The Role of Adipose Tissue in Metabolic Disorders and Molecular Pathways that Affect its Function

Maria Vliora

Supervisors: **Associate Professor Andreas D. Flouris**

Professor Stefania Mitola

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, Greece

Department of Molecular and Translational Medicine, University of Brescia, Italy

June 20th, 2022

Judging Committee Members:

Andreas D. Flouris	Associate Professor, University of Thessaly, Greece
Stefania Mitola	Professor, University of Brescia
Athanasios Jamurtas	Professor, University of Thessaly, Greece
Ioannis Fatouros	Professor, University of Thessaly, Greece
Roberto Ronca	Professor, University of Brescia
George Metsios	Professor, University of Thessaly, Greece
Dimas Konstantinos	Professor, University of Thessaly, Greece

This thesis is part of the PhD program of the Department of Physical Education and Sport Science, University of Thessaly, Greece

and

The PhD Course in Biomedical Sciences and Translational Medicine, of the Department of Molecular and Translational Medicine, University of Brescia, Italy.

Declaration

I, Vliora Maria, 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.

19-06-2022

To Vasilis and Dimosthenis

“The capacity to blunder slightly is the real marvel of DNA. Without this special attribute, we would still be anaerobic bacteria and there would be no music.”

Lewis Thomas

Table of Contents

List of Figures

Abstract (English)

The aim of this thesis is to expand the current knowledge on the role of adipose tissue (AT) on metabolic disorders and to research the molecular pathways that affect its function. In this light, we performed two narrative reviews. Initially, the crosstalk between AT and the vascular system and how this can affect the onset and progression of metabolic diseases was investigated. In a second narrative review, the angiogenic factors that affect AT and pharmacological approaches that could be used in the treatment of obesity and its accompanying co-morbidities were described. Moreover, current bioengineering approaches that could be employed in the fight against obesity and consequent metabolic diseases are suggested. Hence this information led to testing the properties of a new, natural, Histogel-based bioscaffold to support the proliferation and differentiation of white AT to into brown-like AT, a more metabolically active version of fat. As AT is affected by the cytokines of other tissues, irisin, a myokine, and its role on metabolic disorders was explored. Based on accumulating evidence on the implication of irisin in cancer a systematic review was performed, with the aim to elucidate how irisin is involved in the progression of cancer and if it can constitute a valid biomarker for its diagnosis. Finally, the role of irisin on AT and how it affects its metabolic state was investigated. The results of the current thesis suggest that the communication between vascular system and adipose tissue should be considered, when pharmacological approaches are employed for the treatment of metabolic disorders. Moreover, the use of biological scaffolds is suggested, that could result in increased gain of brown adipose tissue (increased metabolic activity) and be part of the treatment

of obesity and accompanying metabolic disorders. The results demonstrate that irisin is involved in cancer by modulating cell proliferation and tumor invasion through several signaling pathways and can also be a good biomarker for the diagnosis of cancer. Finally, the role of irisin in the regulation of lipolysis and thermogenesis *in vitro* is presented. Taken together, these results indicate that adipose tissue is an excellent medium for pharmacological or biomedical interventions for the treatment of metabolic disorders.

Abstract (Italian)

Lo scopo di questa tesi è di espandere le conoscenze attuali sul ruolo del tessuto adiposo (AT) sui disordini metabolici e di ricercare i pathways molecolari che ne influenzano la funzione. In questo contesto abbiamo eseguito due revisioni narrative. Nella prima review abbiamo indagato su come il crosstalk tra AT e sistema vascolare possa influire sull'esordio e sulla progressione dei disordini metabolici. Nella nostra seconda review, abbiamo descritto i fattori angiogenici che influenzano l'AT e gli approcci farmacologici che potrebbero essere utilizzati nella lotta contro l'obesità e le sue co- morbilità associate. Inoltre, proponiamo degli attuali approcci di bioingegneria che potrebbero essere impiegati nella lotta contro l'obesità e i disordini metabolici associati. Queste informazioni ci hanno portato a testare le proprietà di un nuovo, naturale Histogel-based bioscaffold per supportare la proliferazione e il differenziamento del tessuto adiposo bianco in tessuto adiposo brown-like, un tipo di grasso più metabolicamente attivo. Siccome l'AT è influenzato dalle citochine di altri tessuti, noi abbiamo indagato sull'irisina, una miochina, e sul suo ruolo nei disordini metabolici. Basandoci su evidenze crescenti sul ruolo dell'irisina nel cancro abbiamo eseguito una review sistematica con l'obiettivo di chiarire come l'irisina sia coinvolta nella progressione del cancro e se possa costituire un valido biomarker per la sua diagnosi. Infine, abbiamo indagato sul ruolo dell'irisina nell'AT e su come influisca sul suo stato metabolico. I risultati

della presente tesi suggeriscono che la comunicazione tra sistema vascolare e tessuto adiposo dovrebbe essere considerata, quando approcci farmacologici sono impiegati per il trattamento di disordini metabolici. Inoltre, noi proponiamo l'uso di scaffolds biologici nella medicina rigenerativa, che possono risultare nell'incremento di tessuto adiposo bruno (attività metabolica incrementata) ed essere parte del trattamento dell'obesità e dei disordini metabolici associati. Mostriamo inoltre che l'irisina è coinvolta nel cancro modulando la proliferazione cellulare e l'invasività tumorale attraverso alcuni pathways di segnalazione cellulare e che può anche essere un buon biomarker per la diagnosi tumorale. Infine, mostriamo che l'irisina può regolare lipolisi e termogenesi *in vitro*. Complessivamente, questi risultati indicano che il tessuto adiposo è un medium eccellente per interventi farmacologici o biomedicali per il trattamento di disordini metabolici.

Abstract (Greek)

Στόχος της παρούσας διατριβής είναι η διεύρυνση της τρέχουσας γνώσης σχετικά με το ρόλο του λιπώδους ιστού στις μεταβολικές διαταραχές και η έρευνα των μοριακών μονοπατιών που επηρεάζουν τη λειτουργία του. Υπό αυτό το πρίσμα, πραγματοποιήθηκαν δύο βιβλιογραφικές μελέτες. Αρχικά, διερευνήθηκε πώς η αλληλεπίδραση μεταξύ του λιπώδους ιστού και του αγγειακού συστήματος μπορεί να επηρεάσει την εμφάνιση και την εξέλιξη των μεταβολικών ασθενειών. Στη δεύτερη ανασκόπησή που πραγματοποιήθηκε, περιγράφονται οι αγγειογόνοι παράγοντες που επηρεάζουν το λιπώδη ιστό και οι φαρμακολογικές προσεγγίσεις που θα μπορούσαν να χρησιμοποιηθούν στη θεραπεία της παχυσαρκίας και των συνοδών νοσηροτήτων της. Επιπλέον, προτείνονται τρέχουσες προσεγγίσεις της εμβιομηχανικής που θα μπορούσαν να χρησιμοποιηθούν για την καταπολέμηση της παχυσαρκίας και των συνακόλουθων μεταβολικών ασθενειών. Αυτές οι πληροφορίες οδήγησαν στη δοκιμή των ιδιοτήτων ενός νέου, φυσικού, βιολογικού ικριώματος με βάση το Histogel που υποστηρίζει τον πολλαπλασιασμό και τη διαφοροποίηση του λευκού λίπους σε μπεζ, μια μεταβολικά πιο ενεργή εκδοχή του λίπους. Καθώς ο λιπώδης ιστός επηρεάζεται από τις κυτοκίνες άλλων ιστών, ερευνήθηκε η ιρισίνη, μια μυοκίνη, και ο ρόλος της στις μεταβολικές διαταραχές. Με βάση τη βιβλιογραφία σχετικά με το ρόλο της ιρισίνης στον καρκίνο, πραγματοποιήθηκε μια συστηματική ανασκόπηση της βιβλιογραφίας, με στόχο να διευκρινιστεί

πώς η ιρισίνη εμπλέκεται στην εξέλιξη του καρκίνου και εάν μπορεί να αποτελέσει έγκυρο βιοδείκτη για τη διάγνωσή του. Τέλος, διερευνήθηκε ο ρόλος της ιρισίνης στο λιπώδη ιστό και πώς επηρεάζει τη μεταβολική του κατάσταση. Τα αποτελέσματα της παρούσας διατριβής προτείνουν ότι η επικοινωνία μεταξύ αγγειακού συστήματος και λιπώδους ιστού θα πρέπει να λαμβάνεται υπόψη, όταν χρησιμοποιούνται φαρμακολογικές προσεγγίσεις για τη θεραπεία μεταβολικών διαταραχών. Επιπλέον, προτείνεται η χρήση βιολογικών ικριωμάτων, που θα μπορούσαν να οδηγήσουν σε αύξηση του καφέ λιπώδους ιστού (αυξημένη μεταβολική δραστηριότητα) και να αποτελούν μέρος της θεραπείας της παχυσαρκίας και των συνοδών μεταβολικών διαταραχών. Φαίνεται επίσης ότι η ιρισίνη εμπλέκεται στον καρκίνο, ρυθμίζοντας τον κυτταρικό πολλαπλασιασμό και την μετάσταση των όγκων, μέσω διαφόρων σηματοδοτικών μονοπατιών και μπορεί επίσης να είναι ένας καλός βιοδείκτης για τη διάγνωση του καρκίνου. Τέλος, φαίνεται ότι η ιρισίνη μπορεί να ρυθμίσει τη λιπόλυση και τη θερμογένεση *in vitro*. Συνοψίζοντας, τα παραπάνω αποτελέσματά δείχνουν ότι ο λιπώδης ιστός είναι ένα εξαιρετικό μέσο για φαρμακολογικές ή βιοϊατρικές παρεμβάσεις σχετικά με τη θεραπεία των μεταβολικών διαταραχών.

Acknowledgements

During this PhD journey, I was extremely lucky that many people have crossed my way, contributing to this thesis, and making it possible. Looking back to the valuable lessons I have been taught from all you, to the highs and lows that we have shared, and to how much you have all helped me to grow as a person, I need to thank you for being part of this wonderful journey.

Most importantly, finishing this PhD thesis would not have been possible without the immense support of my two supervisors Andreas Flouris and Stefania Mitola.

Andreas, your belief in me was a huge source of motivation to gain some trust in myself. Thank you for the freedom of time, the freedom of choice and mostly the freedom of mind that you have offered me. Science is much different since we have met.

Stefania, I cannot thank you enough for your ever lasting enthusiasm and optimism whenever I presented to you the craziest or weirdest plans, either for my thesis or my life. Thank you for always having an open door in your lab for me, whenever I popped in to perform more experiments.

During the past few years, I had the chance of being part of two very different but equally skilled and unique research teams. I feel so honoured to have been encouraged, inspired, and tolerated by the wonderful people of these two teams.

Thank you, present and past FAME Lab members for all the memories that we have created together. During the past years we've been through easy and tough, but we were always finding a way to gain from all situations. The experiences I've had with you and the friends that I've made, I will treasure for life.

Thank you, Betta, Michela, Cosetta, and Margherita for making my time in Brescia constructive, memorable, and fun. I hope it was not very annoying when I was chasing you around the lab asking desperately for help and I really thank you for always being there to provide it.

Out of the “science world”, there have been many people that have fueled me with passion and inspiration for completing this thesis.

Thank you, Nikos, Akis, Gina and Dimitris. We all started as molecular biologists 18 years ago. Today, you are my extended family that supports me in any choice that I make in life, and I can always depend on.

Thank you Evi, Themis, Kristi, Dimitris, Lia, and Dimitris for being a great distraction from science and constantly giving me reasons to admire you for the most unexpected things.

Grandmother Maria, you will never read these lines but want to thank you, because my choices in life would have never been the same without you in it.

Last, but not least, I would like to thank my family. Thank you, Dad and Brother for always believing in me, for giving me all the support that I could ever ask for and for always being there for me. You are the best!

Maria Vliora

June 19, 2022

Chapter 1

Introduction

The prevalence of obesity worldwide has more than tripled over the past 50 years with a parallel increase in metabolic disorders [1]. Today, according to the World Health Organisation it affects nearly 60% of adults and almost one in three children in Europe [2]. Several clinical studies link the chronic inflammation caused by obesity with metabolic disorders - such as cardiovascular disease, type 2 diabetes, and cancer - and organ tissue complications [2-5]. The main tissue linked with obesity is adipose tissue (AT) [6]. The AT is an active, endocrine organ which secrets multiple humoral factors, adipokines, which are responsible for important metabolic effects [7].

The AT is characterized by high plasticity which allows it to constantly expand and relapse depending on the energy needs or metabolic activity of the organism [8]. Today, five types of adipocytes have been described namely, white, brown (BAT), beige or brown-like, pink (residing in the mammary gland), and bone marrow adipocytes [9-11]. Bone marrow and pink adipocytes seem to have very distinct gene expression patterns and not much literature is available for their metabolic characteristics. On the other hand, white, brown, and beige adipose tissues have received a lot of attention, especially after 2001 when brown adipose tissue was shown to hold an important role to non-shivering thermogenesis [12]. The roles and function of these three types of adipose tissue are described thoroughly in the following chapters.

The AT is highly vascularised which means that it can easily communicate with other tissues in the human body [13]. The vascular network constantly remodels its blood vessels according to the secreted growth factors and metabolites produced by the adipocytes to foster the crosstalk between AT and the other organs [8, 14-16]. This communication requires the crosstalk between AT and the vasculature in the first place.

Angiogenesis is involved in multiple metabolic disorders where AT appears to remodel by altering the metabolic activity of adipocytes [17]. This remodeling is facilitated, among other angiogenic factors, by the vascular endothelial growth factor (VEGF) [18]. The VEGF is a family of proteins, expressed in several cell types, including adipocytes, and stimulates *de novo* formation of circulatory paths and growth of blood vessels [19]. This suggests that AT has an autoregulatory function for angiogenesis, by contributing to the vascular remodeling depending on its metabolic needs [20].

The interplay between these two tissues has generated a lot of interest for the development of alternative treatment approaches other than pharmacological factors. The use of bioscaffolds in regenerative medicine is an approach that has been in use for many years [21]. The use of bioscaffolds for grafting adipocytes that can proliferate into beige adipose tissue with increased metabolic activity could be an excellent approach [22]. Increasing the metabolic rate of obese individuals could lead to efficient body weight loss and eliminate the effects of metabolic disorders.

Cancer is a disorder that rewires the metabolic program of tumors and exceeds the bio energetic and biosynthetic demands of continuous cell growth

[23]. It is the second leading cause of death worldwide and can affect all the different tissues in humans [24]. The WHO, in their latest published report, mention that at least 13 different types of cancer have been linked with obesity [2]. Thus, examining cancer traits along with adipose tissue metabolic activity could be a valid approach in understanding the mechanisms underlying the onset progression of cancer. Indeed, many adipokines have been under consideration for their use as potential therapeutic and diagnostic agents for cancer the past years [25]. Irisin, an adipo-myokine, is one of them and the last years a lot of studies have focused on its involvement in cancer [26].

Irisin was first found to be expressed in skeletal muscle as a cleaved product of fibronectin type III domain-containing protein 5 (FNDC5) after physical activity [27]. Since then, it has been shown that it is also expressed in other tissues as well, such as adipose tissue and cardiac muscle [28]. Irisin is a glycosylated peptide consisting of 112 amino acids, its molecular mass is ≈12kD [27, 28], and has also been shown that can be found as a dimer [29]. Four isoforms of irisin have been discovered with tree of them missing small sequences of amino acids at different positions [30]. These different modifications and isoforms of irisin that have been described, have created controversy over the years about how irisin can properly be detected in the different samples [31]. Today there is accumulating literature on irisin's implication on several types of cancer but still the mechanisms of its action on the different tissues have not been elucidated.

The aim of this thesis is to expand the current knowledge on the role of AT on metabolic disorders and to explore molecular pathways that can affect its function. It is comprised of 8 Chapters. In **Chapters 2 and 3**, the results of two narrative

reviews are described. In the first, the relationship between adipose tissue and the vasculature is described as well as the role of VEGFs in metabolic disorders. In the second review, we describe the angiogenic factors that are being or could be used as pharmacological treatments for metabolic diseases. We also describe the existing literature about bioscaffolds that can potentially be used as vehicles for grafting proliferating adipocytes in obese individuals. In **Chapter 4** we describe the results of testing a Histogel-based bioscaffold in rodents to identify if its properties make it suitable for future use against obesity and other metabolic disorders.

In **Chapter 5** the results of a systematic review of the literature and meta-analysis are presented, considering the implication of irisin in different types of cancer. Information from multiple databases about irisin and its potential use as a biomarker for cancer was collected, while also the literature was screened for possible implication of irisin in tumor progression and metastasis. According to the results, irisin appears to be a valid biomarker in diagnosis of cancer. Moreover, it can affect the progression of the disease through multiple signaling pathways, as discussed in the review.

In **Chapter 6** an attempt to elucidate the mechanism of action of irisin on adipose tissue is made. Thus, a cell line model for adipose tissue was used, NIH-3T3-L1 cell line, treated irisin for different time periods. It is demonstrated how irisin can alter the cellular respiration of these adipocytes and that it modulates certain signaling pathways to regulate the expression of uncoupling protein 1 (UCP1) which is the main protein that induces thermogenesis in adipose tissue. Finally, it is also demonstrated that irisin can regulate the expression of lipogenic genes in 3T3-L1 adipocytes.

In **Chapter 7**, the results are collectively discussed in conjunction with other published data to answer the main research question of this thesis on "What is the role of adipose tissue in metabolic disorders?". **Chapter 8** summarizes the main findings of the studies that were performed.

Chapter 2

Role of VEGFs in metabolic disorders

M di Somma [1], M Vliora [1 2], E Grillo [1], B Castro [3], E Dakou [4], W Schaafsma [3], J Vanparijs [4], M Corsini [1], C Ravelli [1], E Sakellariou [2], S Mitola [5]

1Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.

2FAME Laboratory, Department of Exercise Science, University of Thessaly, Trikala, Greece.

3Histocell, S.L.Parque Tecnológico 801A, 2º, 48160, Derio, Bizkaia, Spain.

4Laboratory of Cell Genetics, Department of Biology, Faculty of Science and Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium.

5Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.

Conception and design of the work: S.M., M.dS., M.V., Data collection: M.dS., M.V., Data analysis and interpretation: M.dS., M.V., E.G., Drafting the article: M.dS., M.V., Critical revision of the article: S.M., E.G., B.C., E.D., W.S., J.V., M.C., C.R., E.S., S.M., Final approval of the version to be published: E.G and S.M.

This work has been published on May 2020, in Angiogenesis Journal, under doi: 10.1007/s10456-019-09700-1 .

Keywords: Adipose tissue; Angiogenesis; BAT; Obesity; VEGF; WAT.

Abstract

Obesity and metabolic disorders are important public health problems. In this review, the role of vasculature network and VEGF in the adipose tissue maintenance and supplementation is discussed. Angiogenesis is a key process

implicated in regulation of tissues homeostasis. Dysregulation of new blood vessels formation may be crucial and contribute to the onset of several pathological conditions, including metabolic syndrome-associated disorders. Adipose tissue homeostasis is fine regulated by vascular network. Vessels support adipose structure. Vasculature modulates the balance between positive and negative regulator factors. In white adipose tissue, Vascular endothelial growth factor (VEGF) controls the metabolic activities of adipocytes promoting the trans-differentiation from white to beige phenotype. Trans-differentiation results in an increasing of energy consumption. VEGF exerts an opposite effect on brown adipose tissue, where VEGF increases oxygen supply and improves energy expenditure inducing the whiting of adipocytes.

Introduction

The vascular network is essential to remove catabolic products and supply nutrients and oxygen for the function, development, and homeostasis of tissues. Blood vessels are formed by two highly regulated process called “vasculogenesis and angiogenesis” which play a central role in embryonic development. Angiogenesis is also involved in adipose tissue (AT) remodelling, bone repair, wound healing, inflammation, and tumour growth [32, 33]. The tight balance between pro- and anti-angiogenic factors maintains the homeostasis of blood vessels and controls the formation of new ones. When the number of signals such as metabolic stress, immune and inflammatory response, hypoxia-inducible factor (HIF) and angiogenic activators increase compared to the inhibitors, the balance is tipped in favour of blood vessels growth (angiogenic

switch) [34]. The amount of pro- and anti-angiogenic molecules is regulated by miRNA [35], by the alternative splice of pre-mRNA of the key genes (such as VEGF-A, VEGFR1, VEGFR2) and by the epigenetic processes [36]. In particular, the alternative splicing produces multiple isoforms of key genes which can have distinct and opposite functions alternative splicing mechanisms [37].

Several evidence have shown the involvement of angiogenesis in metabolic syndrome-associated disorders, such as insulin resistance, hypertension, dyslipidemia, and obesity [38]. In these pathological conditions, the adipose tissue (AT) rapidly and dynamically remodels itself with changing the number, the size, and the metabolic rate of adipocytes (creating a hypertrophy and hyperplasia condition) [17] and induces a hypoxic micro-environment [39]. AT rearrangement is driven by abnormal expression of adipokines [40] and/or multiple angiogenic factors such as free fatty acids (FAs) [41], leptin [42], metalloproteinases (MMPs) [43] and growth factors including VEGF [18].

VEGF/VEGFR2 system

VEGF family comprises seven members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and placental growth factor (PlGF) [44]. Vegf gene expression is influenced by a high number of factors, such as hypoxia, growth factors, cytokines, miRNA, alternative splicing, and epigenetic process [35, 37, 45-48]. VEGFs play an important role in several physiologic and pathologic events, including the induction of vascular leak and permeability, the endothelial

cells proliferation [49], the neuroprotection [50, 51], the haematopoiesis [52] and the differentiation of dendritic cell [52, 53].

VEGF activities are mediated by three specific tyrosine kinase receptors (VEGFR-1, 2 and 3) [54] and by their co-receptors such as neuropilin-1 [55]. VEGFR2 activation is modulated by productive engagement of integrin receptors [56-58], inducing a long-lasting activation of VEGFR2 [57, 58]. Binding of growth factors induces the receptor dimerization, trans-autophosphorylation and protein kinase activation which in turn triggers the fine modulation of intracellular signalling pathways [59, 60].

VEGFR-1 is typically expressed by monocytes, macrophages, haematopoietic stem cells and endothelial cells [61]. VEGFR-1 activities have not been completely characterized, probably due to the lack of highly sensitive assays. Many authors suggest VEGFR-1 to play a negative role in the endothelial cell activation acting as a decoy receptor for VEGF-A [62]. It is worth mentioning that both VEGFR-1 and its soluble form have a higher affinity for VEGF-A compared to VEGFR-2 [54]. PlGF and VEGF-B bind only to VEGFR-1 and show no involvement in embryonic vascular development or physiological angiogenesis in the adult. However, both PlGF and VEGF-B play a role during inflammatory responses and in pathological vascular-dependent diseases including diabetic retinopathy, obesity, and diabetes [63-66]. VEGFR-2 is considered the most active VEGF-receptor in the regulation of many cell and tissue functions such as proliferation, migration, permeability, differentiation, and morphogenesis [49, 67]. It binds several canonical ligands (VEGF-A, VEGF-E, VEGF-C and VEGF-D [61]) with lower affinity in relation to VEGFR-1.

Also, VEGFR2 binds non-canonical ligands (gremlin [59], HIV-1-TAT protein [68]).

Under physiological condition, VEGFR-2 is expressed by vascular and lymphatic endothelial cells [69], neuronal cells and astrocytes, osteoblasts [70], retinal progenitor cells [71], megakaryocytes and haematopoietic cells [72], regulating different biological processes [62, 73]. On the other hand, chemokines and inflammatory stimuli mediate the expression of VEGFR2 into different types of malignancies including melanomas [74], haematological malignancies [75], and breast cancer [76] as well as in diabetic retina [77].

Adipose tissue and its vasculature network

AT is not only an organ specialized in energy storage. In 1987, it was identified as the major site of sex steroid metabolism [78]. Sequentially, the discovery of the cytokine-like factor leptin [79] suggested that the AT transmits information from periphery to central nervous system secreting hormones, called adipokines. Adipokines, such as adiponectin, resistin, tumour necrosis factor α (TNFα), visfatin and obestatin have auto-endocrine, paracrine and endocrine functions. By adipokines production, the AT influences systemic metabolic homeostasis [80].

AT is the most plastic organ in human body, subjected to continuous expanding and regressing. This plasticity requires constant growth, regression, and remodelling of blood vessels, under the control of several metabolites secreted by AT itself. AT is highly vascularized and can be considered a good source for

the isolation of endothelial cell [67]. AT consists of mature adipocytes, surrounded by stromal tissue that consists of different cell types such as preadipocytes, fibroblasts, pericytes, macrophages, adipose stem cells (ASCs) and endothelial cells [43].

In adults, most tissues are in a homeostatic state and normally do not grow; their mass is stable, and the supporting vasculature is quiescent. As previously mentioned, AT is characterized by high plasticity which makes it a perfect model to study pathological conditions and the role of angiogenesis in adult tissue remodelling [81]. The literature suggests that adipocytes, and probably other cell types in the adipose microenvironment, produce at the same time pro- and anti-angiogenic factors which play a central role in the maintenance of vascular homeostasis and vascular regression [8]. The adipose vasculature supports AT in multiple manners. Vascular network provides nutrients and oxygen which is essential for tissue maintenance and removes metabolic products of AT. Indeed, vessels lead the transport of growth factors, cytokines and hormones for modulating adipocyte maintenance and functions. Moreover, they export the AT-derived growth factors, adipokines and cytokines from AT to body tissues regulating physiological functions via endocrine mechanism [63]. Furthermore, vessels facilitate the infiltration of monocytes and neutrophils into the AT [82], which affects adipocyte functions. Inflammation alters the adipose microenvironment producing hypoxic conditions and acidosis that modulates pre-adipocyte differentiation, AT mass and function.

In mammals, two types of AT have been observed, white adipose tissue (WAT) and brown adipose tissue (BAT). Moreover, a cluster of metabolically active

adipocytes can develop in WAT in response to various stimuli. These adipocytes are known as beige or 'brite' (brown in white) adipocytes [83].

Adipose stromal cells in adipose tissue

Within AT, adipocytes originate from the differentiation of adipose stromal cells (ASCs) [84] enabling adipocyte turnover to allow their number to be relatively stable regardless of body fat mass evolution [85]. ASCs, similarly to mesenchymal stem cells (MSCs), display self-renewability, the multipotency to differentiate into diverse tissues [86], and paracrine and immunomodulation properties [87, 88]. ASCs can be expanded in culture for several passages: the adherent derived cell population maintains its mesenchymal phenotype [89]. Several *in vitro* protocols have been described for the differentiation of ASCs into adipogenic lineage [90], osteogenic, chondrogenic and myogenic cells. Although the adipogenic differentiation of ASCs is cost and time consuming, and it eventually yields only low differentiation rates their compared to the mature adipocytes [91], they are easier to handle and may be grown *in vitro* almost infinitely.

ASCs are available in high amounts, are easily accessible and AT removals show little donor site morbidity and are safer and less invasive compared to other biopsies (e.g., bone marrow aspiration). Thus, ASCs become interesting for tissue replacement in Regenerative Medicine to treat severe burn wounds, [92] or in aesthetic plastic surgery and reconstruction surgery. Also, ASC models would help to gain new insights into the development and causes of adipose tissue-related diseases like obesity and diabetes. Obesity-induced

adipocyte death and malfunctioning is responsible for the initiation of a chronic inflammation state in adipose tissue, which underlies the pathophysiology of obesity- and ageing-associated metabolic complications. A high fat diet can induce the release of ASCs [93], from subcutaneous AT, into the blood stream. This specific subpopulation of ASCs driven by CXCL12/CXCR4 axis infiltrates and originates ectopic adipocytes into different sites [94-96].

The role of ASCs in maintaining and remodelling the vascular network also in AT is still a point of discussion. ASCs and ASC-derived adipocytes produce angiogenic molecules. Although ASC-derived adipocytes produce low level of VEGF-A with respect to mature adipocytes, they release a higher amount of VEGF-D and VEGF-C sufficient to support the angiogenic process [96]. Also, hypoxia induces ASCs to release exosomes enriched in VEGF, epidermal growth factor (EGF), fibroblast growth factor (FGF) and their receptors (VEGF-R2, VEGF-R3), and monocyte chemoattractant protein 2 (MCP-2) and MCP-4 [97]. All these proteins can modulate neo-angiogenesis and tissue survival also in AT. Of note, ASCs obtained from diabetic patients or from diabetic rodent models of T2DM have been reported to show impaired angiogenic potential [98, 99].

Several papers point out the ability of ASCs to differentiate into endothelial cells [100], directly contributing to neovascularization. Others show that also in the presence of VEGF ASCs do not develop EC phenotype and do not exhibit tubule forming capabilities in Matrigel tube assay formation [101]. This discrepancy may be due to the heterogeneity of ASCs. Indeed, ASCs are characterized by a mix of progenitor cells. Understanding better the

heterogeneity of ASCs may lead to the development of improved protocols of cell isolation and optimized cell therapy clinical protocols [102].

Vessels support the continuous rearrangement of white adipose tissue

WAT composes around 20% of the total body weight in men and 25% in women [103]. Its main function is to store energy as FAs that are released when the organism needs energy supplementation. White adipocytes are characterized by round shape with large diameter and by the presence of a big lipid droplet in the middle. The lipid droplet pushes all the other components of the cells to the rim and a low number of mitochondria can be observed next to the nucleus and the ribosomes [103]. WAT exists in several areas of neonatal and adult human body and can be divided into two general categories: subcutaneous depot, which is located subcutaneously in the abdomen and thighs, and visceral depot, which is in the abdominal area surrounding vital organs [104].

WAT is supported by a high number of blood vessels and constantly undergoes expansion or shrinkage depending on the balance between energy uptake and consumption of the host [63]. The vascularized area undergoes continuous remodelling and formation of new vessels, and thus facilitates the storage of fatty acids and energy, supporting tissue expansion [81]. In foetal stage, adipose tissue development is characterized by the formation of primitive fat organ composed by vascular structures and few adipocytes. Human preadipocytes secrete PAI-1 inducing a coordinate migration of adipocytes and endothelial cells. In primitive fat organ, adipocytes migrate following the recruited and/or novel formed capillaries during angiogenesis. Furthermore,

PAI-1 ensures the coordination of adipogenesis and angiogenesis in the microenvironment [105, 106].

Specific markers of white adipocytes have been identified in both mice and human, such as homeobox genes (Hox genes, including c8-b9) [107], leptin and amino acid transporter 1 (Asc-1) [108].

Vasculature function in brown adipose tissue

BAT regulates thermogenesis, a process responsible for producing heat in organisms [109]. BAT represents 1% of the body weight in newborn human (around 30 g of BAT) and helps maintaining the body temperature without shivering [110, 111]. BAT can be detected between the shoulder blades, surrounding the kidneys, around the neck, in the supraclavicular area and along the spinal cord [112].

Several evidence showed that BAT regress after the early stages of life. Recently, nuclear medicine studies have shown the presence of metabolically active AT with energy dissipating properties in the supraclavicular, para-spinal, mediastinal and neck regions in adult humans. The amount of BAT is inversely correlated with body fat, and it is sensitive to thermal stimulation [113]. An inverse correlation between BAT activity and average outdoor temperature, beta-blocker medication use, and body-mass index (BMI) has also been reported [111].

The main component of BAT is brown adipocytes. Brown adipocytes derive prenatally from a subset of dermomyotome progenitor myogenic factor 5 (Myf5+) cell lineage. Brown adipocytes are characterized by small lipid droplets,

high number of mitochondria, responsible for the brown colour, central nucleus, and underdeveloped endoplasmic reticulum [114, 115].

BAT is detected in vascularized depots and is densely innervated by the sympathetic nervous system; the nerve fibres directly synapse onto brown adipocytes. Cold, sensed by the central nervous system, stimulates the secretion of noradrenaline which interacts with β3-adrenergic receptors on brown adipocytes. Noradrenergic stimulation activates BAT thermogenic capability, protecting mammals against obesity [116]. Sympathetically activated brown adipocytes (either by thermic stimuli or excess food intake) mobilize FAs from stored triglycerides and increase the release of chemical energy, derived from beta-oxidation. An important feature of the brown adipocytes is the substantially low expression of ATP synthase and the high expression of thermogenin (UCP1, uncoupling protein 1). UCP1 is a fatty acid anion/H + symporter, expressed on the inner membrane of mitochondria, which uncouples the proton gradient, releases heat and reduces ATP synthesis [117]. The phosphate groups of purine nucleotides usually inhibit UCP1 activity. In BAT, the inhibitory effect of purine nucleotides on UCP1 is overridden by the binding of long chain fatty acids (LCFAs) to the cytoplasmic side of UCP1 [118, 119].

Although vasculatures in WAT and BAT share the above-mentioned common functions, the ultimately functional consequence is dependent on the metabolic status of adipocytes. While in WAT vessels regulate the endocrine functions of adipocytes, angiogenesis in BAT increases the oxygen supply and thereby fuel the process of energy consumption, leading to a lean phenotype [120].

Browning of white adipose tissue

Recently, certain adipocytes that express markers of both WAT and BAT have been identified. These cells are called “beige” or “brite” adipocytes. Beige adipocytes are embedded into WAT depots and derived from the trans-differentiation of white-to-brown adipocytes; a process called “browning” [121]. The increase of energy consumption due to cold exposure [122] or exercise [123] promotes the browning process. Several transcriptional and epigenetic regulators are also required for promoting beige adipocyte biogenesis, in response to various environmental stimuli [124]. In contrast to the brown adipocytes present in BAT depots, beige adipocytes that developed in WAT via the browning process come from Myf5− cells that more closely resemble to the white adipocyte precursors [125]. *In vitro* studies suggest the presence of Myf5− mesenchymal precursor cells in WAT depots, which can differentiate into brown adipocytes in response to thermogenic stimuli or β3-adrenergic stimulation [126]. These cells share characteristics of both brown and white adipocytes. In resting condition, beige adipocytes display the morphology of white adipocytes, with large depot of fat stored as a single lipid droplet, while after stimulation their morphology changes [127, 128]. Beige adipocytes express typical BAT proteins like UCP1, PGC-1α (proliferator-activated receptor-co-activator 1-α), PRDM16 (PR domain zinc finger protein 16) and several bone morphogenetic factors (BMPs) [129, 130]. The expressions of transcriptional factors including Cited1, Tmem26, Tbx1, Epsti1 [131] and proteins associated with inflammatory pathways such as CD40 and CD137 are

typical of beige adipocytes and they can be used to distinguish them from brown adipocytes [127, 132].

PGC-1α is highly expressed in tissues where mitochondria are abundant and oxidative metabolism is active, such as BAT, heart and skeletal muscle [122]. It plays an important role in the regulation of thermogenesis, activating the expression of UCP1 and mitochondrial enzymes of the respiratory chain. PGC-1α gene transcription is induced by cAMP-responsive elements and PPARα, in response to adrenergic stimulus in BAT [133]. The PGC-1α expression requires the binding of PRDM16 to the PGC-1α promoter at the PPARα-binding site. PRDM16 has been identified as the key molecular switch that determines the development of brown adipocytes during embryonic development and the presence of beige adipocytes in WAT depots in adult mice. PRDM16 is required for the full thermogenic activation of PGC-1α expression in BAT [134, 135].

VEGF controls the uptake of fatty acids

VEGF-A is highly expressed in AT and its expression increases significantly during adipocyte differentiation [136]. Recently, it was identified an adipocyte precursor cell population (ASCs) within WAT and BAT, as the cell type expressing the highest levels of VEGF-A, with the higher expression in BAT compared to WAT [137]. VEGF-A represents an ASCs promoter for endotheliogenesis and improves proliferation, migration, and cell attachment through VEGFR2 signalling [138]. Due to this effect, several times ASCs were considered for stem cell-based therapies in the field of vascular medicine [139] (Fig. 2.1.a). As previously reported, the endothelium is fundamental for several

hormone, cytokine, and growth factor exchanges across different compartments. One of the most important roles of the endothelium in AT is the transport of LCFAs under the control of VEGF-A and VEGF-B [140]. As described above, the expression of respiration chain genes is transcriptionally controlled by PGC-1α, which also regulates the expression of vegf-b and several of the LCFA transporters [27]. In the FA uptake, three groups of proteins are implicated: fatty acid transport protein (FATPs), the scavenger receptor CD36 and the intracellular fatty acid binding proteins (FABPs). FATP3 and 4 allow the FAs are imported from the blood steam to the ECs under the control of VEGF-B/VEGFR-1/Neuropilin1 system. FATP4 is mainly expressed in the adipocytes under VEGF-B induction [140] and mediates the FA uptake into the adipocyte. FABP4 (adipose-type FABP) acts as intracellular FA handling protein and its expression is induced by VEGF-A/VEGFR2 signalling (Fig. 2.1.b). Nevertheless, the FA importing mechanism in adipocytes remains unexplored [141].

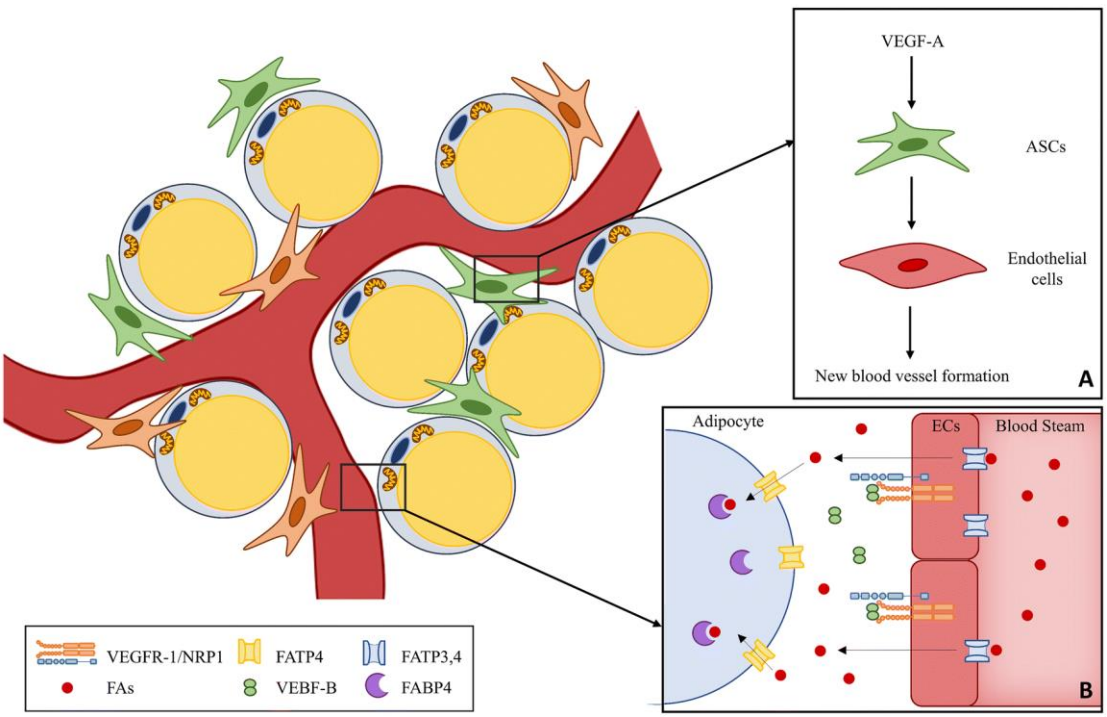


Figure 2.1. VEGF-A and B sustain adipose tissues metabolism. Adipocytes, pericytes (red) and adipose stem cells (green) are supported by blood vessels. VEGF-A promotes the endothelial-differentiation of adipose stem cells, allowing new vessel formation (a). The VEGF-B/VEGFR1 system regulates the fatty acid availability for adipocytes through endothelium. FATP (fatty acid transport protein) 3 and 4 are essential for the FAs transport across the endothelium. Also, adipocytes use FATP4 for FA uptake and the intracellular FABP (fatty acid bind protein) 4 binds internalized FA. VEGF-A/VEGFR2 system promotes FA internalization in the adipocyte (b)

Role of VEGF in WAT

In WAT, a shift to an angiogenic phenotype increases the energy deposition resulting in AT expansion (Fig. 2.2.a). Additionally, vasculature in healthy WAT plays an essential role in controlling the endocrine activities of adipocytes, modulating the release and the transport of adipokines and hormones. Like micro vessels in various endocrine organs, the adipose vasculature contains vasculature fenestrations that are crucial for maintaining the physiological functions of endocrine tissues [142].

