Z., 2018 [45]	24 ms pulses	20 V	1 Hz
Manabe Y., [224]	3 ms pulses	50 V	1 Hz

Martin N.R.W., 2017 [239]		1 V/mm	10 Hz
McArdle F., 2001 [218]	2 ms pulses	30 V/well	1Hz
Nieuwoudt S., 2017 [273]		1.5 V/mm	1Hz, 0.5 Hz
Nikolic´ N., 2012 [165]	Acute=200 ms given every 5th second/ chronic=2 ms	50 V	Acute= 100 Hz/chronic=1 Hz
Løvsletten N., 2019 [247]	2ms pulses	10 V	0,1 Hz
Park S.,2019 [225]	2 ms pulses	11.5 V	1 Hz
Pattamaprapanont P.,2016 [170]	2 ms pulses	30 V	1 Hz
Pattwell DM., 2004 [244]	2 ms in duration for 0.5 of a second every 5 s	30V/well	1 Hz or 50 Hz
Raschke S.,2013 [162]	2 ms pulses	11.5 V	1 Hz
Raschke S. 2013 [171]	2 ms pulses	11.5 V	1 Hz
Sato S., 2019 [181]	2 ms pulses	50 V	100 Hz
Scheler M., 2013 [248]	2ms pulses	4 V and 14 V	5 Hz
Tarum J., 2017 [219]	2 ms pulses	12 V	1 Hz
Thelen M.H., 1997 [243]	6 ms pulses	3 V/cm2	2Hz
Son Y.H., 2019 [242]	1 ms pulse of 2 ms duration	11.5 V	10 Hz
Yue Y., 2019 [226]	24 ms at 976-ms intervals	20 V	1 Hz
Valero-Breton M., 2020 [220]	0.4 ms with 4 s rest between each contraction	15 V	100 Hz
Chaves A.B., 2021 [216]	2 ms	11.5 V	1 Hz
Kugler B.A., 2021 [246]	2 ms pulses	11.5 V	1 Hz
Nakamura T., 2021 [240]	2 ms pulses	1 V/mm	100 Hz
Small L., 2020 [241]	2 ms pulses	30 V	1 Hz
Tamura Y., 2020 [176]	2 ms, twitch and tetanus	13 V, twitch and tetanus	twitch:2 Hz (continuous)

			and tetanus: 66 Hz (5s ON, 5 s OFF)

2.1. Metanalytic findings

Figure S1. Correlation of the duration of stimulation with the difference in expression levels of Akt.

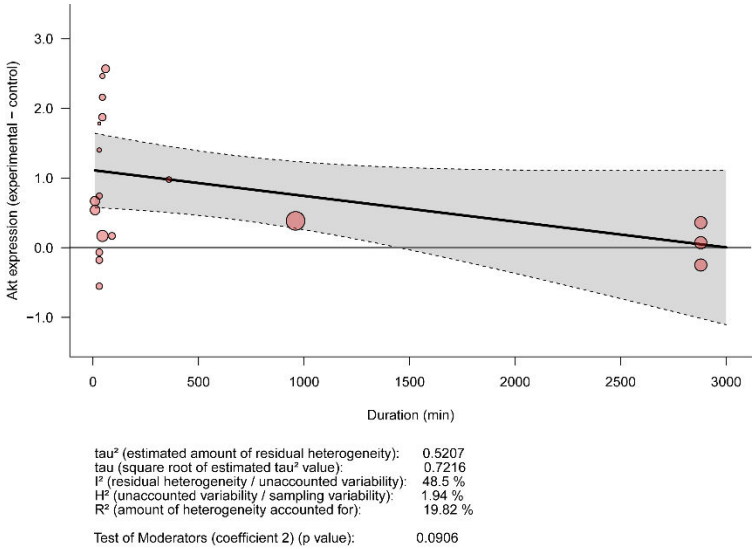


Figure S2. Correlation of the duration of stimulation with the difference in expression levels of Glucose Uptake.

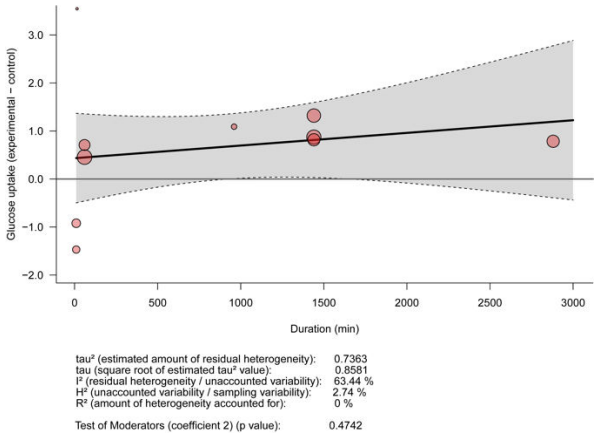


Figure S3. Correlation of the duration of stimulation with the difference in expression levels of GLUT4.

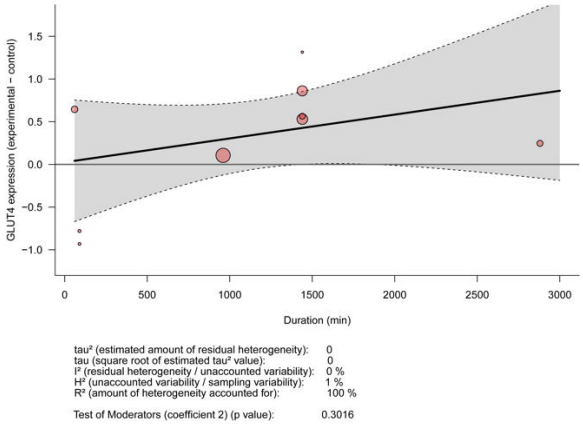


Figure S4. Correlation of the duration of stimulation with the difference in expression levels of PGC1a.

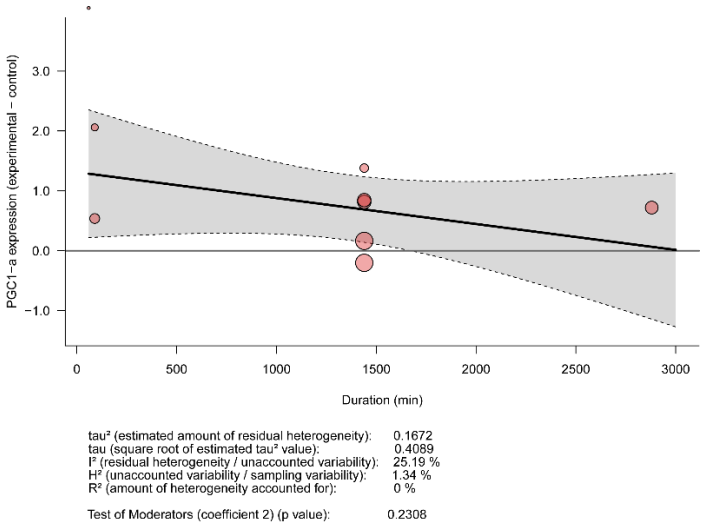
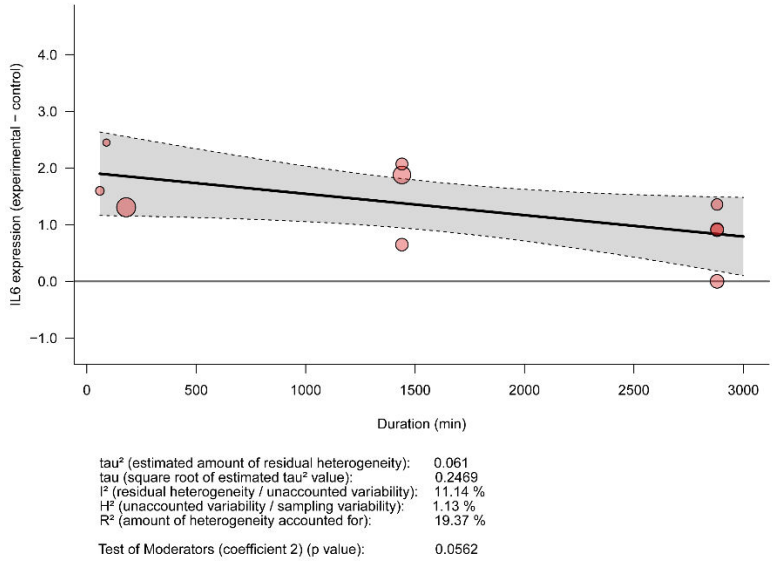


Figure S5. Correlation of the duration of stimulation with the difference in expression levels of IL-6.



References

1. Dinas, P. C., E. Nintou, M. Vliora, A. E. Pravednikova, P. Sakellariou, A. Witkowicz, Z. M. Kachaev, V. V. Kerchev, S. N. Larina, J. Cotton, A. Kowalska, P. Gkiata, A. Bargiota, Z. A. Khachatryan, A. A. Hovhannisyan, M. A. Antonosyan, S. Margaryan, A. Partyka, P. Bogdanski, M. Szulinska, M. Kregielska-Narozna, R. Czepczyński, M. Ruchała, A. Tomkiewicz, L. Yepiskoposyan, L. Karabon, Y. Shidlovskii, G. S. Metsios, and A. D. Flouris. "Prevalence of Uncoupling Protein One Genetic Polymorphisms and Their Relationship with Cardiovascular and Metabolic Health." *PLoS One* 17, no. 4 (2022): e0266386.
2. Nintou, E., E. Karligiotou, M. Vliora, I. G. Fatouros, A. Z. Jamurtas, N. Sakellaridis, K. Dimas, and A. D. Flouris. "Effects of in Vitro Muscle Contraction on Thermogenic Protein Levels in Co-Cultured Adipocytes." *Life (Basel)* 11, no. 11 (2021).
3. Nintou, E., E. Karligiotou, M. Vliora, L. G. Ioannou, and A. D. Flouris. "Characteristics of the Protocols Used in Electrical Pulse Stimulation of Cultured Cells for Mimicking in Vivo Exercise: A Systematic Review, Meta-Analysis, and Meta-Regression." *Int J Mol Sci* 23, no. 21 (2022).
4. Afshin, A., M. H. Forouzanfar, M. B. Reitsma, P. Sur, K. Estep, A. Lee, L. Marczak, A. H. Mokdad, M. Moradi-Lakeh, M. Naghavi, J. S. Salama, T. Vos, K. H. Abate, C. Abbafati, M. B. Ahmed, Z. Al-Aly, A. Alkerwi, R. Al-Raddadi, A. T. Amare, A. Amberbir, A. K. Amegah, E. Amini, S. M. Amrock, R. M. Anjana, J. Ärnlöv, H. Asayesh, A. Banerjee, A. Barac, E. Baye, D. A. Bennett, A. S. Beyene, S. Biadgilign, S. Biryukov, E. Bjertness, D. J. Boneya, I. Campos-Nonato, J. J. Carrero, P. Cecilio, K. Cercy, L. G. Ciobanu, L. Cornaby, S. A. Damtew, L. Dandona, R. Dandona, S. D. Dharmaratne, B. B. Duncan, B. Eshrati, A. Esteghamati, V. L. Feigin, J. C. Fernandes, T. Fürst, T. T. Gebrehiwot, A. Gold, P. N. Gona, A. Goto, T. D. Habtewold, K. T. Hadush, N. Hafezi-Nejad, S. I. Hay, M. Horino, F. Islami, R. Kamal, A. Kasaeian, S. V. Katikireddi, A. P. Kengne, C. N. Kesavachandran, Y. S. Khader, Y. H. Khang, J. Khubchandani, D. Kim, Y. J. Kim, Y. Kinfu, S. Kosen, T. Ku, B. K. Defo, G. A. Kumar, H. J. Larson, M. Leinsalu, X. Liang, S. S. Lim, P. Liu, A. D. Lopez, R. Lozano, A. Majeed, R. Malekzadeh, D. C. Malta, M. Mazidi, C. McAlinden, S. T. McGarvey, D. T. Mengistu, G. A. Mensah, G. B. M. Mensink, H. B. Mezgebe, E. M. Mirrakhimov, U. O. Mueller, J. J. Noubiap, C. M. Obermeyer, F. A. Ogbo, M. O. Owolabi, G. C. Patton, F. Pourmalek, M. Qorbani, A. Rafay, R. K. Rai, C. L. Ranabhat, N. Reinig, S. Safiri, J. A. Salomon, J. R. Sanabria, I. S. Santos, B. Sartorius, M. Sawhney, J. Schmidhuber, A. E. Schutte, M. I. Schmidt, S. G. Sepanlou, M. Shamsizadeh, S. Sheikhbahaei, M. J. Shin, R. Shiri, I. Shiue, H. S. Roba, D. A. S. Silva, J. I.

Silverberg, J. A. Singh, S. Stranges, S. Swaminathan, R. Tabarés-Seisdedos, F. Tadese, B. A. Tedla, B. S. Tegegne, A. S. Terkawi, J. S. Thakur, M. Tonelli, R. Topor-Madry, S. Tyrovolas, K. N. Ukwaja, O. A. Uthman, M. Vaezghasemi, T. Vasankari, V. V. Vlassov, S. E. Vollset, E. Weiderpass, A. Werdecker, J. Wesana, R. Westerman, Y. Yano, N. Yonemoto, G. Yonga, Z. Zaidi, Z. M. Zenebe, B. Zipkin, and C. J. L. Murray. "Health Effects of Overweight and Obesity in 195 Countries over 25 Years." *N Engl J Med* 377, no. 1 (2017): 13-27.