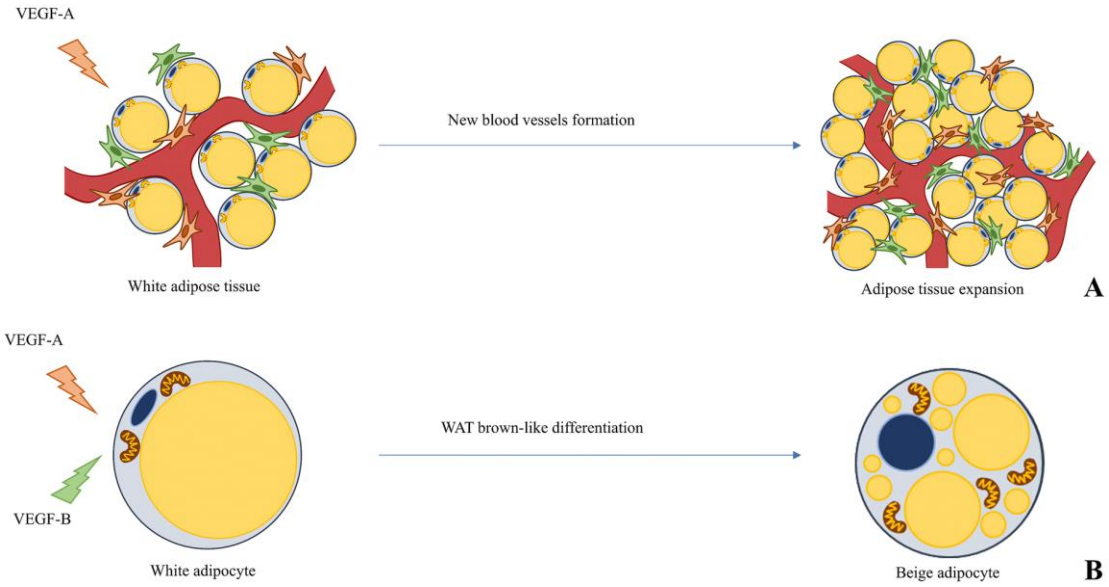


Figure 2.2. VEGF-A and B exert converse effects on WAT. Pro-angiogenic phenotype facilitates the energy deposition resulting in AT expansion. Vessels control the release of adipokine and hormones in WAT (a). VEGF-B modulates mitochondrial thermogenic activity promoting the browning of white adipocytes (b)

The alteration of VEGF-A/VEGFRs system exerts different effects on AT metabolism *in vivo*, depending on the eventually pre-existence of obesity [143]. Inhibition of the VEGF-A/VEGFR-2 system at the beginning of a high fat diet causes exacerbation of metabolic alterations in mice; in contrast, the same inhibition in leptin-deficient (ob/ob) obese mice causes reduction of body weight, improvement of insulin sensitivity and decrease of inflammatory factors [144]. In obese individuals, active adipose angiogenesis in WAT is likely to further promote obesity [145]. Further studies are required to understand the molecular mechanisms leading the role VEGF in the regulation of energy homeostasis [27, 125-144]

In addition, the VEGF-A overexpression in AT (combined with cold exposure and thus β-adrenergic stimulation) induces WAT browning, which increases energy expenditure and beneficially improves metabolism [146]. VEGF-A is probably one of the most potent “browning” factors described [147] and regulates adipocyte differentiation independently of VEGFR1 and VEGFR2. These different activities of VEGF-A may rely on different signalling mechanisms, which remain to be fully elucidated [148]. Moreover, the upregulation of VEGF-B compensates the VEGF-A downregulation, leading to brown-like WAT differentiation [144]. As described in the next chapter, VEGF-B is tightly connected with mitochondrial thermogenic activity (Fig. 2.2.b).

VEGF-A and VEGF-B regulate metabolic activity of brown adipose tissue

Angiogenesis in BAT increases the oxygen supply and improves energy expenditure, leading to a lean phenotype [120] (Fig. 2.3.a). In obese patients, with a high amount of WAT, the inhibition of angiogenesis may represent a therapeutically treatment to avoid the increase of fat mass [146]. As mentioned above, BAT expresses higher amount of VEGF-A compared to WAT, produced by ASCs within both AT types [137].

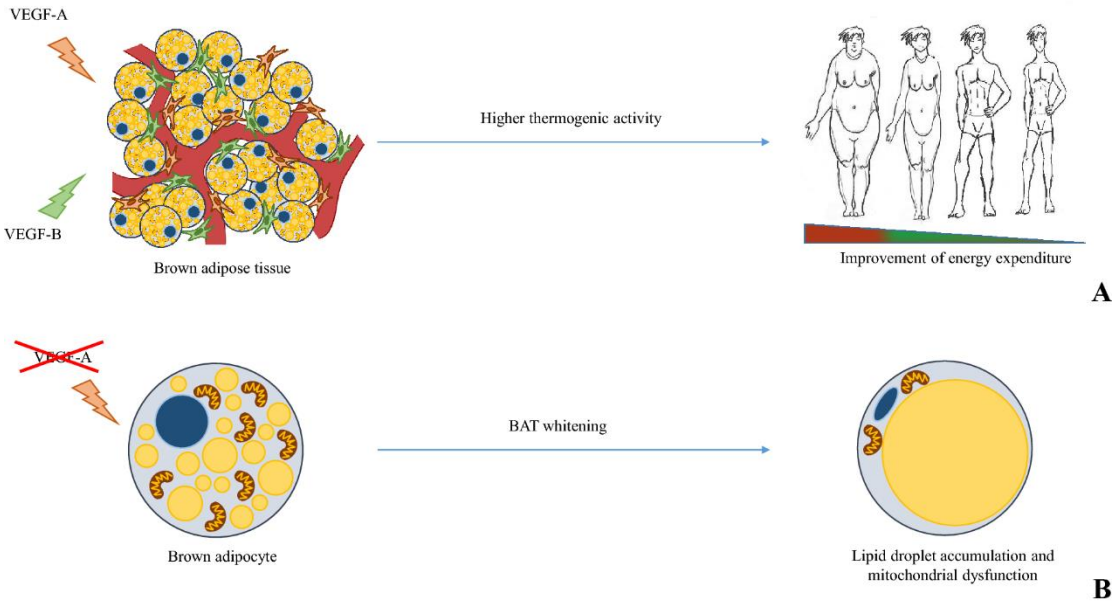


Figure 2.3. VEGF-A and B induce a lean phenotype. VEGF-A-induced angiogenesis increases oxygen supply and improves energy expenditure in BAT. VEGF-B exerts a thermogenic activity in brown adipocytes. In brown adipocytes, PGC-1α transcription factor controls both the expression of mitochondrial genes and of VEGFs (a). VEGF-A reduces blood vessels and alters lipid drop architecture promoting the whitening of BAT (b)

The ablation of endogenous Vegf-a in mice causes decreased vascularity and disrupted lipid architecture in BAT. It is observed as WAT-like lipid droplet accumulation and mitochondrial dysfunction (described as “BAT whitening”) [149] (Fig. 2.3.b). Consequently, these mice are unable to respond to cold, indicating a VEGF-A dependence to the thermogenic activity. Also, VEGF-A stimulates the proliferation of PDGFRα + precursor cells promoting the expression of Prdm16 via p38MAPK pathway [146]. Prdm16 binds and modulates the activity of other transcriptional factors, including c/EBPβ, PPAR-γ, PPAR-α and PGC-1α, promoting the transcription of UCP1. Therefore, Prdm16 is essential for brown fat cell fate [83].

Both VEGF-A and VEGF-B exert metabolic effects on AT increasing the complexity of the VEGF system [144]. The expression of VEGF-A and VEGF-B is strongly modulated by the expression of a cluster of mitochondrial genes. These genes are transcriptionally controlled by PGC-1α. VEGF-A and VEGF-B expression induce the recruitment of blood vessel preventing nutrients and/or oxygen deprivation. A tight interaction between nutrient metabolism and vasculature is clearly defined [150] (Fig. 2.3a).

Conclusions

Obesity represents a major health problem associated with increased mortality and co-morbidities, including many metabolic diseases. Obesity is characterized by an increase in adipose mass due to increased energy intake, decreased energy expenditure, or both. Several elements including lifestyle, environmental, neuro-psychological, genetic, and epigenetic factors contribute to increase in energy intake (calories) and decrease in energy expenditure (metabolic and physical activity). The treatment options for patients with severe obesity are the modification of lifestyle, limiting the intake of total fats and sugars, increasing consumption of fruits and vegetables, practising regular physical activity, bariatric surgery, or pharmacological treatments. Unfortunately, currently drugs available in the market are commonly associated with severe side effects. Indeed, increased risk of psychiatric disorders and non-fatal myocardial infarction or stroke has been described in pharmacologically treated patients. Understanding the origins of obesity and its related pathways is essential for prevention and treatment of metabolic

syndrome-associated disorders. Therefore, several groups are working on new therapeutic approaches including the increase of energy consumption. A new innovative approach would be the induction of trans-differentiation to brown adipocytes within WAT. Beige adipogenesis enhances energy expenditure leading to a lean phenotype, the browning of WAT. Angiogenesis has a significant functional impact on energy metabolism through modulation of adipocyte-related endocrine, paracrine and autocrine mechanisms. Based on preclinical and clinical findings, the effects of angiogenic mediators depend on the metabolic status of the AT and of the patients. Enhanced angiogenesis improves energy consumption in the BAT. To treat obesity and metabolic disorders, the administration of drugs endowed to anti-angiogenic activity reduces the WAT tissue. Unfortunately, high doses of drugs are required to target vessels in the fat mass with the risk to increase systemic side effects. Thus, obese patients with cardiovascular complications might not be recommended for anti-angiogenic therapy [151]. On the other hand, obese patients could benefit from drugs that promote the development of BAT and its vascularization. In these cases, the drug administration may be achievable through in situ delivery. On these bases, cell therapy combined with regenerative medicine represents a promising and potential treatment in metabolic disorders.

Chapter 3

The impact of adipokines on vascular networks in adipose tissue

Vliora Maria[1,2], Ravelli Cosetta [2], Grillo Elisabetta [2], Corsini Michela [2], Flouris Andreas D. [1], and Mitola Stefania [2].

1 FAME Laboratory, Department of Exercise Science, University of Thessaly, Trikala, Greece

2 Department of Molecular and Translational Medicine, University of Brescia, Via Branze 39, Brescia, Italy

Conception and design of the work: M.S., V.M., Data collection: V.M., R.C., G.E., Data analysis and interpretation: V.M., R.C., G.E., Drafting the article: V.M., G.E., Critical revision of the article: V.M., R.C., G.E., C.M, F.A.D., M.S., Final approval of the version to be published: F.A.D., M.S.

This review has been published in Cytokines and Growth Factors Reviews Journal under doi: https://doi.org/10.1016/j.cytogfr.2022.07.008.

This work was supported by H2020-MSCA-RISE-2014 (Grant No. 645640 – SCAFFY) to M.S. and F.A.D.; by Associazione Italiana per la Ricerca sul Cancro (IG AIRC grant n° IG 2021 ID 25726) to M.S., G.E. were supported by Fondazione Umberto Veronesi; C.M. was supported by AIRC fellowships (ID26917).

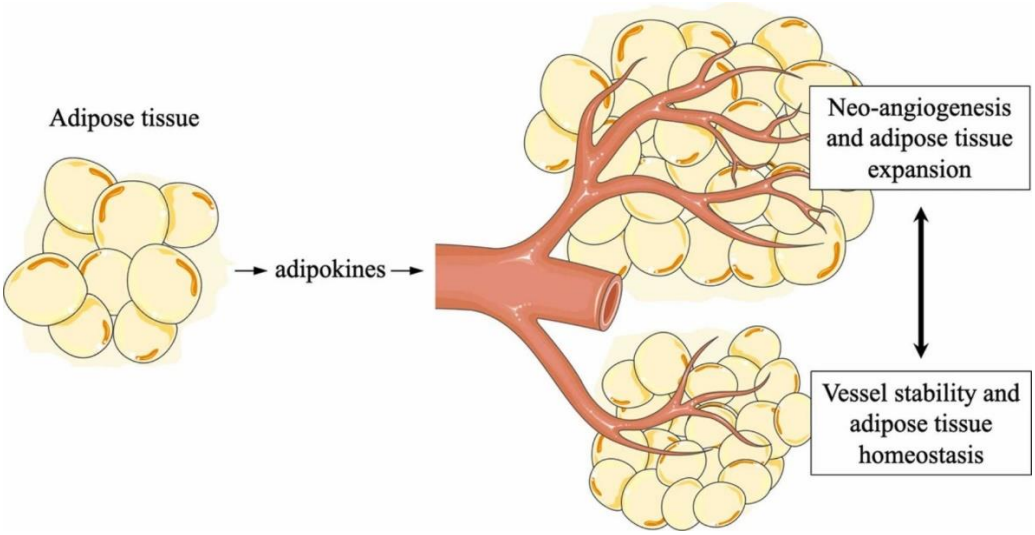


Figure 3. Graphical abstract. The impact of adipokines on vascular networks in adipose tissue.

Abstract

Adipose tissue (AT) is a highly active and plastic endocrine organ. It secretes numerous soluble molecules known as adipokines, which act locally to AT control the remodel and homeostasis or exert pleiotropic functions in different peripheral organs. Aberrant production or loss of certain adipokines contributes to AT dysfunction associated with metabolic disorders, including obesity. The AT plasticity is strictly related to tissue vascularization. Angiogenesis supports the AT expansion, while regression of blood vessels is associated with AT hypoxia, which in turn mediates tissue inflammation, fibrosis, and metabolic dysfunction. Several adipokines can regulate endothelial cell functions and are endowed with either pro- or anti-angiogenic properties. Here we address the role of adipokines in the regulation of angiogenesis. A better understanding of the link between adipokines and angiogenesis will open the way for novel therapeutic approaches to treat obesity and metabolic diseases.

Introduction

Obesity, defined as a body mass index (BMI) ≥30kg/m2 is a chronic, multifactorial disease associated with various life-threatening chronic diseases (https://www.who.int). Thus, prevention and treatment of obesity are key challenges, which are directly linked with the biology and physiology of adipose tissue (AT) [152]. The AT is highly dynamic and comprises multiple cells, including adipocytes, mesenchymal and endothelial cells (ECs), fibroblasts, and immune cells surrounded by extracellular matrix (ECM) [153]. Obesity induces AT expansion and remodeling, characterized by adipocyte hypertrophy and hyperplasia, accumulation of immune cells, hypoxia, and EC activation. This dysfunction is accompanied by lesser vascular density and the consequent development of obesity related metabolic diseases [154, 155].

Recently, an endocrine role has been attributed to AT in addition to its ability to regulate energy expenditure. Indeed, AT secretes several nervous and endocrine factors identified as adipokines [156] which sustain a cross-communication between numerous organs within the body. The production of adipokines is altered by AT hypertrophy and hyperplasia in obese patients, supporting detrimental metabolic alterations leading to insulin resistance, dyslipidemia, and an increased risk of cardiovascular diseases [157].

The expansion and remodeling of AT is regulated by the vascular system, which maintains cellular homeostasis throughout the body via a complex network of capillaries, arteries, and veins [158]. In this narrative review we describe the available evidence regarding the effects of adipokines on the angiogenic

process leading to AT remodeling. We also discuss the cross-communication between AT and angiogenesis and how anti- and pro-angiogenic approaches could be employed to impair the progression of obesity as well as whether AT engineering can improve AT dysfunction in patients with metabolic disorders.

The adipose organ

The AT is an organ specialized in energy storage, endocrine regulation of energy, homeostasis, and thermoregulation. It consists of mature adipocytes surrounded by a stromal vascular cell fraction (SVF) containing pre-adipocytes, macrophages, smooth muscle cells, lymphocytes immune cells, vessels, and a rich innervation of both sensory and sympathetic fibers. The SVF plays important roles in the dynamics of AT development, homeostasis, and inflammation [159]. Extracellular matrix (ECM) provides mechanical support and adsorbs cytokines and growth factors, thus regulating physiological/ pathological processes within AT [17]. In mammals, two major types of AT have been observed, namely white AT (WAT) and brown AT (BAT) [160]. The WAT composes around 20-25% of the total body weight in humans [103]. WAT stores energy in the form of triacylglycerols that are hydrolyzed, releasing fatty acids when the organism needs fuel [161]. White adipocytes have a round shape, a large diameter and are characterized by the presence of a big lipid droplet which pushes all their other components close to the cell membrane. Few mitochondria can be observed next to the nucleus and the ribosomes [103].

In humans, the WAT exists in different depots, characterized by typical patterns of gene expression and, thus, by distinct functions [162]. Indeed, each fat depot

differs in the size of adipocytes, ECM composition and mechanical properties [163]. The BAT is mainly composed of brown adipocytes that derive from Myf5+ precursors and are characterized by many small lipid droplets and a high number of mitochondria, responsible for their brown color [164]. Their nucleus occupies a central position, and the endoplasmic reticulum is not highly developed. BAT regulates thermogenesis, a function that explains the multilocular arrangement of lipids that amplify exponentially the number of fatty acids that can enter the beta-oxidation [165]. Upon sympathetic oxidation, brown adipocytes release chemical energy as heat. Consistent with this, in brown adipocytes there is a high expression of thermogenin (uncoupling protein 1; - UCP1). UCP1 is a fatty acid anion/H+ symporter in the inner membrane of mitochondria. It dissipates the respiration proton gradient by uncoupling cellular respiration and mitochondrial ATP synthesis, thus inducing thermogenesis [117].

Several depots of active BAT have been identified in adult humans [166, 167] including shoulder blades, around the kidneys, neck, and supraclavicular area, and along the spinal cord [112, 168, 169]. Importantly, the localization of BAT and WAT is not exclusive; for example, subcutaneous and visceral depots contain both tissues. Due to its high metabolic activity, the BAT should be considered an organ of pharmaceutical and physiological importance in the adult [170, 171].

Recently “beige” or “brite” adipocytes have been identified [127]. at anatomical sites that correspond to WAT after thermal stimulation [126, 172]. These cells have characteristics of both brown and white adipocytes. In the basal state, they display the morphology of white adipocytes while after stimulation, they

acquire intermediate morphology with multilocular lipid droplets, higher number of mitochondria, and express BAT specific markers, including UCP1[160, 172, 173]. In addition, they express typical beige markers, such as CD137, Tbx1, and Cited-1 [174]. Different developmental lineages have been suggested for beige adipocytes: the transdifferentiation of mature white adipocytes [175, 176] and the maturation of brown or white preadipocytes that are both present in WAT [116, 176, 177]. All these processes could contribute to beige adipocyte development, depending on tissue depot and stimuli [83, 178].

Angiogenesis in AT

Contrary to other organs, AT can also expand in adulthood, up to comprising more than 40% of the total body composition in obese individuals [85]. This high plasticity is paralleled by a coordinated vascular network growth which provides AT with oxygen and nutrients and drains waste products. The continuous growth, regression and remodeling of blood vessels is regulated by metabolites and growth factors known as adipokines, which are secreted by adipocytes [155].

Angiogenesis is a multistep process, regulated by pro- and anti-angiogenic factors. This process can be triggered in response to proliferating and enlarging adipocytes and/or precedes the adipocyte proliferation and enlargement [179] but, of course, it is essential in modulating AT pathophysiology [20, 66]. Adipocytes and other stromal cell types release pro- or anti- angiogenic factors in the microenvironment to maintain vascular homeostasis and induce vascular increase or regression [159]. In expanding WAT, the switch toward an

angiogenic phenotype facilitates energy deposition and vascular density [180]. Instead, in metabolically active BAT, the same angiogenic phenotype may facilitate energy consumption [82]. Also, WAT transition to brite phenotype is associated with an angiogenic switch with a consequent increase in vascular density. Moreover, blood vessels provide protective and niche for adipocyte progenitors that can differentiate into pre-adipocytes and adipocytes [82, 120].

Importantly, angiogenesis can be altered in pathological conditions like obesity, metabolic syndrome, cancers and cardiovascular pathologies [181], characterized by abnormal expression of angiogenic factors and/or by other angiogenic-related conditions including hypoxia, oxidative stress, hormone imbalance and hyperglycemia [182].

Angiogenic modulators released by adipose tissue

Adipokines which include hormones, free fatty acids, growth factors, and cytokines, act either locally or systemically to regulate a wide range of physiological and pathological processes such as immune-system modulation, inflammation, cell differentiation, and angiogenesis [152]. Novel pro/anti-angiogenic adipokines are continuously identified and characterized. AT dysfunction leads in to changes in cellular composition, inability to store the surplus lipids, alteration of insulin sensitivity, and in the secretion of pro-inflammatory and diabetogenic adipokines and cytokines [183, 184].

Surprisingly, dysfunctional AT in patients with obesity and metabolic syndrome often displays increased expression of anti-angiogenic factors. This suggests that the high expression of factors that restrict blood vessel growth is meant to avoid abnormal vessel outgrowth when an AT becomes stabilized. In the next

sections we discuss the role of main adipokines known to regulate EC functions, and potentially involved in the remodeling of AT vasculature. Also, we address the effects of metalloprotease enzymes that indirectly modulate AT angiogenesis (Fig. 3.1.).

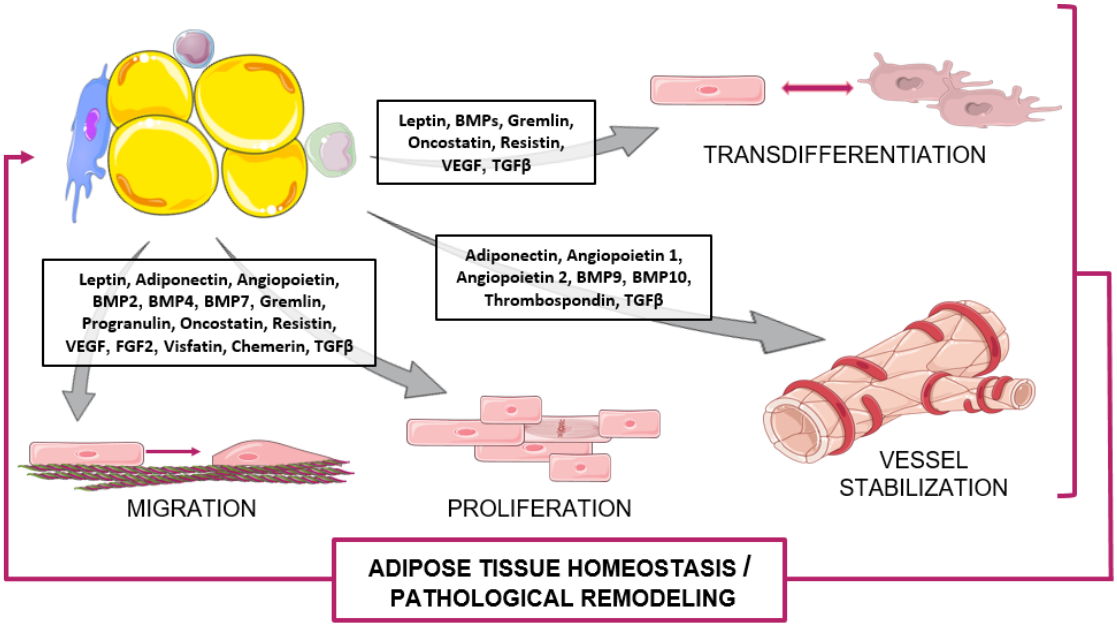


Figure 3.1. Differential roles of adipokines in the remodeling of adipose tissue vascular network

ECM remodeling factors

The ECM of AT is continuously remodeled to accommodate changes in adipocyte size and AT composition during tissue turnover [185]. Proteases and inhibitors of such proteases are locally produced to guide AT remodeling. Among these, metalloproteinases (MMPs) are pivotal enzymes produced by mature adipocytes (especially MMP-12) and the SVF in a depot-dependent manner [185]. Metalloproteases are a protease family involved in the turnover of connective tissue during morphogenesis, development, wound healing,

reproduction, and neovascularization. Also, the activity of MMPs contributes to allow the release of basement membrane components or the activation of latent growth factors [58, 186]. MMPs promote angiogenesis by regulating EC proliferation, migration, and attachment to the ECM, either directly or by releasing growth factors sequestered in the ECM [43, 187]. The proteolytic activity of MMPs is controlled by a family of proteins called tissue inhibitors of metalloproteinases (TIMPs). The imbalance of MMPs vs TIMPs is implicated in many pathological processes and may contribute to altered angiogenesis and AT dysfunction in metabolic diseases and obesity, as it occurs in cancer metastasis, arthritis, inflammation, periodontal diseases, corneal ulceration, and cardiovascular diseases [188].

MMPs are modulated during adipogenic differentiation of 3T3-L1 cells *in vitro*. In addition, proinflammatory adipokines upregulate MMP levels during obesity, with a shift toward increased matrix degradation [189]. Accordingly, MMP pharmacological inhibition leads to a collagen-rich matrix cap around the treated tissue thus restraining AT development in mice [190]. Several studies confirm the role of MMP inhibitors on AT homeostasis *in vivo*. The administration of specific or broad-spectrum MMP inhibitors reduces the subcutaneous or gonadal AT deposits. In this case, MMP inhibitors do not affect the number of adipocytes, but significantly increase the amount of collagen in treated animals. Importantly, MMP inhibition results in a higher blood vessel density in AT [191, 192].

Leptin

Leptin is a 16 kDa protein encoded by obese (Ob) gene [193, 194]. It is mainly secreted by WAT and, at lower levels, by other tissues such as muscle, stomach, and mammary gland. Leptin is an endocrine hormone with multiple properties including the regulation of appetite. For this reason, leptin is also known as the “satiety hormone” [195]. It controls energy expenditure, body weight, thermogenesis, fertility, and immune functions [194] and its serum levels positively correspond with the energy stored in the fat mass [196, 197]. A reduction of circulating leptin follows food restriction. Leptin expression is associated with hypertension, atherosclerosis, myocardial infarction, and stroke [198]. The functions of leptin are mediated by the leptin or obesity receptor (Ob-R), a single membrane-spanning receptor belonging to the class I cytokine receptor family, which is mainly expressed in the hypothalamus and immune cells the JAK/STAT signaling pathway modulates the intracellular cascades activated by leptin in target cells. Loss-of-function mutations in the leptin or Ob-R genes result in severe, early-onset obesity and are associated with altered hematopoiesis, immunity, blood pressure, and angiogenesis [199]. Leptin is a pro-atherogenic, pro-thrombotic molecule and controls vasodilation. In ECs, leptin promotes a pro-inflammatory response, the expression of endothelial nitric oxide synthase (eNOS), and the production of reactive oxygen species (ROS), leading to EC dysfunction [200], proliferation, and survival [201, 202]. In ECs, by binding Ob-R, leptin activates the p38 (MAPK)/Akt/COX-2 and Wnt axes [202, 203]. Moreover, leptin supports angiogenesis in several *in vivo* models [201, 204], upregulating MMP-2/9 and pro-angiogenic factors like fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor A (VEGF-A) and its receptor VEGFR-1 [142]. Also, leptin induces the mobilization of

vascular progenitor cells from the bone marrow, through an Ob-R/NOX2/MMP-9 mechanism [205] and boosts up the angiogenic potential of circulating angiogenic cells through a Ob-R/Src/alphavbeta5 crosstalk [206]. Under other experimental conditions, leptin inhibits EC inflammation, and the endothelial-to-mesenchymal transition (EndMT), thus exerting protective effects on vessel endothelium.

The observation that leptin regulates angiogenesis opens the way to using anti-leptin approaches to treat angiogenic-dependent pathologies. Preclinical studies confirm the effects of therapeutic strategies targeting leptin, such as leptin mutants/antagonists, or neutralizing antibodies in several pathological conditions, including cancers. However, a deeper characterization of leptin antagonists is needed to ameliorate the major drawback of most anti-leptin strategies, including their transport to the hypothalamic nuclei [207]. The fine elucidation of endothelial-specific molecular mechanisms involved in leptin response could contribute to the design of pharmacological approaches to target leptin-driven pro-angiogenic effects without the unwanted associated weight-gain.

Angiogenin

Angiogenin was originally identified in the culture medium of human colon adenocarcinoma cells [208]. As the name suggests, angiogenin is a potent inducer of angiogenesis. Upon receptor binding on the EC surface, angiogenin is internalized and translocated to the nucleus, where it regulates the expression of a variety of pro-angiogenic genes, including MMPs [209]. Its ribonucleolytic activity makes angiogenin unique among the angiogenic factors.

The inhibition of its enzymatic activity or prevention of its internalization pathway ameliorates the angiogenin pro-angiogenic activity [210]. Several inhibitors of angiogenin including the anti-human monoclonal antibody, small chemical compounds, neomycin and neamine, siRNA, antisense, soluble binding proteins, and enzymatic inhibitors have been developed and exert anti-angiogenic/anti-tumor effects in various animal models [211, 212].

Within AT, angiogenin is expressed by both adipocytes and non-adipocyte stromal cells. It is upregulated in cultured AT explants by PPAR agonists [213] and can be used in regenerative fat grafts to improve tissue vascularization [214]. Remarkably, angiogenin levels have been positively correlated with BMI [215]. The reduction of angiogenin expression in the elderly [216] is associated with an impaired angiogenic potential of adipose-derived mesenchymal stromal cells and with coronary artery disease [217]. This data suggests a role of angiogenin in the regulation of AT expansion, further confirming the pivotal role of angiogenesis during AT remodeling in obese patients. On these bases, inhibitors of angiogenin (i.e. monoclonal antibodies, small chemical compounds, neamine and neomycin, enzymatic inhibitors, receptor antagonists [211, 218-220] may have the potential to block angiogenesis in AT, thus restraining its expansion during obesity.

Bone morphogenetic proteins (BMPs)

The bone morphogenetic proteins (BMPs) are small, secreted proteins belonging to the transforming growth factor-beta (TGF-β) superfamily [221]. Originally studied as inducers of bone formation, they are now widely known for their involvement in various morphogenetic and differentiative processes during

development, including the development of AT [222-225]. BMPs are produced by human and mouse adipocytes. In particular, BMP4 and BMP6 control white and brown adipogenesis, respectively. BMPs activate canonical SMAD protein signaling through activation of their cognate serine-threonine kinase receptors. In addition, BMP can also signal through the mitogen-activated protein kinase (MAPK) pathway [226]. The acknowledged role of BMPs in vascular homeostasis derives from the strong connection existing between obesity and cardiovascular diseases. BMPs intervene in both sprouting angiogenesis and vessel maturation steps of the angiogenic process (reviewed in [226]). BMP2, BMP4, and BMP6 have been clearly shown to trigger the pro-angiogenic activation of ECs through the differential activation of their BMP type I receptors ALK3 and ALK2 [227, 228]. BMP2 and BMP6 coordinate tip vs stalk identity of ECs during sprouting angiogenesis. BMP4 stimulates the formation of micro-blood vessels, which serve as stem cell niches during expanding WAT [229]. Meanwhile, BMP4, recruits and commits stem cells to differentiate into preadipocytes. These processes are part of the remodeling of white adipocytes into beige as demonstrated by the expression of Tbx1, Tbx15, Hoxc9, PGC1α, and PRDM16 [224].

Mechanistically, BMP4 elicits a pro-angiogenic responses in ECs through a src-dependent transactivation of VEGFR-2 [229]. BMP7 induces the expansion of BAT, increases the expression of UCP1, the activity of the hormone-sensitive lipase, and energy expenditure [230]. In other words, BMP7 modulates the “browning” of WAT. On the contrary, BMP9 and BMP10 mostly have homeostatic effects on ECs, being involved in the regulation of vessel maintenance and remodeling [226, 231]. BMP7 downregulates and BMP9

upregulates the EMT process respectively [232, 233]. Considering the multiple roles of BMPs in AT, genetic variations, small molecule inhibitors of BMPs, including dorsomorphin and its derivatives, should be considered to influence the plasticity, the systemic metabolic phenotypes, and the vascularization of AT [234].

Gremlin

The activity of BMPs is regulated by extracellular antagonists that sequester BMPs in inactive complexes [235, 236]. Among these, gremlin-1 by binding BMP2/4/7 regulates organogenesis and cancer [237-239]. More recently, gremlin-1 was recognized as an adipokine produced by the human AT [240-242]. It is downregulated during adipogenesis [243] and limits the BMP2/4-dependent brown adipogenesis [240]. Gremlin is more expressed in omental fat than in its subcutaneous counterpart [244]. Local and circulating gremlin is increased in obesity (in human AT from bariatric surgery) and in metabolic diseases, such as type 2 diabetes and non-alcoholic fatty liver disease [242]. Remarkably, gremlin antagonizes insulin action in adipose, skeletal muscle, and liver cells, suggesting a pivotal and detrimental endocrine function of gremlin *in vivo* during obesity [242]. Finally, gremlin levels are normalized by physical exercise, which correlates with reduced cardio-metabolic risk in obese patients [245]. Together, these data indicate a possible role of gremlin as a biomarker and therapeutic target in metabolic disorders.

Gremlin exerts pro-inflammatory and angiogenic responses in ECs both *in vitro* and *in vivo* [246, 247]. The pro-angiogenic activity of gremlin is mediated by the interaction with both low- and high-affinity to heparan sulfate proteoglycans and

VEGFR2 receptors, respectively [59, 235, 248]. Remarkably, gremlin induces EndMT in human pulmonary artery endothelial cells, confirming its involvement in endothelial dysfunction [249]. Also, inhibitors of VEGFR2 block gremlin-driven angiogenesis and fibrosis in kidney disease [59, 250]. These findings should stimulate future research on the role of gremlin in AT angiogenesis, which remains largely unexplored.

Progranulin

Progranulin (PGRN), also known as granulin (GRN)-epithelin precursor, is a secreted glycoprotein which can be fragmented by some matrix metalloproteinases (MMPs) into small homologous subunits, granulins/epithelins [251]. PGRN is a pluripotent growth factor that promotes cell proliferation and tumorigenesis [214]. PGRN is an adipokine, and several pieces of evidence demonstrate that its overexpression is linked to high fat diet (HFD)-induced adipocyte hypertrophy, obesity, insulin resistance, and liver disease [252, 253]. The level of PGRN is higher in obese or diabetic patients and correlates with lipodystrophy syndrome [253-255]. *In vivo* evidence suggests a sexual dimorphism for PGRN, as female mice exhibit higher PGRN expression than males both in subcutaneous and epididymal AT [254]. Taken together, the evidence indicates that PGRN is a promising target for treating HFD-induced obesity [256, 257]. However, it is important to note that AT is not the main source of PGRN in obesity [258].

PGRN is associated with systemic inflammatory markers like C-reactive protein and with AT macrophage infiltration [254]. A recent study showed that PGRN is an inducible protein in response to hypoxia [259]. Accordingly, PGRN is a

pro-angiogenic factor for blood and lymphatic vessels under physiological and pathological conditions [259-261]. It has been found to bind to Ephrin type A receptor 2 receptor with high affinity, prolonging receptor activation and the downstream stimulation of MAPK and Akt, and promotion of capillary morphogenesis [262]. In contrast, its interaction with perlecan may inhibit its function [263]. Based on these findings, the regulation of PGRN expression is of high interest in obesity and metabolic research.

Oncostatin M

Oncostatin M (OSM) is a pleiotropic cytokine produced by several tissues, including AT [264]. Within AT, OSM is produced by the SVF compartment, while it is not expressed in adipocytes. Adipocytes are responsive to OSM as they express the OSM receptor (OSMr), a heterodimeric complex formed by the OSM receptor beta and gp130 monomers. OSM regulates AT metabolic homeostasis as OSM and OSMr are highly induced in the AT of diabetic and obese patients [265] while loss of OSM leads to insulin resistance and AT inflammation in a mouse model [266]. OSM induces EC activation *in vitro*, paralleled by an increase in the expression of the pro-angiogenic molecule FGF-2 [267]. OSM is active also *in vivo* models [264, 268] suggesting its role in pathological angiogenesis in different contexts, like after myocardial infarction and cancers [264, 268]. Moreover, OSM induces the expression of EndMT markers [269], of VEGF, cyclooxygenase-2 (COX2), urokinase-type plasminogen activator (uPA), and angiopoietin 2 (ANG-2) [270] indicating that OSM contributes to the vascularization of growing AT [271]. Inhibition of OSM signaling via anti-receptor antibodies or genetic deletion improves the outcome

of heart failure [272], while the potential role of these approaches in AT angiogenesis remains unclear.

Resistin

Resistin is an adipokine secreted by adipocytes and monocytes, implicated in inflammatory processes including atherosclerosis, non-alcoholic fatty liver disease, and malignancies [273]. In human serum, the physiological concentration of resistin is in a range of 7–22 ng/mL, and higher circulatory levels correlate with autoimmune disorders, as well as metabolic disorders [274-276]. The high levels of resistin in obese patients, suggests its a non-redundant role in energy homeostasis and AT dysfunction [277, 278]. Indeed, high concentration of resistin is often associated with chronic low-grade sub-clinical inflammation accompanied with obesity, which involves macrophage infiltration in the AT. In mice, resistin is a late marker of adipocyte differentiation and its accumulation is necessary for adipogenesis [279]. By binding the adenylyl cyclase-associated protein 1 (CAP1), impairs insulin signaling and oxidative stress response via MAPK pathway [280].

Most of the available literature on the pro-angiogenic role of resistin has focused on cancerous contexts where it often promotes the expression of VEGF [273, 281]. Despite the presence of CAP1 and TLR4 receptors on ECs, the mechanism of action remains to be elucidated [282-284]. If this mechanism is elucidated, resistin may become a prognostic biomarker linking obesity, inflammation, and angiogenesis in dysfunctional AT.

Vascular endothelial growth factors (VEGFs)

Vascular endothelial growth factor (VEGF) is a family of master regulators of blood vessel growth in various physiological and pathological contexts. VEGF family comprises seven members: VEGF-A to -F, and PlGF [285]. VEGF expression is influenced by a high number of factors, such as hypoxia, insulin, growth factors, and several cytokines.

VEGF-A promotes sprouting angiogenesis by inducing vascular permeability, EC migration and proliferation and vessel maturation, both *in vitro* and *in vivo* angiogenesis models. Accordingly, loss of VEGF-A is embryonically lethal due to severe defects in vascular development. VEGFs activity is mediated by 3 transmembrane tyrosine kinase receptors (VEGFR-1, -2, -3). VEGFR3 is mainly expressed in lymphatic vessels, VEGFR2 is the main pro-angiogenic receptor, while VEGFR1 sequesters VEGFs thus limiting the activation of VEGFR2 [286]. In AT, VEGF is produced by mature adipocytes and the SVF [287], where it regulates adipogenesis and angiogenesis [179]. VEGF overexpression in mouse AT increases tissue vascularization and improves insulin sensitivity and glucose tolerance, protecting mice from diet-induced obesity [288]. Accordingly, AT-specific loss of VEGF impairs AT angiogenesis and promotes AT hypoxia, inflammation, and apoptosis in a mouse model [289]. Moreover, inhibition of VEGFR-2 restrains diet-induced AT expansion by decreasing angiogenesis [290]. Finally, genetic ablation or pharmacological inhibition of VEGFR-1 increases angiogenesis and browning in AT, protecting mice from diet-induced obesity. These results confirm the role of VEGFR-1 as a decoy receptor sequestering VEGF from binding pro-angiogenic VEGFR-2 [291]. Together these findings suggest that the role of VEGF in AT may be beneficial, limiting AT dysfunction and obesity. This opens the way for future

anti-angiogenic approaches to prevent AT expansion.Numerous inhibitors of the VEGF/VEGFR2 axis are used in clinics to treat tumors or ocular diseases (i.e bevacizumab or tyrosine kinase inhibitors). Preclinical studies have suggested that targeting this axis may improve obesity and metabolic diseases [292]. However, future clinical trials should assess the potential of these drugs in the treatment of AT angiogenesis and remodeling [292].

Fibroblast growth factors (FGFs)

Fibroblast growth factors are multifunctional secreted growth factors expressed by many cell types. They regulate a plethora of physiological and pathological processes. Among 22 members of the FGF family, FGF-1, FGF-10, and FGF-21 have been recognized as AT-derived adipokines. In light of this role, FGF-1 (regulated by PPARγ) and FGF-10 are essential modulators of white adipogenesis, while FGF-21 activates BAT after cold-exposure and promotes the accumulation of brown adipocytes within BAT during cold-exposure. FGF-1 loss leads to aberrant AT expansion in mice fed with HFD [293]. Also, FGF-21 protects AT from acquiring a dysfunctional state during HFD as demonstrated by resistance to diet-induced obesity of FGF-21 transgenic mice [294]. FGFs are key regulators of angiogenesis [295]. Among FGF adipokines, FGF-21 has been recently classified as a pro-angiogenic stimulus able to induce the pro-angiogenic activation of ECs [296-298] while the angiogenic potential of FGF-1 and FGF-10 needs to be elucidated in order to reveal whether they could contribute to angiogenesis during AT remodeling. Inhibitors of the FGF/FGFR system are available and widely used in preclinical and clinical studies [296, 299, 300]. Once the role of FGFs is clear these drugs could be validated for their effects on FGF-driven AT vascular remodeling.

Visfatin

Visfatin/extracellular-nicotinamide-phosphoribosyltranferase-(eNampt) is a multifaceted adipokine produced preferentially by visceral depots and exerts paracrine pro-adipogenic functions increasing AT mass [299]. Visfatin expression increases in parallel with obesity [301] thus it is not surprising that its plasmatic levels correlate with intra abdominal fat mass. Also, visfatin may promote insulin resistance in peripheral organs [302]. Visfatin promotes EC proliferation, migration and sprouting by activating classical ERK1/2 signaling pathway and induces the expression of VEGF and MMPs [281, 303-305]. *In vivo* visfatin promotes vascular dysfunction and inflammation via TLR4 receptor, the activation of the nod-like-receptor-protein-3 (NLRP3) inflammosome complex and the release of IL-1 beta, the final mediator of EC damage. Accordingly, treatment with MCC 950 (NLRP3-inflammasome inhibitor), or anakinra (interleukin-1-receptor antagonist) reduces the release of IL-1β and the vessel dysfunction [305]. Thus, those targets may become therapeutic strategies for attenuating the adipokine-mediated vascular dysfunction associated with obesity and/or type-2-diabetes. In the future, the molecular basis of visfatin activity on EC should be explored in more detail to better assess its potential role as a therapeutic target for the treatment of AT-related disorders.

Chemerin

Chemerin is a multifunctional adipokine, expressed both by WAT and BAT, that modulates adipogenesis, inflammation, and energy metabolism [306]. Its

plasmatic levels correlate with the BMI [307]. *In vitro* chemerin is overexpressed in differentiated adipocytes compared to pre-adipocytes and promotes the expression of VEGF [308]. Chemerin may contribute to brown-to-white conversion of adipocytes, as high levels of chemerin in the BAT of ob/ob mice is associated with BAT “whitening” [306].

Chemerin induces a pro-inflammatory phenotype in ECs [309] *in vitro* and promotes neo-vessel formation in murine models [310, 311]. However, a recent study shows opposite results demonstrating that chemerin inhibits both physiological and pathological angiogenesis through its main functional receptor CMKLR1 [312]. Also, chemerin binds to other 2 receptors, namely GPR1 and CCRL2, suggesting novel possible functions [313]. The LRH7-G5 peptide antagonist hampers chemerin/GPR1 signaling and has anti-cancer activity in triple-negative breast cancer [314]. Also, the chemokine-like receptor 1 (CMKLR1) antagonist α-NETA counteracts chemerin direct action on ECs [315]. Further studies need to clarify whether chemerin may become a target for the modulation of AT vasculature in metabolic disorders.

Adiponectin

Adiponectin is the most abundant circulating plasma adipokine secreted by mature adipocytes. Adiponectin is widely recognized for its insulin-sensitizing, anti-diabetic, anti-inflammatory, anti-atherogenic, and cardio-protective effects [316]. Consistent with its beneficial effects, adiponectin is abundant in the plasma of lean individuals, while it is downregulated in obese patients [317]. Adiponectin acts through two major functionally distinct ubiquitously expressed G-protein coupled receptors, AdipoR1 and AdipoR2. In the liver, adiponectin

activates glucose transport and inhibits gluconeogenesis via activation of AMPK. On the other hand, it activates fatty acid oxidation and decreases inflammation through the PPARα pathway. Together, these metabolic changes contribute to adiponectin-driven increased insulin sensitivity. In addition, *in vitro* studies demonstrated that adiponectin regulates fat lipid metabolism inhibiting lipolysis [316].

Adiponectin plays a crucial role in the regulation of EC homeostasis, increases nitric oxide (NO) production through the PI3K/Akt pathway, and suppresses ROS generation. Adiponectin induces VEGF-A expression and enhances EC proliferation, migration, and morphogenesis [318-320].

In addition, adiponectin counteracts the vascular inflammatory response by interfering with NF-κB signaling and by down-modulating the expression of EC adhesion molecules ICAM-1, VCAM-1, and E-selectin [321]. Low plasmatic concentration of adiponectin is an independent risk factor for endothelial dysfunction [322]. Consistent with this observation, adiponectin loss is associated with impaired angiogenesis after ischemic stress and its replacement restores post-ischemic angiogenesis [323]. However, a considerable body of literature describes the anti-angiogenic properties of adiponectin [324, 325]. These studies have shown that adiponectin inhibits EC proliferation, migration, and survival and promotes cell apoptosis via MAPK and cAMP-PKA pathways [200, 325, 326]. The different biological activities of adiponectin across different studies may depend on the type of adiponectin employed in each study [327]. Indeed, globular adiponectin, a result of proteolytic cleavage of the full-length protein, strongly induces pro-angiogenic activation of EC, with increased VEGF MMP2-9 expression. On the other hand,

full length adiponectin promoted a negligible angiogenic response [328]. Various adiponectin mimetics, adiponectin receptor agonists, such as AdipoR on, have been developed and tested in a wide spectrum of animal models [329, 330]. On the other hand, very few antagonists of AdipoR including the ceramidase inhibitor, 1S,2R-D-erythro-2-N-myristoylamino-1-phenyl-1-propanol (MAPP) and TNF-a are available at present, but their pharmacological translational potential remains to be assessed [331].

Angiopoietin 2

Angiopoietin 1 and 2 (ANG-1 and ANG-2), the agonistic and antagonistic TIE-2 ligands, respectively are involved in the maintenance of vessel stability[332]. During vessel remodeling, ECs release a high level of ANG-2 which competes with ANG-1 and reduces TIE-2 phosphorylation in the cell–cell junctions. In quiescent vessels, the specific activation of TIE-2 by ANG-1 reduces endothelial permeability and promotes vessel stabilization [333]. ANG modulates the EC responses to other stimuli. In the presence of VEGF, ANG-2 enables EC migration and proliferation and therefore angiogenesis, alternatively ANG-2 prompts EC death and vessel regression [334].

In AT, the functions of the ANGs are poorly defined. In ob/ob mice, the expression of ANG-1 in AT is reduced, while ANG-2 is overexpressed [66]. The expression of ANG-2 is differentially regulated under different metabolic conditions as HFD, fasting, cold exposure and exercise [335]. ANG-2 regulates the alpha5-beta1-dependent transport of fatty acids through the endothelium in subcutaneous AT [336]. ANG2 deletion leads to ectopic fat accumulation in mouse liver and muscle leading to increased insulin resistance. Within AT

results in increased vascularization and reduced inflammation of AT conferring to these animals' resistance to diet-induced obesity and improving their metabolic health [337]. Inhibition of endogenous ANG2 via administration of neutralizing antibodies has opposite effects with decreased vascularization, increased inflammation and fibrosis and exacerbation of HFD-induced metabolic alterations. Together, these changes link ANG2 to angiogenesis during AT expansion and indicate that it plays a role in AT dysfunction *in vivo* [335].

Thrombospondin-1

Thrombospondin-1 (TSP1) is a matricellular protein well characterized for its anti-angiogenic function [338]. TSP-1, by binding the CD36 receptor or the VLDL receptor inhibits intracellular signaling and cell cycle progression inducing EC apoptosis [339]. TSP-1 is normally produced by differentiated adipocytes while it is downregulated in preadipocytes, indicating that *in vivo* it may negatively regulate angiogenesis during AT stabilization. Remarkably, TSP-1 high levels correlate with obesity, adipose inflammation, and insulin resistance [340, 341]. Consistent with its anti-angiogenic properties, TSP-1 sustains HFD-induced muscle fibrosis, suggesting that TSP-1 may promote the acquisition of a fibrotic/mesenchymal phenotype in AT cells. However, loss of TSP-1 expression in mice does not cause AT defects [342]. Thus, further studies are warranted to clarify its function in the regulation of AT vascularization.

Transforming growth factor β

The TGF-β superfamily members are locally produced within AT, where they act as regulator of the differentiation of white and brown adipocytes and AT homeostasis (reviewed in [343]). By activating their cognate cell surface serine/threonine kinase receptors, they also activate SMAD proteins that in turn regulate the expression of adipogenic genes. Generally, while TGF-β promotes pro-fibrotic events leading to AT metabolic dysfunctions [343]. Thus, a fine tuning of TGF-β levels is necessary for AT homeostasis.

TGF-β elicits dose-dependent and context dependent effects of blood vessel outgrowth. Low levels promote angiogenesis, while high levels of TGF-β have anti-angiogenic effects on ECs. Also, the capacity to promote pericytes and smooth muscle cell differentiation of TGF-β suggests that this factor may be involved in vessel stabilization [344, 345]. TGF-β promotes EC transdifferentiation into mesenchymal cells through the EMT and into adipocytes inducing the endothelial-to-adipocyte transdifferentiation. Thus TGF-β being a potential regulator of AT fibrosis and angiogenesis. Antagonists of TGF-β have proven anti-tumor and anti-angiogenic effects in various cancer models [346, 347]. Also, the blockade of TGF-β/smad3 signaling enhances WAT browning and prevents HFD-induced obesity and diabetes in mouse models [348].

Adipokines orchestrate angiogenesis during AT remodeling driving metabolic syndrome

Adipocytes release a wide number of molecules which collaborate with inflammatory cytokines [349] in the regulation of angiogenesis during AT remodeling (Figure 3.2). Also, adipose-derived stromal cells produce VEGF,

FGF, and other angiogenic factors, further contributing to angiogenesis [82]. Although the different subsets of angiogenic molecules produced by different AT depots have been characterized, how these differently regulate vessel biology in different AT depots remains to be elucidated. Among others, high amounts of pro-angiogenic leptin and VEGF are secreted during AT expansion while adiponectin, TSP-1, angiopoietin 2, or other negative regulators of angiogenesis are released in the hypo-plastic AT. Therefore, to activate angiogenesis during AT expansion, both overexpression of pro-angiogenic molecules and downregulation of anti-angiogenic ones are required. Consistently, cold exposure in mice leads to the activation of angiogenesis characterized by the simultaneous upregulation of VEGF and downregulation of TSP-1 [8]. Moreover, adiponectin and gremlin-1 can trigger both pro- and anti-angiogenic responses in ECs, suggesting their context-dependent effects. Thus, the final outcome in terms of AT angiogenesis results from a well-balanced integrated action of all different pro- and anti-angiogenic adipokines.

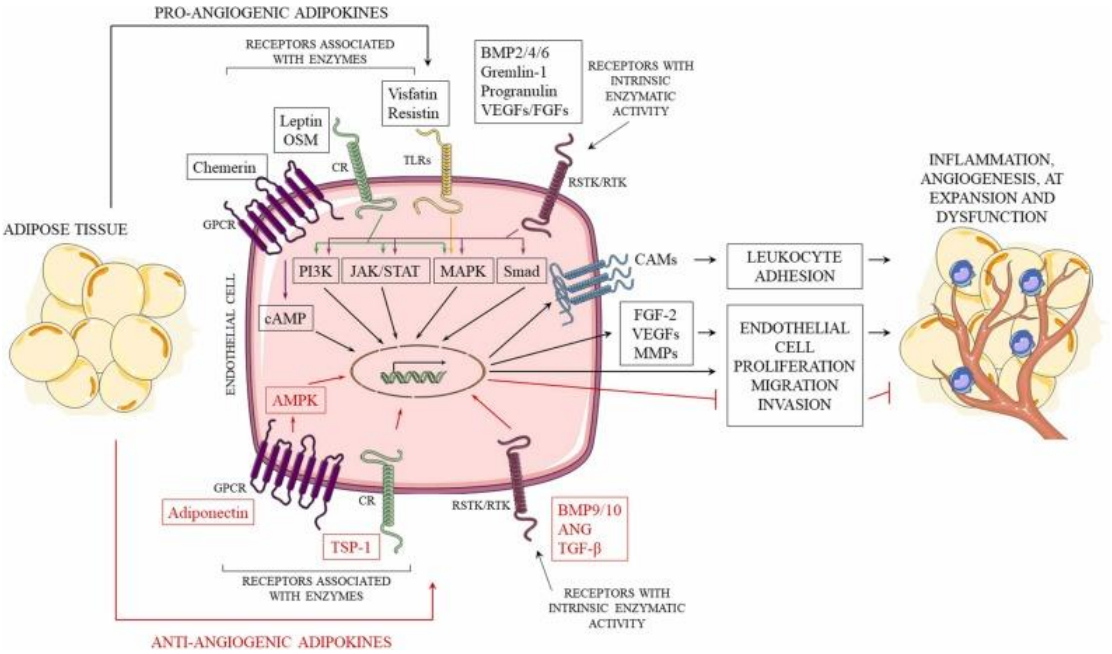


Figure 3.2 Molecular mechanisms involved in adipokines mediated angiogenesis during AT dysfunction.

The effects of pro-angiogenic adipokines often converge to the expression of classical angiogenesis inducers such as VEGF and/or FGF-2 [267] [270, 273, 308, 350], suggesting that they can synergize with these factors to induce AT angiogenesis [142]. In addition, the fact that many different adipokines activate classical angiogenic programs in ECs (i.e. VEGF expression, MMP expression etc.) suggests that redundancy might occur. For example, the overexpression/deletion of adiponectin does not affect body weight, suggesting that the adiponectin system may be redundant [351]. In total, the carefully orchestrated regulation of this diverse adipokine expression in space and time is crucial for a balanced angiogenic process in AT. Perturbations of adipokine expression are indeed hallmarks and biomarkers of AT dysfunction and are associated with profound alterations in AT angiogenesis.

In obesity, WAT expansion is paralleled by angiogenesis which, through a positive loop, promotes adipocyte differentiation [241] and AT expansion [155]. However, hypertrophic WAT is not always accompanied by increased angiogenesis. Paradoxically, lack of angiogenesis is observed even if overexpression of pro-angiogenic molecules occurs. In this condition, the activation of the hypoxia-inducible factor 1-alpha (HIF1α) recruits inflammatory cells and induces ECM production [352]. This, in turn, leads to metabolic alterations (lipid release) and insulin resistance causing AT dysfunction [82]. In this scenario, EC dysfunction play a major pathogenetic role. Increased vascular permeability as well as reduction of basal lamina [353] enhances inflammation and fibrosis [152, 164]. Add to this, leptin, angiogenin, visfatin,

and adiponectin regulate the expression of MMPs controlling ECM remodeling. The above demonstrates a crosstalk between adipocytes and EC in AT, with a negative impact on AT functionality in obese patients [155]. A challenge for future research remains to understand how all adipokines together cooperate to control angiogenesis during AT homeostasis and dysfunction.

Adipose tissue engineering

As obesity has become an epidemic problem, much of the recent efforts have been spent in the engineering of AT not only for the reconstruction of soft tissue but also for its repair and remodeling after injury/chronic inflammation.

Numerous biocompatible/implantable scaffolds for AT engineering have been developed (Figure 3.3).

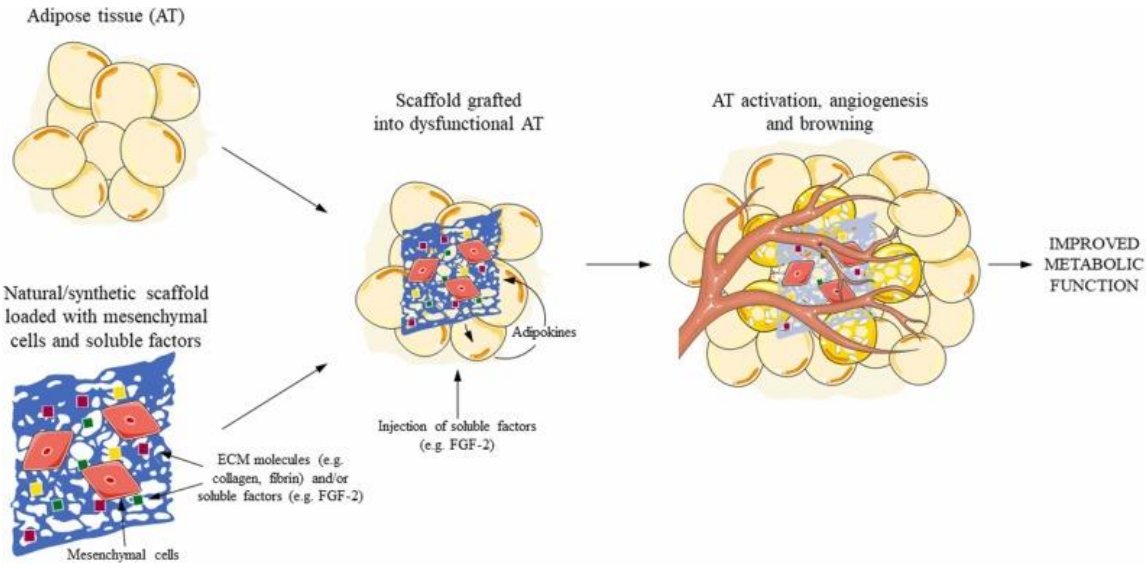


Figure 3.3. Scaffold approaches for adipose tissue engineering.

The synthetic or natural, solid or soft scaffolds should be able to mimic the pre-existing microenvironment of the native ECM, to support the adhesion of cells and their viability and to maintain the structural integrity of the implant until it is replaced with newly formed tissue [354, 355]. Pre-adipocytes or adipose-

derived stem cells have been extensively used as a cell source in combination with different ECM proteins and scaffolds to support the recruitment of vessels [356, 357]. Several synthetic polymeric scaffolds [358] including the poly(lactic-co glycolic) acids (PLGA) [359] the polyethylene tetraphthalate (PET) [360] the non-degradable polytetrafluoroethylene (PTFE) [361] coated with collagen, albumin or fibronectin [362] have been examined. Natural scaffolds such as collagen, fibrin or hyaluronic acid-based materials have been extensively used in tissue engineering due to their low cost, biodegradability and biocompatibility. Given the importance of vascularization in regulating adipogenesis, growth factors have been integrated in scaffolds. Murine models demonstrated that collagen and fibrin matrices induce the regeneration of vascularized AT when implanted in combination with adipose-derived stem cells and that adipogenesis was further stimulated in presence of pro-angiogenic growth factors (i.e., FGF-2). Of note, the exogenous ECMs are fully degraded two weeks post injection.

More recently, decellularized ECM scaffolds from different tissues, including AT, have been tested [363-365]. Characterized by high biocompatibility, AT scaffolds are also composed of all the acellular components of the AT naturally resembling the best environment for cell recruitment, attachment, proliferation, and differentiation [365-367]. Different *in vivo* studies mixed AT scaffolds with methylcellulose, methacrylate glycol chitosan or chondroitin sulfate forming injectable hydrogels. In a few weeks adipose lobule-like structures and vessels were observed and the addition of angiogenic growth factors further increased angiogenesis proving their usefulness for the regeneration and functionality of AT [368, 369].

Conclusion

This review outlines the available evidence regarding the effects of adipokines on the angiogenic process leading to AT remodeling. It also discusses the cross-communication between AT and angiogenesis and how anti- and pro-angiogenic approaches could be employed to impair the progression of obesity. Taken together, the available evidence clearly demonstrates that ECs and blood vessels are directly involved in maintaining the physiological metabolic functions of AT and are pivotal players in AT dysfunction. For this reason, therapeutic intervention on angiogenesis has great potential as a strategy to treat disorders associated with dysfunctional AT such as obesity. Both pharmacological inhibition of angiogenic adipokines and AT engineering represent valuable strategies to improve AT dysfunction in patients with metabolic disorders and may create the potential for novel treatments.

Chapter 4

Natural Histogel-based bio-scaffolds for sustaining angiogenesis in beige adipose tissue.

Margherita Di Somma [1], Wandert Schaafsma [2], Elisabetta Grillo [1], Maria Vliora [1,3], Eleni Dakou [4], Michela Corsini [1], Cosetta Ravelli [1], Roberto Ronca [1], Paraskevi Sakellariou [3], Jef Vanparijs [5], Begona Castro [2] and Stefania Mitola [1]

1 Department of Molecular and Translational Medicine, University of Brescia, 25121 Brescia, Italy

2 Histocell, S.L.Parque Tecnológico 801A, 2o 48160 Derio—BIZKAIA, Spain;

3 FAME Laboratory, Department of Exercise Science, University of Thessaly, 38221 Trikala, Greece

4 Laboratory of Cell Genetics, Department of Biology, Faculty of Science and Bioengineering Sciences, Vrije Universiteit Brussel, 1050 Brussels, Belgium

5 Department of Human Physiology, Faculty of Physical Education and Physical Therapy, Vrije Universiteit Brussel, 1050 Brussels, Belgium

Conceptualization, M.D.S., W.S., E.G., and S.M.; Methodology, M.D.S., W.S., E.G., C.R., M.C., E.K., M.V., E.S., J.V., and R.R.; Formal analysis, M.D.S., W.S., E.G.; Investigation, M.D.S., W.S., E.G., C.R., M.C., E.K., M.V., E.D., E.S., J.V., P.S. and R.R.; Resources, B.C., R.R., J.V. and S.M.; Data curation, M.D.S., W.S.; Writing, original draft preparation, M.D.S., M.V., E.G., and S.M.; Writing, review and editing, M.D.S., E.G., and S.M.; Supervision, S.M.

This study has been published on 18th November 2019, in Cells Journal, under doi: 10.3390/cells8111457.

This work has received funding by H2020-MSCA-RISE-2014 (Grant No. 645640–SCAFFY), by Associazione Italiana per la Ricerca sul Cancro (AIRC) IG 17276 grants and by MFAG-18459.

Keywords: angiogenesis, adipose derived mesenchymal cells, Histogel

Abstract

In the treatment of obesity and its related disorders, one of the measures adopted is weight reduction by controlling nutrition and increasing physical activity. A valid alternative to restore the physiological function of the human body could be the increase of energy consumption by inducing the browning of adipose tissue. To this purpose, we tested the ability of Histogel, a natural mixture of glycosaminoglycans isolated from animal Wharton jelly, to sustain the differentiation of adipose derived mesenchymal cells (ADSCs) into brown-like cells expressing UCP-1. Differentiated cells show a higher energy metabolism compared to undifferentiated mesenchymal cells. Furthermore, Histogel acts as a pro-angiogenic matrix, induces endothelial cell proliferation and sprouting in a three-dimensional gel *in vitro*, and stimulates neovascularization when applied *in vivo* on top of the chicken embryo chorioallantoic membrane or injected subcutaneously in mice. In addition to the pro-angiogenic activity of Histogel, also the ADSC derived beige cells contribute to activating endothelial cells. These data led us to propose Histogel as a promising scaffold for the modulation of the thermogenic behavior of adipose tissue. Indeed, Histogel simultaneously supports the acquisition of brown tissue markers and activates the vasculature process necessary for the correct

function of the thermogenic tissue. Thus, Histogel represents a valid candidate for the development of bio-scaffolds to increase the amount of brown adipose tissue in patients with metabolic disorders.

1. Introduction

Obesity represents a major health problem associated with increased mortality and co-morbidities, including many metabolic diseases [370]. Obesity is characterized by an increase in adipose mass due to increased energy intake, decreased energy expenditure, or both. Several elements including lifestyle, environmental, neuro-psychological, genetic, and epigenetic factors contribute to increase the energy intake (calories) and to reduce the energy expenditure (metabolic and physical activity). The treatment options for patients with severe obesity are the modification of lifestyle, limiting the intake of total fats and sugars, increasing consumption of fruits, vegetables, and whole grains and practicing regular physical activity or pharmacological treatments currently available in the market, which are commonly associated with severe side effects. Indeed, increased risk of psychiatric disorders and non-fatal myocardial infarction or stroke have been described in pharmacologically treated patients [371-373]. In the treatment of obesity, only 1% of patients receive bariatric surgery despite its safety and the better outcomes achieved [374]. Therefore, several groups are working on the development of different and new therapeutic approaches [372]. The increase of energy consumption through the increase of metabolism and thermogenesis of the adipose tissue may be a valid alternative [375].

Adipose tissue (AT) contains adipocytes and pre-adipocytes surrounded by stromal cells (fibroblasts, endothelial cells, macrophages), which make the adipose tissue the most plastic organ in the human body. AT is the major organ that controls the overall energy homeostasis in a living organism, storing the superabundant nutrients in the form of triglycerides, and suppling the nutrients to other tissues through lipolysis [376]. Two different types of adipose tissue have been described in mammalians. White adipose tissue (WAT) stores energy, while brown adipose tissue (BAT) is specialized for energy expenditure. The cellular structure of these tissues well reflects their biological functions: WAT contains adipocytes with a single large lipid droplet and few mitochondria, while BAT cells are characterized by the presence of several small lipid droplets and many mitochondria expressing uncoupling proteins (UCPs). UCP-1 contributes to energy loss as heat; in particular, it controls the dissipation as heat of the proton gradient produced by the mitochondria respiration chain. In humans, BAT mass declines with age, and it is less active in obese patients. The adipose tissue (AT) can be considered an endocrine organ, as both WAT and BAT secrete many cytokines, hormones, and adipokines. Recently, an additional/intermediate AT cell type termed “beige” has been described. Beige adipocytes sporadically reside with white adipocytes and emerge in response to certain environmental cues [127]. Under specific stimuli, beige adipocytes can exert BAT-like or WAT-like functions. Although beige adipocytes express a low level of UCP-1, they retain a remarkable ability to activate the expression of this gene powerfully and to turn on a robust program of respiration and energy expenditure that is equivalent to that of classical brown fat cells [127,

377]. These cells represent a cellular mechanism to provide flexibility in adaptive thermogenesis and metabolism. Thus, the possibility to generate and control the number of beige adipocytes may represent an alternative therapy for obese patients.

Blood vessels play a key role in the regulation of AT behavior. AT is the most plastic organ in the human body, subjected to continuous expansion and regression. This plasticity requires constant growth, regression, and remodeling of blood vessels, under the control of several metabolites secreted by AT itself. The adipose vasculature supports AT in multiple manners. The vascular network provides nutrients and oxygen, which are essential for tissue maintenance, and removes metabolic products of AT. Moreover, it exports the AT derived growth factors, adipokines, and cytokines from AT to body tissues, regulating physiological functions via an endocrine mechanism [20]. Furthermore, similar to mesenchymal cells [378], the adipocyte precursor cells within WAT and BAT express VEGF-A, promoting the angiogenic process and endothelio-genesis through VEGFR2 signaling [138].

Tissue engineering represents an interdisciplinary approach to regenerate damaged tissues, instead of replacing them, by developing biological substitutes that improve or restore tissue functions. Tissue engineering can help to boost human metabolism through the integration of cell biology and biomaterial sciences [379]. With particular attention to adipose tissue, tissue engineering is a promising approach to improve energy balance and metabolic

homeostasis controlling the amount of beige cells [380, 381]. Several biological or synthetic scaffolds have been developed to support and/or promote tissue regeneration or organ repair [382-386]. All the scaffolds share some characteristics including biocompatibility, neglectable immunoreactivity, and suitability for cell growth. Furthermore, scaffolds should be biodegradable, and their derivates should not be toxic to the body.

Here, we tested the ability of a novel bioscaffold able to promote both the differentiation of mesenchymal cells into beige adipose tissue and to sustain the angiogenic process. The Histogel-alginate scaffold promotes adipose tissue derived stem cell (ADSC) differentiation into adipocytes expressing PPARγ, PdK4, and UCP1 proteins supporting vessel recruitment and growth. Our results suggest that Histogel based scaffolds may represent good candidates for the development of scaffolds aimed at regulating energy expenditure in obese patients.

2. Materials and Methods

2.1. Hyaluronan Analysis

Commercial low, high molecular weight HA was electrophoretically analyzed on 1% agarose gel and stained with Stains-All solution HA (25 mg in 500 mL ethanol:water 50:50) overnight in the dark. Then, agarose gels were de-stained with water. The same protocol was used to explore the amount and the molecular weight of HA in Histogel preparations.

2.2. Cell Cultures and Differentiation

ADSCs were isolated from lipoaspirate (Histocell, Spain, in compliance with Certification of Laboratory 4269-E for the manufacture of research drugs (cell therapy products), Spanish Agency of Drugs and Medical Devices (Agencia Española de Medicamentos y Productos Sanitarios, AEMPS) and were cultured in DMEM, complemented with Glutamax, penicillin/streptomycin, 10% FBS, and gentamicin sulfate (identified as basal medium). ADSCs were differentiated for 15 days in basal medium in the presence of 20 nM insulin (Sigma, St. Louis, MO, USA), 5 μM dexamethasone (Sigma), 125 μM indomethacin (Sigma), 1 nM triiodothyronine (T3) and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX). Lipid vesicles formed starting from Day 6 of differentiation [387]. Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cords from healthy informed volunteers and used at early passages (I–IV). Cells were grown on culture plates coated with Porcine Gelatin Type I, in M199 medium supplemented with 20% FCS, endothelial cell growth factor (10 μg/mL), and porcine heparin (100 μg/mL) [388].

2.3. Identification of Angiogenic Factors

Pro- and anti-angiogenic molecules released by ADSCs and ADSC derived beige cells were analyzed using the Human Angiogenesis Antibody Array (R&D System) according to the manufacturer's instructions.

2.4. Quantitative RT-PCR

The expression of brown adipocyte markers was analyzed by RT-PCR. Briefly, total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions from 3 independent differentiation experiments. Two micrograms of total RNA were retro-transcribed with M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) using random hexaprimers in a final 20 μL volume. Quantitative RT-PCR was performed using the iTaqTM Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA). Each PCR reaction was performed in triplicate on one plate, and fluorescence data were recorded using a Viia7 Real Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Relative expression ratios were calculated by the Relative Expression Software Tool. The mRNA expression levels of target genes were normalized to the level of GAPDH transcript.

The following specific primers were used: Hs UCP1_Fw CGCAGGGAAAGAAACAGCAC; Hs UCP1_Rv TTCACGACCTCTGTGGGTTG; HsPdk4_FwATTTAAGAATGCAATGCGGGC; HsPdk4_RvACACCACCTCCTCTGTCTGA; Hs PPARγ_Fw CCGTGGCCGCAGAAATGA; Hs PPARγ_Rv TGATCCCAAAGTTGGTGGGC; MmCD31 FwAAGCCAAGGCCAAACAGA; Mm_CD31_Rv GGGTTTTACTGCATCATTTCC; Mm_CD45_Fw TATCGCGGTGTAAAACTCGTCAA; Mm_CD45_Rv GCTCAGGCCAAGAGACTAACGTT; Hs GAPDH_Fw GAAGGTCGGAGTCAACGGATT; Hs_ GAPDH_Rv TGACGGTGCCATGGAATTTG.

2.5. Mitochondrial Activity

Mitochondrial activity was monitored with Seahorse XFe24 instrument technology analysis. Oxygen consumption rate (OCR) (pmol/minute) was monitored over time before and after sequential injection of oligomycin (100 μM), carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP, 100 μM), and rotenone/antimycin A (50 μM) inhibitors (which inhibit ATPase, the proton gradient, and complex I/III, respectively). Thus, ATP-linked respiration and maximal respiration were measured. Extracellular acidification rate (ECAR; mpH/minute) was also measured to observe glycolytic capability. The colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to analyze the effect of isoproterenol and norepinephrine on the metabolic activity of cells, using the reducing ability of ubiquinone and CyrC and B of the mitochondrial electron transport system. For this, differentiated ADSCs were treated with 10 μM isoproterenol or 1 mM norepinephrine for 24 h. Then, cells were incubated in the presence of 0.2 mg/mL of MTT substrate for 1 h. The amount of metabolized formazan was measured by recording absorbance at 570 nm using a plate reader spectrophotometer (ELx-800 Bio-Tec Instrument).

2.6. EC Sprouting Assay

The collagen gel invasion assay was performed on HUVEC spheroids [389]. Briefly, spheroids were prepared in 20% methylcellulose medium, embedded in collagen gel or collagen gel: Histogel (1:5) in the absence or in the presence of ADSCs or differentiated cells. The formation of radially growing cell sprouts

was observed during the next 24 h. Sprouts were counted and photographed using an Axiovert 200M microscope equipped with an LD A PLAN 20X/0,30PH1 objective (Carl Zeiss, Oberkochen, Germany).

2.7. In vitro Angiogenesis Assay

Wells of µ-Slide Angiogenesis chambers (Ibidi, Martine Marne, Germany) were coated with a 0.8 mm thick layer of gel matrix by adding 10 µL of Cultrex Reduced Growth Factor Basement Membrane Matrix. After gel polymerization, 5000 HUVECs were seeded in M199 added with 5% FCS and treated with conditioned medium of undifferentiated or differentiated ADSCs. Cell viability was confirmed using calcein-AM. In live cells, the non-fluorescent calcein-AM is converted into green-fluorescent calcein. Calcein-AM is a permeant dye. After 5 h, samples were photographed using an inverted Axiovert 200 M epifluorescence microscope equipped with an LD A PLAN 20X/0,30PH1 objective (Carl Zeiss, Oberkochen, Germany). Images were analyzed using ImageJ software with the Angiogenesis plugin to detect total closed structures [67].

2.8. Immunofluorescence Analysis

Cells were seeded on glass coverslips and fixed in 4.0% paraformaldehyde (PFA)/2.0% sucrose in PBS, permeabilized with 0.5% Triton-X100, and saturated with goat serum in PBS. Then, cells were incubated with UCP-1 (sc-6529, SantaCruz, CA, USA), PPARγ (sc-7273, SantaCruz), and ACRP30 (sc-26497, SantaCruz) antibodies. Cells were analyzed using a Zeiss Axiovert

200M epifluorescence microscope equipped with a Plan-Apochromat 63X/1.4 NA oil objective.

2.9. CAM Assay

Alginate beads containing PBS, or 100 ng of recombinant human VEGF-A, or 5% of Histogel, or 30,000 cells were placed on the chicken chorioallantoic membrane (CAM) of fertilized white Leghorn chicken eggs at Day 11 of incubation [390]. After 72 h, newly formed blood vessels converging toward the implant were counted at 5× magnification using an STEMI SR stereo-microscope equipped with an objective f of 100 mm with adapter ring 475,070 (Zeiss, Oberkochen, Germany).

2.10. Murine Angiogenic Assay

All the procedures involving mice and their care conformed to institutional guidelines that complied with national and international laws and policies (EEC Council Directive 86/609, OJ L 358, 12 December 1987). Seven-week-old C57BL/6 mice (Charles River Laboratories International, Inc., Wilmington, MA, USA) were injected subcutaneously with 400 μL of 5% alginic acid (Sigma) containing PBS or 500 ng of VEGF-A, in the absence or in the presence of 5% Histogel solution. One week after injection, mice were sacrificed, and plugs were harvested and processed for RT-qPCR as previously described [246]. The mRNA expression levels of murine CD31 and CD45 were normalized to the levels of human GAPDH. Data are expressed as relative expression ratios (ΔΔCt—fold increase) using one PBS plug as the reference.

2.11. Data Representation and Statistical Analyses

Data are expressed as the mean ± SEM. Statistical analyses were performed using one-way ANOVA followed by Bonferroni's test or Student's t-test. The indicated p-value was set as statistically significant.

3. Results

3.1. Histogel Is a Pro-Angiogenic Bio-Scaffold

Histogel is a natural mixture of glycosaminoglycans including, among others, high grade hyaluronic acid (HA) and chondroitin sulfate obtained from the Wharton jelly found in umbilical cords of animal origin [383]. Histogel modulates inflammation, induces the release of extracellular matrices (ECM), and supports cell recruitment and growth [391]. On these bases, Histogel represents a suitable scaffold to drive cell differentiation. Since hyaluronan is a mix of molecules with different masses and the molecular weight confers different biological properties to hyaluronan preparations, a first set of experiments was performed to compare the amount and the molecular weight of hyaluronan contained in different Histogel preparations. Two different Histogel preparations were analyzed, pre-autoclaved (p.a.) or not, on agarose gel, and stained with Stains-All reagent. The amount of HA was similar in both analyzed batches, and it was around 76–80%. Moreover, the ratio between high and low molecular weight molecules remained approximately constant. Of note, this ratio is not affected by the autoclave sterilization cycles used for the preparation of 5% Histogel working dilutions (Figure 4.1a). Next, we tested the proangiogenic activity of Histogel preparation in several *in vitro* angiogenesis assays. Angiogenesis is a multistep process starting with the activation of endothelial cells (ECs) and the degradation of ECM of the basal membrane.

Then, ECs invade the surrounding tissue, proliferate, and reorganize themselves in capillary-like structures. Among the variety of *in vitro*, ex vivo, and *in vivo* assays that mimic the individual aspects of the angiogenic cascade, *in vitro* models of angiogenesis represent cost effective and rapid tools for testing angiogenic compounds. In particular, the use of 3D culture techniques able to recapitulate all steps of endothelial capillary sprout formation may serve as an effective strategy for these purposes. 3D endothelial cell spheroids were embedded in collagen or collagen:5% Histogel (1:1 ratio) gels. VEGF-A was used as the positive control and as the reference for the angiogenic activity. Figure 4.1b shows that, in keeping with the pro-angiogenic activity of commercial high molecular weight HA (Figure 4.1.a, b), Histogel stimulates the formation of endothelial cell sprouts from aggregates of HUVEC cells embedded in a 3D gel. To assess the *in vivo* pro-angiogenic activity, Histogel loaded alginate beads were implanted onto the chicken embryo CAMs at 11 days of development. After 72 h, a robust angiogenic response was observed around the Histogel implants when compared to alginate or VEGF engrafted embryos (Figure 4.1.c). The number of vessels converging towards the pellets was equal to 7.4 ± 0.6, 33.1 ± 1.0, and 58.3 ± 9.1 for alginate, VEGF, and Histogel implants, respectively. In keeping with these observations, Histogel modulates the recruitment of CD45+ cells and the consequent pro-angiogenic response when injected subcutaneously in mice (Figure 4.1.d). All these data suggest that Histogel is endowed with stronger pro-angiogenic activity if compared to VEGF-A. The pro-angiogenic ability of Histogel makes it a suitable candidate for the development of a bioscaffold for BAT differentiation.