5. Tremmel, Maximilian, Ulf-G. Gerdtham, Peter M. Nilsson, and Sanjib Saha. "Economic Burden of Obesity: A Systematic Literature Review." *International Journal of Environmental Research and Public Health* 14, no. 4 (2017): 435.
6. Huang, L. O., A. Rauch, E. Mazzaferro, M. Preuss, S. Carobbio, C. S. Bayrak, N. Chami, Z. Wang, U. M. Schick, N. Yang, Y. Itan, A. Vidal-Puig, M. den Hoed, S. Mandrup, T. O. Kilpeläinen, and R. J. F. Loos. "Genome-Wide Discovery of Genetic Loci That Uncouple Excess Adiposity from Its Comorbidities." *Nat Metab* 3, no. 2 (2021): 228-43.
7. Scott, R. A., L. J. Scott, R. Mägi, L. Marullo, K. J. Gaulton, M. Kaakinen, N. Pervjakova, T. H. Pers, A. D. Johnson, J. D. Eicher, A. U. Jackson, T. Ferreira, Y. Lee, C. Ma, V. Steinthorsdottir, G. Thorleifsson, L. Qi, N. R. Van Zuydam, A. Mahajan, H. Chen, P. Almgren, B. F. Voight, H. Grallert, M. Müller-Nurasyid, J. S. Ried, N. W. Rayner, N. Robertson, L. C. Karssen, E. M. van Leeuwen, S. M. Willems, C. Fuchsberger, P. Kwan, T. M. Teslovich, P. Chanda, M. Li, Y. Lu, C. Dina, D. Thuillier, L. Yengo, L. Jiang, T. Sparso, H. A. Kestler, H. Chheda, L. Eisele, S. Gustafsson, M. Frånberg, R. J. Strawbridge, R. Benediktsson, A. B. Hreidarsson, A. Kong, G. Sigurðsson, N. D. Kerrison, J. Luan, L. Liang, T. Meitinger, M. Roden, B. Thorand, T. Esko, E. Mihailov, C. Fox, C. T. Liu, D. Rybin, B. Isomaa, V. Lyssenko, T. Tuomi, D. J. Couper, J. S. Pankow, N. Grarup, C. T. Have, M. E. Jørgensen, T. Jørgensen, A. Linneberg, M. C. Cornelis, R. M. van Dam, D. J. Hunter, P. Kraft, Q. Sun, S. Edkins, K. R. Owen, J. R. B. Perry, A. R. Wood, E. Zeggini, J. Tajes-Fernandes, G. R. Abecasis, L. L. Bonnycastle, P. S. Chines, H. M. Stringham, H. A. Koistinen, L. Kinnunen, B. Sennblad, T. W. Mühleisen, M. M. Nöthen, S. Pechlivanis, D. Baldassarre, K. Gertow, S. E. Humphries, E. Tremoli, N. Klopp, J. Meyer, G. Steinbach, R. Wennauer, J. G. Eriksson, S. Männistö, L. Peltonen, E. Tikkanen, G. Charpentier, E. Eury, S. Lobbens, B. Gigante, K. Leander, O. McLeod, E. P. Bottinger, O. Gottesman, D. Ruderfer, M. Blüher, P. Kovacs, A. Tonjes, N. M. Maruthur, C. Scapoli, R. Erbel, K. H. Jöckel, S. Moebus, U. de Faire, A. Hamsten, M. Stumvoll, P. Deloukas, P. J. Donnelly, T. M. Frayling, A. T. Hattersley, S. Ripatti, V. Salomaa, N. L. Pedersen, B. O. Boehm, R. N. Bergman, F. S. Collins, K. L. Mohlke, J. Tuomilehto, T. Hansen, O. Pedersen, I. Barroso, L. Lannfelt, E. Ingelsson, L. Lind, C. M. Lindgren, S. Cauchi, P. Froguel, R. J. F. Loos, B. Balkau, H. Boeing, P. W. Franks, A. Barricarte Gurrea, D. Palli, Y. T. van der Schouw, D. Altshuler, L. C. Groop, C. Langenberg, N. J. Wareham, E. Sijbrands, C. M. van Duijn, J. C. Florez, J. B. Meigs, E. Boerwinkle, C. Gieger, K. Strauch, A. Metspalu, A. D. Morris, C. N. A. Palmer, F. B. Hu, U. Thorsteinsdottir, K. Stefansson, J. Dupuis, A. P. Morris, M. Boehnke, M. I. McCarthy, and I. Prokopenko. "An Expanded Genome-Wide Association Study of Type 2 Diabetes in Europeans." *Diabetes* 66, no. 11 (2017): 2888-902.
8. Shungin, D., T. W. Winkler, D. C. Croteau-Chonka, T. Ferreira, A. E. Locke, R. Mägi, R. J. Strawbridge, T. H. Pers, K. Fischer, A. E. Justice, T. Workalemahu, J. M. W. Wu, M. L. Buchkovich, N. L. Heard-Costa, T. S. Roman, A. W. Drong, C. Song, S. Gustafsson, F. R. Day, T. Esko, T. Fall, Z. Kutalik, J. Luan, J. C. Randall, A. Scherag, S. Vedantam, A. R. Wood, J. Chen, R. Fehrmann, J. Karjalainen, B. Kahali, C. T. Liu, E. M. Schmidt, D. Absher, N. Amin, D. Anderson, M. Beekman, J. L. Bragg-Gresham, S. Buyske, A. Demirkan, G. B. Ehret, M. F. Feitosa, A. Goel, A. U. Jackson, T. Johnson, M. E. Kleber, K. Kristiansson, M. Mangino, I. M. Leach, C. Medina-Gomez, C. D. Palmer, D. Pasko, S. Pechlivanis, M. J. Peters, I. Prokopenko, A. Stančáková, Y. J. Sung, T. Tanaka, A. Teumer, J. V. Van Vliet-Ostaptchouk, L. Yengo, W. Zhang, E. Albrecht, J. Ärnlöv, G. M. Arscott, S. Bandinelli, A. Barrett, C. Bellis, A. J. Bennett, C. Berne, M. Blüher, S. Böhringer, F. Bonnet, Y. Böttcher, M. Bruinenberg, D. B. Carba, I. H.

Caspersen, R. Clarke, E. W. Daw, J. Deelen, E. Deelman, G. Delgado, A. S. Doney, N. Eklund, M. R. Erdos, K. Estrada, E. Eury, N. Friedrich, M. E. Garcia, V. Giedraitis, B. Gigante, A. S. Go, A. Golay, H. Grallert, T. B. Grammer, J. Gräßler, J. Grewal, C. J. Groves, T. Haller, G. Hallmans, C. A. Hartman, M. Hassinen, C. Hayward, K. Heikkilä, K. H. Herzig, Q. Helmer, H. L. Hillege, O. Holmen, S. C. Hunt, A. Isaacs, T. Ittermann, A. L. James, I. Johansson, T. Juliusdottir, I. P. Kalafati, L. Kinnunen, W. Koenig, I. K. Kooner, W. Kratzer, C. Lamina, K. Leander, N. R. Lee, P. Lichtner, L. Lind, J. Lindström, S. Lobbens, M. Lorentzon, F. Mach, P. K. Magnusson, A. Mahajan, W. L. McArdle, C. Menni, S. Merger, E. Mihailov, L. Milani, R. Mills, A. Moayyeri, K. L. Monda, S. P. Mooijaart, T. W. Mühleisen, A. Mulas, G. Müller, M. Müller-Nurasyid, R. Nagaraja, M. A. Nalls, N. Narisu, N. Glorioso, I. M. Nolte, M. Olden, N. W. Rayner, F. Renstrom, J. S. Ried, N. R. Robertson, L. M. Rose, S. Sanna, H. Scharnagl, S. Scholtens, B. Sennblad, T. Seufferlein, C. M. Sitlani, A. V. Smith, K. Stirrups, H. M. Stringham, J. Sundström, M. A. Swertz, A. J. Swift, A. C. Syvänen, B. O. Tayo, B. Thorand, G. Thorleifsson, A. Tomaschitz, C. Troffa, F. V. van Oort, N. Verweij, J. M. Vonk, L. L. Waite, R. Wennauer, T. Wilsgaard, M. K. Wojczynski, A. Wong, Q. Zhang, J. H. Zhao, E. P. Brennan, M. Choi, P. Eriksson, L. Folkersen, A. Franco-Cereceda, A. G. Gharavi, K. Hedman Å, M. F. Hivert, J. Huang, S. Kanoni, F. Karpe, S. Keildson, K. Kiryluk, L. Liang, R. P. Lifton, B. Ma, A. J. McKnight, R. McPherson, A. Metspalu, J. L. Min, M. F. Moffatt, G. W. Montgomery, J. M. Murabito, G. Nicholson, D. R. Nyholt, C. Olsson, J. R. Perry, E. Reinmaa, R. M. Salem, N. Sandholm, E. E. Schadt, R. A. Scott, L. Stolk, E. E. Vallejo, H. J. Westra, K. T. Zondervan, P. Amouyel, D. Arveiler, S. J. Bakker, J. Beilby, R. N. Bergman, J. Blangero, M. J. Brown, M. Burnier, H. Campbell, A. Chakravarti, P. S. Chines, S. Claudi-Boehm, F. S. Collins, D. C. Crawford, J. Danesh, U. de Faire, E. J. de Geus, M. Dörr, R. Erbel, J. G. Eriksson, M. Farrall, E. Ferrannini, J. Ferrières, N. G. Forouhi, T. Forrester, O. H. Franco, R. T. Gansevoort, C. Gieger, V. Gudnason, C. A. Haiman, T. B. Harris, A. T. Hattersley, M. Heliövaara, A. A. Hicks, A. D. Hingorani, W. Hoffmann, A. Hofman, G. Homuth, S. E. Humphries, E. Hyppönen, T. Illig, M. R. Jarvelin, B. Johansen, P. Jousilahti, A. M. Jula, J. Kaprio, F. Kee, S. M. Keinanen-Kiukaanniemi, J. S. Kooner, C. Kooperberg, P. Kovacs, A. T. Kraja, M. Kumari, K. Kuulasmaa, J. Kuusisto, T. A. Lakka, C. Langenberg, L. Le Marchand, T. Lehtimäki, V. Lyssenko, S. Männistö, A. Marette, T. C. Matise, C. A. McKenzie, B. McKnight, A. W. Musk, S. Möhlenkamp, A. D. Morris, M. Nelis, C. Ohlsson, A. J. Oldehinkel, K. K. Ong, L. J. Palmer, B. W. Penninx, A. Peters, P. P. Pramstaller, O. T. Raitakari, T. Rankinen, D. C. Rao, T. K. Rice, P. M. Ridker, M. D. Ritchie, I. Rudan, V. Salomaa, N. J. Samani, J. Saramies, M. A. Sarzynski, P. E. Schwarz, A. R. Shuldiner, J. A. Staessen, V. Steinthorsdottir, R. P. Stolk, K. Strauch, A. Tönjes, A. Tremblay, E. Tremoli, M. C. Vohl, U. Völker, P. Vollenweider, J. F. Wilson, J. C. Witteman, L. S. Adair, M. Bochud, B. O. Boehm, S. R. Bornstein, C. Bouchard, S. Cauchi, M. J. Caulfield, J. C. Chambers, D. I. Chasman, R. S. Cooper, G. Dedoussis, L. Ferrucci, P. Froguel, H. J. Grabe, A. Hamsten, J. Hui, K. Hveem, K. H. Jöckel, M. Kivimaki, D. Kuh, M. Laakso, Y. Liu, W. März, P. B. Munroe, I. Njølstad, B. A. Oostra, C. N. Palmer, N. L. Pedersen, M. Perola, L. Pérusse, U. Peters, C. Power, T. Quertermous, R. Rauramaa, F. Rivadeneira, T. E. Saaristo, D. Saleheen, J. Sinisalo, P. E. Slagboom, H. Snieder, T. D. Spector, K. Stefansson, M. Stumvoll, J. Tuomilehto, A. G. Uitterlinden, M. Uusitupa, P. van der Harst, G. Veronesi, M. Walker, N. J. Wareham, H. Watkins, H. E. Wichmann, G. R. Abecasis, T. L. Assimes, S. I. Berndt, M. Boehnke, I. B. Borecki, P. Deloukas, L. Franke, T. M. Frayling, L. C. Groop, D. J. Hunter, R. C. Kaplan, J. R. O'Connell, L. Qi, D. Schlessinger, D. P. Strachan, U. Thorsteinsdottir, C. M. van Duijn, C. J. Willer, P. M. Visscher, J. Yang, J. N. Hirschhorn, M. C. Zillikens, M. I. McCarthy, E. K. Speliotes, K. E. North, C. S. Fox, I. Barroso, P. W. Franks, E. Ingelsson, I. M. Heid, R. J. Loos, L. A. Cupples, A. P. Morris, C. M. Lindgren, and K. L. Mohlke. "New Genetic Loci Link Adipose and Insulin Biology to Body Fat Distribution." *Nature* 518, no. 7538 (2015): 187-96.

9. Cheng, L., J. Wang, H. Dai, Y. Duan, Y. An, L. Shi, Y. Lv, H. Li, C. Wang, Q. Ma, Y. Li, P. Li, H. Du, and B. Zhao. "Brown and Beige Adipose Tissue: A Novel Therapeutic Strategy for Obesity and Type 2 Diabetes Mellitus." *Adipocyte* 10, no. 1 (2021): 48-65.
10. Heaton, J. M. "The Distribution of Brown Adipose Tissue in the Human." *J Anat* 112, no. Pt 1 (1972): 35-9.
11. Sanchez-Gurmaches, J., and D. A. Guertin. "Adipocyte Lineages: Tracing Back the Origins of Fat." *Biochim Biophys Acta* 1842, no. 3 (2014): 340-51.
12. Cypess, A. M., S. Lehman, G. Williams, I. Tal, D. Rodman, A. B. Goldfine, F. C. Kuo, E. L. Palmer, Y. H. Tseng, A. Doria, G. M. Kolodny, and C. R. Kahn. "Identification and Importance of Brown Adipose Tissue in Adult Humans." *N Engl J Med* 360, no. 15 (2009): 1509-17.
13. Virtanen, K. A., M. E. Lidell, J. Orava, M. Heglind, R. Westergren, T. Niemi, M. Taittonen, J. Laine, N. J. Savisto, S. Enerbäck, and P. Nuutila. "Functional Brown Adipose Tissue in Healthy Adults." *N Engl J Med* 360, no. 15 (2009): 1518-25.
14. Hanssen, M. J., A. A. van der Lans, B. Brans, J. Hoeks, K. M. Jardon, G. Schaart, F. M. Mottaghy, P. Schrauwen, and W. D. van Marken Lichtenbelt. "Short-Term Cold Acclimation Recruits Brown Adipose Tissue in Obese Humans." *Diabetes* 65, no. 5 (2016): 1179-89.
15. Blondin, D. P., F. Frisch, S. Phoenix, B. Guérin, E. Turcotte É, F. Haman, D. Richard, and A. C. Carpentier. "Inhibition of Intracellular Triglyceride Lipolysis Suppresses Cold-Induced Brown Adipose Tissue Metabolism and Increases Shivering in Humans." *Cell Metab* 25, no. 2 (2017): 438-47.
16. Ouellet, V., A. Routhier-Labadie, W. Bellemare, L. Lakhal-Chaieb, E. Turcotte, A. C. Carpentier, and D. Richard. "Outdoor Temperature, Age, Sex, Body Mass Index, and Diabetic Status Determine the Prevalence, Mass, and Glucose-Uptake Activity of 18f-Fdg-Detected Bat in Humans." *J Clin Endocrinol Metab* 96, no. 1 (2011): 192-9.
17. Ikeda, K., and T. Yamada. "Ucp1 Dependent and Independent Thermogenesis in Brown and Beige Adipocytes." *Front Endocrinol (Lausanne)* 11 (2020): 498.
18. Coolbaugh, Crystal L., Bruce M. Damon, Emily C. Bush, E. Brian Welch, and Theodore F. Towse. "Cold Exposure Induces Dynamic, Heterogeneous Alterations in Human Brown Adipose Tissue Lipid Content." *Scientific Reports* 9, no. 1 (2019): 13600.
19. Aldiss, P., J. Betts, C. Sale, M. Pope, H. Budge, and M. E. Symonds. "Exercise-Induced 'Browning' of Adipose Tissues." *Metabolism* 81 (2018): 63-70.
20. Zhang, Qiongyue, Qing Miao, Hongying Ye, Zhaoyun Zhang, Chuantao Zuo, Fengchun Hua, Yihui Guan, and Yiming Li. "The Effects of Thyroid Hormones on Brown Adipose Tissue in Humans: A Pet-Ct Study." *Diabetes/Metabolism Research and Reviews* 30, no. 6 (2014): 513-20.
21. Wu, Zhidan, Pere Puigserver, Ulf Andersson, Chenyu Zhang, Guillaume Adelmant, Vamsi Mootha, Amy Troy, Saverio Cinti, Bradford Lowell, Richard C. Scarpulla, and Bruce M. Spiegelman. "Mechanisms Controlling Mitochondrial Biogenesis and Respiration through the Thermogenic Coactivator Pgc-1." *Cell* 98, no. 1 (1999): 115-24.
22. Smith, Robert E., Jane C. Roberts, and Karl J. Hittelman. "Nonphosphorylating Respiration of Mitochondria from Brown Adipose Tissue of Rats." *Science* 154, no. 3749 (1966): 653-54.
23. Kodela, E., M. Moysidou, S. Karaliota, Y. Koutmani, P. Tsakanikas, K. Kodella, E. A. Karavia, K. E. Kypreos, N. Kostomitsopoulos, and K. P. Karalis. "Strain-Specific Differences in the Effects of Lymphocytes on the Development of Insulin Resistance and Obesity in Mice." *Comp Med* 68, no. 1 (2018): 15-24.
24. Brondani, Letícia A., Tais S. Assmann, Bianca M. de Souza, Ana P. Bouças, Luis H. Canani, and Daisy Crispim. "Meta-Analysis Reveals the Association of Common Variants in the Uncoupling Protein (Ucp) 1–3 Genes with Body Mass Index Variability." *PLOS ONE* 9, no. 5 (2014): e96411.
25. Montesanto, A., A. R. Bonfigli, P. Crocco, P. Garagnani, M. De Luca, M. Boemi, E. Marasco, C. Pirazzini, C. Giuliani, C. Franceschi, G. Passarino, R. Testa, F. Olivieri, and G. Rose. "Genes

Associated with Type 2 Diabetes and Vascular Complications." *Aging (Albany NY)* 10, no. 2 (2018): 178-96.
26. Labruna, G., F. Pasanisi, C. Nardelli, G. Tarantino, D. F. Vitale, R. Bracale, C. Finelli, M. P. Genua, F. Contaldo, and L. Sacchetti. "Ucp1 -3826 Ag+Gg Genotypes, Adiponectin, and Leptin/Adiponectin Ratio in Severe Obesity." *J Endocrinol Invest* 32, no. 6 (2009): 525-9.
27. Nagai, N., N. Sakane, K. Kotani, T. Hamada, K. Tsuzaki, and T. Moritani. "Uncoupling Protein 1 Gene -3826 a/G Polymorphism Is Associated with Weight Loss on a Short-Term, Controlled-Energy Diet in Young Women." *Nutr Res* 31, no. 4 (2011): 255-61.
28. Cha, M. H., B. K. Kang, D. Suh, K. S. Kim, Y. Yang, and Y. Yoon. "Association of Ucp1 Genetic Polymorphisms with Blood Pressure among Korean Female Subjects." *J Korean Med Sci* 23, no. 5 (2008): 776-80.
29. Sakers, Alexander, Mirian Krystel De Siqueira, Patrick Seale, and Claudio J. Villanueva. "Adipose-Tissue Plasticity in Health and Disease." *Cell* 185, no. 3 (2022): 419-46.
30. Scheja, Ludger, and Joerg Heeren. "The Endocrine Function of Adipose Tissues in Health and Cardiometabolic Disease." *Nature Reviews Endocrinology* 15, no. 9 (2019): 507-24.
31. Reilly, Shannon M., and Alan R. Saltiel. "Adapting to Obesity with Adipose Tissue Inflammation." *Nature Reviews Endocrinology* 13, no. 11 (2017): 633-43.
32. Nguyen, M. T., S. Favelyukis, A. K. Nguyen, D. Reichart, P. A. Scott, A. Jenn, R. Liu-Bryan, C. K. Glass, J. G. Neels, and J. M. Olefsky. "A Subpopulation of Macrophages Infiltrates Hypertrophic Adipose Tissue and Is Activated by Free Fatty Acids Via Toll-Like Receptors 2 and 4 and Jnk-Dependent Pathways." *J Biol Chem* 282, no. 48 (2007): 35279-92.
33. Cuevas-Ramos, D., R. Mehta, and C. A. Aguilar-Salinas. "Fibroblast Growth Factor 21 and Browning of White Adipose Tissue." *Front Physiol* 10 (2019): 37.
34. Wang, Q. A., C. Tao, R. K. Gupta, and P. E. Scherer. "Tracking Adipogenesis During White Adipose Tissue Development, Expansion and Regeneration." *Nat Med* 19, no. 10 (2013): 1338-44.
35. Shao, M., L. Vishvanath, N. C. Busbuso, C. Hepler, B. Shan, A. X. Sharma, S. Chen, X. Yu, Y. A. An, Y. Zhu, W. L. Holland, and R. K. Gupta. "De Novo Adipocyte Differentiation from Pdgfrβ+ Preadipocytes Protects against Pathologic Visceral Adipose Expansion in Obesity." *Nature Communications* 9, no. 1 (2018).
36. Park, J., S. Shin, L. Liu, I. Jahan, S. G. Ong, P. Xu, D. C. Berry, and Y. Jiang. "Progenitor-Like Characteristics in a Subgroup of Ucp1+ Cells within White Adipose Tissue." *Dev Cell* 56, no. 7 (2021): 985-99.e4.
37. Vliora, M., E. Grillo, M. Corsini, C. Ravelli, E. Nintou, E. Karligiotou, A. D. Flouris, and S. Mitola. "Irisin Regulates Thermogenesis and Lipolysis in 3t3-L1 Adipocytes." *Biochim Biophys Acta Gen Subj* 1866, no. 4 (2022): 130085.
38. Valente, Angelica, Athanasios Z. Jamurtas, Yiannis Koutedakis, and Andreas D. Flouris. "Molecular Pathways Linking Non-Shivering Thermogenesis and Obesity: Focusing on Brown Adipose Tissue Development." *Biological Reviews* 90, no. 1 (2015): 77-88.
39. Boström, P., J. Wu, M. P. Jedrychowski, A. Korde, L. Ye, J. C. Lo, K. A. Rasbach, E. A. Boström, J. H. Choi, J. Z. Long, S. Kajimura, M. C. Zingaretti, B. F. Vind, H. Tu, S. Cinti, K. Højlund, S. P. Gygi, and B. M. Spiegelman. "A Pgc1-A-Dependent Myokine That Drives Brown-Fat-Like Development of White Fat and Thermogenesis." *Nature* 481, no. 7382 (2012): 463-8.
40. Fisher, ffolliott M., Sandra Kleiner, Nicholas Douris, Elliott C. Fox, Rina J. Mepani, Francisco Verdeguer, Jun Wu, Alexei Kharitonenkov, Jeffrey S. Flier, Eleftheria Maratos-Flier, and Bruce M. Spiegelman. "Fgf21 Regulates Pgc-1α and Browning of White Adipose Tissues in Adaptive Thermogenesis." *Genes & Development* 26, no. 3 (2012): 271-81.
41. Triandafillou, Joan, Cynthia Gwilliam, and Jean Himms-Hagen. "Role of Thyroid Hormone in Cold-Induced Changes in Rat Brown Adipose Tissue Mitochondria." *Canadian Journal of Biochemistry* 60, no. 5 (1982): 530-37.

42. Seale, P., S. Kajimura, W. Yang, S. Chin, L. M. Rohas, M. Uldry, G. Tavernier, D. Langin, and B. M. Spiegelman. "Transcriptional Control of Brown Fat Determination by Prdm16." *Cell Metab* 6, no. 1 (2007): 38-54.
43. Choi, E. W., M. Lee, J. W. Song, K. Kim, J. Lee, J. Yang, S. H. Lee, I. Y. Kim, J. H. Choi, and J. K. Seong. "Fas Mutation Reduces Obesity by Increasing Il-4 and Il-10 Expression and Promoting White Adipose Tissue Browning." *Sci Rep* 10, no. 1 (2020): 12001.
44. van der Vaart, J. I., M. R. Boon, and R. H. Houtkooper. "The Role of Ampk Signaling in Brown Adipose Tissue Activation." *Cells* 10, no. 5 (2021).
45. Li, Z., Y. Yue, F. Hu, C. Zhang, X. Ma, N. Li, L. Qiu, M. Fu, L. Chen, Z. Yao, P. J. Bilan, A. Klip, and W. Niu. "Electrical Pulse Stimulation Induces Glut4 Translocation in C2c12 Myotubes That Depends on Rab8a, Rab13, and Rab14." *Am J Physiol Endocrinol Metab* 314, no. 5 (2018): E478-E93.
46. Gong, H., L. Liu, C. X. Ni, Y. Zhang, W. J. Su, Y. J. Lian, W. Peng, J. P. Zhang, and C. L. Jiang. "Dexamethasone Rapidly Inhibits Glucose Uptake Via Non-Genomic Mechanisms in Contracting Myotubes." *Arch Biochem Biophys* 603 (2016): 102-9.
47. Dinas, Petros C., Argyro Krase, Eleni Nintou, Alexandros Georgakopoulos, Marnie Granzotto, Marinos Metaxas, Elena Karachaliou, Marco Rossato, Roberto Vettor, Panagiotis Georgoulias, Tiago S. Mayor, John Koutsikos, Konstantinos Athanasiou, Leonidas G. Ioannou, Paraskevi Gkiata, Andres E. Carrillo, Yiannis Koutedakis, George S. Metsios, Athanasios Z. Jamurtas, Sofia Chatziioannou, and Andreas D. Flouris. "Human White-Fat Thermogenesis: Experimental and Meta-Analytic Findings." *Temperature* 8, no. 1 (2021): 39-52.
48. Gielen, Stephan, Gerhard Schuler, and Volker Adams. "Cardiovascular Effects of Exercise Training." *Circulation* 122, no. 12 (2010): 1221-38.
49. Nigro, Pasquale, Maria Vamvini, Jiekun Yang, Tiziana Caputo, Li-Lun Ho, Danae Papadopoulos, Nicholas P. Carbone, Royce Conlin, Jie He, Michael F. Hirshman, Joseph D. White, Jacques Robidoux, Robert C. Hickner, Søren Nielsen, Bente K. Pedersen, Manolis Kellis, Roeland J. W. Middelbeek, and Laurie J. Goodyear. "Exercise Training Remodels Inguinal White Adipose Tissue through Adaptations in Innervation, Vascularization and the Extracellular Matrix." *bioRxiv* (2022): 2022.08.09.503375.
50. Picoli, Caroline de Carvalho, Gustavo Renan Gilio, Felipe Henriques, Luana Garcia Leal, Jean Carlos Besson, Magno Alves Lopes, Solange Marta Franzói de Moraes, Luzmarina Hernandes, Miguel Luiz Batista Junior, and Sidney Barnabé Peres. "Resistance Exercise Training Induces Subcutaneous and Visceral Adipose Tissue Browning in Swiss Mice." *Journal of Applied Physiology* 129, no. 1 (2020): 66-74.
51. Otero-Díaz, B., M. Rodríguez-Flores, V. Sánchez-Muñoz, F. Monraz-Preciado, S. Ordoñez-Ortega, V. Becerril-Elias, G. Baay-Guzmán, R. Obando-Monge, E. García-García, B. Palacios-González, M. T. Villarreal-Molina, M. Sierra-Salazar, and B. Antuna-Puente. "Exercise Induces White Adipose Tissue Browning across the Weight Spectrum in Humans." *Front Physiol* 9 (2018): 1781.
52. Mendez-Gutierrez, A., C. M. Aguilera, F. J. Osuna-Prieto, B. Martinez-Tellez, M. C. Rico Prados, F. M. Acosta, J. M. Llamas-Elvira, J. R. Ruiz, and G. Sanchez-Delgado. "Exercise-Induced Changes on Exerkines That Might Influence Brown Adipose Tissue Metabolism in Young Sedentary Adults." *Eur J Sport Sci* 23, no. 4 (2023): 625-36.
53. Vosselman, M. J., J. Hoeks, B. Brans, H. Pallubinsky, E. B. Nascimento, A. A. van der Lans, E. P. Broeders, F. M. Mottaghy, P. Schrauwen, and W. D. van Marken Lichtenbelt. "Low Brown Adipose Tissue Activity in Endurance-Trained Compared with Lean Sedentary Men." *Int J Obes (Lond)* 39, no. 12 (2015): 1696-702.
54. Dreher, Simon I., Martin Irmler, Olga Pivovarova-Ramich, Katharina Kessler, Karsten Jürchott, Carsten Sticht, Louise Fritsche, Patrick Schneeweiss, Jürgen Machann, Andreas F. H. Pfeiffer, Martin Hrabě de Angelis, Johannes Beckers, Andreas L. Birkenfeld, Andreas Peter, Andreas M. Niess, Cora Weigert, and Anja Moller. "Acute and Long-Term Exercise

Adaptation of Adipose Tissue and Skeletal Muscle in Humans: A Matched Transcriptomics Approach after 8-Week Training-Intervention." *International Journal of Obesity* (2023).

55. Tsiloulis, T., A. L. Carey, J. Bayliss, B. Canny, R. C. R. Meex, and M. J. Watt. "No Evidence of White Adipocyte Browning after Endurance Exercise Training in Obese Men." *Int J Obes (Lond)* 42, no. 4 (2018): 721-27.
56. Motiani, P., K. A. Virtanen, K. K. Motiani, J. J. Eskelinen, R. J. Middelbeek, L. J. Goodyear, A. M. Savolainen, J. Kemppainen, J. Jensen, M. U. Din, V. Saunavaara, R. Parkkola, E. Löyttyniemi, J. Knuuti, P. Nuutila, K. K. Kalliokoski, and J. C. Hannukainen. "Decreased Insulin-Stimulated Brown Adipose Tissue Glucose Uptake after Short-Term Exercise Training in Healthy Middle-Aged Men." *Diabetes Obes Metab* 19, no. 10 (2017): 1379-88.
57. de Jong, J. M., O. Larsson, B. Cannon, and J. Nedergaard. "A Stringent Validation of Mouse Adipose Tissue Identity Markers." *Am J Physiol Endocrinol Metab* 308, no. 12 (2015): E1085-105.
58. Dinas, P. C., I. M. Lahart, J. A. Timmons, P. A. Svensson, Y. Koutedakis, A. D. Flouris, and G. S. Metsios. "Effects of Physical Activity on the Link between Pgc-1a and Fndc5 in Muscle, Circulating Irisin and Ucp1 of White Adipocytes in Humans: A Systematic Review." *F1000Res* 6 (2017): 286.
59. Banan Sadeghian, Ramin, Majid Ebrahimi, and Sahar Salehi. "Electrical Stimulation of Microengineered Skeletal Muscle Tissue: Effect of Stimulus Parameters on Myotube Contractility and Maturation." *Journal of Tissue Engineering and Regenerative Medicine* 12, no. 4 (2018): 912-22.
60. Groop, L. "Genetics of the Metabolic Syndrome." *Br J Nutr* 83 Suppl 1 (2000): S39-48.
61. Mirkov, S., J. L. Myers, J. Ramírez, and W. Liu. "Snps Affecting Serum Metabolomic Traits May Regulate Gene Transcription and Lipid Accumulation in the Liver." *Metabolism* 61, no. 11 (2012): 1523-7.
62. Shastry, B. S. "Snp Alleles in Human Disease and Evolution." *J Hum Genet* 47, no. 11 (2002): 561-6.
63. Dinas, P. C., A. Valente, M. Granzotto, M. Rossato, R. Vettor, A. Zacharopoulou, A. E. Carrillo, N. A. Davies, P. Gkiata, A. Z. Jamurtas, Y. Koutedakis, G. S. Metsios, and A. D. Flouris. "Browning Formation Markers of Subcutaneous Adipose Tissue in Relation to Resting Energy Expenditure, Physical Activity and Diet in Humans." *Horm Mol Biol Clin Investig* 31, no. 1 (2017).
64. Valente, A., A. Z. Jamurtas, Y. Koutedakis, and A. D. Flouris. "Molecular Pathways Linking Non-Shivering Thermogenesis and Obesity: Focusing on Brown Adipose Tissue Development." *Biol Rev Camb Philos Soc* 90, no. 1 (2015): 77-88.
65. Chathoth, S., M. H. Ismail, C. Vatte, C. Cyrus, Z. Al Ali, K. A. Ahmed, S. Acharya, A. M. Al Barqi, and A. Al Ali. "Association of Uncoupling Protein 1 (Ucp1) Gene Polymorphism with Obesity: A Case-Control Study." *BMC Med Genet* 19, no. 1 (2018): 203.
66. Franco-Hincapie, L., C. E. Duque, M. V. Parra, N. Gallego, A. Villegas, A. Ruiz-Linares, and G. Bedoya. "[Association between Polymorphism in Uncoupling Proteins and Type 2 Diabetes in a Northwestern Colombian Population]." *Biomedica* 29, no. 1 (2009): 108-18.
67. Jia, J. J., Y. B. Tian, Z. H. Cao, L. L. Tao, X. Zhang, S. Z. Gao, C. R. Ge, Q. Y. Lin, and M. Jois. "The Polymorphisms of Ucp1 Genes Associated with Fat Metabolism, Obesity and Diabetes." *Mol Biol Rep* 37, no. 3 (2010): 1513-22.
68. Lim, J. H., M. M. Ko, T. W. Moon, M. H. Cha, and M. S. Lee. "Association of the Ucp-1 Single Nucleotide Polymorphism a-3826g with the Dampness-Phlegm Pattern among Korean Stroke Patients." *BMC Complement Altern Med* 12 (2012): 180.
69. Pravednikova, Anna E., Sergey Y. Shevchenko, Victor V. Kerchev, Manana R. Skhirtladze, Svetlana N. Larina, Zaur M. Kachaev, Alexander D. Egorov, and Yulii V. Shidlovskii. "Association of Uncoupling Protein (Ucp) Gene Polymorphisms with Cardiometabolic Diseases." *Molecular Medicine* 26, no. 1 (2020): 51.