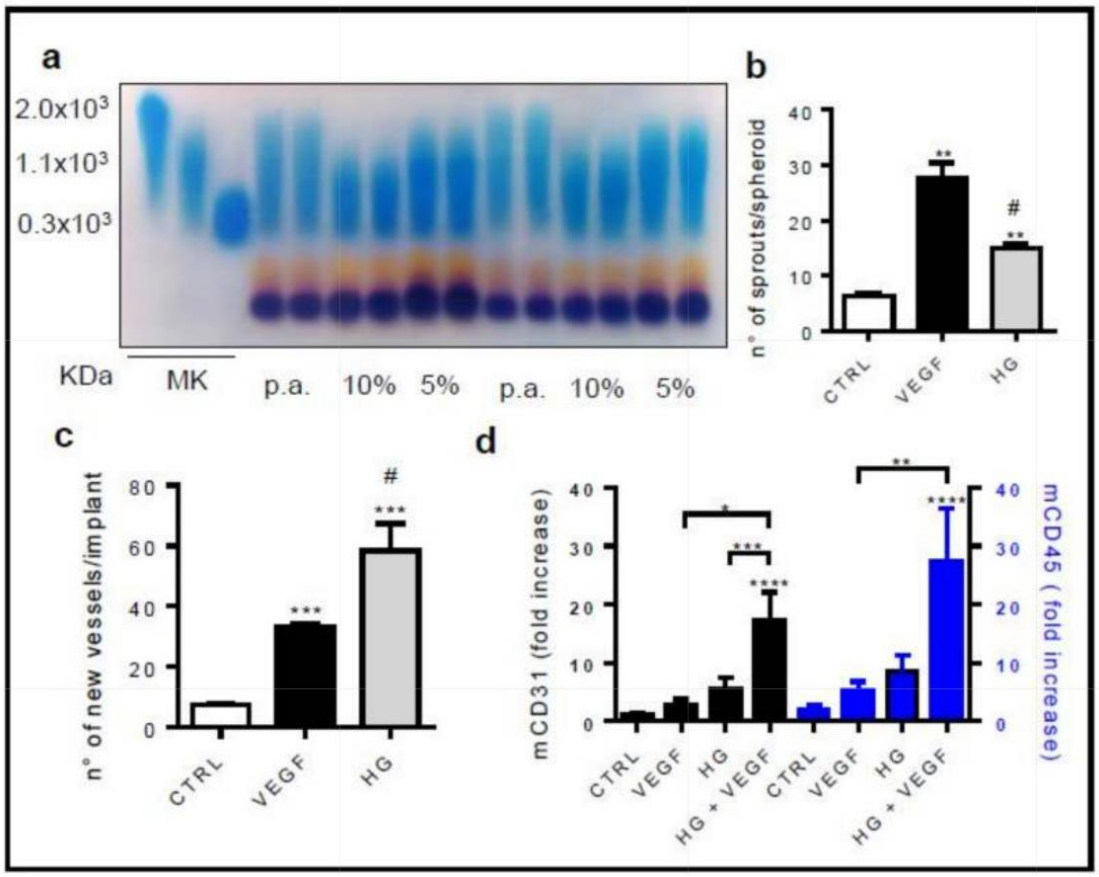


Figure 4.1. Histogel modulates the angiogenic response. (a) Five percent and 10% of two different Histogel preparations were electrophoretically analyzed on agarose gel and stained with Stains-All solution. p.a., pre-autoclaved. Three different standard molecules (2 × 103, 1.1 × 103, 300 kDa) were used as markers of molecular weight (MK). (b) HUVEC spheroids were embedded in collagen or in collagen:5% Histogel 1:1 (HG) gels. Fifty nanograms per milliliter of VEGFA165 were used as the positive control. The formation of radially growing sprouts was evaluated after 24 h of incubation. Data are the mean ± SEM of three independent experiments (** $p < 0.001$ vs. CTRL; # $p < 0.001$ vs. VEGF, one-way ANOVA followed by Bonferroni's test versus the control). (c) Alginate beads containing vehicle, or 100 ng of VEGFA165, or 5% Histogel (v/v 1:1) were implanted on the top of chick embryo chorioallantoic membrane (CAM) at Day 11 of development. After 72 h, newly formed blood vessels

converging toward the implant were counted in ovo at 5× magnification using an STEMI SR stereomicroscope equipped with an objective f equal to 100 mm with adapter ring 475,070 (Carl Zeiss). Data are the mean ± SEM (n = 6–8) (*** $p < 0.0001$ vs. control; # $p < 0.0001$ vs. VEGF, one-way ANOVA followed by Bonferroni's test versus the control). (d) Five percent of liquid alginic acid was mixed with 1.0 μg/mL VEGFA165 in the absence or in the presence of v/v 1:1 of 5% Histogel and injected subcutaneously into the flank of C57BL/6 mice. Plugs with vehicle alone were used as negative controls (CTRL). One week after injection, plugs were harvested. CD31 and CD45 mRNA expression levels were measured by RT-qPCR. Data are the mean ± SEM (n = 10) and are expressed as relative expression ratios (ΔΔCt – fold increase) using one vehicle plug as the reference. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.001$, one-way ANOVA followed by Bonferroni's test versus the control.

3.2. ADSCs Differentiate in Beige Adipocytes

Several protocols for ADSCs' differentiation were tested. ADSCs were maintained for 15 days in commercial specific media (such as StemMACS AdipoDiff Media from Milteny Biotec), or in DMEM supplemented with hBMP7, or supplemented with adipo-growth factors and analyzed for the expression of adipocyte markers including PPARγ, AdipoR, Prdm16, UCP-1, and Pdk4 (Figure 4.2.a). Among all the tested conditions, the custom medium was found to be the most promising in terms of expression of brown tissue markers. Thus, in all the experiments listed below, confluent ADSCs were cultured for 15 days in basal medium complemented with insulin and dexamethasone to stimulate

adipogenic differentiation, indomethacin, and 3-isobutyl-1-methylxanthine (IBMX) to modulate the expression of the PPARγ receptor and with triiodothyronine (T3) to increase UCP-1 expression. Figure 4.2a shows the morphological changes occurring in ADSCs upon differentiation. A clear sign of differentiation was the presence of small lipid droplets in differentiated ADSCs' cytoplasm. Immunofluorescence and RT-PCR analyses for the expression of PPARγ, ACRP30, UCP-1, and PdK4 confirmed that ADSCs acquired brown cell molecular markers during the differentiation protocol (Figure 4.2.b–d). Finally, we tested the metabolic activity of differentiated ADSCs using the Seahorse Cell Mito Stress Test. Although the basal oxygen consumption (OCR) of undifferentiated and differentiated ADSC seemed to be very similar, the maximal mitochondrial activity was significantly increased in differentiated ADSCs as demonstrated by the higher oxygen consumption measured by treating cells with the uncoupling agent FCCP. Furthermore, extracellular acidification increased in differentiated ADSCs compared to control ADSCs (Figure 4.2.e,f). These data were confirmed by the ability of norepinephrine and isoproterenol to positively modulate the mitochondrial activity (Figure 4.2.g) of differentiated ADSCs. Taken together, our results confirm that our protocol was suitable to drive ADSCs differentiation into ADSC derived beige cells.

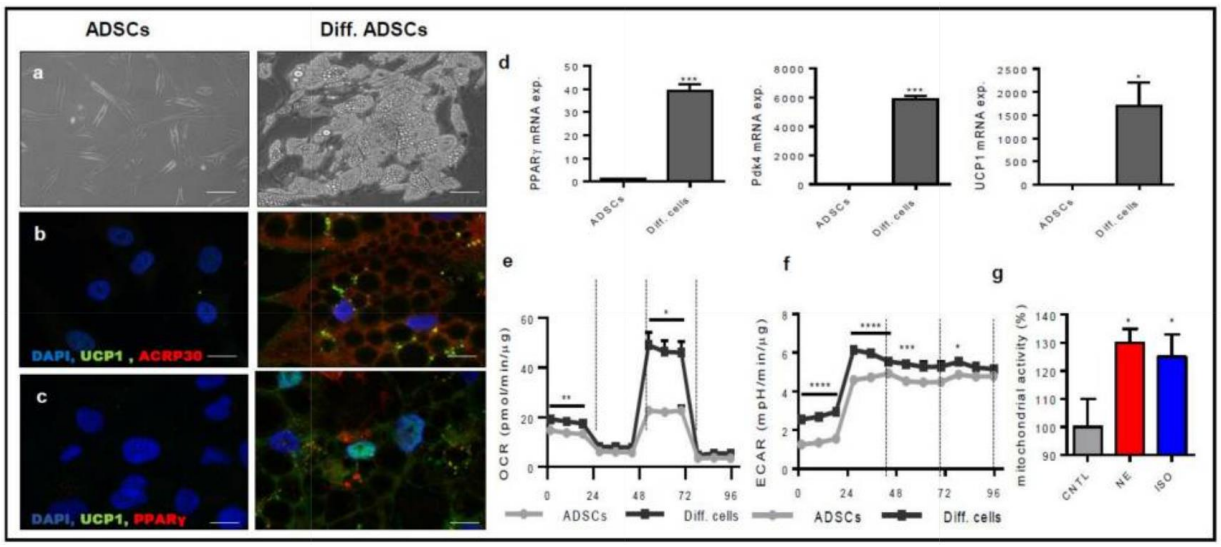


Figure 4.2. ADSCs differentiate into beige-like adipocytes. (a) The morphology of ADSCs and ADSC derived beige cells (Diff.) was analyzed at Day 15 of differentiation (Scale bar 100 μm). (b,c) Immunofluorescent detection of expression levels of UCP-1 (green) and ACRP30 (red) (b) and PPARγ (red) (c). Nuclei were counterstained with DAPI. (Scale bar 10 μm). (d) PPARγ, Pdk4, and UCP1 mRNA expression levels were measured by RT-qPCR analysis. Data are the mean ± SEM (n = 6) and are expressed as relative expression ratios (ΔΔCt – fold increase). (e,f) Mitochondrial energy metabolism was measured using the Agilent Seahorse Cell Mito Stress Test. The oxygen consumption rate (OCR) (e) and extracellular acidification rate (ECAR) (f) of ADSCs and ADSC derived beige cells was recorded before and after treatment with 10 μM oligomycin, 10 μM carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP), and 5 μM rotenone/antimycin A. Data were analyzed according to the Agilent Seahorse XF Cell Mito Stress Test Report Generator. (g) Mitochondrial activity of ADSC derived beige cells was measured by the MTT test in the absence or in the presence of norepinephrine and isoproterenol. * $p < 0.05$; ** $p < 0.01$; *** $p <$

0.005; **** p < 0.001, one-way ANOVA followed by Bonferroni's test versus the control.

3.3. ADSCs-Derived Beige Cells Show Pro-Angiogenic Properties

It is well known that mesenchymal stem cells support vessel recruitment and growth by producing and releasing several growth factors and chemokines. To assess whether ADSC derived beige cells maintain this pro-angiogenic activity, we evaluated their capacity to activate HUVECs in different angiogenic assays. To this, conditioned mediums were collected from ADSCs or ADSC derived beige cells cultured in basal medium for 48 h and tested in the tube formation assay. The conditioned medium of ADSC derived beige cells accelerated the morphogenesis of HUVE cells as demonstrated by the higher number of closed structures formed in 18 h compared to that induced by the conditioned medium of undifferentiated ADSCs (Figure 4.3.a). Of note, VEGF-A, used as a positive control, exerted a pro-angiogenic effect, comparable to the one of the conditioned medium of undifferentiated ADSCs. The presence of pro-angiogenic factors in the conditioned medium of ADSC derived beige cells was confirmed by the human angiogenesis antibody array. For this, all proteins of conditioned medium were labelled with biotinylated antibodies and incubated on a spotted specific capture antibody membrane. The antibody array showed that ADSC derived beige cells continued to express and release in the extracellular environment high levels of several pro-angiogenic molecules including VEGF, FGF, PlGF, and PDGF (Figure 4.3.b). To overcome the possible partial or total degradation of the soluble factors contained in the conditioned medium and to

ensure a continuous release of soluble molecules, we set up an *in vitro* endothelial–ADSC co-culture system in which ADSCs were plated and differentiated for 15 days in the same well used for the co-culture. Then, 3D Cultrex gel was stratified in the well, and HUVECs were plated on it. Again, the ADSC derived beige cells accelerated the morphogenesis of HUVEC cells in terms of the number of closed structures. Of note, HUVECs cultured with undifferentiated ADSCs remained non-organized (Figure 4.4.a,b). ADSC derived beige cells induced more sprouts from HUVEC-formed spheroids when embedded in 3D collagen gel in the co-culture system (Figure 4.4.c,d). Importantly, in both co-culture systems, no physical interaction occurred between HUVEC and ADSCs. Furthermore, to mimic the co-culture system in an *in vivo* assay, ADSCs and ADSC derived beige cells were delivered on the top of the chick embryo chorioallantoic membrane (CAM) at Day 11 of development. In agreement with the *in vitro* results, ADSC derived beige cells supported the recruitment of host vessels from the surrounding tissues into the cell-enriched-3D scaffold on chick embryo CAM (Figure 4.4.e, f).

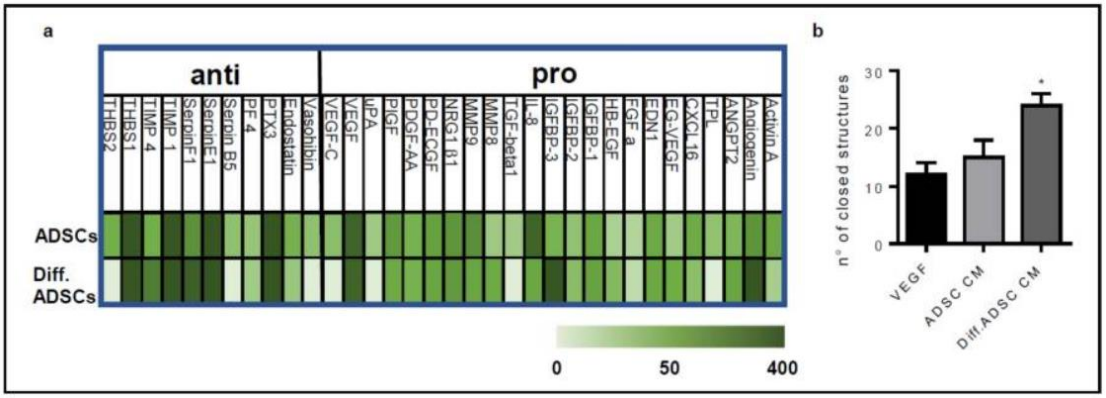


Figure 4.3. Conditioned medium of ADSC derived beige cells contains pro-angiogenic factors. Conditioned media were collected from confluent ADSCs

and ADSC derived beige cells (Diff. ADSCs) cultured in basal medium for 48 h. (a) HUVEC cells (40,000 cells/cm2) were plated on reduced growth factor Cultrex and stimulated with conditioned medium obtained from ADSCs or ADSC derived beige cells. After 5 h, endothelial cell morphogenesis in terms of the formation of closed structures was examined using ImageJ software. Data are the mean ± SEM of three measurements per sample. * $p < 0.05$, Student's t-test. (b) The Proteome Profiler Human Angiogenesis Antibody Array was used to detect angiogenesis related proteins in the conditioned medium of ADSCs and ADSC derived beige cells. Densitometry analysis of positive spot signals was normalized to positive and negative antibody array controls. Data are the mean of duplicate spots and expressed by color code (n = 2). Negative spots in both ADSCs and ADSC derived beige cell conditioned medium were not included in the analysis.

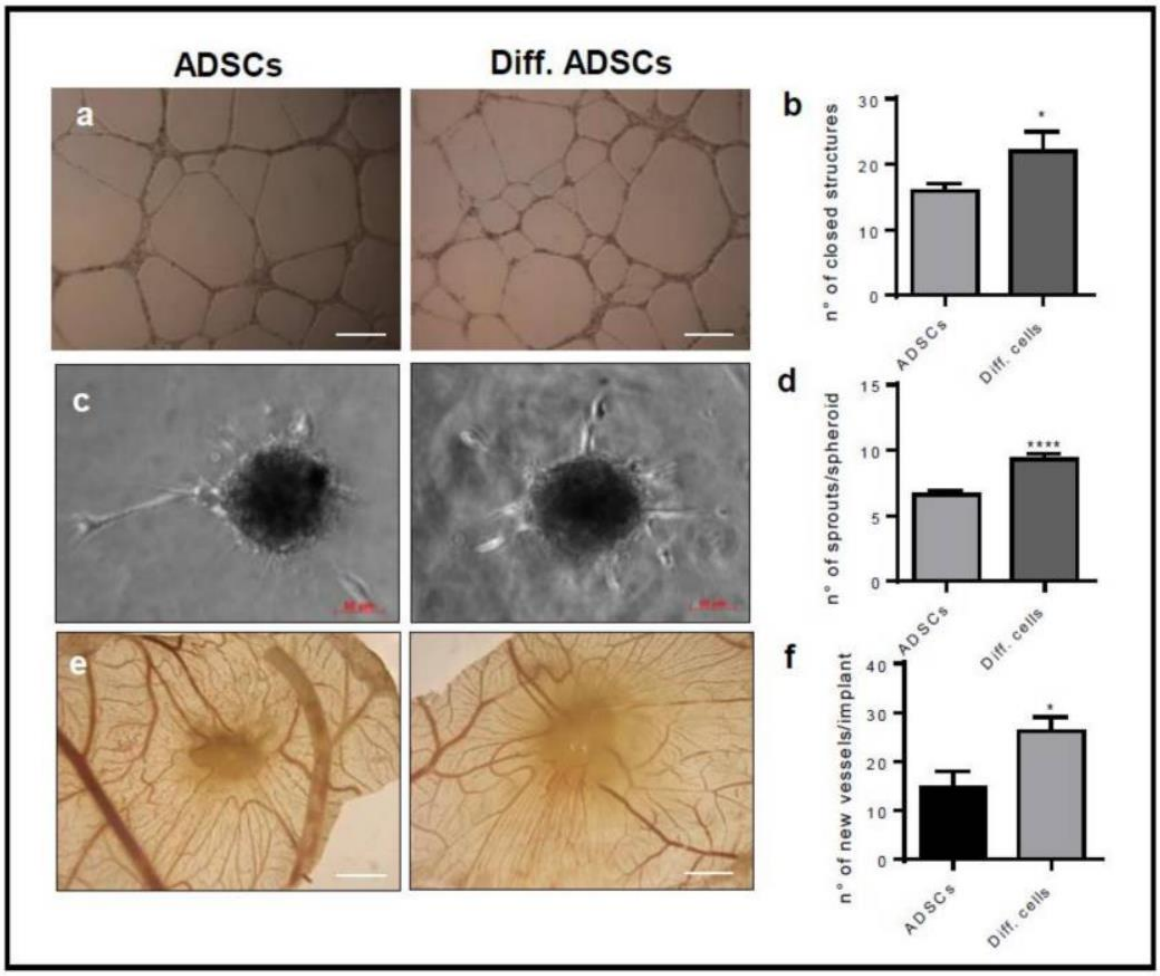


Figure 4.4. The differentiation into ADSC derived beige cells positively modulates the pro-angiogenic ability of ADSCs. (a) Confluent monolayers of ADSCs and ADSC derived beige cells (Diff.cells) were cultured in 24 wells. Two-hundred microliters of growth factor reduced Cultrex were added on the monolayers, and HUVEC cells were plated on gel. After 18 h, the formation of capillary-like structures was examined using a Zeiss Axiovert 200 M microscope (Scale bar 500 μm). Data are the mean ± SEM of three measurements per sample. * $p < 0.05$, Student's t-test. (b) The number of closed structures. (c–d) HUVEC spheroids embedded in collagen gel and plated on an ADSC monolayer. The formation of radially growing sprouts was evaluated after 24 h of incubation. Data are the mean ± SEM of three

independent experiments (* $p < 0.05$, **** $p < 0.001$, one-way ANOVA followed by Bonferroni's test versus the control). (e–f) Alginate beads containing 3 × 104 ADSCs or ADSC derived beige cells were implanted on the top of chick embryo chorioallantoic membranes (CAMs) at Day 11 of development. After 72 h, newly formed blood vessels converging toward the implant were counted in ovo at 5× magnification using an STEMI SR stereomicroscope equipped with an objective f equal to 100 mm with adapter ring 475,070 (Carl Zeiss) (Scale bar 2 mm). Data are the mean ± SEM (n = 6–8) (* $p < 0.05$, one-way ANOVA followed by Bonferroni's test versus the control).

3.4. Histogel Supports the Pro-Angiogenic Activity of ADSC-Derived Beige Cells

Finally, we tested whether the pro-angiogenic ability of Histogel supports beige adipocyte potentiality. ADSC or ADSC derived beige cells were embedded in a co-culture system in a 3D bioscaffold containing a 1:1 ratio of Histogel/collagen on HUVEC sprout growth. Of note, the Histogel/collagen scaffold did not affect cell viability of either ADSCs or ADSC derived beige cells as demonstrated by the ability of their intracellular esterase to hydrolyze calcein-AM also for a long time (Figure 4.5.a). ADSCs and ADSC derived beige cells were seeded and covered by HUVEC spheroids incorporated in the Histogel/collagen bioscaffold prototype. Results demonstrated that brown-like cells, also in the presence of the Histogel based bioscaffold, increased by 33% the angiogenic capacity of HUVEC cells as demonstrated by the higher number of newly formed sprouts (Figure 4.5.b,c). ADSC derived beige cells synergized with the Histogel-

collagen scaffold to induce angiogenesis as demonstrated by the ratio between endothelial sprouts formed in the 3D Histogel/collagen gel with respect to the 3D collagen gel co-cultured respectively with ADSCs or ADSC derived beige cells (Figure 4.5c).

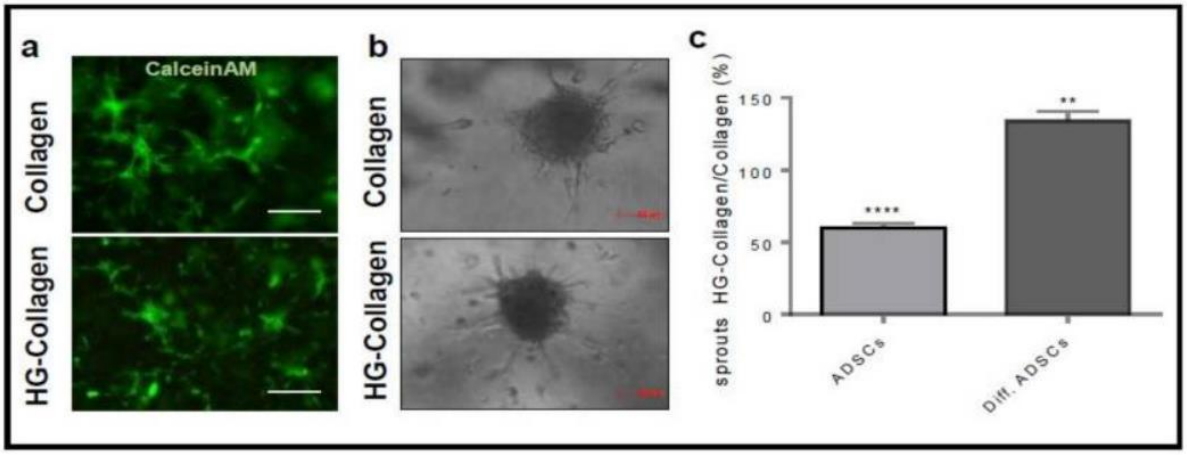


Figure 4.5. Histogel supports the angiogenic ability of ADSC derived beige cells. (a) ADSC derived beige cells were cultured in collagen or collagen:5% Histogel 1:1 for five days. Cells were photographed after 1 h of calcein-AM treatment (Scale bar 200 μm). (b,c) HUVEC spheroids were embedded in collagen gel or in collagen:5% Histogel 1:1 and plated on confluent monolayers of ADSCs and ADSC derived beige cells. The formation of radially growing sprouts was evaluated after 24 h of incubation. Representative figures (b). (c) describes the ratio of the number of sprouts formed from the endothelial cell spheroid co-cultured in 5% Histogel-collagen or in collagen gel with ADSCs or ADSC derived beige cells (ratio ± SEM of three independent experiments (**** $p < 0.0001$, ** $p < 0.01$, Student's t-test versus the control).

4. Discussion

Obesity and its related disorders are mostly preventable conditions, but their treatment has proven to be a complex endeavor that has been mostly

unsuccessful. The countermeasure usually suggested against overweightness and obesity is an increase in physical activity and/or a reduction of energy intake. A valid alternative to restore the normal physiological function of the human body could be the increase of energy consumption. BAT regulates the thermoregulation and metabolism, generating heat via non-shivering thermogenesis. In addition, beige adipocytes located in WAT also have thermogenic properties characterized by the expression of UCP1 [381]. Thus, the increase of brown tissue mass may represent a healthy and practical way to increase energy consumption, thus helping the individuals to control the weight balance [391]. For this purpose, here, we proposed the use of Histogel based bioscaffolds to promote the browning of adipose tissue. Histogel is a natural bioscaffold derived from porcine Wharton jelly. It is non-cytotoxic and non-hemolytic, and it does not induce inflammation [383]. Wharton jelly is a porous connective tissue found in umbilical cords of animal origin, forming a 3D spongy structure of fibrillar collagen and highly hydrated mucopolysaccharides, including hyaluronic acid proteoglycans [392, 393]. The physiological function of Wharton jelly is to prevent the compression of the umbilical cord vessels. Decellularized Wharton jelly is a biocompatible scaffold with mechanical properties suitable to support cell adhesion, proliferation, and reorganization in 3D structures [394], appropriate for tissue engineering [395]. Furthermore, the low cost to obtain it and the “zero waste” material should not be underestimated. Histogel was isolated from porcine farming systems and by transforming them into high-end products with high potentiality for regenerative medicine and cosmetic devices by means of an eco-friendly processing. Here, we showed that Histogel promoted an angiogenic response both *in vitro* and *in vivo*.

Histogel induced the migration, invasion, and reorganization into a tube-like structure of HUVEC cells and supported the recruitment of new blood vessels when implanted on the chorioallantoic membrane of chick embryos. Of note, the angiogenic process is necessary in tissue regeneration; thus, the pro-angiogenic ability of Histogel makes it a good bioscaffold for tissue engineering.

Biodegradability and porosity are critical features to keep in mind when designing bioscaffolds for tissue engineering. A good bioscaffold must remain in the tissue for the time necessary to support the engraftment of implanted cells, the recruitment of cells from surrounding tissue, and to sustain the metabolism of cells. Thus, the bioscaffold must remain in the host for a long time and not be degraded too quickly. To delay Histogel degradation and to achieve a longer permanence of Histogel in the host, we combined it with alginate or type I collagen gels. Both matrixes are currently used in the production of scaffolds for tissue engineering. Alginate is a polymer of mannuronic and glucuronic acid, extracted from brown algae [396]. Alginate has found numerous applications in biomedical sciences and engineering, such as controlled drug delivery for cartilage repair and regeneration [397]. Mammals lack alginase; thus, alginate results in being a non-degradable polymer. Therefore, in our protocol, alginate depolymerization was only dependent on the presence of monovalent ions in the microenvironment [398]. One critical drawback of alginate is its inherent lack of cell adhesivity [399]. In our system, this drawback was overcome by the high adhesivity of Histogel. Then, we tested Histogel-Type I collagen combinations. Type I collagen is a biodegradable material with remarkable water retention ability, low antigenicity, and cytocompatibility. Collagen is an efficient scaffold used for bone repair. It is also

employed in regenerative medicine to promote regeneration of skin, cartilage, or ligaments [400]. The presented results supported that both Histogel based materials were biocompatible and provided good mechanical support, creating a scaffold suitable for cell adhesion and proliferation. Histogel based scaffolds allowed adhesion, migration, and endothelial cell reorganization and supported the adhesion and the survival of undifferentiated and differentiated ADSCs *in vitro* and *in vivo*.

Both histogel-alginate and Histogel-type I collagen gel scaffolds well supported on the one hand the differentiation of adipose tissue derived stem cells (ADSCs) into brown-like adipose cells expressing PPARγ, PdK4, and UCP-1 proteins and on the other hand the recruitment of blood vessels. The expression of specific adipose markers and the increase of OCR in differentiated ADSCs confirmed that our models well supported ADSCs differentiation. As expected, norepinephrine and isoproterenol increased the mitochondrial activity of ADSC derived beige cells [401, 402].

Blood vessels are a necessary requirement to support BAT maintenance. In human adults, BAT consists of brown adipocytes, adipocyte progenitor cells, and blood vessels. BAT is a highly vascularized tissue located in the thorax in quantities inversely proportional to the size of the animal. Therefore, to increase brown adipose tissue or to promote the browning of WAT, it is essential to support the growth of new blood vessels. In our model, the ADSC derived beige cells produced and released in the microenvironment several pro-angiogenic factors, which, in association with the pro-angiogenic behavior of the Histogel based scaffolds, may contribute to supporting the vascularization of BAT.

The regeneration of BAT has been largely ignored in tissue engineering. Here, we proposed the use of biological scaffolds to support the proliferation and differentiation of the adipose tissue resident stem cells into a brown-like tissue and to allow the recruitment of vessels from the surrounding tissues. The employment of such devices will result in heavy BAT mass gain and efficient body weight loss. This would substantially improve the already existing applications in regenerative medicine and metabolic disease treatments.

Chapter 5

Implication of irisin in different types of cancer: A Systematic Review and Meta-analysis.

Maria Vliora[1,2], Eleni Nintou[1], Eleni Karligiotou[1], Leonidas Ioannou[1], Stefania Mitola[2], Andreas D. Flouris[1]

1.FAME Laboratory, Department of Physical Education and Sport Science, University of Thessaly, Trikala, Greece

2. Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

Conception and design of the work: M.V., A.D.F., Data collection: M.V., E.N., Data analysis and interpretation: M.V., E.K., L.I., Drafting the article: M.V., Critical revision of the article: E.N., E.K., L.I., E.G., S.M., A.D.F, Final approval of the version to be published: A.D.F.

Abstract

Cancer is a set of diseases characterized by several hallmark properties, such as increased angiogenesis, proliferation, invasion, and metastasis. The increased angiogenic activity constantly supplies the tumors with nutrients and a plethora of cytokines to ensure cell survival. Along these cytokines, is a newly discovered protein, called irisin, that is released in the circulation after physical exercise. Irisin is released after proteolytic cleavage from another protein that is expressed on the membrane of skeletal muscles and recently it has been

subject of investigation in several types of cancer. Here, we conducted a systematic review and meta-analysis to investigate its implication in different types of cancer. Our results suggest that irisin expression is decreased in cancer patients thus, it can be used as a valid biomarker for the diagnosis of several types of cancer. In addition, it seems that irisin has an important role in tumor progression and metastasis since it is involved in multiple signaling pathways that promote cell proliferation and migration.

1. Introduction

Cancer is the second leading cause of death, and according to World Health Organization the leading cause of death before the age of 70 around the world [403]. As it is a set of diseases, cancer can affect different tissues of the human body, characterized, among others, by irregular growth of cells, increased angiogenesis, resistance in cell death, increased proliferative activity, invasion, and metastasis [404]. The increased angiogenic activity and extended vasculature network constantly supply tumor cells with oxygen and nutrients that are crucial for their survival [404]. Along with intake of nutrients, the cytokines that are released in the circulation by tumors are delivered to the tissues they normally target [405].

One of these cytokines is irisin, discovered in 2012, that is released in the circulation after its proteolytic cleavage from a larger transmembrane protein, fibronectin type III domain-containing protein 5 (FNDC5) [27]. When released, it binds to α_v integrin receptors and initiates signaling cascades that mediate

the beneficial effects physical of exercise [406]. Irisin was originally described to participate in the process called browning of white adipose tissue and the induction of thermogenesis [27]. Further studies though, have demonstrated that irisin has a protective role against pathological conditions that are accompanied by chronic inflammation [407, 408]. Inflammation supports multiple cancer hallmark processes by the recruitment of inflammatory cells and the modulation of bioactive molecules in the tumor microenvironment the tumors [404]. Thus, inflammation caused by physical exercise has important role in the prevention and management of cancer [409]. Since irisin is a myokine that is released after physical exercise [410], research has focused on the potential role of irisin in different types of cancer.

Recent studies have shown decreased irisin expression in tissues that are affected by cancer in comparison with healthy tissues [411-414]. Also, the signaling pathways that are affected by the presence of irisin in different types of cancer have been investigated [415-417]. Even though there is increasing literature about the role of irisin in the occurrence and development of tumors or cancer prognosis the molecular mechanisms remain to be elucidated. For its biological behavior irisin may become a novel prognostic maker and/or a suitable target for the development of anti cancer drugs [418].

Our incomplete understanding about the involvement of irisin in tumor progression and prognosis, limits the potential of establishing of a new biomarker hence, the development of novel therapeutic treatments. It is important to research the potential of irisin as a biomarker since we need a wide range when designing new drugs against cancer to be able to validate their activity and effects [419]. To the best of our knowledge this is the first systematic

review that gleans all the information about irisin and its involvement in cancer, including *in vitro* and *in vivo* studies that have been conducted upon its discovery. The primary aim of our study was to discuss the possibility of irisin being used as a therapeutic agent for different types of cancers as well as its capability of comprising an independent prognostic factor for developing new pharmacological treatments. Moreover, our meta-analysis strongly supports the circulating irisin as a tumoral biomarker. Finally, we discuss how different doses of irisin can affect cancer cell viability.

2. Methodology

To reduce bias and the likelihood of duplication and to maximize the validity of the method used, our systematic review was registered in the international prospective register for systematic reviews (PROSPERO) database, with registration number CRD42019119590. This systematic review also fulfills the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (Figure 5.1).

2.1.1. Search strategy and selection criteria

Following PRISMA guidelines, PubMed central, Embase and Cochrane libraries databases were searched, from date of their inception to March 9th, 2021, for studies that researched any implication of irisin in different types of cancer. A search update, via alerts, was conducted until August 2021. Studies that included the investigation of irisin on any biological model (cell cultures, animals, and humans) in relation to any type of cancer were included. No date or study design limits were applied. The search algorithm that was used can be found in the Supplement 1 (1.1). We excluded reviews, conference

proceedings, editorials, magazine articles, opinion papers, letters to editor or papers that were not in English language, but we screened the reference list of such articles and the reviewed publications to retrieve relevant papers. Across all searches we included articles that consisted of original research published in peer reviewed journals. The searching process was conducted independently by two investigators (MV and EN) and any conflicts were resolved with consensus.

The screening of the titles, abstracts, and full texts for eligibility as well as the selection of the included studies was conducted by two investigators (MV and EN). Any conflict was resolved by a referee investigator (ADF). We included all methodological designs, using healthy or non-affected control-groups and/or interventional studies that showed the function of irisin in different signaling pathways in cancer; no sample size pre-set criterion was considered for the included studies. The list of included and excluded papers is available in the Supplement 2.

2.1.2. Risk of Bias Assessment

The included studies were evaluated for risk of bias by MV and EK. The selected publications consisted of both *in vivo* and *in vitro* investigations. For assessment of risk of bias and internal validity, the tool developed from the Office for Human and Animal Studies (OHAT) was used [420]. OHAT is a tool based on eleven (11) questions (corresponding to 7 categories of bias) and four different answers (“+ +” for definitely low, “+” for probably low, “-” or “NR” for probably high or not reported, and “- -” for definitely high risk of bias). For ease of comparison the scores were recorded as +3, +2, +1 and 0 respectively [421].

The maximum total score for case control and cohort studies was 21, for experimental animal studies and randomized controlled trials was 27, and for cross sectional studies was 24.

As mentioned above, the tool is designed to assess bias of multiple study designs but not studies conducted *in vitro*. Despite our thorough investigation in the literature, we did not identify a tool purposed for *in vitro* studies. Thus, we modified the OHAT tool to fit the purpose of our *in vitro* studies. On this scope, we examined bias in six out of the seven categories proposed by the OHAT tool and we slightly modified the questions according to the original ones to fit the purpose of *in vitro* studies. The scoring system was kept as in the original tool and the maximum score in the *in vitro* studies was 30. The full version of the tool as well as the modified for *in vitro* studies tool can be found in Supplement 1, section 2.1.

2.1.3. Data extraction

The data extraction was performed by MV, while EN performed data extraction on a random sample (40%) of the selected publications. Any conflicts in the data extraction were resolved through consensus and supervision by a third investigator (ADF). When necessary additional information was requested by the corresponding authors of the selected papers via email. For all studies we extracted author name(s), year of publication, type of cancer studied, analysed samples (cell line, animal strain, or human) and health condition of participants (healthy/diseased) (in the cases of human studies). In the case of *in vivo* studies, the type of cancer, the type of assay that was used to measure irisin concentration and the kit or antibody that was used were recorded. For the *in*

vitro studies, the type of cancer model, the type (modified/ non modified) of irisin that was used, the dose of irisin and period of treatment were recorded. (Supplement 1, Tables S3-S5)

2.2. Meta-analyses

2.2.1. Differences in irisin protein level and cell viability

We performed meta-analyses to calculate the differences between irisin levels in healthy tissues and tissues affected with cancer, and the blood irisin levels between healthy and diseased individuals. For the *in vitro* studies we calculated the difference in cell viability between treated and untreated cells for different doses of irisin, at different time points. In the cases where exact values were not reported in the text, we used WebPlotDigitizer (v4.5, 2021) to extract the information from the available graphs [422]. Also, we calculated the differences in cell viability between treated and untreated cells, in relation to time of treatment. For cell proliferation, migration, and invasion, there were not enough data that we could use to perform meta-analysis. The doses have been categorized into three groups, namely low (<5 nM), physiological (5-10 nM), and pharmacological (>10 nM) dose, according to the literature [423]. 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 a uniform scale. For the same reason, a random-effect model was used to account for heterogeneity due to the differences in study populations, assays used, types of cancer and cell lines. All analyses were conducted using the “metafor” package in the R language (Rstudio,

version 1.3.1093, PBC, Boston, MA, USA). The level of significance was set at an alpha level of $p < 0.05$.

2.2.2. Rating of overall study effect

To rate the overall study effect of included studies in our systematic review, we used the Rating of Overall Study Effect (ROSE, www.rose.reviews), which is a newly developed meta-analysis tool. In this type of meta-analysis, each article receives a score based on the study's qualitative or quantitative data, which is then adjusted based on the number of the participants or replications of each *in vitro* experiment. The final ROSE score for all the assessed publications varies from -3.5 (high negative effect) to 3.5 (high positive effect). The studies were independently assessed by two independent investigators (MV and EK) based on qualitative or quantitative data. We performed two ROSE meta-analyses, one for each of the two different research questions that were addressed in this review: "Can irisin be used as a biomarker?" and "Does irisin have any role in the development and progression of cancer?". The papers that provided information for both questions were evaluated in both meta-analyses using the relevant information each time.

3. Results

3.1.1. Screening of publications

In total 235 records were identified through the search that we performed in the three databases (120 from Embase, 97 from PubMed and 18 from Cochrane Library). Of these, 99 were duplicates and were removed. From the remaining 136 publications, 49 were excluded as reviews, commentaries, conference

proceedings or in language different than English. Finally, the abstracts of the remaining 87 papers were screened and 55 were found to be irrelevant to the aim of this systematic review. A total of 32 publications were included in this review while 2 more articles were retrieved by manual searching in the references of the screened articles or via alerts from the searched databases. A list of the included and excluded papers can be found in Supplement 2. The searching procedure results are illustrated in a PRISMA flowchart (Figure 5.1).