70. Brondani, L. A., T. S. Assmann, G. C. Duarte, J. L. Gross, L. H. Canani, and D. Crispim. "The Role of the Uncoupling Protein 1 (Ucp1) on the Development of Obesity and Type 2 Diabetes Mellitus." *Arq Bras Endocrinol Metabol* 56, no. 4 (2012): 215-25.
71. Esterbauer, H., H. Oberkofler, Y. M. Liu, D. Breban, E. Hell, F. Krempler, and W. Patsch. "Uncoupling Protein-1 Mrna Expression in Obese Human Subjects: The Role of Sequence Variations at the Uncoupling Protein-1 Gene Locus." *J Lipid Res* 39, no. 4 (1998): 834-44.
72. Hayakawa, T., Y. Nagai, M. Taniguchi, H. Yamashita, T. Takamura, T. Abe, G. Nomura, and K. Kobayashi. "Phenotypic Characterization of the Beta3-Adrenergic Receptor Mutation and the Uncoupling Protein 1 Polymorphism in Japanese Men." *Metabolism* 48, no. 5 (1999): 636-40.
73. Ramis, J. M., J. L. Gonzalez-Sanchez, A. M. Proenza, M. T. Martinez-Larrad, C. Fernandez-Perez, A. Palou, and M. Serrano-Rios. "The Arg64 Allele of the Beta 3-Adrenoceptor Gene but Not the -3826g Allele of the Uncoupling Protein 1 Gene Is Associated with Increased Leptin Levels in the Spanish Population." *Metabolism* 53, no. 11 (2004): 1411-6.
74. Forga, Ll, M. Corbalan, A. Marti, C. Fuentes, M. A. Martinez-Gonzalez, and A. Martinez. "[Influence of the Polymorphism 03826 a --> G in the Ucp1 Gene on the Components of Metabolic Syndrome]." *An Sist Sanit Navar* 26, no. 2 (2003): 231-6.
75. Oh, H. H., K. S. Kim, S. M. Choi, H. S. Yang, and Y. Yoon. "The Effects of Uncoupling Protein-1 Genotype on Lipoprotein Cholesterol Level in Korean Obese Subjects." *Metabolism* 53, no. 8 (2004): 1054-9.
76. Heilbronn, L. K., K. L. Kind, E. Pancewicz, A. M. Morris, M. Noakes, and P. M. Clifton. "Association of -3826 G Variant in Uncoupling Protein-1 with Increased Bmi in Overweight Australian Women." *Diabetologia* 43, no. 2 (2000): 242-4.
77. Zhang, Y., N. Meng, Z. Lv, H. Li, and Y. Qu. "The Gene Polymorphisms of Ucp1 but Not Ppar Γ and Tcf7l2 Are Associated with Diabetic Retinopathy in Chinese Type 2 Diabetes Mellitus Cases." *Acta Ophthalmol* 93, no. 3 (2015): e223-9.
78. Flouris, A. D., P. C. Dinas, A. Valente, C. M. B. Andrade, N. H. Kawashita, and P. Sakellariou. "Exercise-Induced Effects on Ucp1 Expression in Classical Brown Adipose Tissue: A Systematic Review." *Horm Mol Biol Clin Investig* 31, no. 2 (2017).
79. Abdullahi, A., P. Chen, M. Stanojcic, A. R. Sadri, N. Coburn, and M. G. Jeschke. "Il-6 Signal from the Bone Marrow Is Required for the Browning of White Adipose Tissue Post Burn Injury." *Shock* 47, no. 1 (2017): 33-39.
80. Fukuyama, K., T. Ohara, Y. Hirota, K. Maeda, S. Kuno, M. Zenibayashi, T. Teranishi, K. Kouyama, E. Maeda, N. Sakamoto, and M. Kasuga. "Association of the -112a>C Polymorphism of the Uncoupling Protein 1 Gene with Insulin Resistance in Japanese Individuals with Type 2 Diabetes." *Biochem Biophys Res Commun* 339, no. 4 (2006): 1212-6.
81. Mori, H., H. Okazawa, K. Iwamoto, E. Maeda, M. Hashiramoto, and M. Kasuga. "A Polymorphism in the 5' Untranslated Region and a Met229-->Leu Variant in Exon 5 of the Human Ucp1 Gene Are Associated with Susceptibility to Type Ii Diabetes Mellitus." *Diabetologia* 44, no. 3 (2001): 373-6.
82. Herrmann, Stefan-Martin, Ji-Guang Wang, Jan A. Staessen, Ercan Kertmen, Klaus Schmidt-Petersen, Walter Zidek, Martin Paul, and Eva Brand. "Uncoupling Protein 1 and 3 Polymorphisms Are Associated with Waist-to-Hip Ratio." *Journal of Molecular Medicine* 81, no. 5 (2003): 327-32.
83. Soo Kim, Kil, Dae-Yeon Cho, Young Joo Kim, Sun Mi Choi, Jong Yeol Kim, Seung Uoo Shin, and Yoo Sik Yoon. "The Finding of New Genetic Polymorphism of Ucp-1 a-1766g and Its Effects on Body Fat Accumulation." *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1741, no. 1 (2005): 149-55.
84. Shin, H. D., K. S. Kim, M. H. Cha, and Y. Yoon. "The Effects of Ucp-1 Polymorphisms on Obesity Phenotypes among Korean Female Subjects." *Biochem Biophys Res Commun* 335, no. 2 (2005): 624-30.

85. Pei, X., L. Liu, J. Cai, W. Wei, Y. Shen, Y. Wang, Y. Chen, P. Sun, M. U. Imam, Z. Ping, and X. Fu. "Haplotype-Based Interaction of the Ppargc1a and Ucp1 Genes Is Associated with Impaired Fasting Glucose or Type 2 Diabetes Mellitus." *Medicine (Baltimore)* 96, no. 23 (2017): e6941.
86. Vimaleswaran, K. S., V. Radha, S. Ghosh, P. P. Majumder, M. R. Rao, and V. Mohan. "A Haplotype at the Ucp1 Gene Locus Contributes to Genetic Risk for Type 2 Diabetes in Asian Indians (Cures-72)." *Metab Syndr Relat Disord* 8, no. 1 (2010): 63-8.
87. Cha, Min Ho, Byoung Kab Kang, Dongchul Suh, Kil Soo Kim, Young Yang, and Yoosik Yoon. "Association of Ucp1 Genetic Polymorphisms with Blood Pressure among Korean Female Subjects." *Journal of Korean medical science* 23, no. 5 (2008): 776-80.
88. de Souza, B. M., L. A. Brondani, A. P. Bouças, D. A. Sortica, C. K. Kramer, L. H. Canani, C. B. Leitão, and D. Crispim. "Associations between Ucp1 -3826a/G, Ucp2 -866g/a, Ala55val and Ins/Del, and Ucp3 -55c/T Polymorphisms and Susceptibility to Type 2 Diabetes Mellitus: Case-Control Study and Meta-Analysis." *PLoS One* 8, no. 1 (2013): e54259.
89. Malczewska-Malec, M., I. Wybranska, I. Leszczynska-Golabek, L. Partyka, J. Hartwich, A. Jabrocka, B. Kiec-Wilk, M. Kwasniak, M. Motyka, and A. Dembinska-Kiec. "Analysis of Candidate Genes in Polish Families with Obesity." *Clin Chem Lab Med* 42, no. 5 (2004): 487-93.
90. Schaffler, A., K. D. Palitzsch, E. Watzlawek, W. Drobnik, H. Schwer, J. Scholmerich, and G. Schmitz. "Frequency and Significance of the a-->G (-3826) Polymorphism in the Promoter of the Gene for Uncoupling Protein-1 with Regard to Metabolic Parameters and Adipocyte Transcription Factor Binding in a Large Population-Based Caucasian Cohort." *Eur J Clin Invest* 29, no. 9 (1999): 770-9.
91. Balkau, Beverley, John E. Deanfield, Jean-Pierre Després, Jean-Pierre Bassand, Keith A. A. Fox, Sidney C. Smith, Jr., Philip Barter, Chee-Eng Tan, Luc Van Gaal, Hans-Ulrich Wittchen, Christine Massien, and Steven M. Haffner. "International Day for the Evaluation of Abdominal Obesity (Idea): A Study of Waist Circumference, Cardiovascular Disease, and Diabetes Mellitus in 168,000 Primary Care Patients in 63 Countries." *Circulation* 116, no. 17 (2007): 1942-51.
92. WHO. "Global Atlas on Cardiovascular Disease Prevention and Control. Geneva: World Health Organization;World Heart Federation; World Stroke Organization." (2011).
93. Higgins JP, and J. Thomas. *Cochrane Handbook for Systematic Reviews of Interventions*, Version 6: Cochrane collaboration 2019.
94. de Almeida Brondani, Letícia, Bianca Marmontel de Souza, Taís Silveira Assmann, Ana Paula Bouças, Andrea Carla Bauer, Luís Henrique Canani, and Daisy Crispim. "Association of the Ucp Polymorphisms with Susceptibility to Obesity: Case–Control Study and Meta-Analysis." *Molecular biology reports* 41, no. 8 (2014): 5053-67.
95. Liu, Xujia, Zehua Jiang, Guihua Zhang, Tsz Kin Ng, and Zhenggen Wu. "Association of Ucp1 and Ucp2 Variants with Diabetic Retinopathy Susceptibility in Type-2 Diabetes Mellitus Patients: A Meta-Analysis." *BMC ophthalmology* 21, no. 1 (2021): 1-12.
96. WHO. "Cardiovascular Diseases (Cvds)." 2017.
97. ———. "Noncommunicable Diseases." 2018.
98. Clarke, Geraldine M., Carl A. Anderson, Fredrik H. Pettersson, Lon R. Cardon, Andrew P. Morris, and Krina T. Zondervan. "Basic Statistical Analysis in Genetic Case-Control Studies." *Nature Protocols* 6, no. 2 (2011): 121-33.
99. Lunetta, K. L. "Genetic Association Studies." *Circulation* 118, no. 1 (2008): 96-101.
100. Namipashaki, A., Z. Razaghi-Moghadam, and N. Ansari-Pour. "The Essentiality of Reporting Hardy-Weinberg Equilibrium Calculations in Population-Based Genetic Association Studies." *Cell J* 17, no. 2 (2015): 187-92.
101. Li, Z., Z. Zhang, Z. He, W. Tang, T. Li, Z. Zeng, L. He, and Y. Shi. "A Partition-Ligation-Combination-Subdivision Em Algorithm for Haplotype Inference with Multiallelic Markers: Update of the Shesis (Http://Analysis.Bio-X.Cn)." *Cell Res* 19, no. 4 (2009): 519-23.

102. Shi, Y. Y., and L. He. "Shesis, a Powerful Software Platform for Analyses of Linkage Disequilibrium, Haplotype Construction, and Genetic Association at Polymorphism Loci." *Cell Res* 15, no. 2 (2005): 97-8.
103. Kim, Hae-Young. "Statistical Notes for Clinical Researchers: Chi-Squared Test and Fisher's Exact Test." *Restorative dentistry & endodontics* 42, no. 2 (2017): 152-55.
104. Feise, R. J. "Do Multiple Outcome Measures Require P-Value Adjustment?" *BMC Med Res Methodol* 2 (2002): 8.
105. Perneger, T. V. "What's Wrong with Bonferroni Adjustments." *BMJ* 316, no. 7139 (1998): 1236-8.
106. Rothman, K. J. "No Adjustments Are Needed for Multiple Comparisons." *Epidemiology* 1, no. 1 (1990): 43-6.
107. ———. "Six Persistent Research Misconceptions." *J Gen Intern Med* 29, no. 7 (2014): 1060-4.
108. Moher, D., A. Liberati, J. Tetzlaff, D. G. Altman, and Prisma Group. "Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The Prisma Statement." *PLoS Med* 6, no. 7 (2009): e1000097.
109. Bracale, Renata, Giuseppe Labruna, Carmine Finelli, Aurora Daniele, Lucia Sacchetti, Giovannangelo Oriani, F. Contaldo, and Fabrizio Pasanisi. "The Absence of Polymorphisms in Adrb3, Ucp1, Pparγ, and Adipoq Genes Protects Morbid Obese Patients toward Insulin Resistance." *Journal of endocrinological investigation* 35 (2012): 2-4.
110. Brondani, Leticia A, Tais S Assmann, Bianca M de Souza, Ana P Boucas, Luis H Canani, and Daisy Crispim. "Meta-Analysis Reveals the Association of Common Variants in the Uncoupling Protein (Ucp) 1–3 Genes with Body Mass Index Variability." *PLoS One* 9, no. 5 (2014): e96411.
111. Brondani, L. A., B. M. de Souza, T. S. Assmann, A. P. Bouças, A. C. Bauer, L. H. Canani, and D. Crispim. "Association of the Ucp Polymorphisms with Susceptibility to Obesity: Case-Control Study and Meta-Analysis." *Mol Biol Rep* 41, no. 8 (2014): 5053-67.
112. Chen, Y., X. Wang, Z. Shen, P. Fan, R. Liu, Y. Liu, R. Ren, L. Ma, and H. Bai. "Effect of the Beta-3 Adrenergic Receptor Trp64arg and Uncoupling Protein 1-3826 a>G Genotypes on Lipid and Apolipoprotein Levels in Overweight/Obese and Non-Obese Chinese Subjects." *Lipids Health Dis* 14 (2015): 34.
113. Csernus, K., G. Pauler, É Erhardt, É Lányi, and D. Molnár. "Effects of Energy Expenditure Gene Polymorphisms on Obesity-Related Traits in Obese Children." *Obes Res Clin Pract* 9, no. 2 (2015): 133-40.
114. Dhall, M., M. M. Chaturvedi, U. Rai, and S. Kapoor. "Sex-Dependent Effects of the Ucp1 -3826 a/G Polymorphism on Obesity and Blood Pressure." *Ethn Dis* 22, no. 2 (2012): 181-4.
115. Gagnon, J., F. Lago, Y. C. Chagnon, L. Pérusse, I. Näslund, L. Lissner, L. Sjöström, and C. Bouchard. "DNA Polymorphism in the Uncoupling Protein 1 (Ucp1) Gene Has No Effect on Obesity Related Phenotypes in the Swedish Obese Subjects Cohorts." *Int J Obes Relat Metab Disord* 22, no. 6 (1998): 500-5.
116. Hamada, T., K. Kotani, N. Nagai, K. Tsuzaki, Y. Matsuoka, Y. Sano, M. Fujibayashi, N. Kiyohara, S. Tanaka, M. Yoshimura, K. Egawa, Y. Kitagawa, Y. Kiso, T. Moritani, and N. Sakane. "Low-Calorie Diet-Induced Reduction in Serum Hdl Cholesterol Is Ameliorated in Obese Women with the -3826 G Allele in the Uncoupling Protein-1 Gene." *Tohoku J Exp Med* 219, no. 4 (2009): 337-42.
117. Jin, P., Z. Li, X. Xu, J. He, J. Chen, X. Xu, X. Du, X. Bai, B. Zhang, X. He, L. Lu, J. Zhu, Y. Shi, and H. Zou. "Analysis of Association between Common Variants of Uncoupling Proteins Genes and Diabetic Retinopathy in a Chinese Population." *BMC Med Genet* 21, no. 1 (2020): 25.
118. Kieć-Wilk, B., I. Wybrańska, M. Malczewska-Malec, L. Leszczyńska-Gołabek, L. Partyka, S. Niedbał, A. Jabrocka, and A. Dembińska-Kieć. "Correlation of the -3826a >G Polymorphism in the Promoter of the Uncoupling Protein 1 Gene with Obesity and Metabolic Disorders in Obese Families from Southern Poland." *J Physiol Pharmacol* 53, no. 3 (2002): 477-90.

119. Kotani, K., S. Fujiwara, K. Tsuzaki, Y. Sano, N. Nagai, T. Yamada, and N. Sakane. "The Association between the Uncoupling Protein-1 Gene a-3826g Polymorphism and High-Density Lipoprotein Cholesterol in a General Japanese Population: A Consideration of the Obesity Status." *J Clin Med Res* 3, no. 6 (2011): 319-24.
120. Kotani, K., N. Sakane, K. Saiga, S. Adachi, H. Shimohiro, H. Mu, and Y. Kurozawa. "Relationship between a-3826g Polymorphism in the Promoter of the Uncoupling Protein-1 Gene and High-Density Lipoprotein Cholesterol in Japanese Individuals: A Cross-Sectional Study." *Arch Med Res* 39, no. 1 (2008): 142-6.
121. Lim, Ji Hye, Mi Mi Ko, Tae-Woong Moon, Min Ho Cha, and Myeong Soo Lee. "Association of the Ucp-1 Single Nucleotide Polymorphism a-3826g with the Dampness-Phlegm Pattern among Korean Stroke Patients." *BMC complementary and alternative medicine* 12 (2012): 180-80.
122. Lin, E., D. Pei, Y. J. Huang, C. H. Hsieh, and L. S. Wu. "Gene-Gene Interactions among Genetic Variants from Obesity Candidate Genes for Nonobese and Obese Populations in Type 2 Diabetes." *Genet Test Mol Biomarkers* 13, no. 4 (2009): 485-93.
123. Lindholm, E., M. Klannemark, E. Agardh, L. Groop, and C. D. Agardh. "Putative Role of Polymorphisms in Ucp1-3 Genes for Diabetic Nephropathy." *J Diabetes Complications* 18, no. 2 (2004): 103-7.
124. Mottagui-Tabar, S., J. Hoffstedt, A. J. Brookes, H. Jiao, P. Arner, and I. Dahlman. "Association of Adrb1 and Ucp3 Gene Polymorphisms with Insulin Sensitivity but Not Obesity." *Horm Res* 69, no. 1 (2008): 31-6.
125. Nakatochi, M., Y. Ushida, Y. Yasuda, Y. Yoshida, S. Kawai, R. Kato, T. Nakashima, M. Iwata, Y. Kuwatsuka, M. Ando, N. Hamajima, T. Kondo, H. Oda, M. Hayashi, S. Kato, M. Yamaguchi, S. Maruyama, S. Matsuo, and H. Honda. "Identification of an Interaction between Vwf Rs7965413 and Platelet Count as a Novel Risk Marker for Metabolic Syndrome: An Extensive Search of Candidate Polymorphisms in a Case-Control Study." *PLoS One* 10, no. 2 (2015): e0117591.
126. Nicoletti, C. F., A. P. de Oliveira, M. J. Brochado, B. P. de Oliveira, M. A. Pinhel, J. S. Marchini, J. E. dos Santos, W. Salgado Junior, W. A. Silva Junior, and C. B. Nonino. "Ucp1 -3826 a>G Polymorphism Affects Weight, Fat Mass, and Risk of Type 2 Diabetes Mellitus in Grade Iii Obese Patients." *Nutrition* 32, no. 1 (2016): 83-7.
127. Nieters, A., N. Becker, and J. Linseisen. "Polymorphisms in Candidate Obesity Genes and Their Interaction with Dietary Intake of N-6 Polyunsaturated Fatty Acids Affect Obesity Risk in a Sub-Sample of the Epic-Heidelberg Cohort." *Eur J Nutr* 41, no. 5 (2002): 210-21.
128. Proenza, A. M., C. M. Poissonnet, M. Ozata, S. Ozen, S. Guran, A. Palou, and A. D. Strosberg. "Association of Sets of Alleles of Genes Encoding Beta3-Adrenoreceptor, Uncoupling Protein 1 and Lipoprotein Lipase with Increased Risk of Metabolic Complications in Obesity." *Int J Obes Relat Metab Disord* 24, no. 1 (2000): 93-100.
129. Rudofsky, G., Jr., A. Schrödter, O. E. Voron'ko, A. Schlotterer, P. M. Humpert, J. Tafel, P. P. Nawroth, A. Bierhaus, and A. Hamann. "Promoter Polymorphisms of Ucp1, Ucp2, and Ucp3 Are Not Associated with Diabetic Microvascular Complications in Type 2 Diabetes." *Horm Metab Res* 39, no. 4 (2007): 306-9.
130. Rudofsky, G., Jr., A. Schroedter, A. Schlotterer, O. E. Voron'ko, M. Schlimme, J. Tafel, B. H. Isermann, P. M. Humpert, M. Morcos, A. Bierhaus, P. P. Nawroth, and A. Hamann. "Functional Polymorphisms of Ucp2 and Ucp3 Are Associated with a Reduced Prevalence of Diabetic Neuropathy in Patients with Type 1 Diabetes." *Diabetes Care* 29, no. 1 (2006): 89-94.
131. Sale, M. M., F. C. Hsu, N. D. Palmer, C. J. Gordon, K. L. Keene, H. M. Borgerink, A. J. Sharma, R. N. Bergman, K. D. Taylor, M. F. Saad, and J. M. Norris. "The Uncoupling Protein 1 Gene, Ucp1, Is Expressed in Mammalian Islet Cells and Associated with Acute Insulin Response to Glucose in African American Families from the Iras Family Study." *BMC Endocr Disord* 7 (2007): 1.

132. Sámano, R., C. Huesca-Gómez, R. López-Marure, A. K. Hernández-Cabrera, A. Rodríguez-Ventura, M. Tolentino, R. M. Morales, and R. Gamboa. "Association between Ucp Polymorphisms and Adipokines with Obesity in Mexican Adolescents." *J Pediatr Endocrinol Metab* 31, no. 5 (2018): 561-68.
133. Sivenius, K., R. Valve, V. Lindi, L. Niskanen, M. Laakso, and M. Uusitupa. "Synergistic Effect of Polymorphisms in Uncoupling Protein 1 and Beta3-Adrenergic Receptor Genes on Long-Term Body Weight Change in Finnish Type 2 Diabetic and Non-Diabetic Control Subjects." *Int J Obes Relat Metab Disord* 24, no. 4 (2000): 514-9.
134. Sramkova, D., S. Krejbichova, J. Vcelak, M. Vankova, P. Samalikova, M. Hill, H. Kvasnickova, K. Dvorakova, K. Vondra, V. Hainer, and B. Bendlova. "The Ucp1 Gene Polymorphism a-3826g in Relation to Dm2 and Body Composition in Czech Population." *Exp Clin Endocrinol Diabetes* 115, no. 5 (2007): 303-7.
135. Sun, H., J. T. Zhang, X. R. Xie, T. Li, X. Y. Li, N. N. Wang, J. P. Li, Z. H. Deng, and C. C. Qiu. "Association of Uncoupling Protein Gene Polymorphisms with Essential Hypertension in a Northeastern Han Chinese Population." *J Hum Hypertens* 33, no. 7 (2019): 524-30.
136. Tiwari, A. K., P. Prasad, K. T. B, K. M. Kumar, A. C. Ammini, A. Gupta, and R. Gupta. "Oxidative Stress Pathway Genes and Chronic Renal Insufficiency in Asian Indians with Type 2 Diabetes." *J Diabetes Complications* 23, no. 2 (2009): 102-11.
137. Verdi, H., S. T. Kınık, H. P. Baysan-Çebi, Y. Y. Yalçın, A. C. Yazıcı-Güvercin, B. Aydın, N. B. Tütüncü, and F. B. Ataç. "Uncoupling Protein Gene Ucp1-3826a/G, Ucp2 Ins/Del and Ucp3-55c/T Polymorphisms in Obese Turkish Children." *Turk J Pediatr* 62, no. 6 (2020): 921-29.
138. Vimaleswaran, K. S., V. Radha, R. Deepa, and V. Mohan. "Absence of Association of Metabolic Syndrome with Ppargc1a, Pparg and Ucp1 Gene Polymorphisms in Asian Indians." *Metab Syndr Relat Disord* 5, no. 2 (2007): 153-62.
139. Yiew, S. K., L. Y. Khor, M. L. Tan, C. L. Pang, V. Y. Chai, S. S. Kanachamy, and Y. H. Say. "No Association between Peroxisome Proliferator-Activated Receptor and Uncoupling Protein Gene Polymorphisms and Obesity in Malaysian University Students." *Obes Res Clin Pract* 4, no. 4 (2010): e247-342.
140. Zhang, Y., N. Meng, Z. Lv, H. Li, and Y. Qu. "The Gene Polymorphisms of Ucp1 but Not Ppar Gamma and Tcf7l2 Are Associated with Diabetic Retinopathy in Chinese Type 2 Diabetes Mellitus Cases." *Acta Ophthalmol* 93, no. 3 (2015): e223-9.
141. Zietz, B., E. Watzlawek, K. D. Palitzsch, J. Schölmerich, and A. Schäffler. "Gg-Genotype in the Promotor Region of Uncoupling-Protein-1 Gene Is Associated with Lower Level of Dehydroepiandrosterone in Type 2 Diabetes." *Exp Clin Endocrinol Diabetes* 109, no. 2 (2001): 102-6.
142. Schwartz, D. A. "Environmental Genomics and Human Health." *G Ital Med Lav Ergon* 33, no. 1 (2011): 31-4.
143. Yoneshiro, T., T. Ogawa, N. Okamoto, M. Matsushita, S. Aita, T. Kameya, Y. Kawai, T. Iwanaga, and M. Saito. "Impact of Ucp1 and B3ar Gene Polymorphisms on Age-Related Changes in Brown Adipose Tissue and Adiposity in Humans." *International Journal of Obesity* 37, no. 7 (2013): 993-98.
144. Ward, Lucas D., and Manolis Kellis. "Haploreg V4: Systematic Mining of Putative Causal Variants, Cell Types, Regulators and Target Genes for Human Complex Traits and Disease." *Nucleic acids research* 44, no. D1 (2016): D877-D81.
145. Vegiopoulos, A., M. Rohm, and S. Herzig. "Adipose Tissue: Between the Extremes." *Embo j* 36, no. 14 (2017): 1999-2017.
146. Bartelt, A., and J. Heeren. "Adipose Tissue Browning and Metabolic Health." *Nat Rev Endocrinol* 10, no. 1 (2014): 24-36.
147. Cinti, S. "Adipose Tissues and Obesity." *Ital J Anat Embryol* 104, no. 2 (1999): 37-51.
148. *Meta-Analytic Interval Estimation for Standardized and Unstandardized Mean Differences*.