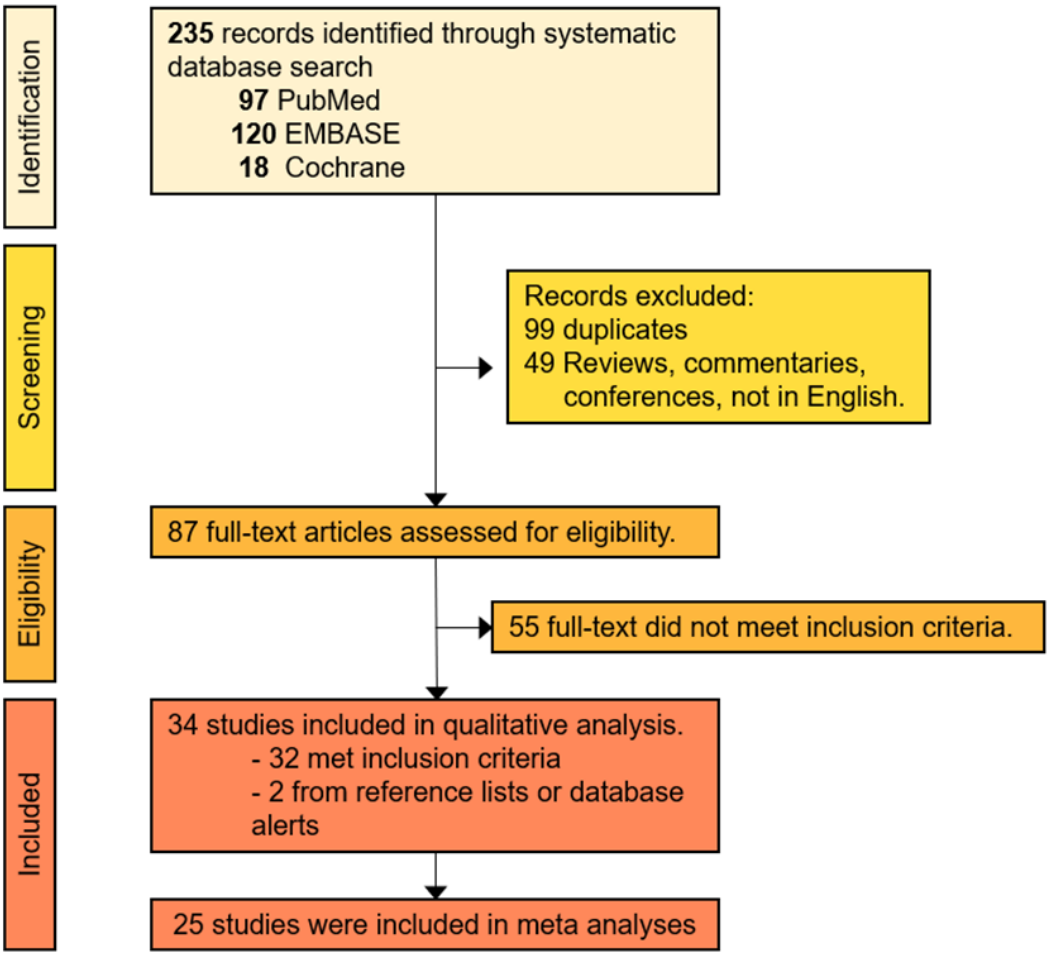


Figure 5.1. PRISMA flowchart. Selection process of papers included in the systematic review.

Out of the 34 publications that were selected for this systematic review, 24 [412-414, 417, 424-443] described *in vivo* studies of which five [413, 414, 424, 432,

438] contained immunohistochemistry results referring to the presence of irisin on tissue samples. The remaining 19 articles presented results referring to assays using blood sample extracts. Finally, 12 [415-417, 432, 444-450] articles presented results from *in vitro* experiments. In two [417, 432] of the articles, both *in vivo* and *in vitro* experiments were conducted.

3.1.2. Risk of bias assessment

For the case-control studies, the included papers scored between 12 and 21 (out of a max score of 21) on the OHAT risk assessment. For 11 out of the 18 studies in this category, detection bias was recorded, and we could not be confident on the outcome assessment. Moreover, other sources of bias were also detected such as groups with low number of participants or exogenous reasons for cancer development. For the rest of the study designs (cohort studies, experimental animal studies, cross sectional studies and randomised controlled trials), the articles scored as “definitely low risk of bias”.

Out of the 25 assessed studies, 20 (80%) scored a probably low or definitely low risk of bias on the complete reporting item. Overall, the studies included in this review present a moderate risk of bias. The full scoring of the included papers can be found in Supplement 1, Tables S2 and S3.

3.1.3. Data extraction

In vivo studies

In total 3073 human participant samples have been investigated in the 24 publications referring to *in vivo* studies and included in this systematic review,

of which 825 (26.85%) belonged to healthy individuals and 2248 (73.15%) to patients diagnosed with different types of cancer. Also, one publication with 60 animals (mice) was included in this systematic review, of which 12 were healthy and 48 were cancer induced (Supplement 1, Table S1).

Irisin serum level was decreased in cancer patients in 58% of the studies. In the rest of the studies irisin was found unaltered or there was no control group to compare. One study reported increased irisin serum protein level in renal cancer. In the *in vivo* study that was performed on laboratory animals (mice), irisin serum protein level was increased. (Supplement 1, Table S4). Ten types of cancer were investigated. Interestingly out of the 19 studies that assessed irisin protein level in serum eleven different ELISA kits we used for producing the results (Supplement 1, Table S4).

Of the five studies that used immunohistochemistry to locate irisin on tissue samples, three used the same antibody and one did not report the source of antibody that was used. In four out of the five studies, increased irisin immunoreactivity was reported in the specimens affected by cancer. (Supplement 1, Table S5).

In vitro studies

The subjects in these studies were different cancer cell lines that were treated with various concentrations of irisin or other substances in the presence or absence of irisin. In total 34 cell lines were used to investigate the role of irisin in 10 types of cancer. Three studies revealed that irisin can increase chemosensitivity of cancer cell lines to other pharmacological treatments. Five studies used modified (glycosylated) irisin from different manufacturers for

treating cells, three used non-modified irisin while the rest used anti-irisin antibodies for detection of the protein (Supplement 1, Table S6).

Nine out of 12 studies reported the inhibiting role of irisin on tumor progression through decreased cell proliferation, migration, invasion, and viability that are all hallmarks of cancer. Several signaling pathways are described, suggesting a specific role for irisin in tumor progression. Interestingly, four studies suggest that irisin acts through modulation of PI3K/AKT signaling pathway which, in turn, can control Snail or NF-kB signaling cascades (Supplement 1, Table S6). In Figure 5.2. we present the signaling pathways that are affected from irisin as presented in the publications of this review.

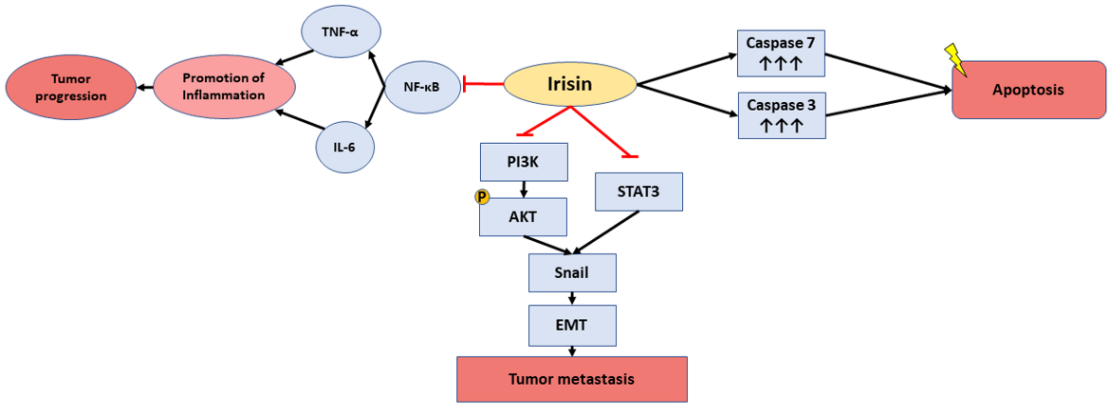


Figure 5.2. Signaling pathways that are affected by irisin in different types of cancer.

3.2. Meta-analysis Results

In total we conducted five meta-analyses, two regarding the *in vivo* studies, calculating the difference in irisin concentration in serum or tissue between healthy and diseased participants. Three meta-analyses were conducted to calculate the effect of low, physiological, and pharmacological doses of irisin on cell viability.

3.2.1. Standardized mean differences

For the *in vivo* studies that measured irisin concentration in plasma, our meta-analysis results showed that irisin decreased significantly ($p<0.01$) in cancer patients (Supplement 1, Figure S1). In contrast, in the studies that assessed irisin concentration on tissues, irisin level was not altered ($p<0.01$) after immunohistochemical localization (Supplement 1, Figure S2).

When cancer cell lines were treated with low doses of irisin cell viability tended to decrease but not significantly ($p>0.05$). We observed though a significant decrease between 24 and 48 hours ($p<0.05$) indicating that longer exposure in low doses of irisin can induce cell death (Supplement 1, Figure S3). With physiological and pharmacological doses, we observed a significant decrease in cell viability after treatment, at 24h and 48h respectively ($p<0.01$) (Supplement 1, Figures S4 and S5). Overall, we show that the highest the dose of irisin used, the more effective is the decrease in cell viability and, as expected, time of treatment is also related to cell viability.

3.2.2. ROSE meta-analysis

The ROSE platform was used to evaluate the overall study effect for the two research questions of this review: “Can irisin be used as a biomarker?” and “Does irisin have any role in the development and progression of cancer?”. In

the first meta-analysis, 23 publications contained information that could address the research question, and the overall ROSE score was 2.5 (MV=2.24 and EK=2.69), indicating a moderately positive effect. This suggests that irisin can be used as a biomarker with moderate confidence. As for the second research question, 17 publications contained relevant information, resulting in an overall ROSE score of 2.1 (MV=2.24 and EK=1.91), also indicating a moderately positive effect. This suggests moderate confidence that irisin has a role in the development and progression of cancer.

4. Discussion

Sedentary life, obesity and poor nutrition are believed to increase the risk for developing cancer [451, 452]. The mechanisms underlying this relationship though have not been established yet [377]. Exercise has been linked with reduced risk for cancer development and tumor progression [453]. The effects of exercise in chronic inflammation, cytokine production and immune system can mediate the positive effects of exercise on cancer [454]. Irisin is one of the cytokines that are produced during exercise and increasing literature indicates that it may have an important role in prognosis and treatment of various types of cancers [455, 456]. Our findings in this review confirm that irisin could be used as a biomarker and/or a prognostic factor for cancer. We also show that irisin is also implicated in tumor progression through different signaling pathways.

The *in vivo* studies that assessed irisin levels in healthy and diseased patients show that irisin significantly decreases in patients with prostate [426], osteosarcoma [427], bladder [430], breast [435], colorectal [442], liver [440],

and gastric cancers [437]. In addition, the meta-analyses we performed showed that overall irisin decreases in patients with cancer independently of the type of cancer or the assay that was employed to detect irisin in serum samples, since a random effects model was applied to our analysis. These indicate that irisin could be a good diagnostic factor for cancer.

The fact that irisin is decreased in different types of cancer may indicate the interactive relationship of irisin and cancer cachexia. Cancer cachexia is a wasting syndrome that is accompanied with weight and muscle loss [457]. The loss of muscle mass can lead to decreased amount of irisin in the circulation or on the affected tissues [458]. Irisin on the other hand can increase the expression of uncoupling protein 1, a protein that increases thermogenesis and energy consumption in adipose tissue [459]. Indeed Castro et al. [429] showed that changes in myokines is associated may induce tumor evasion and inflammation.

Furthermore, in our systematic review we also show that irisin is involved in different signaling pathways that may reveal the mechanisms through which irisin may be implicated in the different types of cancer. A hallmark of cancer is the endothelial to mesenchymal transition (EMT) which is the phenotypic conversion of endothelial to mesenchymal cells and is involved in the regulation of processes such as tissue fibrosis, promotion of metastasis and chemotherapy resistance [460]. Irisin was showed to inhibit EMT through the modulation of STAT3/Snail pathway in osteosarcoma [415] and by mediating the PI3K/Akt/Snail signaling pathway [449]. PI3K/Akt signaling pathway is also downregulated in pancreatic and liver cancer [416, 417, 450] by inducing cell apoptosis. Moreover, Zhang Z. at al. [441] in their study showed that serum

irisin is decreased in breast cancer patients with spinal metastasis compared to non-metastatic patients. Finally, irisin has been shown to inhibit NF-kB signaling by increasing chemosensitivity and inducing cell apoptosis [416] which prevents tumor progression. Also, as we show in our meta-analysis, cell viability is decreased in the presence of irisin, which makes irisin a promising agent that could be used in the treatment of cancer.

4.1. Strengths and limitations

To the best of our knowledge this is the first systematic review with meta-analysis conducted about the implication of irisin in different types of cancer. In our analysis we gathered and analysed information from 34 studies including information about 13 types of cancer and multiple subtypes of them. Also, our meta-analysis included information for 34 different cell lines that were employed in these studies. We included both *in vivo* and *in vitro* studies in our analysis to investigate both the possibility of irisin being a valid biomarker for cancer diagnosis and the effect of irisin in cancer development.

We cannot disregard the fact that in our meta-analysis we included rather diverse information, such as type of cancer, different assays for detecting irisin or measuring cell viability, different antibodies, and different types of irisin (modified and non-modified). We tried to overcome these differences by applying random effects model in our analysis. Nevertheless, we could not overcome limitations such as the lack of clinical history of the patients, the lack of correlation with other health and lifestyle conditions, the gender of the participants or small sample size in some of the studies, and the use of only

animal models or *in vitro* experiments to produce the results in the eligible studies. Thus, in future studies these factors should be considered.

5. Conclusion

We conclude that irisin decreases in cancer patients and can be used as a valid biomarker for diagnosis of several types of cancer. Also, irisin can inhibit cell viability in a time and dose dependent manner which makes it a good candidate for the development of treatments against cancer. Finally, irisin can inhibit EMT and modulate tumor progression by its implication in multiple signaling pathways.

Chapter 6

Irisin regulates thermogenesis and lipolysis in 3T3-L1 adipocytes.

Maria Vliora [1], Elisabetta Grillo [2], Michela Corsini [2], Cosetta Ravelli [2], Eleni Nintou [3], Eleni Karligiotou [3], Andreas D Flouris [3], Stefania Mitola [4].

1FAME Laboratory, Department of Exercise Science, University of Thessaly, Trikala, Greece; Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy. Electronic address: mvliora@uth.gr.
2Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.
3FAME Laboratory, Department of Exercise Science, University of Thessaly, Trikala, Greece.
4Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy. Electronic address: stefania.mitola@unibs.it.

Conceptualization, M.V., A.D.F., S.M.; Methodology, M.V., E.G., M.C. ; Formal analysis, M.V., E.G., A.D.F. ; Investigation, M.V., E.G.,C.R., ; Resources, M.C.,S.M.; Data curation, M.V., E.G., A.D.F.; Writing, original draft preparation, M.V., E.G., A.D.F. and S.M.; Writing, review and editing, M.V., E.G., C.R., E.N., E.K., M.C., A.D.F., and S.M.; Supervision, A.D.F., S.M.

This work has been published on April 2022, in Biochimica et Biophysica Acta (BBA) - General Subjects Journal under doi: 10.1016/j.bbagen.2022.130085 .

Keywords: Irisin, browning, adipocytes, cell metabolism, signalling

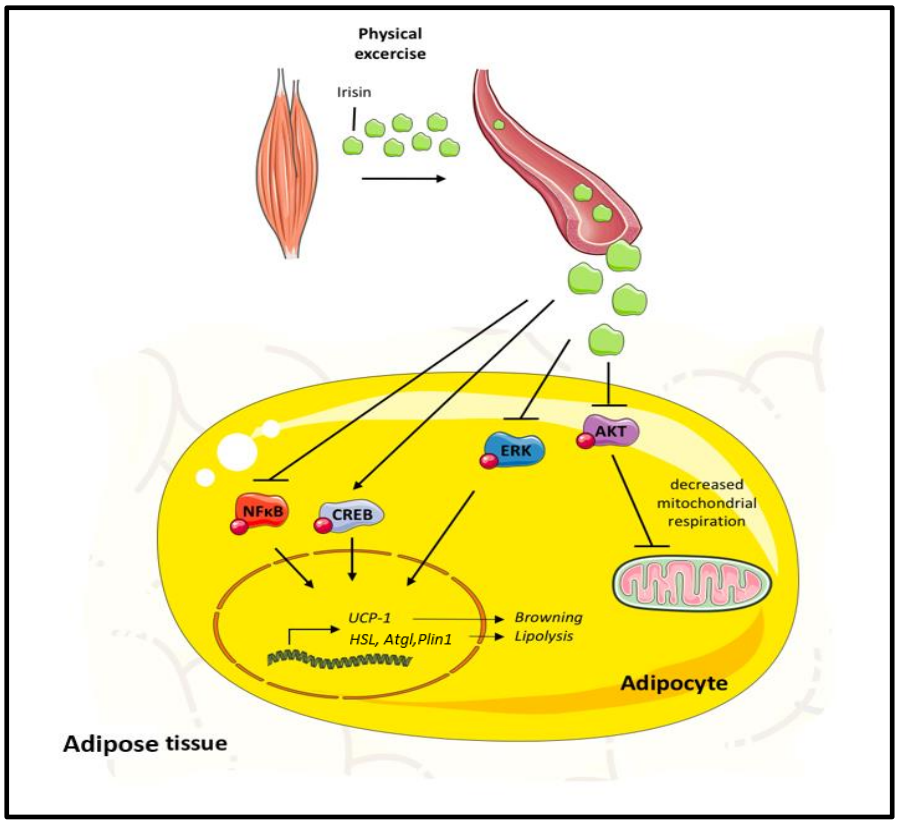


Figure 6. Graphical abstract. Intracellular pathways regulated by irisin in 3T3-L1derived adipocytes.

Abstract

Adipose tissue plays a pivotal role in the development and progression of the metabolic syndrome which along with its complications is an epidemic of the 21st century. Brown-like adipocytes reside within white adipose tissue (WAT) and respond to environmental cues releasing cytokines. Irisin is an adipo-myokine secreted mainly by skeletal muscle and targeting, among others, adipose tissue. In brown adipose tissue it upregulates uncoupling protein-1 (UCP1) which is responsible for mitochondrial non-shivering thermogenesis. Here we analysed the effects of irisin on the metabolic activity of 3T3-L1 derived adipocytes. Irisin affects mitochondrial respiration and lipolysis in a time-dependent manner through the regulation of PI3K-AKT pathway. Irisin also

induces the expression of UCP1 and the regulation of NF-κB, and CREB and ERK pathways. Our data supports the role of irisin in the induction of non-shivering thermogenesis, the regulation of energy expenditure and lipolysis in adipocytes. Thus, irisin may be an attractive therapeutic target in the treatment of obesity and related metabolic disorders.

1. Introduction

Metabolic syndrome and its complications have become an epidemic in the twenty-first century and a major public health issue. Adipose tissue (AT) plays a pivotal role in the development and progression of the metabolic syndrome [461, 462]. AT is the most plastic organ in the human body and can constantly expand and regress [376]. It is the major organ that regulates energy homeostasis in living organisms and responds to environmental cues. Brown-like adipocytes reside within white adipose tissue (WAT) and can emerge through trans-differentiation after cold exposure, physical activity, and/or drug stimulation. This process is called browning of AT [121, 160] and it is characterized by the increase of non-shivering thermogenesis, which depends on uncoupling protein 1 (UCP1) and increased lipolysis [463]. Thus, AT browning leads to in heat production and/or energy dissipation and has been considered as a possible strategy for the treatment of obesity [179].

Adipocytes play a central role in energy balance. Alteration of the mechanical, metabolic and secretory functions of adipocytes contribute to the pathogenesis of metabolic diseases, including obesity. For years, adipocytes were considered only as storage cells for triglycerides, with a passive role in the

development of obesity. Today they are instead appreciated for their mechanical support, their thermal insulation function as well as the production of several hormone-like molecules, identified as adipokines [464, 465]. Notably, the release of adipokines changes, depending on AT localization, on its cellular composition, and on the interaction with vascular structures as well as following the onset of obesity [466].

Irisin is an adipo-myokine secreted mainly by skeletal muscle and by AT, after the proteolytic cleavage of fibronectin type III domain-containing protein 5 (FNDC5) [466]. Physical activity induces the expression of irisin through the activation of the transcriptional factor peroxisome proliferator-activated receptor γ (PPARγ), the coactivator-1α (PGC-1α) and of FNDC5 [467, 468]. These pathways are involved in the browning of WAT [469]. The concentration of irisin into the bloodstream is also affected by diet and hormones, while its uptake and clearance are not yet well characterized [470].

The biological activity of irisin is not limited to adipocytes, since it can modulate several cellular responses, including proliferation, differentiation, and cell growth in normal and cancerous cells [471]. Irisin binding to α-integrin receptor [472] activates the intracellular integrin-dependent signaling pathway, including the phosphorylation of focal adhesion kinase (FAK), extracellular signal-regulated kinases (ERK and MAPK), and the activator of transcription 3 (STAT3) in several cells including cardiomyocytes, hippocampal neuronal cells, and osteoblasts [423, 473, 474]. Moreover, irisin is involved in whole-body metabolism and thermoregulation by affecting thyroid hormone secretion [475, 476]. In brown adipose tissue (BAT), it upregulates the expression of PGC-1a, leading to increased UCP1 expression and mitochondrial respiration [477].

UCP1 expression leads to non-shivering thermogenesis which takes place in the inner mitochondrial membrane of brown adipose tissue (BAT), WAT, and skeletal muscle[476] and can constitute up to 5% of the basal metabolic rate [478]. The mitochondrial expression of UCP1 separates the oxidative phosphorylation from ATP synthesis with energy being dissipated as heat, a process referred to as the mitochondrial proton leak [463, 479].

Despite the accumulating information about irisin, our knowledge of its mechanistic effects on adipocytes remains poor and limits our understanding of how the thermogenic process occurs after irisin release in the circulation. Here, we examine the role of hr-irisin on non-shivering thermogenesis and energy expenditure using the well-characterized 3T3-L1 derived adipocytes model [480]. We also monitor the time-dependent effects of hr-irisin on mitochondrial respiration and characterize the metabolic signaling pathways and the lipolytic process modulated by irisin. Our findings set the basis for the potential use of irisin as a therapeutic target in combating obesity and related metabolic disorders.

2. Materials and Methods

2.1 Reagents

All reagents were of analytical grade. DMEM media and fetal calf serum were purchased from GIBCO Life Technologies (Grand Island, NY). Insulin, dexamethasone, 3-isobutyl-methyl-xanthine, Oil Red O, EtOH Triton-X100, BriJ, and protease inhibitor were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant human irisin was purchased from (Cayman Chemical, Michigan, USA). Anti-phospho-NF-κB [sc101748; working dilution (wd) 1:1000],

anti-NF-κB (sc109, wd 1:1000), anti-AKT (sc 1619, wd 1:1000), anti UCP- (sc 6529, wd 1:1000), anti-CREB (sc 186, wd 1:1000) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-ERK1,2 (#4695, wd 1:2000), anti-phospho-AKT (#4060, wd 1:2000) and anti-phospho-ERK1/2 (#4370, wd 1:2000) antibodies were purchased from Cell Signaling Technology (Beverly, MA). Donkey HRP-labeled secondary antibodies (anti-mouse Cat #SA1-100, wd 1:5000; anti rabbit Cat #31458, wd 1:5000; anti goat Cat #A16005, wd 1:5000) were purchased from Thermo Fisher Scientific.

2.2 Cell lines

Murine 3T3-L1 fibroblasts (ATCC, Manassas, VA) were maintained at no higher than 90% confluence in DMEM with 10% Bovine Serum (BS) and antibiotics (preadipocyte medium). To induce adipocyte differentiation, cell lines reaching the confluence received a differentiating medium, consisting of DMEM supplemented with 10% fetal bovine serum (FBS), 10 μg/ml insulin, 1 μM dexamethasone and 0.5 mM 3-isobutyl-methyl-xanthine (IBMX), for two days. The medium was then changed to 10% FBS-DMEM containing 10 μg/mL insulin. After the course of three to five days, differentiated adipocytes could be observed under the light microscope (accumulated lipid droplets in the cytoplasm) until day 12 to 15 of differentiation.

2.3 Oil Red O staining

Staining with Oil Red O was performed to confirm the differentiation of 3T3-L1 cells. On day 12 of differentiation the cell monolayer was fixed for 24-hours (h) with phosphate buffered formalin (10%). After rinsing with water and EtOH 70%, the monolayer was stained with the Oil Red O solution (water-based solution of

60% saturated Oil Red O in isopropanol) for 15-min. Excess stain was removed with EtOH 70% followed by a last wash with water before observation under an inverted photomicroscope (Zeiss, Axiovert 200M).

2.4 Extracellular mitochondrial flux assay

3T3-L1 preadipocytes were seeded on Seahorse XFe24 culture plates (Agilent, Santa Clara, CA, USA) in a density of 30,000 cells/well and differentiated into mature adipocytes as described above. Then, cells were treated with 20 nM hr-irisin (Boström et al., 2012) (Cayman Chemical, Michigan, USA) for 2-h and 4-h. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) measurements were performed at 9-min intervals (3-min mixing, 3-min waiting and 3-min measuring) using a Seahorse XFe24 Extracellular Flux Analyzer (XFe Wave software) [481]. Seahorse XF Mito-Stress Test (Agilent, # 103015-100) was used to measure key parameters of mitochondrial function. Sequential treatments with oligomycin (1 μM), FCCP (0.5 μM) and rotenone/antimycin A (0.5 μM) were performed to enable quantification of basal OCR, ATP-coupled OCR, proton leak, and maximal respiration. Finally, cells were lysed, and total proteins quantified by Bradford assay. Results are expressed as pmol of OCR / min for μg of proteins.

2.5 ATP measurement

ATP quantification was performed on 1x103 cells in growth medium with ATP Determination Kit (Molecular Probes, Thermo Fisher Scientific, #A22066) following manufacturer's instructions. The bioluminescent signal was measured with EnSight Multimode Plate Reader (PerkinElmer, Waltham, Massachusetts, United States).

2.6 RT-qPCR

Total RNA was extracted using TRIzol Reagent (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's instructions. Two μg of total RNA were retro-transcribed with MMLV reverse transcriptase (Invitrogen, Thermo Fisher Scientific) using random hexaprimers. Then, cDNA was analyzed by quantitative PCR using the following primers:

Plin1_For_TTACCTAGCTGCTTTCTCGGTG,

Plin1_Rev_CACAGGCAGCTGCAGAACTC;

Lipe_For_GCTGGGCTGTCAAGCACTGT,

Lipe_Rev_GTAACTGGGTAGGCTGCCAT;

ATGL_For_ACAGGGCTACAGAGATGGACT,

ATGL_Rev_AGGCTGCAATTGATCCTCCTC;

mGAPDH_For_CATGGCCTTCCGTGTTCCTAC,

mGAPDH_Rev_TTGCTGTTGAAGTCGCAGGAG.

mUCP1_For_AGGCTTCCAGTACCATTAGGT,

mUCP1_Rev_CTGAGTGAGGCAAAGCTGATTT.

Quantitative RT-PCR was performed using the iTaqTM Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA), by ViiA7 Real-Time PCR System (Life Technologies), and data were analyzed with Viia7 Real-Time Software (Life Technologies). 2-ΔΔCt was calculated using murine_GAPDH as housekeeping. Data are expressed as relative expression ratios. All samples were analysed in triplicates.

2.7 Western Blot

Whole-cell lysates were prepared in lysis buffer containing 1% Triton-X100, 0.1 BriJ, 1mM sodium orthovanadate, and protease inhibitor cocktail. 50 μg of total proteins were separated by SDS-PAGE and probed with specific antibodies and donkey HRP-labeled secondary antibodies. Chemiluminescent signal was acquired by ChemiDoc Imaging System (BioRad). All experiments have been performed in duplicates.

2.8 Statistical analysis

Statistical analysis was performed using SPSS 22.0 (IBM, Armonk, NY, USA). Paired samples t-tests were used to assess the differences in proton leak, basal OCR, and mean ATP production. Repeated measures ANOVA was performed to assess differences in protein expression, gene expression, and ATP production. Post-hoc tests incorporating Bonferroni adjustment were performed for multiple comparisons. All values are reported as mean±SD. The level of significance was set at $p<0.05$.

3. Results

3.1 3T3-L1 differentiated cells show higher cellular respiration

In a first set of experiments, we differentiated murine 3T3-L1 cells into adipose cells and analysed their metabolic potential. To this, confluent cells were treated with differentiating medium for 12 days. As show in Figure 6.1.A-C, the morphological changes of differentiated 3T3-L1 cells are clearly visible. These

cells are filled with lipid droplets (Oil red O stained) visible under the microscope (Figure 6.1.C).

Seahorse Mito-Stress Test was employed to measure key parameters of mitochondrial function, including basal respiration, ATP-linked respiration, spare capacity, proton leak, and maximal respiration by directly measuring the OCR of undifferentiated and 3T3-L1 derived adipocytes (Figure 6.1.D-J). As expected, differentiation induced an increase in the metabolic capacity of 3T3-L1 cells as demonstrated by the significantly higher basal OCR (Figure 6.1.E) and a simultaneous increase of the ECAR. Differentiation also significantly increased the maximal mitochondrial potential as demonstrated by the higher OCR measured upon treatment with the uncoupling agent FCCP (Figure 6.1.H) and the proton leak (Figure 6.1.G). These results confirm that our protocol was suitable to drive 3T3-L1 cell differentiation into mature adipocytes.

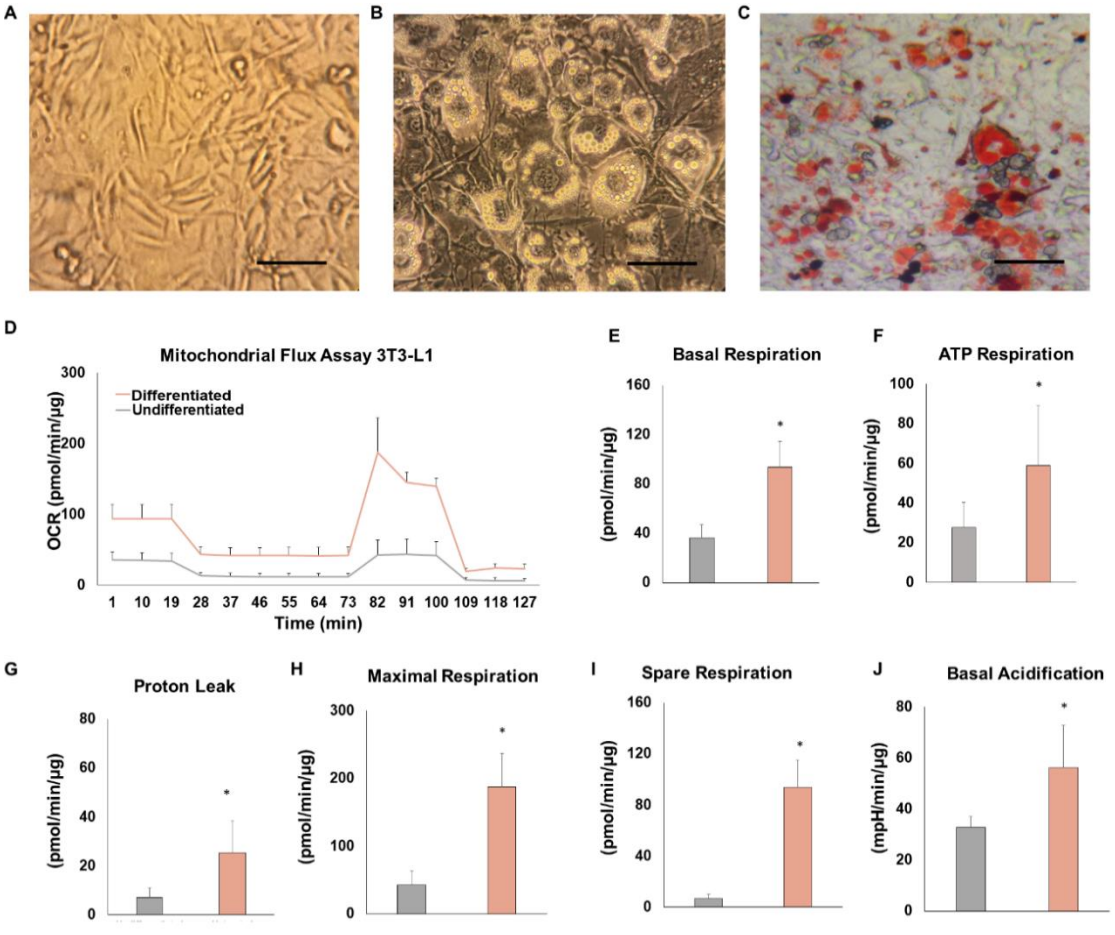


Figure 6.1. The differentiation of 3T3-L1 cells into adipose cells increases their mitochondrial metabolism. A-B) Representative images of undifferentiated (A) and differentiated 3T3-L1 cells (B) stuffed with lipid droplets (scale bar 20μm). C) Lipid droplets stained with oil O Red (scale bar 20μm). D) Seahorse Mito Stress Test performed on undifferentiated and differentiated 3T3-L1 cells. Sequential treatments with Oligomycin, FCCP and Rotenone/Antimycin A were performed to enable quantification of E) basal respiration, F) ATP-linked respiration, G) proton leak, H) maximal respiration, I) spare respiration and J) basal ECAR. Data were analyzed according to the Agilent Seahorse XF Cell Mito Stress Test Report Generator. * indicates statistically significant differences ($p<0.05$). Experiments were performed in triplicates.

3.2 Irisin regulates browning of 3T3-L1 derived adipocytes through PI3K-Akt pathway

To understand the effects of irisin on 3T3-L1 derived adipocytes, cells were treated for different times with 20 nM of hr-irisin. Then, 50 µg of the whole lysate were assessed for the activated intracellular signaling (Figure 6.2.). Irisin rapidly increased the phosphorylation level of the transcriptional factor cAMP response element-binding protein (CREB) (Figure 6.2.B), while nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (Figure 6.2.C) was downregulated. Also, the activation of their upstream modulators including protein kinase B (AKT) and extracellular regulated kinase (ERK), were reduced after the treatment with irisin (Figure 6.2.D-E). Irisin treatment induced a fast and transitory increase of the expression of the brown adipogenic marker UCP1 both at mRNA and protein levels (Figure 6.2.F-G and S1), supporting the role of irisin in thermogenesis. Furthermore, the prolongation of irisin stimulation restored the UCP1 expression in 3T3-L1-derived adipocytes (Figure S1).

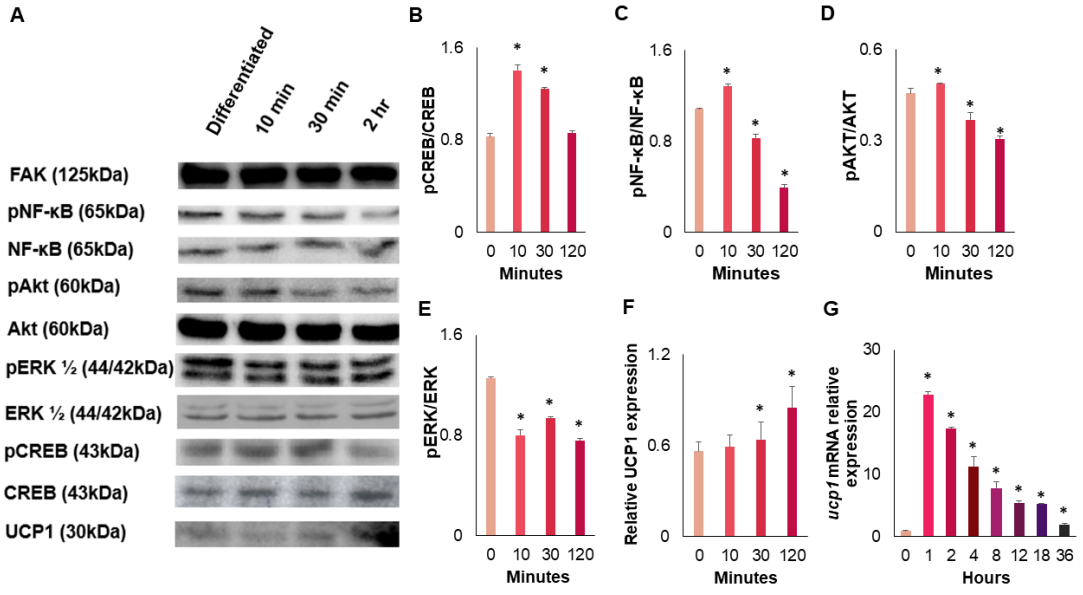


Figure 6.2. Irisin downstream effect on protein expression. A) Western blot

analysis of 50μg of total protein lysates (all experiments were performed in duplicates). B-E) Phosphorylated vs unphosphorylated relative ratios for pCREB/CREB, pNF-κB/NF-κB, pAKT/AKT, and pERK/ERK were calculated by WB densitometry. F) Relative densitometric analysis of UCP1 protein normalized to FAK. G) mRNA expression of ucp1 calculated by RT-qPCR. * indicates statistically significant differences ($p<0.05$) from untreated cells.

3.3 Irisin modulates cellular thermogenesis by reducing cellular respiration

The effects of irisin on UCP1 expression prompted us to delve into the molecular mechanisms involved in the modulation of AT metabolism. To this, we analyzed the OCR, an index of OXPHOS, in 20 nM irisin-treated adipocytes by Seahorse Mito-Stress Test (Figure 6.3.A). Results showed that irisin rapidly affected the adipocyte metabolism. Indeed, the basal OCR was significantly reduced in the first 2-h of treatment [53±19.3 vs. 110±51.2 pmol/min/μg of untreated cells ($p<0.05$)] and returned to basal levels upon prolonged stimulation 74±7.6 pmol/min/μg (Figure 6.3.A-D). Importantly, to this point the amount of irisin in cell supernatant did not change over the stimulation (Figure S2).

The energy map combining OCR with ECAR, a readout of cell glycolytic activity (Figure 6.3.B) indicated that 2-h of treatment with irisin results in a more quiescent metabolic profile, suggesting that irisin suppresses ATP production. Again, the energy phenotype was partially rescued at 4-h of treatment (Figure 6.3.A). Also, proton leak and ATP-linked respiration show the same rapid and transitory increase (Figure 6.3.C-E). Accordingly, irisin stimulation induced the

expression of lipogenic genes including adipose triglyceride lipase (ATGL) and of the hormone-sensitive lipase (HSL). Perilipin (Plin1) continued to increase until 24-h after irisin treatment (Figure 6.4.A). The production of ATP increased during irisin stimulation (Figure 6.4.B). Of note, 20 nM of irisin ensured a stimulation with a large excess of soluble stimulus. Indeed, the amount of irisin in cell supernatant was almost constant during the experimental timeframe (Figure S2).