149. Qiu, Y., L. Sun, X. Hu, X. Zhao, H. Shi, Z. Liu, and X. Yin. "Compromised Browning Plasticity of Primary Subcutaneous Adipocytes Derived from Overweight Chinese Adults." *Diabetol Metab Syndr* 12 (2020): 91.
150. Wang, W., M. Kissig, S. Rajakumari, L. Huang, H. W. Lim, K. J. Won, and P. Seale. "Ebf2 Is a Selective Marker of Brown and Beige Adipogenic Precursor Cells." *Proc Natl Acad Sci U S A* 111, no. 40 (2014): 14466-71.
151. Rao, R. R., J. Z. Long, J. P. White, K. J. Svensson, J. Lou, I. Lokurkar, M. P. Jedrychowski, J. L. Ruas, C. D. Wrann, J. C. Lo, D. M. Camera, J. Lachey, S. Gygi, J. Seehra, J. A. Hawley, and B. M. Spiegelman. "Meteorin-Like Is a Hormone That Regulates Immune-Adipose Interactions to Increase Beige Fat Thermogenesis." *Cell* 157, no. 6 (2014): 1279-91.
152. Heinonen, I., K. K. Kalliokoski, J. C. Hannukainen, D. J. Duncker, P. Nuutila, and J. Knuuti. "Organ-Specific Physiological Responses to Acute Physical Exercise and Long-Term Training in Humans." *Physiology (Bethesda)* 29, no. 6 (2014): 421-36.
153. Stanford, K. I., R. J. Middelbeek, K. L. Townsend, M. Y. Lee, H. Takahashi, K. So, K. M. Hitchcox, K. R. Markan, K. Hellbach, M. F. Hirshman, Y. H. Tseng, and L. J. Goodyear. "A Novel Role for Subcutaneous Adipose Tissue in Exercise-Induced Improvements in Glucose Homeostasis." *Diabetes* 64, no. 6 (2015): 2002-14.
154. Golbidi, S., and I. Laher. "Exercise Induced Adipokine Changes and the Metabolic Syndrome." *J Diabetes Res* 2014 (2014): 726861.
155. Cao, L., E. Y. Choi, X. Liu, A. Martin, C. Wang, X. Xu, and M. J. During. "White to Brown Fat Phenotypic Switch Induced by Genetic and Environmental Activation of a Hypothalamic-Adipocyte Axis." *Cell Metab* 14, no. 3 (2011): 324-38.
156. Dewal, R. S., and K. I. Stanford. "Effects of Exercise on Brown and Beige Adipocytes." *Biochim Biophys Acta Mol Cell Biol Lipids* 1864, no. 1 (2019): 71-78.
157. Flouris, Andreas D., Petros C. Dinas, Angelica Valente, Cláudia Marlise Balbinotti Andrade, Nair Honda Kawashita, and Paraskevi Sakellariou. "Exercise-Induced Effects on Ucp1 Expression in Classical Brown Adipose Tissue: A Systematic Review." 31, no. 2 (2017).
158. Knudsen, J. G., M. Murholm, A. L. Carey, R. S. Biensø, A. L. Basse, T. L. Allen, J. Hidalgo, B. A. Kingwell, M. A. Febbraio, J. B. Hansen, and H. Pilegaard. "Role of Il-6 in Exercise Training- and Cold-Induced Ucp1 Expression in Subcutaneous White Adipose Tissue." *PLoS One* 9, no. 1 (2014): e84910.
159. Daskalopoulou, S. S., A. B. Cooke, Y. H. Gomez, A. F. Mutter, A. Filippaios, E. T. Mesfum, and C. S. Mantzoros. "Plasma Irisin Levels Progressively Increase in Response to Increasing Exercise Workloads in Young, Healthy, Active Subjects." *Eur J Endocrinol* 171, no. 3 (2014): 343-52.
160. Jedrychowski, M. P., C. D. Wrann, J. A. Paulo, K. K. Gerber, J. Szpyt, M. M. Robinson, K. S. Nair, S. P. Gygi, and B. M. Spiegelman. "Detection and Quantitation of Circulating Human Irisin by Tandem Mass Spectrometry." *Cell Metab* 22, no. 4 (2015): 734-40.
161. Wu, J., P. Boström, L. M. Sparks, L. Ye, J. H. Choi, A. H. Giang, M. Khandekar, K. A. Virtanen, P. Nuutila, G. Schaart, K. Huang, H. Tu, W. D. van Marken Lichtenbelt, J. Hoeks, S. Enerbäck, P. Schrauwen, and B. M. Spiegelman. "Beige Adipocytes Are a Distinct Type of Thermogenic Fat Cell in Mouse and Human." *Cell* 150, no. 2 (2012): 366-76.
162. Raschke, S., K. Eckardt, K. Bjørklund Holven, J. Jensen, and J. Eckel. "Identification and Validation of Novel Contraction-Regulated Myokines Released from Primary Human Skeletal Muscle Cells." *PLoS One* 8, no. 4 (2013): e62008.
163. Scheler, M., M. Irmler, S. Lehr, S. Hartwig, H. Staiger, H. Al-Hasani, J. Beckers, M. H. de Angelis, H. U. Häring, and C. Weigert. "Cytokine Response of Primary Human Myotubes in an in Vitro Exercise Model." *Am J Physiol Cell Physiol* 305, no. 8 (2013): C877-86.
164. Grygiel-Górniak, B., and M. Puszczewicz. "A Review on Irisin, a New Protagonist That Mediates Muscle-Adipose-Bone-Neuron Connectivity." *Eur Rev Med Pharmacol Sci* 21, no. 20 (2017): 4687-93.

165. Nikolić, N., S. S. Bakke, E. T. Kase, I. Rudberg, I. Flo Halle, A. C. Rustan, G. H. Thoresen, and V. Aas. "Electrical Pulse Stimulation of Cultured Human Skeletal Muscle Cells as an in Vitro Model of Exercise." *PLoS One* 7, no. 3 (2012): e33203.
166. Chen, W., M. R. Nyasha, M. Koide, M. Tsuchiya, N. Suzuki, Y. Hagiwara, M. Aoki, and M. Kanzaki. "In Vitro Exercise Model Using Contractile Human and Mouse Hybrid Myotubes." *Sci Rep* 9, no. 1 (2019): 11914.
167. Burch, N., A. S. Arnold, F. Item, S. Summermatter, G. Brochmann Santana Santos, M. Christe, U. Boutellier, M. Toigo, and C. Handschin. "Electric Pulse Stimulation of Cultured Murine Muscle Cells Reproduces Gene Expression Changes of Trained Mouse Muscle." *PLoS One* 5, no. 6 (2010): e10970.
168. Brown, A. E., D. E. Jones, M. Walker, and J. L. Newton. "Abnormalities of Ampk Activation and Glucose Uptake in Cultured Skeletal Muscle Cells from Individuals with Chronic Fatigue Syndrome." *PLoS One* 10, no. 4 (2015): e0122982.
169. Christensen, C. S., D. P. Christensen, M. Lundh, M. S. Dahllof, T. N. Haase, J. M. Velasquez, M. J. Laye, T. Mandrup-Poulsen, and T. P. Solomon. "Skeletal Muscle to Pancreatic Beta-Cell Cross-Talk: The Effect of Humoral Mediators Liberated by Muscle Contraction and Acute Exercise on Beta-Cell Apoptosis." *J Clin Endocrinol Metab* 100, no. 10 (2015): E1289-98.
170. Pattamaprapanont, P., C. Garde, O. Fabre, and R. Barrès. "Muscle Contraction Induces Acute Hydroxymethylation of the Exercise-Responsive Gene Nr4a3." *Front Endocrinol (Lausanne)* 7 (2016): 165.
171. Raschke, S., M. Elsen, H. Gassenhuber, M. Sommerfeld, U. Schwahn, B. Brockmann, R. Jung, U. Wisloff, A. E. Tjonna, T. Raastad, J. Hallen, F. Norheim, C. A. Drevon, T. Romacho, K. Eckardt, and J. Eckel. "Evidence against a Beneficial Effect of Irisin in Humans." *PLoS One* 8, no. 9 (2013): e73680.
172. Ishiuchi, Y., H. Sato, K. Tsujimura, H. Kawaguchi, T. Matsuwaki, K. Yamanouchi, M. Nishihara, and T. Nedachi. "Skeletal Muscle Cell Contraction Reduces a Novel Myokine, Chemokine (C-X-C Motif) Ligand 10 (Cxcl10): Potential Roles in Exercise-Regulated Angiogenesis." *Biosci Biotechnol Biochem* 82, no. 1 (2018): 97-105.
173. Li, Ling-Jie, Jin Ma, Song-Bo Li, Xue-Fei Chen, and Jing Zhang. "Electric Pulse Stimulation Inhibited Lipid Accumulation on C2c12 Myotubes Incubated with Oleic Acid and Palmitic Acid." *Archives of Physiology and Biochemistry* 127, no. 4 (2021): 344-50.
174. Nieuwoudt, S., A. Mulya, C. E. Fealy, E. Martelli, S. Dasarathy, S. V. Naga Prasad, and J. P. Kirwan. "In Vitro Contraction Protects against Palmitate-Induced Insulin Resistance in C2c12 Myotubes." *Am J Physiol Cell Physiol* (2017): ajpcell.00123.2017.
175. Li, H., M. Dong, W. Liu, C. Gao, Y. Jia, X. Zhang, X. Xiao, Q. Liu, and H. Lin. "Peripheral Il-6/Stat3 Signaling Promotes Beiging of White Fat." *Biochim Biophys Acta Mol Cell Res* 1868, no. 10 (2021): 119080.
176. Tamura, Y., K. Kouzaki, T. Kotani, and K. Nakazato. "Electrically Stimulated Contractile Activity-Induced Transcriptomic Responses and Metabolic Remodeling in C2c12 Myotubes: Twitch Vs. Tetanic Contractions." *Am J Physiol Cell Physiol* 319, no. 6 (2020): C1029-C44.
177. Encyclopedia of DNA Elements at UCSC, https://genome.ucsc.edu/ENCODE/.
178. Zebisch, K., V. Voigt, M. Wabitsch, and M. Brandsch. "Protocol for Effective Differentiation of 3t3-L1 Cells to Adipocytes." *Anal Biochem* 425, no. 1 (2012): 88-90.
179. Pandurangan, M., D. Jeong, T. Amna, H. Van Ba, and I. Hwang. "Co-Culture of C2c12 and 3t3-L1 Preadipocyte Cells Alters the Gene Expression of Calpains, Caspases and Heat Shock Proteins." *In Vitro Cell Dev Biol Anim* 48, no. 9 (2012): 577-82.
180. Marotta, M., R. Bragós, and A. M. Gómez-Foix. "Design and Performance of an Electrical Stimulator for Long-Term Contraction of Cultured Muscle Cells." *Biotechniques* 36, no. 1 (2004): 68-73.
181. Sato, S., M. Nomura, I. Yamana, A. Uchiyama, Y. Furuichi, Y. Manabe, and N. L. Fujii. "A New in Vitro Muscle Contraction Model and Its Application for Analysis of Mtorc1 Signaling

in Combination with Contraction and Beta-Hydroxy-Beta-Methylbutyrate Administration." *Biosci Biotechnol Biochem* 83, no. 10 (2019): 1851-57.
182. Chan, F. K., K. Moriwaki, and M. J. De Rosa. "Detection of Necrosis by Release of Lactate Dehydrogenase Activity." *Methods Mol Biol* 979 (2013): 65-70.
183. Wood, E. J. "Molecular Cloning. A Laboratory Manual: By T Maniatis, E F Fritsch and J Sambrook. Pp 545. Cold Spring Harbor Laboratory, New York. 1982. ." *Biochemical Education* 11, no. 2 (1983): 82.
184. Karpen, Samuel C. "P Value Problems." *American Journal of Pharmaceutical Education* 81, no. 9 (2017): 6570.
185. Cohen, Jacob. "Statistical Power Analysis for the Behavioral Sciences " (1988).
186. Huh, J. Y. "The Role of Exercise-Induced Myokines in Regulating Metabolism." *Arch Pharm Res* 41, no. 1 (2018): 14-29.
187. Xu, X., Z. Ying, M. Cai, Z. Xu, Y. Li, S. Y. Jiang, K. Tzan, A. Wang, S. Parthasarathy, G. He, S. Rajagopalan, and Q. Sun. "Exercise Ameliorates High-Fat Diet-Induced Metabolic and Vascular Dysfunction, and Increases Adipocyte Progenitor Cell Population in Brown Adipose Tissue." *Am J Physiol Regul Integr Comp Physiol* 300, no. 5 (2011): R1115-25.
188. Lira, V. A., C. R. Benton, Z. Yan, and A. Bonen. "Pgc-1alpha Regulation by Exercise Training and Its Influences on Muscle Function and Insulin Sensitivity." *Am J Physiol Endocrinol Metab* 299, no. 2 (2010): E145-61.
189. Nedachi, T., H. Fujita, and M. Kanzaki. "Contractile C2c12 Myotube Model for Studying Exercise-Inducible Responses in Skeletal Muscle." *Am J Physiol Endocrinol Metab* 295, no. 5 (2008): E1191-204.
190. Pettersson-Klein, A. T., M. Izadi, D. M. S. Ferreira, I. Cervenka, J. C. Correia, V. Martinez-Redondo, M. Southern, M. Cameron, T. Kamenecka, L. Z. Agudelo, M. Porsmyr-Palmertz, U. Martens, B. Lundgren, M. Otrocka, A. Jenmalm-Jensen, P. R. Griffin, and J. L. Ruas. "Small Molecule Pgc-1alpha1 Protein Stabilizers Induce Adipocyte Ucp1 Expression and Uncoupled Mitochondrial Respiration." *Mol Metab* 9 (2018): 28-42.
191. Shabalina, I. G., N. Petrovic, J. M. de Jong, A. V. Kalinovich, B. Cannon, and J. Nedergaard. "Ucp1 in Brite/Beige Adipose Tissue Mitochondria Is Functionally Thermogenic." *Cell Rep* 5, no. 5 (2013): 1196-203.
192. Rohleder, N., M. Aringer, and M. Boentert. "Role of Interleukin-6 in Stress, Sleep, and Fatigue." *Ann N Y Acad Sci* 1261 (2012): 88-96.
193. Aboouf, M. A., J. Armbruster, M. Thiersch, M. Gassmann, A. Gödecke, E. Gnaiger, G. Kristiansen, A. Bicker, T. Hankeln, H. Zhu, and T. A. Gorr. "Myoglobin, Expressed in Brown Adipose Tissue of Mice, Regulates the Content and Activity of Mitochondria and Lipid Droplets." *Biochim Biophys Acta Mol Cell Biol Lipids* 1866, no. 12 (2021): 159026.
194. Kristóf, E., Á Klusóczki, R. Veress, A. Shaw, Z. S. Combi, K. Varga, F. Győry, Z. Balajthy, P. Bai, Z. Bacso, and L. Fésüs. "Interleukin-6 Released from Differentiating Human Beige Adipocytes Improves Browning." *Exp Cell Res* 377, no. 1-2 (2019): 47-55.
195. Tamura, K., N. Goto-Inoue, K. Miyata, Y. Furuichi, N. L. Fujii, and Y. Manabe. "Effect of Treatment with Conditioned Media Derived from C2c12 Myotube on Adipogenesis and Lipolysis in 3t3-L1 Adipocytes." *PLoS One* 15, no. 8 (2020): e0237095.
196. Pilkington, Anna-Claire, Henry A. Paz, and Umesh D. Wankhade. "Beige Adipose Tissue Identification and Marker Specificity—Overview." *Frontiers in Endocrinology* 12, no. 8 (2021).
197. Pérez-Sotelo, D., A. Roca-Rivada, I. Baamonde, J. Baltar, A. I. Castro, E. Domínguez, M. Collado, F. F. Casanueva, and M. Pardo. "Lack of Adipocyte-Fndc5/Irisin Expression and Secretion Reduces Thermogenesis and Enhances Adipogenesis." *Scientific Reports* 7, no. 1 (2017): 16289.
198. Shan, Tizhong, Xinrong Liang, Pengpeng Bi, and Shihuan Kuang. "Myostatin Knockout Drives Browning of White Adipose Tissue through Activating the Ampk-Pgc1α-Fndc5 Pathway in Muscle." *The FASEB Journal* 27, no. 5 (2013): 1981-89.

199. Glassman, A. R. "Are P Values Enough?" *JAMA Ophthalmol* 138, no. 5 (2020): 567-68.
200. Dufau, J., J. X. Shen, M. Couchet, T. De Castro Barbosa, N. Mejhert, L. Massier, E. Griseti, E. Mouisel, E. Z. Amri, V. M. Lauschke, M. Rydén, and D. Langin. "In Vitro and Ex Vivo Models of Adipocytes." *Am J Physiol Cell Physiol* 320, no. 5 (2021): C822-c41.
201. Samuelson, I., and A. Vidal-Puig. "Studying Brown Adipose Tissue in a Human in Vitro Context." *Front Endocrinol (Lausanne)* 11 (2020): 629.
202. Carter, S., and T. P. J. Solomon. "In Vitro Experimental Models for Examining the Skeletal Muscle Cell Biology of Exercise: The Possibilities, Challenges and Future Developments." *Pflugers Arch* 471, no. 3 (2019): 413-29.
203. Pudkasam, Supa, Kathy Tangalakis, Nanthapan Chinlumprasert, Vasso Apostolopoulos, and Lily Stojanovska. "Breast Cancer and Exercise: The Role of Adiposity and Immune Markers." *Maturitas* 105 (2017): 16-22.
204. Colberg, Sheri R., Ronald J. Sigal, Jane E. Yardley, Michael C. Riddell, David W. Dunstan, Paddy C. Dempsey, Edward S. Horton, Kristin Castorino, and Deborah F. Tate. "Physical Activity/Exercise and Diabetes: A Position Statement of the American Diabetes Association." *Diabetes Care* 39, no. 11 (2016): 2065-79.
205. Harding, Amy T., and Belinda R. Beck. "Exercise, Osteoporosis, and Bone Geometry." *Sports* 5, no. 2 (2017): 29.
206. Wewege, Michael A., Jeanette M. Thom, Kerry-Anne Rye, and Belinda J. Parmenter. "Aerobic, Resistance or Combined Training: A Systematic Review and Meta-Analysis of Exercise to Reduce Cardiovascular Risk in Adults with Metabolic Syndrome." *Atherosclerosis* 274 (2018): 162-71.
207. Flouris, A. D., C. Bouziotas, A. D. Christodoulos, and Y. Koutedakis. "Longitudinal Preventive-Screening Cutoffs for Metabolic Syndrome in Adolescents." *Int J Obes (Lond)* 32, no. 10 (2008): 1506-12.
208. Muscella, Antonella, Erika Stefàno, and Santo Marsigliante. "The Effects of Exercise Training on Lipid Metabolism and Coronary Heart Disease." *American Journal of Physiology-Heart and Circulatory Physiology* 319, no. 1 (2020): H76-H88.
209. da Costa Daniele, Thiago Medeiros, Pedro Felipe Carvalhedo de Bruin, Robson Salviano de Matos, Gabriela Sales de Bruin, Cauby Maia Chaves, and Veralice Meireles Sales de Bruin. "Exercise Effects on Brain and Behavior in Healthy Mice, Alzheimer's Disease and Parkinson's Disease Model—a Systematic Review and Meta-Analysis." *Behavioural Brain Research* 383 (2020): 112488.
210. Wang, Qiaoyun, and Wenli Zhou. "Roles and Molecular Mechanisms of Physical Exercise in Cancer Prevention and Treatment." *Journal of Sport and Health Science* 10, no. 2 (2021): 201-10.
211. Lambernd, S., A. Taube, A. Schober, B. Platzbecker, S. W. Gorgens, R. Schlich, K. Jeruschke, J. Weiss, K. Eckardt, and J. Eckel. "Contractile Activity of Human Skeletal Muscle Cells Prevents Insulin Resistance by Inhibiting Pro-Inflammatory Signalling Pathways." *Diabetologia* 55, no. 4 (2012): 1128-39.
212. Song, Yang, Jennifer Soto, Binru Chen, Li Yang, and Song Li. "Cell Engineering: Biophysical Regulation of the Nucleus." *Biomaterials* 234 (2020): 119743.
213. Orfanos, Z., M. P. Godderz, E. Soroka, T. Godderz, A. Rumyantseva, P. F. van der Ven, T. J. Hawke, and D. O. Furst. "Breaking Sarcomeres by in Vitro Exercise." *Sci Rep* 6 (2016): 19614.
214. Evers-van Gogh, I. J., S. Alex, R. Stienstra, A. B. Brenkman, S. Kersten, and E. Kalkhoven. "Electric Pulse Stimulation of Myotubes as an in Vitro Exercise Model: Cell-Mediated and Non-Cell-Mediated Effects." *Sci Rep* 5 (2015): 10944.
215. Beiter, T., J. Hudemann, C. Burgstahler, A. M. Niess, and B. Munz. "Effects of Extracellular Orotic Acid on Acute Contraction-Induced Adaptation Patterns in C2c12 Cells." *Mol Cell Biochem* 448, no. 1-2 (2018): 251-63.
216. Chaves, A. B., E. R. Miranda, J. T. Mey, B. K. Blackburn, K. N. Z. Fuller, B. Stearns, A. Ludlow, D. L. th Williamson, J. A. Houmard, and J. M. Haus. "Exercise Reduces the Protein

Abundance of Txnip and Its Interacting Partner Redd1 in Skeletal Muscle: Potential Role for a Pka-Mediated Mechanism." *J Appl Physiol (1985)* 132, no. 2 (2022): 357-66.

217. Feng, Y. Z., N. Nikolic, S. S. Bakke, E. T. Kase, K. Guderud, J. Hjelmesaeth, V. Aas, A. C. Rustan, and G. H. Thoresen. "Myotubes from Lean and Severely Obese Subjects with and without Type 2 Diabetes Respond Differently to an in Vitro Model of Exercise." *Am J Physiol Cell Physiol* 308, no. 7 (2015): C548-56.
218. MCARDLE, A. "Contractile Activity-Induced Oxidative Stress: Cellular Origin and Adaptive Responses." *Am J Physiol Cell Physiol* 280 (2001): 621-27.
219. Tarum, J., M. Folkesson, P. J. Atherton, and F. Kadi. "Electrical Pulse Stimulation: An in Vitro Exercise Model for the Induction of Human Skeletal Muscle Cell Hypertrophy. A Proof-of-Concept Study." *Exp Physiol* 102, no. 11 (2017): 1405-13.
220. Valero-Breton, M., G. Warnier, M. Castro-Sepulveda, L. Deldicque, and H. Zbinden-Foncea. "Acute and Chronic Effects of High Frequency Electric Pulse Stimulation on the Akt/Mtor Pathway in Human Primary Myotubes." *Front Bioeng Biotechnol* 8 (2020): 565679.
221. Laurens, Claire, Anisha Parmar, Enda Murphy, Deborah Carper, Benjamin Lair, Pauline Maes, Julie Vion, Nathalie Boulet, Coralie Fontaine, Marie Marquès, Dominique Larrouy, Isabelle Harant, Claire Thalamas, Emilie Montastier, Sylvie Caspar-Bauguil, Virginie Bourlier, Geneviève Tavernier, Jean-Louis Grolleau, Anne Bouloumié, Dominique Langin, Nathalie Viguerie, Fabrice Bertile, Stéphane Blanc, Isabelle de Glisezinski, Donal O'Gorman, and Cedric Moro. "Growth and Differentiation Factor 15 Is Secreted by Skeletal Muscle During Exercise and Promotes Lipolysis in Humans." *JCI Insight* 5, no. 6 (2020).
222. Lambertucci, R. H., R. Silveira Ldos, S. M. Hirabara, R. Curi, G. Sweeney, and T. C. Pithon-Curi. "Effects of Moderate Electrical Stimulation on Reactive Species Production by Primary Rat Skeletal Muscle Cells: Cross Talk between Superoxide and Nitric Oxide Production." *J Cell Physiol* 227, no. 6 (2012): 2511-8.
223. Nikolić, N., S. W. Görgens, G. H. Thoresen, V. Aas, J. Eckel, and K. Eckardt. "Electrical Pulse Stimulation of Cultured Skeletal Muscle Cells as a Model for In vitro Exercise - Possibilities and Limitations." *Acta Physiol (Oxf)* 220, no. 3 (2017): 310-31.
224. Manabe, Y., S. Miyatake, M. Takagi, M. Nakamura, A. Okeda, T. Nakano, M. F. Hirshman, L. J. Goodyear, and N. L. Fujii. "Characterization of an Acute Muscle Contraction Model Using Cultured C2c12 Myotubes." *PLoS One* 7, no. 12 (2012): e52592.
225. Park, S., K. D. Turner, D. Zheng, J. J. Brault, K. Zou, A. B. Chaves, T. S. Nielsen, C. J. Tanner, J. T. Treebak, and J. A. Houmard. "Electrical Pulse Stimulation Induces Differential Responses in Insulin Action in Myotubes from Severely Obese Individuals." *J Physiol* 597, no. 2 (2019): 449-66.
226. Yue, Yingying, Chang Zhang, Xuejiao Zhang, Shitian Zhang, Qian Liu, Fang Hu, Xiaoting Lv, Hanqi Li, Jianming Yang, Xinli Wang, Liming Chen, Zhi Yao, Hongquan Duan, and Wenyan Niu. "An Ampk/Axin1-Rac1 Signaling Pathway Mediates Contraction-Regulated Glucose Uptake in Skeletal Muscle Cells." *American Journal of Physiology-Endocrinology and Metabolism* 318, no. 3 (2020): E330-E42.
227. Page, Matthew J, Joanne E McKenzie, Patrick M Bossuyt, Isabelle Boutron, Tammy C Hoffmann, Cynthia D Mulrow, Larissa Shamseer, Jennifer M Tetzlaff, Elie A Akl, Sue E Brennan, Roger Chou, Julie Glanville, Jeremy M Grimshaw, Asbjørn Hróbjartsson, Manoj M Lalu, Tianjing Li, Elizabeth W Loder, Evan Mayo-Wilson, Steve McDonald, Luke A McGuinness, Lesley A Stewart, James Thomas, Andrea C Tricco, Vivian A Welch, Penny Whiting, and David Moher. "The Prisma 2020 Statement: An Updated Guideline for Reporting Systematic Reviews." *BMJ* 372 (2021): n71.
228. Pereira, Marcelo G., Vanessa A. Voltarelli, Gabriel C. Tobias, Lara de Souza, Gabriela S. Borges, Ailma O. Paixão, Ney R. de Almeida, Thomas Scott Bowen, Marilene Demasi, Elen H. Miyabara, and Patricia C. Brum. "Aerobic Exercise Training and in Vivo Akt Activation

Counteract Cancer Cachexia by Inducing a Hypertrophic Profile through Eif-2Α Modulation." *Cancers* 14, no. 1 (2022): 28.
229. Rohatgi, A. "Webplotdigitizer. 4.5.".
230. Weir, Christopher J., Isabella Butcher, Valentina Assi, Stephanie C. Lewis, Gordon D. Murray, Peter Langhorne, and Marian C. Brady. "Dealing with Missing Standard Deviation and Mean Values in Meta-Analysis of Continuous Outcomes: A Systematic Review." *BMC Medical Research Methodology* 18, no. 1 (2018): 25.
231. Barlow, J., and T. P. J. Solomon. "Conditioned Media from Contracting Skeletal Muscle Potentiates Insulin Secretion and Enhances Mitochondrial Energy Metabolism of Pancreatic Beta-Cells." *Metabolism* 91 (2019): 1-9.
232. Connor, M. K., I. Irrcher, and D. A. Hood. "Contractile Activity-Induced Transcriptional Activation of Cytochrome C Involves Sp1 and Is Proportional to Mitochondrial Atp Synthesis in C2c12 Muscle Cells." *J Biol Chem* 276, no. 19 (2001): 15898-904.
233. Fernandez-Verdejo, R., A. M. Vanwynsberghe, T. Hai, L. Deldicque, and M. Francaux. "Activating Transcription Factor 3 Regulates Chemokine Expression in Contracting C2c12 Myotubes and in Mouse Skeletal Muscle after Eccentric Exercise." *Biochem Biophys Res Commun* 492, no. 2 (2017): 249-54.
234. Fujita, H., K. Shimizu, and E. Nagamori. "Novel Method for Measuring Active Tension Generation by C2c12 Myotube Using Uv-Crosslinked Collagen Film." *Biotechnol Bioeng* 106, no. 3 (2010): 482-9.
235. Furuichi, Y., Y. Manabe, M. Takagi, M. Aoki, and N. L. Fujii. "Evidence for Acute Contraction-Induced Myokine Secretion by C2c12 Myotubes." *PLoS One* 13, no. 10 (2018): e0206146.
236. Guigni, Blas A., Dennis K. Fix, Joseph J. Bivona 3rd, Bradley M. Palmer, James A. Carson, and Michael J. Toth. "Electrical Stimulation Prevents Doxorubicin-Induced Atrophy and Mitochondrial Loss in Cultured Myotubes." *American Journal of Physiology-Cell Physiology* 317, no. 6 (2019): C1213-C28.
237. Horie, M., E. Warabi, S. Komine, S. Oh, and J. Shoda. "Cytoprotective Role of Nrf2 in Electrical Pulse Stimulated C2c12 Myotube." *PLoS One* 10, no. 12 (2015): e0144835.
238. Lee, J. O., W. S. Byun, M. J. Kang, J. A. Han, J. Moon, M. J. Shin, H. J. Lee, J. H. Chung, J. S. Lee, C. G. Son, K. H. Song, T. W. Kim, E. S. Lee, H. M. Kim, C. H. Chung, K. R. W. Ngoei, N. X. Y. Ling, J. S. Oakhill, S. Galic, L. Murray-Segal, B. E. Kemp, K. M. Kim, S. Lim, and H. S. Kim. "The Myokine Meteorin-Like (Metrnl) Improves Glucose Tolerance in Both Skeletal Muscle Cells and Mice by Targeting Ampkalpha2." *FEBS J* 287, no. 10 (2020): 2087-104.
239. Martin, N. R. W., M. C. Turner, R. Farrington, D. J. Player, and M. P. Lewis. "Leucine Elicits Myotube Hypertrophy and Enhances Maximal Contractile Force in Tissue Engineered Skeletal Muscle in Vitro." *J Cell Physiol* 232, no. 10 (2017): 2788-97.
240. Nakamura, T., S. Takagi, D. Okuzaki, S. Matsui, and T. Fujisato. "Hypoxia Transactivates Cholecystokinin Gene Expression in 3d-Engineered Muscle." *J Biosci Bioeng* 132, no. 1 (2021): 64-70.
241. Small, L., A. Altintas, R. C. Laker, A. Ehrlich, P. Pattamaprapanont, J. Villarroel, N. J. Pillon, J. R. Zierath, and R. Barres. "Contraction Influences Per2 Gene Expression in Skeletal Muscle through a Calcium-Dependent Pathway." *J Physiol* 598, no. 24 (2020): 5739-52.
242. Son, Y. H., S. M. Lee, S. H. Lee, J. H. Yoon, J. S. Kang, Y. R. Yang, and K. S. Kwon. "Comparative Molecular Analysis of Endurance Exercise in Vivo with Electrically Stimulated in Vitro Myotube Contraction." *J Appl Physiol (1985)* 127, no. 6 (2019): 1742-53.
243. Thelen, Marc H.M. "Electrical Stimulation of C2c12 Myotubes Induces Contractions and Represses Thyroid-Hormone-Dependent Transcription of the Fast-Type Sarcoplasmic-Reticulum Ca2+-Atpase Gene." *Biochemistry Journal* 321 (1997): 845-48.