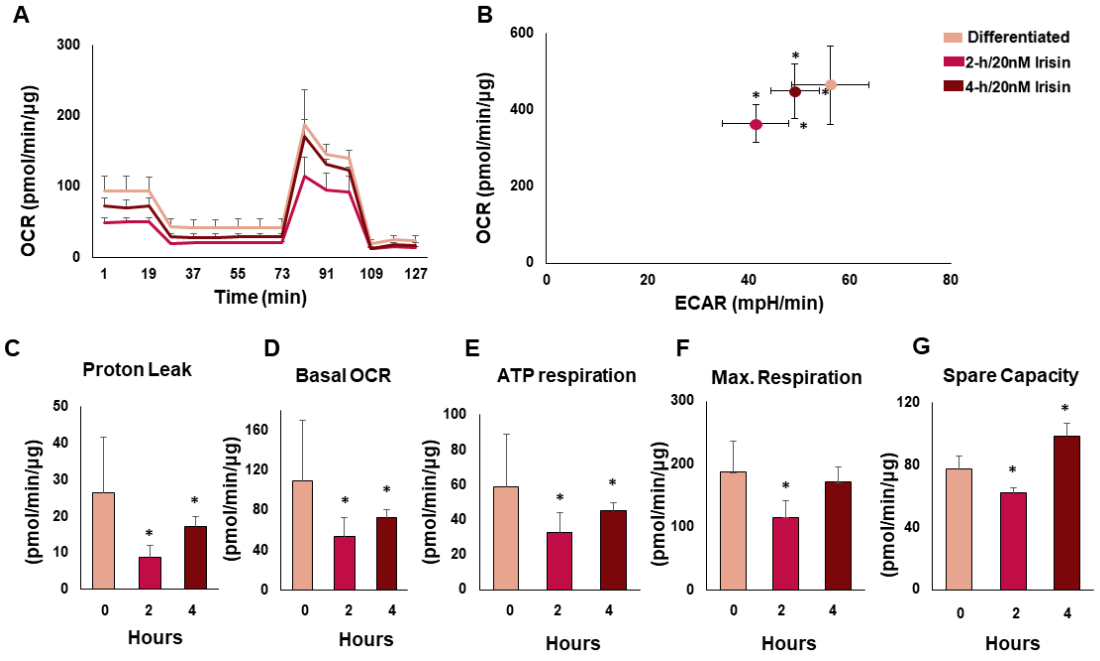


Figure 6.3. Irisin reduces mitochondrial activity in adipose-derived 3T3 cells. A) Seahorse Cell Mito Stress Test performed on adipose-derived 3T3 cells in the absence or in the presence of 20 nM of irisin. Oxygen consumption rate (OCR) was recorded over time before and after sequential addition of 1 µM oligomycin (Oligom), 0.5 µM FCCP and 1 µM Rotenone/Antimycin-A (Rot/Anti-A). B) Stressed energy phenotype of 3T3-L1 differentiated adipocytes with different periods of treatment with hr-irisin. Normalized OCR and ECAR were plotted to reveal overall basal metabolic profiles for differently treated cells. (C,

D, E) Proton leak, basal OCR and mean ATP-linked OCR were calculated. Basal OCR was calculated by subtracting non-mitochondrial respiration from baseline OCR. Note: * indicates statistical significance ($p<0.05$) from untreated cells. Experiments were performed in triplicates.

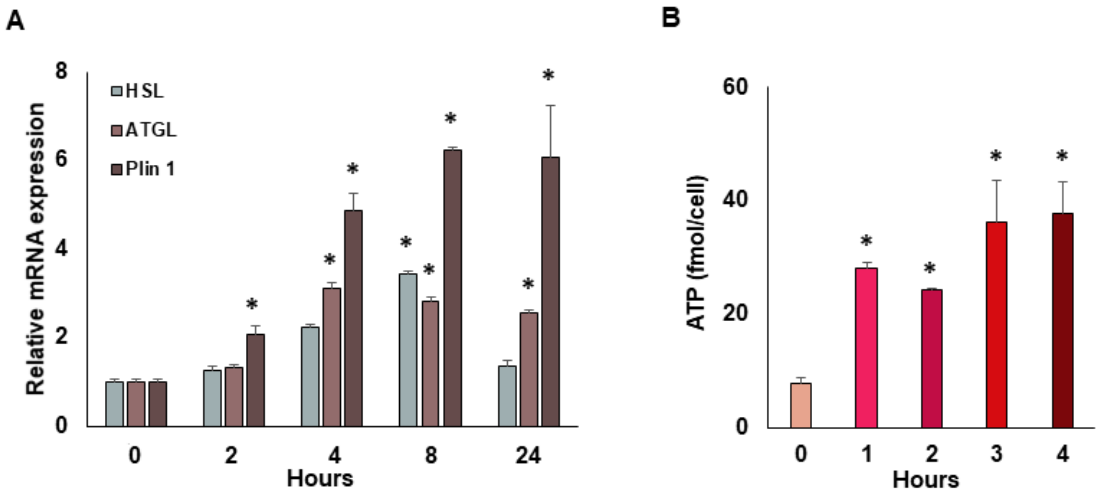


Figure 6.4. Irisin affects the expression of lipogenic genes A) Relative expression of HSL, ATGL and Plin1 during treatment with irisin (0-h to 24-h). B) ATP concentration at different time points during 4-h. * indicates statistically significant difference ($p<0.05$) from untreated cells. Experiments were performed in triplicates.

4. Discussion

Upon its discovery, irisin has been a subject of intense investigation [468]. Since irisin is an exercise-induced hormone [27] and passes a signal to AT, it is important to identify the molecular mechanisms that mediate its effects. Irisin holds an essential role in the upregulation of UCP1 [418, 469, 482], which is abundant in the inner membrane of the BAT mitochondria. However, the mechanism through which irisin causes the upregulation of UCP1 remains

unclear. Furthermore, the increase of irisin after physical exercise is another factor that needs to be clarified, since our current knowledge is based on measurements that took place across different time points, utilizing various exercise protocols [483]. As a result, controversial data about the clearance of irisin from the circulation exist in the literature. The present study addresses these gaps by defining a timeframe for the effect of irisin on adipocytes.

We found that irisin regulates UCP1 gene in adipose derived 3T3-L1 cells inducing a fast and transitory increase in its expression, while it induces a slow and long-term upregulation of lipolytic genes. These data suggest that prolonged treatment with irisin could induce AT browning. Classically, UCP1 gene expression is under the control of free fatty acids released upon extensive lipolysis [117]. However, more recent studies demonstrated that thermogenesis and UCP1 expression is independent from lipolysis, suggesting that the two processes may be involved in AT browning through distinct mechanisms [484]. Our results support this notion, showing that irisin induces a fast upregulation of ucp1 gene that precedes, and is likely independent from, the upregulation of lipolytic genes. Moreover, we elucidated the intracellular signaling activated by irisin, which involves NF-κB (p65), AKT, pCREB, and pERK.

Adipocyte differentiation is under the control of PPARγ, which is responsible for regulating the expression of genes involved in adipogenesis and lipid storage [27, 485]. PPARγ, which has an important role in adipocyte differentiation [486], interacts with the p65 subunit of NF-κB by inhibiting NF-κB transcriptional activity [487]. NF-κB is a physiological regulator of mitochondrial respiration [488]. We observed that phosphorylation of the p65 subunit of NF-κB, which enhances the selective NF-κB-mediated gene expression [489], was

attenuated at 30-min and 2-h of hr-irisin treatment (Figure 6.3.B). The decreased phosphorylation of p65 after irisin administration in the medium indicates that irisin induces adipocyte differentiation through the regulation NF-κB signaling pathway. The concomitant increase of UCP1 expression observed in this study further demonstrates the possible role of irisin in the induction of the browning process.

Irisin is involved in the molecular pathway that drives the expression of UCP1 which decreases the proton gradient developed in oxidative phosphorylation and generates heat [490]. As revealed in our results, 2-h of irisin treatment on 3T3-L1 adipocytes moved the energy phenotype of the cells to a more quiescent state, indicating that the metabolic processes that produce energy in the form of ATP (oxidative phosphorylation and glycolysis) are initially restricted upon the presence of irisin. Of note, the ATP levels that we measured represent accumulated (produced and non-consumed) ATP in the cell medium. Accordingly, we found that ATP accumulates in the medium during irisin stimulation Taken together, our findings suggest that during exercise, and consequent release of irisin, 3T3-L1 cells release ATP in the extracellular environment. This extracellular ATP could have an autocrine effect (e.g., on insulin stimulated glucose uptake) [491] or be taken up by the muscle tissue as an extra fuel source [477].

The quiescent state that the cells are transitioning to after irisin administration could also indicate a role of irisin on fatigue development. Here we examined the effects of irisin on adipocytes, but if the present results are also confirmed in muscle cells, this would suggest that irisin acts as an autocrine agent to induce fatigue, potentially as a protective mechanism. Also, it would be

promising to use a systemic or an *in vitro* co-culture approach to understand the crosstalk between the muscle and adipose tissues and its potential role on fatigue development [492].

AKT regulates glycolysis and oxidative phosphorylation in AT through the glucose transporter [493], which mediates β3-adrenergic signaling followed by increased lipolysis and glucose uptake [494]. Our results are in line with previous findings demonstrating that reduced AKT levels in AT can induce browning of WAT [495]. Increased UCP1 expression accompanied by reduced activation of AKT upon irisin stimulation indicates that irisin targets the PI3K-AKT pathway in AT. The CREB is a downstream target of PI3K-AKT pathway [496] and a transcriptional regulator of UCP1 [497]. CREB was activated rapidly after irisin treatment, and its activation was present even after 2-h of treatment. This confirms the involvement of irisin in UCP1 upregulation and its role in thermogenesis. Accordingly, irisin induces the expression of lipolytic genes, including ATGL, HSL and Plin1, which are necessary for thermogenesis.

Extracellular signal kinases (ERK ½) mediate an inhibitory effect of the expression of UCP1 as a response to their phosphorylation of tumour necrosis factor-alpha (TNF-α) [498]. During 2-h stimulation with irisin, we observed that ERK ½ phosphorylation was reduced which indicates that irisin may modulate TNF-α activity. As we did not directly measure the expression of TNF-α, potential confirmation by future studies will demonstrate the role of irisin in the browning of white adipocytes through ERK signaling. Finally, we also measured the expression of FAK which participates in adipocyte differentiation, and its disruption impairs adipocyte survival *in vitro* in 3T3-L1 adipocytes [499, 500].

We did not observe significant changes in the expression of FAK following irisin stimulation, indicating that it is not a molecular target of irisin in adipose tissue.

Following previous methodology [27], we treated our cells with 20 nM hr-irisin. This may be considered relatively high compared to the levels of irisin in human blood plasma reported in some studies. The literature data on circulating irisin levels are neither concordant nor conclusive, as the results depend on the affinity of the antibody use [31]. Further analyses are necessary to establish the normative range of irisin in human blood plasma induced by physical activity [31]. Therefore, we based our experimental design on the available literature of *in vitro* experiments [27], considering that *in vitro* experiments require higher concentrations of stimuli as there is no constant source of the cytokine as in the *in vivo* environment.

5. Conclusion

In summary, in this study we demonstrate that irisin regulates the mitochondrial respiration in 3T3-L1 derived adipocytes and exerts its effects 2-h after induction in the cellular environment. The effects of irisin occur through the modulation of intracellular signaling pathways, the expression of lipolytic genes, as well as the upregulation of UCP1. Taken together, our findings demonstrate the potential role of irisin in the induction of non-shivering thermogenesis and the regulation of energy expenditure and lipolysis in WAT adipocytes. Since AT metabolism holds a crucial role in combating obesity and related metabolic disorders, irisin can be an attractive therapeutic target that needs to be investigated in future studies.

Chapter 7

Discussion

In this thesis we show that AT holds a crucial role for the onset and development of metabolic disorders. Its attribute of communicating and exchanging biochemical signals with the surrounding tissues makes it a great target for therapeutical interventions against cancer, obesity, and other diseases of the metabolic system.

In mammals, the adipose tissues are contained in a multi-depot organ: the adipose organ [9]. Each tissue presents a unique gene expression pattern which can be altered depending on the depot it is in and its interaction with other tissues [107]. WAT is known to store energy in the form of lipids.

BAT, on the other hand, is a metabolically active tissue that consumes energy to produce heat. *"It helps us to defend our body temperature in a comfortable manner."* as Dr. Barbara Cannon has stated, and this happens through the increased expression of uncoupling protein1 (UCP1) [131]. The UCP1 is highly expressed in the mitochondria of BAT and decreases the proton gradient resulting from oxidative phosphorylation. This process takes place when BAT is activated due to external stimuli such as, cold exposure or pharmacological agents such as menthol or capsaicin [501, 502]. Activation of BAT could be a very promising approach in combating obesity and other metabolic disorders,

since it is capable of expending huge amounts of energy compared to its mass within the human body[503]. The problem with BAT though, is that *“We know where it can be found, but it's not always there in every single person. There's a region in the neck and the shoulders, and that is where you typically find it, but not everybody has it there.”* according to Dr. Aaron Cypess.

Beige adipocytes reside within WAT and can trans-differentiate and acquire characteristics of brown adipocytes with a process called “browning” of WAT [504]. They present increased mitochondrial biogenesis and activity, and they express UCP1. Thus, these cells start consuming energy, after environmental stimulation, that is stored in the nearby WAT by inducing lipolysis [484]. Since beige adipocytes were described to be able to acquire a brown phenotype, they have launched immense interest as a target for treating metabolic diseases. In this light, we conducted the review presented in Chapter 2 to investigate how the communication between AT and the vascular network can induce energy expenditure in WAT. Since the available pharmacological treatments for obesity and its comorbidities are either ineffective or associated with critical side effects, the induction of browning in WAT can be a promising approach for treatment.

In Chapter 3, in the second narrative review that was conducted, the angiogenic factors that can affect AT and can eventually be used as targets for altering its metabolic activity were investigated. A lot of these factors have shown potential in the treatment of metabolic disorders[155]. In some cases, though the amount of substance that needs to be used for effective response of AT can be prohibitive for their use in drug development [505]. Thus, an alternative approach for the induction of browning into WAT was explored. The use of

biodegradable bioscaffolds for transplanting proliferating adipocytes and endothelial cells seems to be an auspicious approach for increasing energy expenditure in obese individuals.

As mentioned above, not everyone has BAT that could be activated. It has been shown that obese individuals tend to have lower mass of BAT. Thus, the use of bioscaffolds can be a good approach to resolve this problem. Combining the information from the two reviews, a bioscaffold was tested for its capacity to facilitate angiogenic and adipogenic properties *in vivo*. Consequently, the study described in Chapter 4 was conducted, where a natural, biodegradable Histogel-based bioscaffold. HistoGel™ was tested. Indeed, it was shown that it can foster the migration, invasion, and reorganization of endothelial cells and promote blood vessel recruitment, which makes it a great candidate for use in increasing energy consumption.

We may never be able to completely cure metabolic diseases such as cancer. There is growing evidence though, that we can prevent and treat a much greater proportion of cases than currently happens. In the prevention and early prognosis of cancer many factors are being studied either as diagnostic/prognostic biomarkers or as pharmacological treatments for the disease [506-508]. One of these factors is irisin. Upon its discovery, a lot of contradiction has occurred due to the differences of its detected concentrations in the circulation. Indeed, the different studies have demonstrated results that show huge differences in the concentration of irisin and the size of the detected protein. This can be attributed to many different factors. The very small size of the protein as well as the fact that it can be found in different forms (monomer or dimer) [29] could be the reason for the various sizes that are being detected

in the literature. Considering the physiological amount of irisin in plasma, the different commercially available kits can produce quite different results. Thus, when comparing physiological to pathological results, it would be wise to refer to differences in percentage of irisin concentration or comparing the gold standard method for measuring its concentration (mass spectrometry) [509].

Irisin is a protein that targets AT and multiple tissues and has been investigated for its effect in several types of cancer [406]. As described in the systematic review presented in Chapter 5, irisin expression is reduced in cancer patients which makes it a good biomarker for diagnosis of the disease. Moreover, it seems that it can tackle a few of the hallmarks of cancer and increase sensitivity to chemotherapy, which also makes it a promising therapeutic agent. Of course, we cannot perceive cancer as one disease. It is a general definition for a series of diseases, so we need to be very cautious when we suggest a general approach [510]. Irisin though, in our meta-analysis seems to have this favourable effect in multiple types of cancer which makes it an excellent candidate for future studies.

To understand how irisin communicates with AT, the experiments described in Chapter 6 were performed. Since irisin was initially described to target adipose tissue, an experimental *in vitro* model of AT, NIH-3T3-L1 derived adipocytes, was used to test the effect on irisin on it. We showed that irisin can affect mitochondrial respiration and lipolysis in a time-dependent manner through the regulation of PI3K-AKT pathway. It can also regulate signaling pathways where NF-κB, CREB and ERK proteins are involved in. Finally, we show that irisin induces the expression of UCP1 in 3T3-L1 adipocytes thus increases the energy expenditure of these cells.

Our studies are not free of limitations. Metabolic disorders include a wide range of pathologies that each needs a separate approach. Moreover, each individual needs personalized treatment since metabolic disorders do not occur from a singular event, or factor. They can occur due to genetic background, organ, or mitochondrial dysfunction [511]. All these are influenced by a plethora of factors in the environment such as nutrition, physical activity, lifestyle choices or ambient temperature [512]. Thus, following one strategy for their treatment is far from reasonable. Therefore, in our review the common ground between metabolic diseases was investigated, and this is the dysfunction of AT. The use of bioscaffolds for the employment of factors that could increase energy expenditure or as a vehicle for delivering specific substances [513] sounds promising, but before their use *in vivo,* their testing *in vitro* or on laboratory animals is crucial. Moreover, we should always make sure of the biodegradability of the proposed bioscaffolds [514] before we use them since the induction of angiogenesis could gradually lead to opposite results.

The possible use of irisin in the diagnosis and treatment of cancer is proposed. The current literature indeed points to that conclusion, but more studies need to be conducted both *in vivo* and *in vitro* to understand the mechanisms that underlie the function of irisin. Today we are aware that irisin is naturally produced in our body after physical exercise, but a lot of information is missing about the type of exercise that is required for its expression [410]. We also are not aware of the dose of irisin that is required for sufficient response from the AT. These are certainly questions that need to be addressed in future research.

The combined results of the abovementioned studies indicate the critical importance of AT in metabolic disorders and how it could be an excellent target

for pharmacological interventions. This thesis emphasizes the significance of the cross-communication of AT with other tissues and how this crosstalk could be employed in the development of drugs and strategies that could be beneficial against metabolic diseases. The immense value of angiogenesis in this crosstalk is also stressed since it is the delivery system for all the growth factors and cytokines that transfer between AT and tissues that target it. Two main strategies for future interventions are proposed, which are use of natural Histogel-based bioscaffolds and the employment of irisin as a therapeutic agent. Probably, the combined use of these two approaches could decipher more effective results. Of course, more research is required before we proceed to human *in vivo* experiments but nonetheless these two strategies constitute encouraging approaches in the battle against metabolic disorders.

While conducting the above-mentioned research, there were many times that I hesitated in making bold statements about the produced results. I was constantly seeking for confirmation in the existing literature to explain my research, which is appropriate but not always convenient since results do not always follow the obvious path. This, many times has created controversy inside me on how these results could and should be interpreted. This also stimulated me to dive into the literature and understand how there is always a path to follow to reach into conclusions. Having to grow out of my molecular biology background and consider a wider image of science, has led me to writing this thesis with confidence, but most of all, to feeding my passion to explore new scientific skylines.

Chapter 8

Key findings

1. The crosstalk between AT and angiogenesis holds a crucial role for the onset and development of metabolic disorders.
2. Therapeutic intervention on angiogenesis has great potential as a strategy to treat disorders associated with dysfunctional AT like obesity.
3. Angiogenic factors and the use of natural bioscaffolds for the induction energy expenditure in WAT can be alternative approaches for combating obesity and other metabolic disorders.
4. Histogel based scaffolds allow adhesion, migration, and endothelial cell reorganization and support the adhesion and the survival of undifferentiated and differentiated ADSCs *in vitro* and *in vivo*.
5. Histogel-based bioscaffolds are a safe and promising bioengineering approach for improving energy consumption.
6. Irisin can be used as a valid biomarker for diagnosis of several types of cancer.
7. Irisin can potentially be used in the development of treatment in different types of cancer.
8. Irisin alters cellular respiration in 3T3-L1 adipocytes after physical activity.
9. Irisin regulates the expression of lipogenic genes in 3T3-L1 adipocytes.

Viaggiate che sennò poi diventate razzisti	Travel, otherwise, you will become racist
E finite per credere	And you end up believing
Che la vostra pelle sia l'unica ad aver ragione	that your skin is the only one that's right.
Che la vostra lingua è la più romantica	That your language is the most romantic
E che siete stati i primi ad essere i primi	And that you were the first to be the first.
Viaggiate che se non viaggiate	Travel, that if you don't travel
Poi non vi si fortificano i pensieri	Then your thoughts won't be strengthened
Non vi riempite di idee	won't get filled with ideas
Vi nascono i sogni con le gambe fragili	Your dreams will be born with fragile legs
E poi finite per credere alle televisioni	And then you will end up believing in tv-shows
E a quelli che inventano nemici	and to those who invent enemies
Che calzano a pennello con i vostri incubi	That fit perfectly with your nightmares
Per farvi vivere di terrore, senza più saluti	To make you live in terror, with no more acknowledgement
Né grazie né prego né si figuri	neither gratitude nor pray nor imagination
Viaggiate che viaggiare insegna a dare il buongiorno a tutti	Travel, that traveling teaches to say good morning to everyone
A prescindere da quale sole proveniamo	Regardless of which sun we come from
Viaggiate che viaggiare insegna a dare la buonanotte a tutti	Travel, traveling teaches everyone to say goodnight
A prescindere dalle tenebre che ci portiamo dentro	Regardless of the darkness we carry inside
Viaggiate che viaggiare insegna a resistere, a non dipendere	Travel that traveling teaches you to resist, not to depend
Ad accettare gli altri non solo per quello che sono	To accept others not just for who they are
Ma anche per quello che non potranno mai essere	But also, for what they can never be
A conoscere di cosa siamo capaci	To know what we are capable of
A sentirsi parte di una famiglia	To feel part of a family
Oltre frontiere, oltre confini	Beyond borders, beyond confinements
Oltre tradizioni e cultura	Beyond traditions and culture
Viaggiare insegna a essere oltre	Traveling teaches us to be beyond
Viaggiate che sennò poi finite per credere	Travel that otherwise you end up believing
Che siete fatti solo per un panorama	That you are made only for one view
E invece dentro di voi	And instead, inside of you
Esistono paesaggi meravigliosi	There are wonderful landscapes
Ancora da visitare	Still to visit

Gio Evan

References

1. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. The New England journal of medicine. 2017;377(1):13-27. Epub 2017/06/13. doi: 10.1056/NEJMoa1614362. PubMed PMID: 28604169; PubMed Central PMCID: PMCPMC5477817.
2. WHO. WHO European Region Obesity Report. ISBN: 9789289057738. 2022.
3. Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis RJ, et al. Body-mass index and mortality among 1.46 million white adults. The New England journal of medicine. 2010;363(23):2211-9. Epub 2010/12/03. doi: 10.1056/NEJMoa1000367. PubMed PMID: 21121834; PubMed Central PMCID: PMCPMC3066051.
4. Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K. Body Fatness and Cancer--Viewpoint of the IARC Working Group. The New England journal of medicine. 2016;375(8):794-8. Epub 2016/08/25. doi: 10.1056/NEJMsr1606602. PubMed PMID: 27557308; PubMed Central PMCID: PMCPMC6754861.
5. Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L, Thompson A, et al. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. Lancet (London, England). 2011;377(9771):1085-95. Epub 2011/03/15. doi: 10.1016/s0140-6736(11)60105-0. PubMed PMID: 21397319; PubMed Central PMCID: PMCPMC3145074.
6. Fuster JJ, Ouchi N, Gokce N, Walsh K. Obesity-Induced Changes in Adipose Tissue Microenvironment and Their Impact on Cardiovascular Disease. Circ Res. 2016;118(11):1786-807. doi: 10.1161/CIRCRESAHA.115.306885. PubMed PMID: 27230642.
7. Felipe Henriques AHBaMLBJ. Adipose Tissue Inflammation and Metabolic Disorders. 2019;Adipose Tissue. doi: DOI: 10.5772/intechopen.88631.
8. Xue Y, Petrovic N, Cao R, Larsson O, Lim S, Chen S, et al. Hypoxia-independent angiogenesis in adipose tissues during cold acclimation. Cell metabolism. 2009;9(1):99-109. Epub 2009/01/02. doi: 10.1016/j.cmet.2008.11.009. PubMed PMID: 19117550.
9. Cinti S. The adipose organ. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2005;73(1):9-15. doi: https://doi.org/10.1016/j.plefa.2005.04.010.
10. Horowitz MC, Berry R, Holtrup B, Sebo Z, Nelson T, Fretz JA, et al. Bone marrow adipocytes. Adipocyte. 2017;6(3):193-204. Epub 2017/09/06. doi: 10.1080/21623945.2017.1367881. PubMed PMID: 28872979; PubMed Central PMCID: PMCPMC5638373.
11. Cinti S. Pink Adipocytes. Trends in endocrinology and metabolism: TEM. 2018;29(9):651-66. Epub 2018/07/19. doi: 10.1016/j.tem.2018.05.007. PubMed PMID: 30017740.
12. Nedergaard J, Golozoubova V, Matthias A, Asadi A, Jacobsson A, Cannon B. UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. Biochimica et biophysica acta. 2001;1504(1):82-106. Epub 2001/03/10. doi: 10.1016/s0005-2728(00)00247-4. PubMed PMID: 11239487.
13. Gu P, Xu A. Interplay between adipose tissue and blood vessels in obesity and vascular dysfunction. Reviews in endocrine & metabolic disorders. 2013;14(1):49-58. Epub 2013/01/04. doi: 10.1007/s11154-012-9230-8. PubMed PMID: 23283583.

14. Sabaratnam R, Svenningsen P. Adipocyte-Endothelium Crosstalk in Obesity. Frontiers in Endocrinology. 2021;12. doi: 10.3389/fendo.2021.681290.
15. Romacho T, Elsen M, Röhrborn D, Eckel J. Adipose tissue and its role in organ crosstalk. Acta physiologica (Oxford, England). 2014;210(4):733-53. Epub 2014/02/06. doi: 10.1111/apha.12246. PubMed PMID: 24495317.
16. Seldin MM, Wong GW. Regulation of tissue crosstalk by skeletal muscle-derived myonectin and other myokines. Adipocyte. 2012;1(4):200-2. doi: 10.4161/adip.20877. PubMed PMID: 23700534.
17. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. The Journal of clinical investigation. 2011;121(6):2094-101. Epub 2011/06/03. doi: 10.1172/jci45887. PubMed PMID: 21633177; PubMed Central PMCID: PMCPMC3104761.
18. Panina YA, Yakimov AS, Komleva YK, Morgun AV, Lopatina OL, Malinovskaya NA, et al. Plasticity of Adipose Tissue-Derived Stem Cells and Regulation of Angiogenesis. Frontiers in physiology. 2018;9:1656. Epub 2018/12/12. doi: 10.3389/fphys.2018.01656. PubMed PMID: 30534080; PubMed Central PMCID: PMCPMC6275221.
19. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science (New York, NY). 1983;219(4587):983-5. Epub 1983/02/25. doi: 10.1126/science.6823562. PubMed PMID: 6823562.
20. Cao Y. Angiogenesis modulates adipogenesis and obesity. The Journal of clinical investigation. 2007;117(9):2362-8. Epub 2007/09/06. doi: 10.1172/jci32239. PubMed PMID: 17786229; PubMed Central PMCID: PMCPMC1963348.
21. Hosseinkhani M, Mehrabani D, Karimfar MH, Bakhtiyari S, Manafi A, Shirazi R. Tissue engineered scaffolds in regenerative medicine. World J Plast Surg. 2014;3(1):3-7. PubMed PMID: 25489516.
22. Hendley MA, Isely C, Murphy KP, Hall HE, Annamalai P, Gower RM. Scaffold Implant Into the Epididymal Adipose Tissue Protects Mice From High Fat Diet Induced Ectopic Lipid Accumulation and Hyperinsulinemia. Frontiers in Bioengineering and Biotechnology. 2020;8. doi: 10.3389/fbioe.2020.00562.
23. Coller HA. Is cancer a metabolic disease? Am J Pathol. 2014;184(1):4-17. Epub 10/17. doi: 10.1016/j.ajpath.2013.07.035. PubMed PMID: 24139946.
24. Nagai H, Kim YH. Cancer prevention from the perspective of global cancer burden patterns. J Thorac Dis. 2017;9(3):448-51. doi: 10.21037/jtd.2017.02.75. PubMed PMID: 28449441.
25. Booth A, Magnuson A, Fouts J, Foster M. Adipose tissue, obesity and adipokines: role in cancer promotion. Hormone molecular biology and clinical investigation. 2015;21(1):57-74. Epub 2015/03/18. doi: 10.1515/hmbci-2014-0037. PubMed PMID: 25781552.
26. Sumsuzzman DM, Jin Y, Choi J, Yu J-H, Lee TH, Hong Y. Pathophysiological role of endogenous irisin against tumorigenesis and metastasis: Is it a potential biomarker and therapeutic? Tumor Biology. 2019;41(12):1010428319892790. doi: 10.1177/1010428319892790.
27. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012;481(7382):463-8. Epub 2012/01/13. doi: 10.1038/nature10777. PubMed PMID: 22237023; PubMed Central PMCID: PMCPMC3522098.
28. Senesi P, Luzi L, Terruzzi I. Adipokines, Myokines, and Cardiokines: The Role of Nutritional Interventions. International journal of molecular sciences. 2020;21(21). Epub 2020/11/12. doi: 10.3390/ijms21218372. PubMed PMID: 33171610; PubMed Central PMCID: PMCPMC7664629.
29. Schumacher MA, Chinnam N, Ohashi T, Shah RS, Erickson HP. The structure of irisin reveals a novel intersubunit β-sheet fibronectin type III (FNIII) dimer: implications for receptor

activation. J Biol Chem. 2013;288(47):33738-44. Epub 10/10. doi: 10.1074/jbc.M113.516641. PubMed PMID: 24114836.
30. Uniprot. https://www.uniprot.org/uniprot/Q8NAU1. 2022.
31. Albrecht E, Norheim F, Thiede B, Holen T, Ohashi T, Schering L, et al. Irisin – a myth rather than an exercise-inducible myokine. Scientific Reports. 2015;5(1):8889. doi: 10.1038/srep08889.
32. Kazerounian S, Lawler J. Integration of pro- and anti-angiogenic signals by endothelial cells. Journal of cell communication and signaling. 2018;12(1):171-9. Epub 2017/12/22. doi: 10.1007/s12079-017-0433-3. PubMed PMID: 29264709; PubMed Central PMCID: PMCPMC5842194.
33. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature. 2000;407(6801):249-57. Epub 2000/09/23. doi: 10.1038/35025220. PubMed PMID: 11001068.
34. Iruela-Arispe LM, Dvorak HF. Angiogenesis: a Dynamic Balance of Stimulators and Inhibitors. Thromb Haemost. 1997;78(07):672-7.
35. Tiwari A, Mukherjee B, Dixit M. MicroRNA Key to Angiogenesis Regulation: MiRNA Biology and Therapy. Current cancer drug targets. 2018;18(3):266-77. Epub 2017/07/04. doi: 10.2174/1568009617666170630142725. PubMed PMID: 28669338.
36. Turunen MP, Ylä-Herttuala S. Epigenetic regulation of key vascular genes and growth factors. Cardiovascular research. 2011;90(3):441-6. Epub 2011/04/19. doi: 10.1093/cvr/cvr109. PubMed PMID: 21498421.
37. Bowler E, Oltean S. Alternative Splicing in Angiogenesis. International journal of molecular sciences. 2019;20(9). Epub 2019/04/28. doi: 10.3390/ijms20092067. PubMed PMID: 31027366; PubMed Central PMCID: PMCPMC6540211.
38. R S. Oxidative stress, inflammation and angiogenesis in the metabolic syndrome. Springer Netherlands. 2009. doi: https ://doi.org/10.1007/978-1-4020-9701-0

39. Di Stefano AB, Massihnia D, Grisafi F, Castiglia M, Toia F, Montesano L, et al. Adipose tissue, angiogenesis and angio-MIR under physiological and pathological conditions. European journal of cell biology. 2019;98(2-4):53-64. Epub 2018/12/12. doi: 10.1016/j.ejcb.2018.11.005. PubMed PMID: 30527802.
40. Virtue S, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome — An allostatic perspective. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2010;1801(3):338-49. doi: https://doi.org/10.1016/j.bbalip.2009.12.006.
41. Bråkenhielm E, Cao R, Gao B, Angelin B, Cannon B, Parini P, et al. Angiogenesis inhibitor, TNP-470, prevents diet-induced and genetic obesity in mice. Circ Res. 2004;94(12):1579-88. Epub 2004/05/25. doi: 10.1161/01.res.0000132745.76882.70. PubMed PMID: 15155527.
42. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998;395(6704):763-70. doi: 10.1038/27376.
43. Christiaens V, Lijnen HR. Angiogenesis and development of adipose tissue. Molecular and cellular endocrinology. 2010;318(1-2):2-9. Epub 2009/08/19. doi: 10.1016/j.mce.2009.08.006. PubMed PMID: 19686803.
44. Holmes DI, Zachary I. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome biology. 2005;6(2):209. Epub 2005/02/08. doi: 10.1186/gb-2005-6-2-209. PubMed PMID: 15693956; PubMed Central PMCID: PMCPMC551528.
45. Mukhopadhyay D, Tsiokas L, Zhou XM, Foster D, Brugge JS, Sukhatme VP. Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation.

Nature. 1995;375(6532):577-81. Epub 1995/06/15. doi: 10.1038/375577a0. PubMed PMID: 7540725.
46. Veikkola T, Alitalo K. VEGFs, receptors and angiogenesis. Seminars in cancer biology. 1999;9(3):211-20. Epub 1999/05/27. doi: 10.1006/scbi.1998.0091. PubMed PMID: 10343072.
47. Akagi Y, Liu W, Xie K, Zebrowski B, Shaheen RM, Ellis LM. Regulation of vascular endothelial growth factor expression in human colon cancer by interleukin-1beta. British journal of cancer. 1999;80(10):1506-11. Epub 1999/07/17. doi: 10.1038/sj.bjc.6690553. PubMed PMID: 10408390; PubMed Central PMCID: PMCPMC2363101.
48. Goradel NH, Mohammadi N, Haghi-Aminjan H, Farhood B, Negahdari B, Sahebkar A. Regulation of tumor angiogenesis by microRNAs: State of the art. Journal of cellular physiology. 2019;234(2):1099-110. Epub 2018/08/03. doi: 10.1002/jcp.27051. PubMed PMID: 30070704.
49. Cébe-Suarez S, Zehnder-Fjällman A, Ballmer-Hofer K. The role of VEGF receptors in angiogenesis; complex partnerships. Cellular and molecular life sciences : CMLS. 2006;63(5):601-15. Epub 2006/02/09. doi: 10.1007/s00018-005-5426-3. PubMed PMID: 16465447; PubMed Central PMCID: PMCPMC2773843.
50. Geiseler SJ, Morland C. The Janus Face of VEGF in Stroke. International journal of molecular sciences. 2018;19(5). Epub 2018/05/08. doi: 10.3390/ijms19051362. PubMed PMID: 29734653; PubMed Central PMCID: PMCPMC5983623.
51. Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A, et al. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. The Journal of clinical investigation. 2003;111(12):1843-51. Epub 2003/06/19. doi: 10.1172/jci17977. PubMed PMID: 12813020; PubMed Central PMCID: PMCPMC161428.
52. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature. 1996;380(6573):439-42. Epub 1996/04/04. doi: 10.1038/380439a0. PubMed PMID: 8602242.
53. Lu J, Zhao J, Zhao J, Ma J, Liu K, Yang H, et al. VEGF-A-induced immature DCs not mature DCs differentiation into endothelial-like cells through ERK1/2-dependent pathway. Cell biochemistry and function. 2011;29(4):294-302. Epub 2011/04/27. doi: 10.1002/cbf.1752. PubMed PMID: 21520151.
54. Waltenberger J, Claesson-Welsh L, Siegbahn A, Shibuya M, Heldin CH. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. J Biol Chem. 1994;269(43):26988-95. Epub 1994/10/28. PubMed PMID: 7929439.
55. Soker S, Miao HQ, Nomi M, Takashima S, Klagsbrun M. VEGF165 mediates formation of complexes containing VEGFR-2 and neuropilin-1 that enhance VEGF165-receptor binding. Journal of cellular biochemistry. 2002;85(2):357-68. Epub 2002/04/12. doi: 10.1002/jcb.10140. PubMed PMID: 11948691.
56. Soldi R, Mitola S, Strasly M, Defilippi P, Tarone G, Bussolino F. Role of alphavbeta3 integrin in the activation of vascular endothelial growth factor receptor-2. The EMBO journal. 1999;18(4):882-92. Epub 1999/02/18. doi: 10.1093/emboj/18.4.882. PubMed PMID: 10022831; PubMed Central PMCID: PMCPMC1171181.
57. Mitola S, Brenchio B, Piccinini M, Tertoolen L, Zammataro L, Breier G, et al. Type I collagen limits VEGFR-2 signaling by a SHP2 protein-tyrosine phosphatase-dependent mechanism 1. Circ Res. 2006;98(1):45-54. Epub 2005/12/13. doi: 10.1161/01.RES.0000199355.32422.7b. PubMed PMID: 16339483.
58. Ravelli C, Grillo E, Corsini M, Coltrini D, Presta M, Mitola S. β3 Integrin Promotes Long-Lasting Activation and Polarization of Vascular Endothelial Growth Factor Receptor 2 by Immobilized Ligand. Arteriosclerosis, thrombosis, and vascular biology. 2015;35(10):2161-71. Epub 2015/08/22. doi: 10.1161/atvbaha.115.306230. PubMed PMID: 26293466; PubMed Central PMCID: PMCPMC4894810.