244. Pattwell, D. M., A. McArdle, J. E. Morgan, T. A. Patridge, and M. J. Jackson. "Release of Reactive Oxygen and Nitrogen Species from Contracting Skeletal Muscle Cells." *Free Radic Biol Med* 37, no. 7 (2004): 1064-72.
245. Broholm, C., M. J. Laye, C. Brandt, R. Vadalasetty, H. Pilegaard, B. K. Pedersen, and C. Scheele. "Lif Is a Contraction-Induced Myokine Stimulating Human Myocyte Proliferation." *J Appl Physiol (1985)* 111, no. 1 (2011): 251-9.
246. Kugler, B. A., W. Deng, B. Francois, M. Anderson, J. M. Hinkley, J. A. Houmard, P. N. Gona, and K. Zou. "Distinct Adaptations of Mitochondrial Dynamics to Electrical Pulse Stimulation in Lean and Severely Obese Primary Myotubes." *Med Sci Sports Exerc* 53, no. 6 (2021): 1151-60.
247. Løvsletten, Nils, Arild Rustan, Claire Laurens, Hege Thoresen, Cedric Moro, and Nataša Nikolić. "Primary Defects in Lipid Handling and Resistance to Exercise in Myotubes from Obese Donors with and without Type 2 Diabetes." *Applied Physiology, Nutrition, and Metabolism* 45 (2019).
248. Scheler, M., M. H. de Angelis, H. Al-Hasani, H. U. Haring, C. Weigert, and S. Lehr. "Methods for Proteomics-Based Analysis of the Human Muscle Secretome Using an in Vitro Exercise Model." *Methods Mol Biol* 1295 (2015): 55-64.
249. Kubis, H. P., R. J. Scheibe, J. D. Meissner, G. Hornung, and G. Gros. "Fast-to-Slow Transformation and Nuclear Import/Export Kinetics of the Transcription Factor Nfatc1 During Electrostimulation of Rabbit Muscle Cells in Culture." *J Physiol* 541, no. Pt 3 (2002): 835-47.
250. Miyatake, Shouta, Philip J. Bilan, Nicolas J. Pillon, and Amira Klip. "Contracting C2c12 Myotubes Release Ccl2 in an Nf-Kb-Dependent Manner to Induce Monocyte Chemoattraction." *American Journal of Physiology-Endocrinology and Metabolism* 310, no. 2 (2016): E160-E70.
251. Richter, E. A., and N. B. Ruderman. "Ampk and the Biochemistry of Exercise: Implications for Human Health and Disease." *Biochem J* 418, no. 2 (2009): 261-75.
252. Sylow, Lykke, Maximilian Kleinert, Erik A. Richter, and Thomas E. Jensen. "Exercise-Stimulated Glucose Uptake — Regulation and Implications for Glycaemic Control." *Nature Reviews Endocrinology* 13, no. 3 (2017): 133-48.
253. Mann, Gagandeep, Michael C. Riddell, and Olasunkanmi A. J. Adegoke. "Effects of Acute Muscle Contraction on the Key Molecules in Insulin and Akt Signaling in Skeletal Muscle in Health and in Insulin Resistant States." *Diabetology* 3, no. 3 (2022): 423-46.
254. Munoz-Canoves, P., C. Scheele, B. K. Pedersen, and A. L. Serrano. "Interleukin-6 Myokine Signaling in Skeletal Muscle: A Double-Edged Sword?" *FEBS J* 280, no. 17 (2013): 4131-48.
255. Neufer, P. Darrell, Marcas M Bamman, Deborah M Muoio, Claude Bouchard, Dan M Cooper, Bret H Goodpaster, Frank W Booth, Wendy M Kohrt, Robert E Gerszten, Mark P Mattson, Russell T Hepple, William E Kraus, Michael B Reid, Sue C Bodine, John M Jakicic, Jerome L Fleg, John P Williams, Lyndon Joseph, Mary Evans, Padma Maruvada, Mary Rodgers, Mary Roary, Amanda T Boyce, Jonelle K Drugan, James I Koenig, Richard H Ingraham, Danuta Krotoski, Mary Garcia-Cazarin, Joan A McGowan, and Maren R Laughlin. "Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits." *Cell Metabolism* 22, no. 1 (2015): 4-11.
256. Muise, Eric S., Hong-Ping Guan, Jinqi Liu, Andrea R. Nawrocki, Xiaodong Yang, Chuanlin Wang, Carlos G. Rodríguez, Dan Zhou, Judith N. Gorski, Marc M. Kurtz, Danqing Feng, Kenneth J. Leavitt, Lan Wei, Robert R. Wilkening, James M. Apgar, Shiyao Xu, Ku Lu, Wen Feng, Ying Li, Huaibing He, Stephen F. Previs, Xiaolan Shen, Margaret van Heek, Sandra C. Souza, Mark J. Rosenbach, Tesfaye Biftu, Mark D. Erion, David E. Kelley, Daniel M. Kemp, Robert W. Myers, and Iyassu K. Sebhat. "Pharmacological Ampk Activation Induces Transcriptional Responses Congruent to Exercise in Skeletal and Cardiac Muscle, Adipose Tissues and Liver." *PLoS One* 14, no. 2 (2019): e0211568.

257. Sakamoto, Kei, David E. W. Arnolds, Ingvar Ekberg, Anders Thorell, and Laurie J. Goodyear. "Exercise Regulates Akt and Glycogen Synthase Kinase-3 Activities in Human Skeletal Muscle." *Biochemical and Biophysical Research Communications* 319, no. 2 (2004): 419-25.
258. Pedersen, B. K., A. Steensberg, C. Fischer, C. Keller, P. Keller, P. Plomgaard, E. Wolsk-Petersen, and M. Febbraio. "The Metabolic Role of Il-6 Produced During Exercise: Is Il-6 an Exercise Factor?" *Proceedings of the Nutrition Society* 63, no. 2 (2004): 263-67.
259. Uguccioni, Giulia, Donna D'Souza, and David A. Hood. "Regulation of Ppar<Svg Style="Vertical-Align:-4.698pt;Width:11.35px;" Id="M1" Height="16.012501" Version="1.1" Viewbox="0 0 11.35 16.012501" Width="11.35" Xmlns:Xlink="Http://Www.W3.Org/1999/Xlink" Xmlns="Http://Www.W3.Org/2000/Svg"> <G Transform="Matrix(.022,-0,0,-.022,.062,10.1)"><Path Id="X1d6fe" D="M478 372q0 -39 -31 -97t-61 -98t-78 -98q-45 -55 -73 -102q-13 -79 -13 -197q-11 -11 -43.5 -25.5t-53.5 -15.5l-15 17q5 35 26 101.5t47 123.5q8 72 -1.5 174.5t-37.5 178.5q-14 37 -29 37q-20 0 -67 -65l-25 21q37 60 73 90.5t63 30.5q47 0 72 -112q13 -56 17.5 -141.5T0.5 -143.5h2q155 193 155 297q0 26 -12 47q-5 8 -5 15q0 16 12.5 27t29.5 11q21 0 34 -21t13 -55z" /></G> </Svg> Coactivator-1<Svg Style="Vertical-Align:-0.216pt;Width:12.8625px;" Id="M2" Height="10.4375" Version="1.1" Viewbox="0 0 12.8625 10.4375" Width="12.8625" Xmlns:Xlink="Http://Www.W3.Org/1999/Xlink" Xmlns="Http://Www.W3.Org/2000/Svg"> <G Transform="Matrix(.022,-0,0,-.022,.062,10.1)"><Path Id="X1d6fc" D="M545 106q-67 -118 -134 -118q-24 0 -40 37.5t-30 129.5h-2q-47 -72 -103 -119.5t-108 -47.5q-47 0 -76 45.5t-29 119.5q0 113 85 204t174 91q47 0 70 -33.5t43 -119.5h3q32 47 80 140l55 13l10 -9q-47 -80 -138 -201q17 -99 27.5 -136t22.5 -37q23 0 69 61zm333 204 Q-14 98 -31 149.5t-50 51.5q-49 0 -94 -70t-45 -164q0 -55 15.5 -86t40.5 -31q70 0 164 150z" /></G> </Svg> Function and Expression in Muscle: Effect of Exercise." *PPAR Research* 2010 (2010): 937123.
260. O'Neill, H. M. "Ampk and Exercise: Glucose Uptake and Insulin Sensitivity." *Diabetes Metab J* 37, no. 1 (2013): 1-21.
261. Chowdhury, Subrata, Logan Schulz, Biagio Palmisano, Parminder Singh, Julian M. Berger, Vijay K. Yadav, Paula Mera, Helga Ellingsgaard, Juan Hidalgo, Jens Brüning, and Gerard Karsenty. "Muscle-Derived Interleukin 6 Increases Exercise Capacity by Signaling in Osteoblasts." *The Journal of Clinical Investigation* 130, no. 6 (2020): 2888-902.
262. Kistner, Timothy M., Bente K. Pedersen, and Daniel E. Lieberman. "Interleukin 6 as an Energy Allocator in Muscle Tissue." *Nature Metabolism* 4, no. 2 (2022): 170-79.
263. Fischer, Christian P. "Interleukin-6 in Acute Exercise and Training: What Is the Biological Relevance." *Exerc immunol rev* 12, no. 6-33 (2006): 41.
264. Röhling, Martin, Christian Herder, Theodor Stemper, and Karsten Müssig. "Influence of Acute and Chronic Exercise on Glucose Uptake." *Journal of Diabetes Research* 2016 (2016): 2868652.
265. Viswanathan, M., N. D. Berkman, D. M. Dryden, and L. Hartling. "Ahrq Methods for Effective Health Care." In *Assessing Risk of Bias and Confounding in Observational Studies of Interventions or Exposures: Further Development of the Rti Item Bank*. Rockville (MD): Agency for Healthcare Research and Quality (US), 2013.
266. Margulis, A. V., M. Pladevall, N. Riera-Guardia, C. Varas-Lorenzo, L. Hazell, N. D. Berkman, M. Viswanathan, and S. Perez-Gutthann. "Quality Assessment of Observational Studies in a Drug-Safety Systematic Review, Comparison of Two Tools: The Newcastle-Ottawa Scale and the Rti Item Bank." *Clin Epidemiol* 6 (2014): 359-68.
267. Al-Saleh, M. A., S. Armijo-Olivo, N. Thie, H. Seikaly, P. Boulanger, J. Wolfaardt, and P. Major. "Morphologic and Functional Changes in the Temporomandibular Joint and Stomatognathic System after Transmandibular Surgery in Oral and Oropharyngeal Cancers: Systematic Review." *J Otolaryngol Head Neck Surg* 41, no. 5 (2012): 345-60.
268. 2019, RevMan Web. "Review Manager Web (Revman Web)." The Cochrane Collaboration, 2019.

269. Bracale, R., G. Labruna, C. Finelli, A. Daniele, L. Sacchetti, G. Oriani, F. Contaldo, and F. Pasanisi. "The Absence of Polymorphisms in Adrb3, Ucp1, Pparγ, and Adipoq Genes Protects Morbid Obese Patients toward Insulin Resistance." *J Endocrinol Invest* 35, no. 1 (2012): 2-4.
270. Brondani, L. A., G. C. Duarte, L. H. Canani, and D. Crispim. "The Presence of at Least Three Alleles of the Adrb3 Trp64arg (C/T) and Ucp1 -3826a/G Polymorphisms Is Associated with Protection to Overweight/Obesity and with Higher High-Density Lipoprotein Cholesterol Levels in Caucasian-Brazilian Patients with Type 2 Diabetes." *Metab Syndr Relat Disord* 12, no. 1 (2014): 16-24.
271. Dong, C., Y. Lv, L. Xie, R. Yang, L. Chen, L. Zhang, T. Long, H. Yang, X. Mao, Q. Fan, X. Chen, and H. Zhang. "Association of Ucp1 Polymorphisms with Type 2 Diabetes Mellitus and Their Interaction with Physical Activity and Sedentary Behavior." *Gene* 739 (2020): 144497.
272. Lee, I. H., Y. J. Lee, H. Seo, Y. S. Kim, J. O. Nam, B. D. Jeon, and T. D. Kwon. "Study of Muscle Contraction Induced by Electrical Pulse Stimulation and Nitric Oxide in C2c12 Myotube Cells." *J Exerc Nutrition Biochem* 22, no. 1 (2018): 22-28.
273. Nieuwoudt, S., A. Mulya, C. E. Fealy, E. Martelli, S. Dasarathy, S. V. Naga Prasad, and J. P. Kirwan. "In Vitro Contraction Protects against Palmitate-Induced Insulin Resistance in C2c12 Myotubes." *Am J Physiol Cell Physiol* 313, no. 5 (2017): C575-C83.

Annex 1

University of Thessaly
Department of Physical Education and Sport Science

IInternal Ethics Committee

Trikala: 9/ July / 2012
Protocol Number.: 610

Application for approval of research entitled: Prevalence of UCP1 genetic variants and their connection with obesity and cardio-metabolic disease.

Scientist responsible – supervisor: Dr. Andreas D. Flouris

Main researcher – student:

Institution & Department: FAME Laboratory, Centre for Research and Technology Thessaly, Department of Physical Education and Sport Science, University of Thessaly.

The proposed research relates to a:

Research grant ☐ Postgraduate thesis ☐ Undergraduate thesis ☐ Independent research ■

Contact phone:
Contact email:

The Internal Ethics Committee (IEC) of the Department of PE and Sport Science (DPESS), University of Thessaly, examined the proposal in its 4-3/20-6-2012 meeting and approves the implementation of the proposed research.

The Chair of the IEC – DPESS

Athanasios Tsiokanos, PhD

Annex 2

ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ
ΤΜΗΜΑ ΕΠΙΣΤΗΜΗΣ ΦΥΣΙΚΗΣ ΑΓΩΓΗΣ ΚΑΙ ΑΘΛΗΤΙΣΜΟΥ

Εσωτερική Επιτροπή Δεοντολογίας

Τρίκαλα: 6/12/2017
Αριθμ. Πρωτ:1306

Βεβαίωση έγκρισης της πρότασης για διεξαγωγή Έρευνας με τίτλο: Μελέτη των παραγόντων και των σηματοδοτικών μονοπατιών που ενέχονται στη φαιοποίηση του λευκού λιπώδους ιστού και στην επίδραση της άσκησης στο λιπώδη ιστό

Επιστημονικώς υπεύθυνος-η / επιβλέπων-ουσα: Ανδρέας Φλουρής
Ιδιότητα: Επίτιμος Διδάσκων στη Φυσιολογία του Ανθρώπου
Ίδρυμα: Πανεπιστήμιο Θεσσαλίας
Τμήμα: Τμήμα Επιστήμης Φυσικής Αγωγής και Αθλητισμού

Κύριος ερευνητής-τρια / φοιτητής-τρια: Ελένη Νίντου
Πρόγραμμα Σπουδών: Διδακτορικό δίπλωμα
Ίδρυμα: Πανεπιστήμιο Θεσσαλίας
Τμήμα: Τμήμα Επιστήμης Φυσικής Αγωγής και Αθλητισμού

Η προτεινόμενη έρευνα θα είναι: Διδακτορική διατριβή

Τηλ. επικοινωνίας:
Email επικοινωνίας:

Η Εσωτερική Επιτροπή Δεοντολογίας του Τ.Ε.Φ.Α.Α., Πανεπιστημίου Θεσσαλίας μετά την υπ. Αριθμ. 3-1/6-12/2017 συνεδρίασή της εγκρίνει τη διεξαγωγή της προτεινόμενης έρευνας.

Ο Πρόεδρος της
Εσωτερικής Επιτροπής
Δεοντολογίας – ΤΕΦΑΑ

Τσιόκανος Αθανάσιος
Αναπληρωτής Καθηγητής

Αφαίρεση προσωπικών δεδομένων
(Υπηρεσία Βιβλιοθήκης & Πληροφόρησης Πανεπιστημίου Θεσσαλίας)