59. Mitola S, Ravelli C, Moroni E, Salvi V, Leali D, Ballmer-Hofer K, et al. Gremlin is a novel agonist of the major proangiogenic receptor VEGFR2. Blood. 2010;116(18):3677-80. Epub 2010/07/28. doi: 10.1182/blood-2010-06-291930. PubMed PMID: 20660291.
60. Roskoski R, Jr. VEGF receptor protein-tyrosine kinases: structure and regulation. Biochemical and biophysical research communications. 2008;375(3):287-91. Epub 2008/08/06. doi: 10.1016/j.bbrc.2008.07.121. PubMed PMID: 18680722.
61. Holmes K, Roberts OL, Thomas AM, Cross MJ. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. Cellular signalling. 2007;19(10):2003-12. Epub 2007/07/31. doi: 10.1016/j.cellsig.2007.05.013. PubMed PMID: 17658244.
62. Shibuya M. Vascular endothelial growth factor receptor-1 (VEGFR-1/Flt-1): a dual regulator for angiogenesis. Angiogenesis. 2006;9(4):225-30; discussion 31. Epub 2006/11/17. doi: 10.1007/s10456-006-9055-8. PubMed PMID: 17109193.
63. Cao Y. Positive and negative modulation of angiogenesis by VEGFR1 ligands. Science signaling. 2009;2(59):re1. Epub 2009/02/27. doi: 10.1126/scisignal.259re1. PubMed PMID: 19244214.
64. Nguyen QD, De Falco S, Behar-Cohen F, Lam WC, Li X, Reichhart N, et al. Placental growth factor and its potential role in diabetic retinopathy and other ocular neovascular diseases. Acta ophthalmologica. 2018;96(1):e1-e9. Epub 2016/11/23. doi: 10.1111/aos.13325. PubMed PMID: 27874278; PubMed Central PMCID: PMCPMC5811779.
65. Takahashi H, Shibuya M. The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. Clinical Science. 2005;109(3):227-41. doi: 10.1042/cs20040370.
66. Voros G, Maquoi E, Demeulemeester D, Clerx N, Collen D, Lijnen HR. Modulation of angiogenesis during adipose tissue development in murine models of obesity. Endocrinology. 2005;146(10):4545-54. Epub 2005/07/16. doi: 10.1210/en.2005-0532. PubMed PMID: 16020476.
67. Nowak-Sliwinska P, Alitalo K, Allen E, Anisimov A, Aplin AC, Auerbach R, et al. Consensus guidelines for the use and interpretation of angiogenesis assays. Angiogenesis. 2018;21(3):425-532. Epub 2018/05/17. doi: 10.1007/s10456-018-9613-x. PubMed PMID: 29766399; PubMed Central PMCID: PMCPMC6237663.
68. Mitola S, Soldi R, Zanon I, Barra L, Gutierrez MI, Berkhout B, et al. Identification of Specific Molecular Structures of Human Immunodeficiency Virus Type 1 Tat Relevant for Its Biological Effects on Vascular Endothelial Cells. Journal of Virology. 2000;74(1):344-53. doi: doi:10.1128/JVI.74.1.344-353.2000.
69. Bosisio D, Ronca R, Salvi V, Presta M, Sozzani S. Dendritic cells in inflammatory angiogenesis and lymphangiogenesis. Current opinion in immunology. 2018;53:180-6. Epub 2018/06/08. doi: 10.1016/j.coi.2018.05.011. PubMed PMID: 29879585.
70. Deckers MM, Karperien M, van der Bent C, Yamashita T, Papapoulos SE, Löwik CW. Expression of vascular endothelial growth factors and their receptors during osteoblast differentiation. Endocrinology. 2000;141(5):1667-74. Epub 2000/05/10. doi: 10.1210/endo.141.5.7458. PubMed PMID: 10803575.
71. Yang X, Cepko CL. Flk-1, a Receptor for Vascular Endothelial Growth Factor (VEGF), Is Expressed by Retinal Progenitor Cells. The Journal of Neuroscience. 1996;16(19):6089-99. doi: 10.1523/jneurosci.16-19-06089.1996.
72. Casella I, Feccia T, Chelucci C, Samoggia P, Castelli G, Guerriero R, et al. Autocrine-paracrine VEGF loops potentiate the maturation of megakaryocytic precursors through Flt1 receptor. Blood. 2003;101(4):1316-23. Epub 2002/10/31. doi: 10.1182/blood-2002-07-2184. PubMed PMID: 12406876.

73. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nature medicine. 2003;9(6):669-76. Epub 2003/06/05. doi: 10.1038/nm0603-669. PubMed PMID: 12778165.
74. Pisacane AM, Risio M. VEGF and VEGFR-2 immunohistochemistry in human melanocytic naevi and cutaneous melanomas. Melanoma Research. 2005;15(1).
75. List AF. Vascular Endothelial Growth Factor Signaling Pathway as an Emerging Target in Hematologic Malignancies. The Oncologist. 2001;6(S5):24-31. doi: 10.1634/theoncologist.6-suppl_5-24.
76. Youssoufian H, Hicklin DJ, Rowinsky EK. Review: monoclonal antibodies to the vascular endothelial growth factor receptor-2 in cancer therapy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2007;13(18 Pt 2):5544s-8s. Epub 2007/09/19. doi: 10.1158/1078-0432.ccr-07-1107. PubMed PMID: 17875787.
77. Witmer AN, Blaauwgeers HG, Weich HA, Alitalo K, Vrensen GF, Schlingemann RO. Altered expression patterns of VEGF receptors in human diabetic retina and in experimental VEGF-induced retinopathy in monkey. Investigative ophthalmology & visual science. 2002;43(3):849-57. Epub 2002/02/28. PubMed PMID: 11867607.
78. Siiteri PK. Adipose tissue as a source of hormones. The American journal of clinical nutrition. 1987;45(1 Suppl):277-82. Epub 1987/01/01. doi: 10.1093/ajcn/45.1.277. PubMed PMID: 3541569.
79. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994;372(6505):425-32. Epub 1994/12/01. doi: 10.1038/372425a0. PubMed PMID: 7984236.
80. Aldhahi W, Hamdy O. Adipokines, inflammation, and the endothelium in diabetes. Current diabetes reports. 2003;3(4):293-8. doi: 10.1007/s11892-003-0020-2.
81. Rupnick MA, Panigrahy D, Zhang CY, Dallabrida SM, Lowell BB, Langer R, et al. Adipose tissue mass can be regulated through the vasculature. Proceedings of the National Academy of Sciences of the United States of America. 2002;99(16):10730-5. Epub 2002/08/01. doi: 10.1073/pnas.162349799. PubMed PMID: 12149466; PubMed Central PMCID: PMCPMC125027.
82. Cao Y. Adipose tissue angiogenesis as a therapeutic target for obesity and metabolic diseases. Nature reviews Drug discovery. 2010;9(2):107-15. Epub 2010/02/02. doi: 10.1038/nrd3055. PubMed PMID: 20118961.
83. Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. Nature medicine. 2013;19(10):1252-63. Epub 2013/10/09. doi: 10.1038/nm.3361. PubMed PMID: 24100998.
84. Wang QA, Tao C, Gupta RK, Scherer PE. Tracking adipogenesis during white adipose tissue development, expansion and regeneration. Nature medicine. 2013;19(10):1338-44. Epub 2013/09/03. doi: 10.1038/nm.3324. PubMed PMID: 23995282; PubMed Central PMCID: PMCPMC4075943.
85. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. Nature. 2008;453(7196):783-7. Epub 2008/05/06. doi: 10.1038/nature06902. PubMed PMID: 18454136.
86. Girousse A, Gil-Ortega M, Bourlier V, Bergeaud C, Sastourné-Arrey Q, Moro C, et al. The Release of Adipose Stromal Cells from Subcutaneous Adipose Tissue Regulates Ectopic Intramuscular Adipocyte Deposition. Cell reports. 2019;27(2):323-33.e5. Epub 2019/04/11. doi: 10.1016/j.celrep.2019.03.038. PubMed PMID: 30970240.
87. Sengenès C, Miranville A, Maumus M, de Barros S, Busse R, Bouloumié A. Chemotaxis and differentiation of human adipose tissue CD34+/CD31- progenitor cells: role of stromal derived factor-1 released by adipose tissue capillary endothelial cells. Stem cells (Dayton, Ohio). 2007;25(9):2269-76. Epub 2007/05/26. doi: 10.1634/stemcells.2007-0180. PubMed PMID: 17525234.

88. Maumus M, Peyrafitte JA, D'Angelo R, Fournier-Wirth C, Bouloumié A, Casteilla L, et al. Native human adipose stromal cells: localization, morphology and phenotype. International Journal of Obesity. 2011;35(9):1141-53. doi: 10.1038/ijo.2010.269.
89. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Molecular biology of the cell. 2002;13(12):4279-95. Epub 2002/12/12. doi: 10.1091/mbc.e02-02-0105. PubMed PMID: 12475952; PubMed Central PMCID: PMCPMC138633.
90. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue engineering. 2001;7(2):211-28. Epub 2001/04/17. doi: 10.1089/107632701300062859. PubMed PMID: 11304456.
91. Flynn L, Woodhouse KA. Adipose tissue engineering with cells in engineered matrices. Organogenesis. 2008;4(4):228-35. Epub 2009/04/02. doi: 10.4161/org.4.4.7082. PubMed PMID: 19337402; PubMed Central PMCID: PMCPMC2634327.
92. Tan SS, Ng ZY, Zhan W, Rozen W. Role of Adipose-derived Stem Cells in Fat Grafting and Reconstructive Surgery. Journal of cutaneous and aesthetic surgery. 2016;9(3):152-6. Epub 2016/10/21. doi: 10.4103/0974-2077.191672. PubMed PMID: 27761084; PubMed Central PMCID: PMCPMC5064678.
93. Bellows CF, Zhang Y, Simmons PJ, Khalsa AS, Kolonin MG. Influence of BMI on level of circulating progenitor cells. Obesity (Silver Spring, Md). 2011;19(8):1722-6. Epub 2011/02/05. doi: 10.1038/oby.2010.347. PubMed PMID: 21293449; PubMed Central PMCID: PMCPMC3143489.
94. Gil-Ortega M, Fernández-Alfonso MS, Somoza B, Casteilla L, Sengenès C. Ex vivo microperfusion system of the adipose organ: a new approach to studying the mobilization of adipose cell populations. International Journal of Obesity. 2014;38(9):1255-62. doi: 10.1038/ijo.2013.243.
95. Gil-Ortega M, Garidou L, Barreau C, Maumus M, Breasson L, Tavernier G, et al. Native adipose stromal cells egress from adipose tissue in vivo: evidence during lymph node activation. Stem cells (Dayton, Ohio). 2013;31(7):1309-20. Epub 2013/03/28. doi: 10.1002/stem.1375. PubMed PMID: 23533182.
96. Volz AC, Omengo B, Gehrke S, Kluger PJ. Comparing the use of differentiated adipose-derived stem cells and mature adipocytes to model adipose tissue in vitro. Differentiation; research in biological diversity. 2019;110:19-28. Epub 2019/10/01. doi: 10.1016/j.diff.2019.09.002. PubMed PMID: 31568881.
97. Han Y, Ren J, Bai Y, Pei X, Han Y. Exosomes from hypoxia-treated human adipose-derived mesenchymal stem cells enhance angiogenesis through VEGF/VEGF-R. The international journal of biochemistry & cell biology. 2019;109:59-68. Epub 2019/02/03. doi: 10.1016/j.biocel.2019.01.017. PubMed PMID: 30710751.
98. El-Ftesi S, Chang EI, Longaker MT, Gurtner GC. Aging and diabetes impair the neovascular potential of adipose-derived stromal cells. Plastic and reconstructive surgery. 2009;123(2):475-85. Epub 2009/02/03. doi: 10.1097/PRS.0b013e3181954d08. PubMed PMID: 19182604; PubMed Central PMCID: PMCPMC2878769.
99. Rennert RC, Sorkin M, Januszyk M, Duscher D, Kosaraju R, Chung MT, et al. Diabetes impairs the angiogenic potential of adipose-derived stem cells by selectively depleting cellular subpopulations. Stem Cell Research & Therapy. 2014;5(3):79. doi: 10.1186/scrt468.
100. Miranville A, Heeschen C, Sengenès C, Curat CA, Busse R, Bouloumié A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. Circulation. 2004;110(3):349-55. Epub 2004/07/09. doi: 10.1161/01.cir.0000135466.16823.d0. PubMed PMID: 15238461.
101. Vishnubalaji R, Manikandan M, Al-Nbaheen M, Kadalmani B, Aldahmash A, Alajez NM. In vitro differentiation of human skin-derived multipotent stromal cells into putative

endothelial-like cells. BMC developmental biology. 2012;12:7. Epub 2012/01/28. doi: 10.1186/1471-213x-12-7. PubMed PMID: 22280443; PubMed Central PMCID: PMCPMC3280173.
102. Santos Rizzo Zuttion MS, Dias Câmara DA, Dariolli R, Takimura C, Wenceslau C, Kerkis I. In vitro heterogeneity of porcine adipose tissue-derived stem cells. Tissue & cell. 2019;58:51-60. Epub 2019/05/28. doi: 10.1016/j.tice.2019.04.001. PubMed PMID: 31133246.
103. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, et al. Identification and importance of brown adipose tissue in adult humans. The New England journal of medicine. 2009;360(15):1509-17. Epub 2009/04/10. doi: 10.1056/NEJMoa0810780. PubMed PMID: 19357406; PubMed Central PMCID: PMCPMC2859951.
104. Bjørndal B, Burri L, Staalesen V, Skorve J, Berge RK. Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. Journal of obesity. 2011;2011:490650. Epub 2011/03/16. doi: 10.1155/2011/490650. PubMed PMID: 21403826; PubMed Central PMCID: PMCPMC3042633.
105. Hausman GJ, Richardson RL. Adipose tissue angiogenesis1,2. Journal of Animal Science. 2004;82(3):925-34. doi: 10.2527/2004.823925x.
106. Crandall DL, Busler DE, McHendry-Rinde B, Groeling TM, Kral JG. Autocrine regulation of human preadipocyte migration by plasminogen activator inhibitor-1. The Journal of clinical endocrinology and metabolism. 2000;85(7):2609-14. Epub 2000/07/21. doi: 10.1210/jcem.85.7.6678. PubMed PMID: 10902815.
107. Yamamoto Y, Gesta S, Lee KY, Tran TT, Saadatirad P, Kahn CR. Adipose Depots Possess Unique Developmental Gene Signatures. Obesity. 2010;18(5):872-8. doi: https://doi.org/10.1038/oby.2009.512.
108. Ussar S, Lee KY, Dankel SN, Boucher J, Haering MF, Kleinridders A, et al. ASC-1, PAT2, and P2RX5 are cell surface markers for white, beige, and brown adipocytes. Science translational medicine. 2014;6(247):247ra103. Epub 2014/08/01. doi: 10.1126/scitranslmed.3008490. PubMed PMID: 25080478; PubMed Central PMCID: PMCPMC4356008.
109. Matthias A, Ohlson KB, Fredriksson JM, Jacobsson A, Nedergaard J, Cannon B. Thermogenic responses in brown fat cells are fully UCP1-dependent. UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty scid-induced thermogenesis. J Biol Chem. 2000;275(33):25073-81. Epub 2000/05/29. doi: 10.1074/jbc.M000547200. PubMed PMID: 10825155.
110. Bouillaud F, Ricquier D, Gulik-Krzywicki T, Gary-Bobo CM. The possible proton translocating activity of the mitochondrial uncoupling protein of brown adipose tissue. FEBS letters. 1983;164(2):272-6. doi: https://doi.org/10.1016/0014-5793(83)80300-7.
111. Cypess AM, Kahn CR. The role and importance of brown adipose tissue in energy homeostasis. Current opinion in pediatrics. 2010;22(4):478-84. Epub 2010/05/22. doi: 10.1097/MOP.0b013e32833a8d6e. PubMed PMID: 20489634; PubMed Central PMCID: PMCPMC3593062.
112. Cypess AM, White AP, Vernochet C, Schulz TJ, Xue R, Sass CA, et al. Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nature medicine. 2013;19(5):635-9. Epub 2013/04/23. doi: 10.1038/nm.3112. PubMed PMID: 23603815; PubMed Central PMCID: PMCPMC3650129.
113. During MJ, Liu X, Huang W, Magee D, Slater A, McMurphy T, et al. Adipose VEGF Links the White-to-Brown Fat Switch With Environmental, Genetic, and Pharmacological Stimuli in Male Mice. Endocrinology. 2015;156(6):2059-73. Epub 2015/03/13. doi: 10.1210/en.2014-1905. PubMed PMID: 25763639; PubMed Central PMCID: PMCPMC4430610.
114. Avram AS, Avram MM, James WD. Subcutaneous fat in normal and diseased states: 2. Anatomy and physiology of white and brown adipose tissue. Journal of the American Academy

of Dermatology. 2005;53(4):671-83. Epub 2005/10/04. doi: 10.1016/j.jaad.2005.05.015. PubMed PMID: 16198791.
115. Schulz TJ, Huang TL, Tran TT, Zhang H, Townsend KL, Shadrach JL, et al. Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(1):143-8. Epub 2010/12/22. doi: 10.1073/pnas.1010929108. PubMed PMID: 21173238; PubMed Central PMCID: PMCPMC3017184.
116. Wang W, Seale P. Control of brown and beige fat development. Nature reviews Molecular cell biology. 2016;17(11):691-702. Epub 2016/10/21. doi: 10.1038/nrm.2016.96. PubMed PMID: 27552974; PubMed Central PMCID: PMCPMC5627770.
117. Fedorenko A, Lishko PV, Kirichok Y. Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. Cell. 2012;151(2):400-13. Epub 2012/10/16. doi: 10.1016/j.cell.2012.09.010. PubMed PMID: 23063128; PubMed Central PMCID: PMCPMC3782081.
118. Nicholls DG. The physiological regulation of uncoupling proteins. Biochimica et biophysica acta. 2006;1757(5-6):459-66. Epub 2006/05/27. doi: 10.1016/j.bbabio.2006.02.005. PubMed PMID: 16725104.
119. Shabalina IG, Jacobsson A, Cannon B, Nedergaard J. Native UCP1 displays simple competitive kinetics between the regulators purine nucleotides and fatty acids. J Biol Chem. 2004;279(37):38236-48. Epub 2004/06/23. doi: 10.1074/jbc.M402375200. PubMed PMID: 15208325.
120. Cao Y. Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity. Cell metabolism. 2013;18(4):478-89. Epub 2013/09/17. doi: 10.1016/j.cmet.2013.08.008. PubMed PMID: 24035587.
121. Enerbäck S. Human brown adipose tissue. Cell metabolism. 2010;11(4):248-52. Epub 2010/04/09. doi: 10.1016/j.cmet.2010.03.008. PubMed PMID: 20374955.
122. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell. 1998;92(6):829-39. Epub 1998/04/07. doi: 10.1016/s0092-8674(00)81410-5. PubMed PMID: 9529258.
123. Rodrigues K, Pereira RM, de Campos TDP, de Moura RF, da Silva ASR, Cintra DE, et al. The Role of Physical Exercise to Improve the Browning of White Adipose Tissue via POMC Neurons. Frontiers in cellular neuroscience. 2018;12:88. Epub 2018/04/13. doi: 10.3389/fncel.2018.00088. PubMed PMID: 29643769; PubMed Central PMCID: PMCPMC5882867.
124. Inagaki T, Sakai J, Kajimura S. Transcriptional and epigenetic control of brown and beige adipose cell fate and function. Nature reviews Molecular cell biology. 2016;17(8):480-95. Epub 2016/06/03. doi: 10.1038/nrm.2016.62. PubMed PMID: 27251423; PubMed Central PMCID: PMCPMC4956538.
125. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, et al. PRDM16 controls a brown fat/skeletal muscle switch. Nature. 2008;454(7207):961-7. Epub 2008/08/23. doi: 10.1038/nature07182. PubMed PMID: 18719582; PubMed Central PMCID: PMCPMC2583329.
126. Giralt M, Villarroya F. White, brown, beige/brite: different adipose cells for different functions? Endocrinology. 2013;154(9):2992-3000. Epub 2013/06/21. doi: 10.1210/en.2013-1403. PubMed PMID: 23782940.
127. Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell. 2012;150(2):366-76. Epub 2012/07/17. doi: 10.1016/j.cell.2012.05.016. PubMed PMID: 22796012; PubMed Central PMCID: PMCPMC3402601.
128. Sharp LZ, Shinoda K, Ohno H, Scheel DW, Tomoda E, Ruiz L, et al. Human BAT possesses molecular signatures that resemble beige/brite cells. PloS one. 2012;7(11):e49452. Epub 2012/11/21. doi: 10.1371/journal.pone.0049452. PubMed PMID: 23166672; PubMed

Central PMCID: PMCPMC3500293 Pharma Co.), the authors declare that they have no conflicts of interest, including employment, consultancy, patents, products in development or marketed products. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.
129. Bartelt A, Heeren J. Adipose tissue browning and metabolic health. Nature reviews Endocrinology. 2014;10(1):24-36. Epub 2013/10/23. doi: 10.1038/nrendo.2013.204. PubMed PMID: 24146030.
130. Bowers RR, Lane MD. A role for bone morphogenetic protein-4 in adipocyte development. Cell cycle (Georgetown, Tex). 2007;6(4):385-9. Epub 2007/02/23. doi: 10.4161/cc.6.4.3804. PubMed PMID: 17314508.
131. Ikeda K, Maretich P, Kajimura S. The Common and Distinct Features of Brown and Beige Adipocytes. Trends in endocrinology and metabolism: TEM. 2018;29(3):191-200. Epub 2018/01/26. doi: 10.1016/j.tem.2018.01.001. PubMed PMID: 29366777; PubMed Central PMCID: PMCPMC5826798.
132. Waldén TB, Hansen IR, Timmons JA, Cannon B, Nedergaard J. Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. American journal of physiology Endocrinology and metabolism. 2012;302(1):E19-31. Epub 2011/08/11. doi: 10.1152/ajpendo.00249.2011. PubMed PMID: 21828341.
133. Hondares E, Mora O, Yubero P, Rodriguez de la Concepción M, Iglesias R, Giralt M, et al. Thiazolidinediones and rexinoids induce peroxisome proliferator-activated receptor-coactivator (PGC)-1alpha gene transcription: an autoregulatory loop controls PGC-1alpha expression in adipocytes via peroxisome proliferator-activated receptor-gamma coactivation. Endocrinology. 2006;147(6):2829-38. Epub 2006/03/04. doi: 10.1210/en.2006-0070. PubMed PMID: 16513826.
134. Hondares E, Rosell M, Díaz-Delfín J, Olmos Y, Monsalve M, Iglesias R, et al. Peroxisome proliferator-activated receptor α (PPARα) induces PPARγ coactivator 1α (PGC-1α) gene expression and contributes to thermogenic activation of brown fat: involvement of PRDM16. J Biol Chem. 2011;286(50):43112-22. Epub 2011/10/29. doi: 10.1074/jbc.M111.252775. PubMed PMID: 22033933; PubMed Central PMCID: PMCPMC3234861.
135. Ishibashi J, Seale P. Functions of Prdm16 in thermogenic fat cells. Temperature. 2015;2(1):65-72. doi: 10.4161/23328940.2014.974444.
136. Fukumura D, Ushiyama A, Duda DG, Xu L, Tam J, Krishna V, et al. Paracrine regulation of angiogenesis and adipocyte differentiation during in vivo adipogenesis. Circ Res. 2003;93(9):e88-97. Epub 2003/10/04. doi: 10.1161/01.res.0000099243.20096.fa. PubMed PMID: 14525808; PubMed Central PMCID: PMCPMC2755542.
137. Mahdaviani K, Chess D, Wu Y, Shirihai O, Aprahamian TR. Autocrine effect of vascular endothelial growth factor-A is essential for mitochondrial function in brown adipocytes. Metabolism: clinical and experimental. 2016;65(1):26-35. Epub 2015/12/20. doi: 10.1016/j.metabol.2015.09.012. PubMed PMID: 26683794; PubMed Central PMCID: PMCPMC4684900.
138. Almalki SG, Agrawal DK. ERK signaling is required for VEGF-A/VEGFR2-induced differentiation of porcine adipose-derived mesenchymal stem cells into endothelial cells. Stem Cell Research & Therapy. 2017;8(1):113. doi: 10.1186/s13287-017-0568-4.
139. Khan S, Villalobos MA, Choron RL, Chang S, Brown SA, Carpenter JP, et al. Fibroblast growth factor and vascular endothelial growth factor play a critical role in endotheliogenesis from human adipose-derived stem cells. Journal of vascular surgery. 2017;65(5):1483-92. Epub 2016/08/16. doi: 10.1016/j.jvs.2016.04.034. PubMed PMID: 27514438.
140. Hagberg CE, Falkevall A, Wang X, Larsson E, Huusko J, Nilsson I, et al. Vascular endothelial growth factor B controls endothelial fatty acid uptake. Nature. 2010;464(7290):917-21. Epub 2010/03/17. doi: 10.1038/nature08945. PubMed PMID: 20228789.

141. Hagberg C, Mehlem A, Falkevall A, Muhl L, Eriksson U. Endothelial fatty acid transport: role of vascular endothelial growth factor B. Physiology (Bethesda, Md). 2013;28(2):125-34. Epub 2013/03/05. doi: 10.1152/physiol.00042.2012. PubMed PMID: 23455771; PubMed Central PMCID: PMCPMC3768092.
142. Cao R, Brakenhielm E, Wahlestedt C, Thyberg J, Cao Y. Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(11):6390-5. Epub 2001/05/10. doi: 10.1073/pnas.101564798. PubMed PMID: 11344271; PubMed Central PMCID: PMCPMC33478.
143. Sun K, Wernstedt Asterholm I, Kusminski CM, Bueno AC, Wang ZV, Pollard JW, et al. Dichotomous effects of VEGF-A on adipose tissue dysfunction. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(15):5874-9. Epub 2012/03/28. doi: 10.1073/pnas.1200447109. PubMed PMID: 22451920; PubMed Central PMCID: PMCPMC3326476.
144. Elias I, Franckhauser S, Bosch F. New insights into adipose tissue VEGF-A actions in the control of obesity and insulin resistance. Adipocyte. 2013;2(2):109-12. Epub 2013/06/28. doi: 10.4161/adip.22880. PubMed PMID: 23805408; PubMed Central PMCID: PMCPMC3661112.
145. Harwood HJ, Jr. The adipocyte as an endocrine organ in the regulation of metabolic homeostasis. Neuropharmacology. 2012;63(1):57-75. Epub 2011/12/28. doi: 10.1016/j.neuropharm.2011.12.010. PubMed PMID: 22200617.
146. Wang B, Fu X, Liang X, Deavila JM, Wang Z, Zhao L, et al. Retinoic acid induces white adipose tissue browning by increasing adipose vascularity and inducing beige adipogenesis of PDGFRα(+) adipose progenitors. Cell discovery. 2017;3:17036. Epub 2017/10/13. doi: 10.1038/celldisc.2017.36. PubMed PMID: 29021914; PubMed Central PMCID: PMCPMC5633810.
147. Park J, Kim M, Sun K, An YA, Gu X, Scherer PE. VEGF-A-Expressing Adipose Tissue Shows Rapid Beiging and Enhanced Survival After Transplantation and Confers IL-4-Independent Metabolic Improvements. Diabetes. 2017;66(6):1479-90. Epub 2017/03/04. doi: 10.2337/db16-1081. PubMed PMID: 28254844; PubMed Central PMCID: PMCPMC5440018.
148. Liu Y, Berendsen AD, Jia S, Lotinun S, Baron R, Ferrara N, et al. Intracellular VEGF regulates the balance between osteoblast and adipocyte differentiation. The Journal of clinical investigation. 2012;122(9):3101-13. Epub 2012/08/14. doi: 10.1172/jci61209. PubMed PMID: 22886301; PubMed Central PMCID: PMCPMC3428080.
149. Jo DH, Park SW, Cho CS, Powner MB, Kim JH, Fruttiger M, et al. Intravitreally Injected Anti-VEGF Antibody Reduces Brown Fat in Neonatal Mice. PloS one. 2015;10(7):e0134308. Epub 2015/08/01. doi: 10.1371/journal.pone.0134308. PubMed PMID: 26226015; PubMed Central PMCID: PMCPMC4520452.
150. Scarpulla RC. Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. Biochimica et biophysica acta. 2011;1813(7):1269-78. Epub 2010/10/12. doi: 10.1016/j.bbamcr.2010.09.019. PubMed PMID: 20933024; PubMed Central PMCID: PMCPMC3035754.
151. Cao Y. Molecular mechanisms and therapeutic development of angiogenesis inhibitors. Advances in cancer research. 2008;100:113-31. Epub 2008/07/16. doi: 10.1016/s0065-230x(08)00004-3. PubMed PMID: 18620094.
152. Colleluori G, Perugini J, Giordano A, Cinti S. From Obesity to Diabetes: The Role of the Adipose Organ. Handbook of experimental pharmacology. 2022. Epub 2022/01/20. doi: 10.1007/164_2021_572. PubMed PMID: 35044536.
153. Ruiz-Ojeda FJ, Méndez-Gutiérrez A, Aguilera CM, Plaza-Díaz J. Extracellular Matrix Remodeling of Adipose Tissue in Obesity and Metabolic Diseases. International journal of molecular sciences. 2019;20(19). Epub 2019/10/05. doi: 10.3390/ijms20194888. PubMed PMID: 31581657; PubMed Central PMCID: PMCPMC6801592.

154. Nijhawans P, Behl T, Bhardwaj S. Angiogenesis in obesity. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2020;126:110103. Epub 2020/03/23. doi: 10.1016/j.biopha.2020.110103. PubMed PMID: 32200253.
155. Herold J, Kalucka J. Angiogenesis in Adipose Tissue: The Interplay Between Adipose and Endothelial Cells. Frontiers in physiology. 2020;11:624903. Epub 2021/02/27. doi: 10.3389/fphys.2020.624903. PubMed PMID: 33633579; PubMed Central PMCID: PMCPMC7900516.
156. Booth A, Magnuson A, Fouts J, Foster MT. Adipose tissue: an endocrine organ playing a role in metabolic regulation. Hormone molecular biology and clinical investigation. 2016;26(1):25-42. Epub 2016/02/26. doi: 10.1515/hmbci-2015-0073. PubMed PMID: 26910750.
157. Taylor EB. The complex role of adipokines in obesity, inflammation, and autoimmunity. Clinical science (London, England : 1979). 2021;135(6):731-52. Epub 2021/03/18. doi: 10.1042/cs20200895. PubMed PMID: 33729498; PubMed Central PMCID: PMCPMC7969664.
158. Eelen G, de Zeeuw P, Treps L, Harjes U, Wong BW, Carmeliet P. Endothelial Cell Metabolism. Physiological reviews. 2018;98(1):3-58. Epub 2017/11/24. doi: 10.1152/physrev.00001.2017. PubMed PMID: 29167330; PubMed Central PMCID: PMCPMC5866357.
159. Chun SY, Lim JO, Lee EH, Han MH, Ha YS, Lee JN, et al. Preparation and Characterization of Human Adipose Tissue-Derived Extracellular Matrix, Growth Factors, and Stem Cells: A Concise Review. Tissue engineering and regenerative medicine. 2019;16(4):385-93. Epub 2019/08/16. doi: 10.1007/s13770-019-00199-7. PubMed PMID: 31413942; PubMed Central PMCID: PMCPMC6675808.
160. Valente A, Jamurtas AZ, Koutedakis Y, Flouris AD. Molecular pathways linking non-shivering thermogenesis and obesity: focusing on brown adipose tissue development. Biological reviews of the Cambridge Philosophical Society. 2015;90(1):77-88. Epub 2014/04/09. doi: 10.1111/brv.12099. PubMed PMID: 24708171.
161. Trayhurn P, Beattie JH. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. The Proceedings of the Nutrition Society. 2001;60(3):329-39. Epub 2001/10/30. doi: 10.1079/pns200194. PubMed PMID: 11681807.
162. Perrini S, Laviola L, Cignarelli A, Melchiorre M, De Stefano F, Caccioppoli C, et al. Fat depot-related differences in gene expression, adiponectin secretion, and insulin action and signalling in human adipocytes differentiated in vitro from precursor stromal cells. Diabetologia. 2008;51(1):155-64. Epub 2007/10/26. doi: 10.1007/s00125-007-0841-7. PubMed PMID: 17960360.
163. Unamuno X, Gómez-Ambrosi J, Becerril S, Álvarez-Cienfuegos FJ, Ramírez B, Rodríguez A, et al. Changes in mechanical properties of adipose tissue after bariatric surgery driven by extracellular matrix remodelling and neovascularization are associated with metabolic improvements. Acta biomaterialia. 2022;141:264-79. Epub 2022/01/11. doi: 10.1016/j.actbio.2022.01.007. PubMed PMID: 35007786.
164. Cinti S. Adipose Organ Development and Remodeling. Comprehensive Physiology. 2018;8(4):1357-431. Epub 2018/09/15. doi: 10.1002/cphy.c170042. PubMed PMID: 30215863.
165. de Jong JMA, Sun W, Pires ND, Frontini A, Balaz M, Jespersen NZ, et al. Author Correction: Human brown adipose tissue is phenocopied by classical brown adipose tissue in physiologically humanized mice. Nature metabolism. 2019;1(9):927. Epub 2020/08/05. doi: 10.1038/s42255-019-0119-7. PubMed PMID: 32747808.
166. Heaton JM. The distribution of brown adipose tissue in the human. Journal of anatomy. 1972;112(Pt 1):35-9. Epub 1972/05/01. PubMed PMID: 5086212; PubMed Central PMCID: PMCPMC1271341.

167. Leitner BP, Huang S, Brychta RJ, Duckworth CJ, Baskin AS, McGehee S, et al. Mapping of human brown adipose tissue in lean and obese young men. Proceedings of the National Academy of Sciences of the United States of America. 2017;114(32):8649-54. Epub 2017/07/26. doi: 10.1073/pnas.1705287114. PubMed PMID: 28739898; PubMed Central PMCID: PMCPMC5559032.
168. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. American journal of physiology Endocrinology and metabolism. 2007;293(2):E444-52. Epub 2007/05/03. doi: 10.1152/ajpendo.00691.2006. PubMed PMID: 17473055.
169. Ran C, Albrecht DS, Bredella MA, Yang J, Yang J, Liang SH, et al. PET Imaging of Human Brown Adipose Tissue with the TSPO Tracer [(11)C]PBR28. Molecular imaging and biology. 2018;20(2):188-93. Epub 2017/10/07. doi: 10.1007/s11307-017-1129-z. PubMed PMID: 28983743; PubMed Central PMCID: PMCPMC6394407.
170. Dinas PC, Valente A, Granzotto M, Rossato M, Vettor R, Zacharopoulou A, et al. Browning formation markers of subcutaneous adipose tissue in relation to resting energy expenditure, physical activity and diet in humans. Hormone molecular biology and clinical investigation. 2017;31(1). Epub 2017/07/06. doi: 10.1515/hmbci-2017-0008. PubMed PMID: 28678735.
171. Moonen MPB, Nascimento EBM, van Marken Lichtenbelt WD. Human brown adipose tissue: Underestimated target in metabolic disease? Biochimica et biophysica acta Molecular and cell biology of lipids. 2019;1864(1):104-12. Epub 2018/05/29. doi: 10.1016/j.bbalip.2018.05.012. PubMed PMID: 29802972.
172. Pilkington AC, Paz HA, Wankhade UD. Beige Adipose Tissue Identification and Marker Specificity-Overview. Front Endocrinol (Lausanne). 2021;12:599134. Epub 2021/03/30. doi: 10.3389/fendo.2021.599134. PubMed PMID: 33776911; PubMed Central PMCID: PMCPMC7996049.
173. Pfeifer A, Hoffmann LS. Brown, beige, and white: the new color code of fat and its pharmacological implications. Annual review of pharmacology and toxicology. 2015;55:207-27. Epub 2014/08/26. doi: 10.1146/annurev-pharmtox-010814-124346. PubMed PMID: 25149919.
174. Singh R, Parveen M, Basgen JM, Fazel S, Meshesha MF, Thames EC, et al. Increased Expression of Beige/Brown Adipose Markers from Host and Breast Cancer Cells Influence Xenograft Formation in Mice. Molecular cancer research : MCR. 2016;14(1):78-92. Epub 2015/10/16. doi: 10.1158/1541-7786.mcr-15-0151. PubMed PMID: 26464213; PubMed Central PMCID: PMCPMC4749269.
175. Himms-Hagen J, Melnyk A, Zingaretti MC, Ceresi E, Barbatelli G, Cinti S. Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. American journal of physiology Cell physiology. 2000;279(3):C670-81. Epub 2000/08/16. doi: 10.1152/ajpcell.2000.279.3.C670. PubMed PMID: 10942717.
176. Vitali A, Murano I, Zingaretti MC, Frontini A, Ricquier D, Cinti S. The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes. Journal of lipid research. 2012;53(4):619-29. Epub 2012/01/25. doi: 10.1194/jlr.M018846. PubMed PMID: 22271685; PubMed Central PMCID: PMCPMC3307639.
177. Wang QA, Scherer PE. The AdipoChaser mouse: A model tracking adipogenesis in vivo. Adipocyte. 2014;3(2):146-50. Epub 2014/04/11. doi: 10.4161/adip.27656. PubMed PMID: 24719789; PubMed Central PMCID: PMCPMC3979880.
178. Sanchez-Gurmaches J, Guertin DA. Adipocyte lineages: tracing back the origins of fat. Biochimica et biophysica acta. 2014;1842(3):340-51. Epub 2013/06/12. doi: 10.1016/j.bbadis.2013.05.027. PubMed PMID: 23747579; PubMed Central PMCID: PMCPMC3805734.

179. di Somma M, Vliora M, Grillo E, Castro B, Dakou E, Schaafsma W, et al. Role of VEGFs in metabolic disorders. Angiogenesis. 2020;23(2):119-30. Epub 2019/12/20. doi: 10.1007/s10456-019-09700-1. PubMed PMID: 31853841.
180. Dallabrida SM, Rupnick MA. Vascular endothelium in tissue remodeling: implications for heart failure. Cold Spring Harbor symposia on quantitative biology. 2002;67:417-27. Epub 2003/07/16. doi: 10.1101/sqb.2002.67.417. PubMed PMID: 12858567.
181. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011;473(7347):298-307. Epub 2011/05/20. doi: 10.1038/nature10144. PubMed PMID: 21593862; PubMed Central PMCID: PMCPMC4049445.
182. Mahjoub S, Masrour-Roudsari J. Role of oxidative stress in pathogenesis of metabolic syndrome. Caspian journal of internal medicine. 2012;3(1):386-96. Epub 2012/01/01. PubMed PMID: 26557292; PubMed Central PMCID: PMCPMC4600138.
183. Blüher M. Adipose tissue dysfunction contributes to obesity related metabolic diseases. Best practice & research Clinical endocrinology & metabolism. 2013;27(2):163-77. Epub 2013/06/05. doi: 10.1016/j.beem.2013.02.005. PubMed PMID: 23731879.
184. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, et al. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. International journal of molecular sciences. 2019;20(9). Epub 2019/05/16. doi: 10.3390/ijms20092358. PubMed PMID: 31085992; PubMed Central PMCID: PMCPMC6539070.
185. Kim M, Lee C, Park J. Extracellular matrix remodeling facilitates obesity-associated cancer progression. Trends in cell biology. 2022. Epub 2022/03/22. doi: 10.1016/j.tcb.2022.02.008. PubMed PMID: 35307288.
186. Zimmermann LA, Correns A, Furlan AG, Spanou CES, Sengle G. Controlling BMP growth factor bioavailability: The extracellular matrix as multi skilled platform. Cellular signalling. 2021;85:110071. Epub 2021/07/05. doi: 10.1016/j.cellsig.2021.110071. PubMed PMID: 34217834.
187. Mitola S, Strasly M, Prato M, Ghia P, Bussolino F. IL-12 regulates an endothelial cell-lymphocyte network: effect on metalloproteinase-9 production. Journal of immunology (Baltimore, Md : 1950). 2003;171(7):3725-33. Epub 2003/09/23. doi: 10.4049/jimmunol.171.7.3725. PubMed PMID: 14500672.
188. Sang QX. Complex role of matrix metalloproteinases in angiogenesis. Cell research. 1998;8(3):171-7. Epub 1998/10/29. doi: 10.1038/cr.1998.17. PubMed PMID: 9791730.
189. Chavey C, Mari B, Monthouel MN, Bonnafous S, Anglard P, Van Obberghen E, et al. Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation. J Biol Chem. 2003;278(14):11888-96. Epub 2003/01/17. doi: 10.1074/jbc.M209196200. PubMed PMID: 12529376.
190. Lijnen HR, Maquoi E, Hansen LB, Van Hoef B, Frederix L, Collen D. Matrix metalloproteinase inhibition impairs adipose tissue development in mice. Arteriosclerosis, thrombosis, and vascular biology. 2002;22(3):374-9. Epub 2002/03/09. doi: 10.1161/hq0302.104522. PubMed PMID: 11884277.
191. Alessi MC, Peiretti F, Morange P, Henry M, Nalbone G, Juhan-Vague I. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. Diabetes. 1997;46(5):860-7. Epub 1997/05/01. doi: 10.2337/diab.46.5.860. PubMed PMID: 9133556.
192. Demeulemeester D, Collen D, Lijnen HR. Effect of matrix metalloproteinase inhibition on adipose tissue development. Biochemical and biophysical research communications. 2005;329(1):105-10. Epub 2005/02/22. doi: 10.1016/j.bbrc.2005.01.103. PubMed PMID: 15721280.
193. Gaillard RC, Spinedi E, Chautard T, Pralong FP. Cytokines, leptin, and the hypothalamo-pituitary-adrenal axis. Annals of the New York Academy of Sciences. 2000;917:647-57. Epub 2001/03/28. doi: 10.1111/j.1749-6632.2000.tb05430.x. PubMed PMID: 11268393.

194. Obradovic M, Sudar-Milovanovic E, Soskic S, Essack M, Arya S, Stewart AJ, et al. Leptin and Obesity: Role and Clinical Implication. Front Endocrinol (Lausanne). 2021;12:585887. Epub 2021/06/05. doi: 10.3389/fendo.2021.585887. PubMed PMID: 34084149; PubMed Central PMCID: PMCPMC8167040.
195. Comninos AN, Jayasena CN, Dhillo WS. The relationship between gut and adipose hormones, and reproduction. Human reproduction update. 2014;20(2):153-74. Epub 2013/11/01. doi: 10.1093/humupd/dmt033. PubMed PMID: 24173881.
196. Hulver MW, Houmard JA. Plasma leptin and exercise: recent findings. Sports medicine (Auckland, NZ). 2003;33(7):473-82. Epub 2003/05/24. doi: 10.2165/00007256-200333070-00001. PubMed PMID: 12762824.
197. Thomas T. Leptin: a potential mediator for protective effects of fat mass on bone tissue. Joint bone spine. 2003;70(1):18-21. Epub 2003/03/18. doi: 10.1016/s1297-319x(02)00005-2. PubMed PMID: 12639613.
198. Meadows KL. Ischemic stroke and select adipose-derived and sex hormones: a review. Hormones (Athens, Greece). 2018;17(2):167-82. Epub 2018/06/08. doi: 10.1007/s42000-018-0034-4. PubMed PMID: 29876798.
199. de Candia P, Prattichizzo F, Garavelli S, Alviggi C, La Cava A, Matarese G. The pleiotropic roles of leptin in metabolism, immunity, and cancer. The Journal of experimental medicine. 2021;218(5). Epub 2021/04/16. doi: 10.1084/jem.20191593. PubMed PMID: 33857282; PubMed Central PMCID: PMCPMC8056770 work. G. Matarese reported grants from Merck, personal fees from Merck, grants from Biogen, personal fees from Biogen, personal fees from Roche, personal fees from Novartis, and grants from IBSA outside the submitted work. No other disclosures were reported.
200. Adya R, Tan BK, Randeva HS. Differential effects of leptin and adiponectin in endothelial angiogenesis. Journal of diabetes research. 2015;2015:648239. Epub 2015/02/05. doi: 10.1155/2015/648239. PubMed PMID: 25650072; PubMed Central PMCID: PMCPMC4310451.
201. Bouloumié A, Drexler HC, Lafontan M, Busse R. Leptin, the product of Ob gene, promotes angiogenesis. Circ Res. 1998;83(10):1059-66. Epub 1998/11/14. doi: 10.1161/01.res.83.10.1059. PubMed PMID: 9815153.
202. Garonna E, Botham KM, Birdsey GM, Randi AM, Gonzalez-Perez RR, Wheeler-Jones CP. Vascular endothelial growth factor receptor-2 couples cyclo-oxygenase-2 with pro-angiogenic actions of leptin on human endothelial cells. PloS one. 2011;6(4):e18823. Epub 2011/05/03. doi: 10.1371/journal.pone.0018823. PubMed PMID: 21533119; PubMed Central PMCID: PMCPMC3078934.
203. Yu F, Fu R, Liu L, Wang X, Wu T, Shen W, et al. Leptin-Induced Angiogenesis of EA.Hy926 Endothelial Cells via the Akt and Wnt Signaling Pathways In Vitro and In Vivo. Frontiers in pharmacology. 2019;10:1275. Epub 2019/11/19. doi: 10.3389/fphar.2019.01275. PubMed PMID: 31736756; PubMed Central PMCID: PMCPMC6836761.
204. Anagnostoulis S, Karayiannakis AJ, Lambropoulou M, Efthimiadou A, Polychronidis A, Simopoulos C. Human leptin induces angiogenesis in vivo. Cytokine. 2008;42(3):353-7. Epub 2008/05/02. doi: 10.1016/j.cyto.2008.03.009. PubMed PMID: 18448353.
205. Schroeter MR, Stein S, Heida NM, Leifheit-Nestler M, Cheng IF, Gogiraju R, et al. Leptin promotes the mobilization of vascular progenitor cells and neovascularization by NOX2-mediated activation of MMP9. Cardiovascular research. 2012;93(1):170-80. Epub 2011/11/09. doi: 10.1093/cvr/cvr275. PubMed PMID: 22065732.
206. Heida NM, Leifheit-Nestler M, Schroeter MR, Müller JP, Cheng IF, Henkel S, et al. Leptin enhances the potency of circulating angiogenic cells via src kinase and integrin (alpha)vbeta5: implications for angiogenesis in human obesity. Arteriosclerosis, thrombosis, and vascular biology. 2010;30(2):200-6. Epub 2009/11/17. doi: 10.1161/atvbaha.109.192807. PubMed PMID: 19910644; PubMed Central PMCID: PMCPMC2824857.

207. Zabeau L, Peelman F, Tavernier J. Antagonizing leptin: current status and future directions. Biological chemistry. 2014;395(5):499-514. Epub 2014/02/14. doi: 10.1515/hsz-2013-0283. PubMed PMID: 24523306.
208. Fett JW, Strydom DJ, Lobb RR, Alderman EM, Bethune JL, Riordan JF, et al. Isolation and characterization of angiogenin, an angiogenic protein from human carcinoma cells. Biochemistry. 1985;24(20):5480-6. Epub 1985/09/24. doi: 10.1021/bi00341a030. PubMed PMID: 4074709.
209. Miyake M, Goodison S, Lawton A, Gomes-Giacoia E, Rosser CJ. Angiogenin promotes tumoral growth and angiogenesis by regulating matrix metallopeptidase-2 expression via the ERK1/2 pathway. Oncogene. 2015;34(7):890-901. Epub 2014/02/25. doi: 10.1038/onc.2014.2. PubMed PMID: 24561529; PubMed Central PMCID: PMCPMC4317372.
210. Smith BD, Raines RT. Genetic selection for peptide inhibitors of angiogenin. Protein engineering, design & selection : PEDS. 2008;21(5):289-94. Epub 2008/03/01. doi: 10.1093/protein/gzm089. PubMed PMID: 18308863; PubMed Central PMCID: PMCPMC2587640.
211. Kao RY, Jenkins JL, Olson KA, Key ME, Fett JW, Shapiro R. A small-molecule inhibitor of the ribonucleolytic activity of human angiogenin that possesses antitumor activity. Proceedings of the National Academy of Sciences of the United States of America. 2002;99(15):10066-71. Epub 2002/07/16. doi: 10.1073/pnas.152342999. PubMed PMID: 12118120; PubMed Central PMCID: PMCPMC126625.
212. Sheng J, Xu Z. Three decades of research on angiogenin: a review and perspective. Acta biochimica et biophysica Sinica. 2016;48(5):399-410. Epub 2015/12/26. doi: 10.1093/abbs/gmv131. PubMed PMID: 26705141; PubMed Central PMCID: PMCPMC4888354.
213. Klimcakova E, Moro C, Mazzucotelli A, Lolmède K, Viguerie N, Galitzky J, et al. Profiling of adipokines secreted from human subcutaneous adipose tissue in response to PPAR agonists. Biochemical and biophysical research communications. 2007;358(3):897-902. Epub 2007/05/22. doi: 10.1016/j.bbrc.2007.05.012. PubMed PMID: 17511960.
214. He Y, Yu X, Chen Z, Li L. Stromal vascular fraction cells plus sustained release VEGF/Ang-1-PLGA microspheres improve fat graft survival in mice. Journal of cellular physiology. 2019;234(5):6136-46. Epub 2018/09/22. doi: 10.1002/jcp.27368. PubMed PMID: 30238985.
215. Silha JV, Krsek M, Sucharda P, Murphy LJ. Angiogenic factors are elevated in overweight and obese individuals. International journal of obesity (2005). 2005;29(11):1308-14. Epub 2005/06/15. doi: 10.1038/sj.ijo.0802987. PubMed PMID: 15953938.
216. Efimenko A, Dzhoyashvili N, Kalinina N, Kochegura T, Akchurin R, Tkachuk V, et al. Adipose-derived mesenchymal stromal cells from aged patients with coronary artery disease keep mesenchymal stromal cell properties but exhibit characteristics of aging and have impaired angiogenic potential. Stem cells translational medicine. 2014;3(1):32-41. Epub 2013/12/20. doi: 10.5966/sctm.2013-0014. PubMed PMID: 24353175; PubMed Central PMCID: PMCPMC3902283.
217. Yang J, Li X, Xu D. Research Progress on the Involvement of ANGPTL4 and Loss-of-Function Variants in Lipid Metabolism and Coronary Heart Disease: Is the "Prime Time" of ANGPTL4-Targeted Therapy for Coronary Heart Disease Approaching? Cardiovascular drugs and therapy. 2021;35(3):467-77. Epub 2020/06/06. doi: 10.1007/s10557-020-07001-0. PubMed PMID: 32500296.
218. Gho YS, Chae CB. Anti-angiogenin activity of the peptides complementary to the receptor-binding site of angiogenin. J Biol Chem. 1997;272(39):24294-9. Epub 1997/09/26. doi: 10.1074/jbc.272.39.24294. PubMed PMID: 9305884.
219. Ibaragi S, Yoshioka N, Li S, Hu MG, Hirukawa S, Sadow PM, et al. Neamine inhibits prostate cancer growth by suppressing angiogenin-mediated rRNA transcription. Clinical

cancer research : an official journal of the American Association for Cancer Research. 2009;15(6):1981-8. Epub 2009/03/12. doi: 10.1158/1078-0432.ccr-08-2593. PubMed PMID: 19276260; PubMed Central PMCID: PMCPMC2670466.
220. Kishimoto K, Liu S, Tsuji T, Olson KA, Hu GF. Endogenous angiogenin in endothelial cells is a general requirement for cell proliferation and angiogenesis. Oncogene. 2005;24(3):445-56. Epub 2004/11/24. doi: 10.1038/sj.onc.1208223. PubMed PMID: 15558023.
221. Kirkbride KC, Townsend TA, Bruinsma MW, Barnett JV, Blobe GC. Bone morphogenetic proteins signal through the transforming growth factor-beta type III receptor. J Biol Chem. 2008;283(12):7628-37. Epub 2008/01/11. doi: 10.1074/jbc.M704883200. PubMed PMID: 18184661.
222. Blázquez-Medela AM, Jumabay M, Boström KI. Beyond the bone: Bone morphogenetic protein signaling in adipose tissue. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2019;20(5):648-58. Epub 2019/01/05. doi: 10.1111/obr.12822. PubMed PMID: 30609449; PubMed Central PMCID: PMCPMC6447448.
223. Margoni A, Fotis L, Papavassiliou AG. The transforming growth factor-beta/bone morphogenetic protein signalling pathway in adipogenesis. The international journal of biochemistry & cell biology. 2012;44(3):475-9. Epub 2012/01/10. doi: 10.1016/j.biocel.2011.12.014. PubMed PMID: 22226816.
224. Qian S, Tang Y, Tang QQ. Adipose tissue plasticity and the pleiotropic roles of BMP signaling. J Biol Chem. 2021;296:100678. Epub 2021/04/20. doi: 10.1016/j.jbc.2021.100678. PubMed PMID: 33872596; PubMed Central PMCID: PMCPMC8131923.
225. Schulz TJ, Tseng YH. Emerging role of bone morphogenetic proteins in adipogenesis and energy metabolism. Cytokine & growth factor reviews. 2009;20(5-6):523-31. Epub 2009/11/10. doi: 10.1016/j.cytogfr.2009.10.019. PubMed PMID: 19896888; PubMed Central PMCID: PMCPMC2799934.
226. Kulikauskas MR, X S, Bautch VL. The versatility and paradox of BMP signaling in endothelial cell behaviors and blood vessel function. Cellular and molecular life sciences : CMLS. 2022;79(2):77. Epub 2022/01/20. doi: 10.1007/s00018-021-04033-z. PubMed PMID: 35044529; PubMed Central PMCID: PMCPMC8770421.
227. Benn A, Hiepen C, Osterland M, Schütte C, Zwijsen A, Knaus P. Role of bone morphogenetic proteins in sprouting angiogenesis: differential BMP receptor-dependent signaling pathways balance stalk vs. tip cell competence. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2017;31(11):4720-33. Epub 2017/07/25. doi: 10.1096/fj.201700193RR. PubMed PMID: 28733457; PubMed Central PMCID: PMCPMC5636702.
228. Chen WC, Chung CH, Lu YC, Wu MH, Chou PH, Yen JY, et al. BMP-2 induces angiogenesis by provoking integrin α6 expression in human endothelial progenitor cells. Biochemical pharmacology. 2018;150:256-66. Epub 2018/02/20. doi: 10.1016/j.bcp.2018.02.021. PubMed PMID: 29458046.
229. Tang Y, Qian SW, Wu MY, Wang J, Lu P, Li X, et al. BMP4 mediates the interplay between adipogenesis and angiogenesis during expansion of subcutaneous white adipose tissue. Journal of molecular cell biology. 2016;8(4):302-12. Epub 2016/04/01. doi: 10.1093/jmcb/mjw019. PubMed PMID: 27030507.
230. Saini S, Duraisamy AJ, Bayen S, Vats P, Singh SB. Role of BMP7 in appetite regulation, adipogenesis, and energy expenditure. Endocrine. 2015;48(2):405-9. Epub 2014/09/03. doi: 10.1007/s12020-014-0406-8. PubMed PMID: 25178649.
231. Desroches-Castan A, Tillet E, Bouvard C, Bailly S. BMP9 and BMP10: Two close vascular quiescence partners that stand out. Developmental dynamics : an official publication of the American Association of Anatomists. 2022;251(1):178-97. Epub 2021/07/10. doi: 10.1002/dvdy.395. PubMed PMID: 34240497.

232. Ge W, Mi Y, Xu S, Li T, Lu Y, Jiang J. rhBMP-7 suppresses TGF-β1-induced endothelial to mesenchymal transition in circulating endothelial cells by regulating Smad5. Molecular medicine reports. 2020;21(1):478-84. Epub 2020/01/16. doi: 10.3892/mmr.2019.10842. PubMed PMID: 31939623.
233. Szulcek R, Sanchez-Duffhues G, Rol N, Pan X, Tsonaka R, Dickhoff C, et al. Exacerbated inflammatory signaling underlies aberrant response to BMP9 in pulmonary arterial hypertension lung endothelial cells. Angiogenesis. 2020;23(4):699-714. Epub 2020/08/20. doi: 10.1007/s10456-020-09741-x. PubMed PMID: 32813135; PubMed Central PMCID: PMCPMC7524846.
234. Hong CC, Yu PB. Applications of small molecule BMP inhibitors in physiology and disease. Cytokine & growth factor reviews. 2009;20(5-6):409-18. Epub 2009/11/17. doi: 10.1016/j.cytogfr.2009.10.021. PubMed PMID: 19914855; PubMed Central PMCID: PMCPMC2813719.
235. Mitola S, Ravelli C, Corsini M, Gianoncelli A, Galvagni F, Ballmer-Hofer K, et al. Production and Biochemical Characterization of Dimeric Recombinant Gremlin-1. International journal of molecular sciences. 2022;23(3). Epub 2022/02/16. doi: 10.3390/ijms23031151. PubMed PMID: 35163075; PubMed Central PMCID: PMCPMC8835488.
236. Topol LZ, Bardot B, Zhang Q, Resau J, Huillard E, Marx M, et al. Biosynthesis, post-translation modification, and functional characterization of Drm/Gremlin. J Biol Chem. 2000;275(12):8785-93. Epub 2000/03/18. doi: 10.1074/jbc.275.12.8785. PubMed PMID: 10722723.
237. Choudhuri KSR, Mishra S. Structural basis of BMP-2 and BMP-7 interactions with antagonists Gremlin-1 and Noggin in Glioblastoma tumors. Journal of computational chemistry. 2020;41(30):2544-61. Epub 2020/09/17. doi: 10.1002/jcc.26407. PubMed PMID: 32935366.
238. Neckmann U, Wolowczyk C, Hall M, Almaas E, Ren J, Zhao S, et al. GREM1 is associated with metastasis and predicts poor prognosis in ER-negative breast cancer patients. Cell communication and signaling : CCS. 2019;17(1):140. Epub 2019/11/07. doi: 10.1186/s12964-019-0467-7. PubMed PMID: 31694641; PubMed Central PMCID: PMCPMC6836336.
239. Walsh DW, Godson C, Brazil DP, Martin F. Extracellular BMP-antagonist regulation in development and disease: tied up in knots. Trends in cell biology. 2010;20(5):244-56. Epub 2010/03/02. doi: 10.1016/j.tcb.2010.01.008. PubMed PMID: 20188563.
240. Gustafson B, Hammarstedt A, Hedjazifar S, Hoffmann JM, Svensson PA, Grimsby J, et al. BMP4 and BMP Antagonists Regulate Human White and Beige Adipogenesis. Diabetes. 2015;64(5):1670-81. Epub 2015/01/22. doi: 10.2337/db14-1127. PubMed PMID: 25605802.
241. Hammarstedt A, Gogg S, Hedjazifar S, Nerstedt A, Smith U. Impaired Adipogenesis and Dysfunctional Adipose Tissue in Human Hypertrophic Obesity. Physiological reviews. 2018;98(4):1911-41. Epub 2018/08/02. doi: 10.1152/physrev.00034.2017. PubMed PMID: 30067159.
242. Hedjazifar S, Khatib Shahidi R, Hammarstedt A, Bonnet L, Church C, Boucher J, et al. The Novel Adipokine Gremlin 1 Antagonizes Insulin Action and Is Increased in Type 2 Diabetes and NAFLD/NASH. Diabetes. 2020;69(3):331-41. Epub 2019/12/29. doi: 10.2337/db19-0701. PubMed PMID: 31882566.
243. Wade FM, Wakade C, Mahesh VB, Brann DW. Differential expression of the peripheral benzodiazepine receptor and gremlin during adipogenesis. Obesity research. 2005;13(5):818-22. Epub 2005/05/28. doi: 10.1038/oby.2005.93. PubMed PMID: 15919833.
244. Hoggard N, Cruickshank M, Moar KM, Bashir S, Mayer CD. Using gene expression to predict differences in the secretome of human omental vs. subcutaneous adipose tissue. Obesity (Silver Spring, Md). 2012;20(6):1158-67. Epub 2012/01/31. doi: 10.1038/oby.2012.14. PubMed PMID: 22286531.

245. Saeidi A, Seifi-Ski-Shahr F, Soltani M, Daraei A, Shirvani H, Laher I, et al. Resistance training, gremlin 1 and macrophage migration inhibitory factor in obese men: a randomised trial. Archives of physiology and biochemistry. 2020:1-9. Epub 2020/12/29. doi: 10.1080/13813455.2020.1856142. PubMed PMID: 33370549.
246. Corsini M, Moroni E, Ravelli C, Andrés G, Grillo E, Ali IH, et al. Cyclic adenosine monophosphate-response element-binding protein mediates the proangiogenic or proinflammatory activity of gremlin. Arteriosclerosis, thrombosis, and vascular biology. 2014;34(1):136-45. Epub 2013/11/16. doi: 10.1161/atvbaha.113.302517. PubMed PMID: 24233491.
247. Stabile H, Mitola S, Moroni E, Belleri M, Nicoli S, Coltrini D, et al. Bone morphogenic protein antagonist Drm/gremlin is a novel proangiogenic factor. Blood. 2007;109(5):1834-40. Epub 2006/11/02. doi: 10.1182/blood-2006-06-032276. PubMed PMID: 17077323.
248. Chiodelli P, Mitola S, Ravelli C, Oreste P, Rusnati M, Presta M. Heparan sulfate proteoglycans mediate the angiogenic activity of the vascular endothelial growth factor receptor-2 agonist gremlin. Arteriosclerosis, thrombosis, and vascular biology. 2011;31(12):e116-27. Epub 2011/09/17. doi: 10.1161/atvbaha.111.235184. PubMed PMID: 21921258.
249. Zhang Y, Zhang M, Xie W, Wan J, Tao X, Liu M, et al. Gremlin-1 is a key regulator of endothelial-to-mesenchymal transition in human pulmonary artery endothelial cells. Experimental cell research. 2020;390(1):111941. Epub 2020/03/08. doi: 10.1016/j.yexcr.2020.111941. PubMed PMID: 32145252.
250. Marquez-Exposito L, Lavoz C, Rodrigues-Diez RR, Rayego-Mateos S, Orejudo M, Cantero-Navarro E, et al. Gremlin Regulates Tubular Epithelial to Mesenchymal Transition via VEGFR2: Potential Role in Renal Fibrosis. Frontiers in pharmacology. 2018;9:1195. Epub 2018/11/06. doi: 10.3389/fphar.2018.01195. PubMed PMID: 30386246; PubMed Central PMCID: PMCPMC6199372.
251. Zanocco-Marani T, Bateman A, Romano G, Valentinis B, He ZH, Baserga R. Biological activities and signaling pathways of the granulin/epithelin precursor. Cancer research. 1999;59(20):5331-40. Epub 1999/10/28. PubMed PMID: 10537317.
252. Ebert T, Gebhardt C, Scholz M, Wohland T, Schleinitz D, Fasshauer M, et al. Relationship Between 12 Adipocytokines and Distinct Components of the Metabolic Syndrome. The Journal of clinical endocrinology and metabolism. 2018;103(3):1015-23. Epub 2018/01/13. doi: 10.1210/jc.2017-02085. PubMed PMID: 29325128.
253. Klöting N, Fasshauer M, Dietrich A, Kovacs P, Schön MR, Kern M, et al. Insulin-sensitive obesity. American journal of physiology Endocrinology and metabolism. 2010;299(3):E506-15. Epub 2010/06/24. doi: 10.1152/ajpendo.00586.2009. PubMed PMID: 20570822.
254. Schmid A, Hochberg A, Kreiß AF, Gehl J, Patz M, Thomalla M, et al. Role of progranulin in adipose tissue innate immunity. Cytokine. 2020;125:154796. Epub 2019/08/28. doi: 10.1016/j.cyto.2019.154796. PubMed PMID: 31454754.
255. Miehle K, Ebert T, Kralisch S, Hoffmann A, Kratzsch J, Schlögl H, et al. Progranulin is increased in human and murine lipodystrophy. Diabetes research and clinical practice. 2016;120:1-7. Epub 2016/08/09. doi: 10.1016/j.diabres.2016.07.017. PubMed PMID: 27497155.
256. Matsubara T, Mita A, Minami K, Hosooka T, Kitazawa S, Takahashi K, et al. PGRN is a key adipokine mediating high fat diet-induced insulin resistance and obesity through IL-6 in adipose tissue. Cell metabolism. 2012;15(1):38-50. Epub 2012/01/10. doi: 10.1016/j.cmet.2011.12.002. PubMed PMID: 22225875.
257. Kraemer MS, Moehlecke M, Rheinheimer J, Canani LH, Leitão CB, Nicoletto BB. Plasma progranulin levels in obese patients before and after Roux-en-Y gastric bariatric surgery: a longitudinal study. Surgery for obesity and related diseases : official journal of the American

Society for Bariatric Surgery. 2020;16(11):1655-60. Epub 2020/08/26. doi: 10.1016/j.soard.2020.06.056. PubMed PMID: 32839122.
258. Brock J, Schmid A, Karrasch T, Pfefferle P, Schlegel J, Busse I, et al. Progranulin serum levels and gene expression in subcutaneous vs visceral adipose tissue of severely obese patients undergoing bariatric surgery. Clinical endocrinology. 2019;91(3):400-10. Epub 2019/05/19. doi: 10.1111/cen.14040. PubMed PMID: 31102282.
259. Guerra RR, Kriazhev L, Hernandez-Blazquez FJ, Bateman A. Progranulin is a stress-response factor in fibroblasts subjected to hypoxia and acidosis. Growth factors (Chur, Switzerland). 2007;25(4):280-5. Epub 2007/12/20. doi: 10.1080/08977190701781222. PubMed PMID: 18092235.
260. Li G, Dong T, Yang D, Gao A, Luo J, Yang H, et al. Progranulin promotes lymphangiogenesis through VEGF-C and is an independent risk factor in human esophageal cancers. Human pathology. 2018;75:116-24. Epub 2018/02/17. doi: 10.1016/j.humpath.2018.02.008. PubMed PMID: 29452214.
261. Xu B, Chen X, Ding Y, Chen C, Liu T, Zhang H. Abnormal angiogenesis of placenta in progranulin-deficient mice. Molecular medicine reports. 2020;22(4):3482-92. Epub 2020/09/19. doi: 10.3892/mmr.2020.11438. PubMed PMID: 32945448; PubMed Central PMCID: PMCPMC7453605.
262. Neill T, Buraschi S, Goyal A, Sharpe C, Natkanski E, Schaefer L, et al. EphA2 is a functional receptor for the growth factor progranulin. The Journal of cell biology. 2016;215(5):687-703. Epub 2016/12/03. doi: 10.1083/jcb.201603079. PubMed PMID: 27903606; PubMed Central PMCID: PMCPMC5146997.
263. Gonzalez EM, Mongiat M, Slater SJ, Baffa R, Iozzo RV. A novel interaction between perlecan protein core and progranulin: potential effects on tumor growth. J Biol Chem. 2003;278(40):38113-6. Epub 2003/08/06. doi: 10.1074/jbc.C300310200. PubMed PMID: 12900424.
264. Araujo AM, Abaurrea A, Azcoaga P, López-Velazco JI, Manzano S, Rodriguez J, et al. Stromal oncostatin M cytokine promotes breast cancer progression by reprogramming the tumor microenvironment. The Journal of clinical investigation. 2022;132(7). Epub 2022/02/23. doi: 10.1172/jci148667. PubMed PMID: 35192545; PubMed Central PMCID: PMCPMC8970678.
265. Sanchez-Infantes D, White UA, Elks CM, Morrison RF, Gimble JM, Considine RV, et al. Oncostatin m is produced in adipose tissue and is regulated in conditions of obesity and type 2 diabetes. The Journal of clinical endocrinology and metabolism. 2014;99(2):E217-25. Epub 2013/12/04. doi: 10.1210/jc.2013-3555. PubMed PMID: 24297795; PubMed Central PMCID: PMCPMC3913819.
266. Elks CM, Zhao P, Grant RW, Hang H, Bailey JL, Burk DH, et al. Loss of Oncostatin M Signaling in Adipocytes Induces Insulin Resistance and Adipose Tissue Inflammation in Vivo. J Biol Chem. 2016;291(33):17066-76. Epub 2016/06/22. doi: 10.1074/jbc.M116.739110. PubMed PMID: 27325693; PubMed Central PMCID: PMCPMC5016111.
267. Wijelath ES, Carlsen B, Cole T, Chen J, Kothari S, Hammond WP. Oncostatin M induces basic fibroblast growth factor expression in endothelial cells and promotes endothelial cell proliferation, migration and spindle morphology. Journal of cell science. 1997;110 (Pt 7):871-9. Epub 1997/04/01. doi: 10.1242/jcs.110.7.871. PubMed PMID: 9133674.
268. Zhang X, Zhu D, Wei L, Zhao Z, Qi X, Li Z, et al. OSM Enhances Angiogenesis and Improves Cardiac Function after Myocardial Infarction. BioMed research international. 2015;2015:317905. Epub 2015/07/07. doi: 10.1155/2015/317905. PubMed PMID: 26146616; PubMed Central PMCID: PMCPMC4471304.
269. Marden G, Wan Q, Wilks J, Nevin K, Feeney M, Wisniacki N, et al. The role of the oncostatin M/OSM receptor β axis in activating dermal microvascular endothelial cells in systemic sclerosis. Arthritis research & therapy. 2020;22(1):179. Epub 2020/08/02. doi:

10.1186/s13075-020-02266-0. PubMed PMID: 32736577; PubMed Central PMCID: PMCPMC7393919.
270. Rychli K, Kaun C, Hohensinner PJ, Rega G, Pfaffenberger S, Vyskocil E, et al. The inflammatory mediator oncostatin M induces angiopoietin 2 expression in endothelial cells in vitro and in vivo. Journal of thrombosis and haemostasis : JTH. 2010;8(3):596-604. Epub 2010/01/22. doi: 10.1111/j.1538-7836.2010.03741.x. PubMed PMID: 20088942; PubMed Central PMCID: PMCPMC2857505.
271. Rega G, Kaun C, Demyanets S, Pfaffenberger S, Rychli K, Hohensinner PJ, et al. Vascular endothelial growth factor is induced by the inflammatory cytokines interleukin-6 and oncostatin m in human adipose tissue in vitro and in murine adipose tissue in vivo. Arteriosclerosis, thrombosis, and vascular biology. 2007;27(7):1587-95. Epub 2007/05/26. doi: 10.1161/atvbaha.107.143081. PubMed PMID: 17525365.
272. Pöling J, Gajawada P, Richter M, Lörchner H, Polyakova V, Kostin S, et al. Therapeutic targeting of the oncostatin M receptor-β prevents inflammatory heart failure. Basic research in cardiology. 2014;109(1):396. Epub 2013/12/03. doi: 10.1007/s00395-013-0396-3. PubMed PMID: 24292852.
273. Pang L, Zhang Y, Yu Y, Zhang S. Resistin promotes the expression of vascular endothelial growth factor in ovary carcinoma cells. International journal of molecular sciences. 2013;14(5):9751-66. Epub 2013/05/09. doi: 10.3390/ijms14059751. PubMed PMID: 23652833; PubMed Central PMCID: PMCPMC3676810.
274. Deb A, Deshmukh B, Ramteke P, Bhati FK, Bhat MK. Resistin: A journey from metabolism to cancer. Translational oncology. 2021;14(10):101178. Epub 2021/07/23. doi: 10.1016/j.tranon.2021.101178. PubMed PMID: 34293684; PubMed Central PMCID: PMCPMC8319804.
275. Raghu P, Ghosh S, Soundarya K, Haseeb A, Aruna B, Ehtesham NZ. Dimerization of human recombinant resistin involves covalent and noncovalent interactions. Biochemical and biophysical research communications. 2004;313(3):642-6. Epub 2003/12/31. doi: 10.1016/j.bbrc.2003.11.156. PubMed PMID: 14697240.
276. Malvi P, Chaube B, Singh SV, Mohammad N, Vijayakumar MV, Singh S, et al. Elevated circulatory levels of leptin and resistin impair therapeutic efficacy of dacarbazine in melanoma under obese state. Cancer & metabolism. 2018;6:2. Epub 2018/03/24. doi: 10.1186/s40170-018-0176-5. PubMed PMID: 29568521; PubMed Central PMCID: PMCPMC5859707.
277. Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN inflammation. 2013;2013:139239. Epub 2014/01/24. doi: 10.1155/2013/139239. PubMed PMID: 24455420; PubMed Central PMCID: PMCPMC3881510.
278. Sadashiv, Tiwari S, Paul BN, Kumar S, Chandra A, Dhananjai S, et al. Over expression of resistin in adipose tissue of the obese induces insulin resistance. World journal of diabetes. 2012;3(7):135-41. Epub 2012/07/21. doi: 10.4239/wjd.v3.i7.135. PubMed PMID: 22816026; PubMed Central PMCID: PMCPMC3399912.
279. Sánchez-Solana B, Laborda J, Baladrón V. Mouse resistin modulates adipogenesis and glucose uptake in 3T3-L1 preadipocytes through the ROR1 receptor. Molecular endocrinology (Baltimore, Md). 2012;26(1):110-27. Epub 2011/11/15. doi: 10.1210/me.2011-1027. PubMed PMID: 22074948; PubMed Central PMCID: PMCPMC5417166.
280. Chen C, Jiang J, Lü JM, Chai H, Wang X, Lin PH, et al. Resistin decreases expression of endothelial nitric oxide synthase through oxidative stress in human coronary artery endothelial cells. American journal of physiology Heart and circulatory physiology. 2010;299(1):H193-201. Epub 2010/05/04. doi: 10.1152/ajpheart.00431.2009. PubMed PMID: 20435848; PubMed Central PMCID: PMCPMC2904138.
281. Tsai CH, Liu SC, Chung WH, Wang SW, Wu MH, Tang CH. Visfatin Increases VEGF-dependent Angiogenesis of Endothelial Progenitor Cells during Osteoarthritis Progression.

Cells. 2020;9(5). Epub 2020/05/30. doi: 10.3390/cells9051315. PubMed PMID: 32466159; PubMed Central PMCID: PMCPMC7291153.
282. Lee S, Lee HC, Kwon YW, Lee SE, Cho Y, Kim J, et al. Adenylyl cyclase-associated protein 1 is a receptor for human resistin and mediates inflammatory actions of human monocytes. Cell metabolism. 2014;19(3):484-97. Epub 2014/03/13. doi: 10.1016/j.cmet.2014.01.013. PubMed PMID: 24606903; PubMed Central PMCID: PMCPMC3969988.
283. Mu H, Ohashi R, Yan S, Chai H, Yang H, Lin P, et al. Adipokine resistin promotes in vitro angiogenesis of human endothelial cells. Cardiovascular research. 2006;70(1):146-57. Epub 2006/03/07. doi: 10.1016/j.cardiores.2006.01.015. PubMed PMID: 16515776.
284. Robertson SA, Rae CJ, Graham A. Induction of angiogenesis by murine resistin: putative role of PI3-kinase and NO-dependent pathways. Regulatory peptides. 2009;152(1-3):41-7. Epub 2008/08/30. doi: 10.1016/j.regpep.2008.07.008. PubMed PMID: 18722482.
285. Shibuya M. VEGF-VEGFR Signals in Health and Disease. Biomolecules & therapeutics. 2014;22(1):1-9. Epub 2014/03/07. doi: 10.4062/biomolther.2013.113. PubMed PMID: 24596615; PubMed Central PMCID: PMCPMC3936422.
286. Claesson-Welsh L. Signal transduction by vascular endothelial growth factor receptors. Biochemical Society transactions. 2003;31(Pt 1):20-4. Epub 2003/01/28. doi: 10.1042/bst0310020. PubMed PMID: 12546646.
287. Lemoine AY, Ledoux S, Larger E. Adipose tissue angiogenesis in obesity. Thromb Haemost. 2013;110(4):661-8. Epub 2013/04/19. doi: 10.1160/th13-01-0073. PubMed PMID: 23595655.
288. Elias I, Franckhauser S, Ferré T, Vilà L, Tafuro S, Muñoz S, et al. Adipose tissue overexpression of vascular endothelial growth factor protects against diet-induced obesity and insulin resistance. Diabetes. 2012;61(7):1801-13. Epub 2012/04/24. doi: 10.2337/db11-0832. PubMed PMID: 22522611; PubMed Central PMCID: PMCPMC3379662.
289. Sung HK, Doh KO, Son JE, Park JG, Bae Y, Choi S, et al. Adipose vascular endothelial growth factor regulates metabolic homeostasis through angiogenesis. Cell metabolism. 2013;17(1):61-72. Epub 2013/01/15. doi: 10.1016/j.cmet.2012.12.010. PubMed PMID: 23312284.
290. Tam J, Duda DG, Perentes JY, Quadri RS, Fukumura D, Jain RK. Blockade of VEGFR2 and not VEGFR1 can limit diet-induced fat tissue expansion: role of local versus bone marrow-derived endothelial cells. PloS one. 2009;4(3):e4974. Epub 2009/04/01. doi: 10.1371/journal.pone.0004974. PubMed PMID: 19333381; PubMed Central PMCID: PMCPMC2659427.
291. Seki T, Hosaka K, Fischer C, Lim S, Andersson P, Abe M, et al. Ablation of endothelial VEGFR1 improves metabolic dysfunction by inducing adipose tissue browning. The Journal of experimental medicine. 2018;215(2):611-26. Epub 2018/01/07. doi: 10.1084/jem.20171012. PubMed PMID: 29305395; PubMed Central PMCID: PMCPMC5789413.
292. Liu HM, Wang CH, Chang ZY, Huang TH, Lee TY. Losartan Attenuates Insulin Resistance and Regulates Browning Phenomenon of White Adipose Tissue in ob/ob Mice. Current issues in molecular biology. 2021;43(3):1828-43. Epub 2021/12/11. doi: 10.3390/cimb43030128. PubMed PMID: 34889901; PubMed Central PMCID: PMCPMC8929071.
293. Jonker JW, Suh JM, Atkins AR, Ahmadian M, Li P, Whyte J, et al. A PPARγ-FGF1 axis is required for adaptive adipose remodelling and metabolic homeostasis. Nature. 2012;485(7398):391-4. Epub 2012/04/24. doi: 10.1038/nature10998. PubMed PMID: 22522926; PubMed Central PMCID: PMCPMC3358516.
294. Ohta H, Itoh N. Roles of FGFs as Adipokines in Adipose Tissue Development, Remodeling, and Metabolism. Front Endocrinol (Lausanne). 2014;5:18. Epub 2014/03/08. doi: 10.3389/fendo.2014.00018. PubMed PMID: 24605108; PubMed Central PMCID: PMCPMC3932445.

295. Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine & growth factor reviews. 2005;16(2):159-78. Epub 2005/05/03. doi: 10.1016/j.cytogfr.2005.01.004. PubMed PMID: 15863032.
296. Dai Q, Fan X, Meng X, Sun S, Su Y, Ling X, et al. FGF21 promotes ischaemic angiogenesis and endothelial progenitor cells function under diabetic conditions in an AMPK/NAD+-dependent manner. Journal of cellular and molecular medicine. 2021;25(6):3091-102. Epub 2021/02/19. doi: 10.1111/jcmm.16369. PubMed PMID: 33599110; PubMed Central PMCID: PMCPMC7957202.
297. Huang W, Shao M, Liu H, Chen J, Hu J, Zhu L, et al. Fibroblast growth factor 21 enhances angiogenesis and wound healing of human brain microvascular endothelial cells by activating PPARγ. Journal of pharmacological sciences. 2019;140(2):120-7. Epub 2019/07/01. doi: 10.1016/j.jphs.2019.03.010. PubMed PMID: 31255518.
298. Yaqoob U, Jagavelu K, Shergill U, de Assuncao T, Cao S, Shah VH. FGF21 promotes endothelial cell angiogenesis through a dynamin-2 and Rab5 dependent pathway. PloS one. 2014;9(5):e98130. Epub 2014/05/23. doi: 10.1371/journal.pone.0098130. PubMed PMID: 24848261; PubMed Central PMCID: PMCPMC4029959.
299. Hui Q, Jin Z, Li X, Liu C, Wang X. FGF Family: From Drug Development to Clinical Application. International journal of molecular sciences. 2018;19(7). Epub 2018/06/29. doi: 10.3390/ijms19071875. PubMed PMID: 29949887; PubMed Central PMCID: PMCPMC6073187.
300. Leali D, Bianchi R, Bugatti A, Nicoli S, Mitola S, Ragona L, et al. Fibroblast growth factor 2-antagonist activity of a long-pentraxin 3-derived anti-angiogenic pentapeptide. Journal of cellular and molecular medicine. 2010;14(8):2109-21. Epub 2009/07/25. doi: 10.1111/j.1582-4934.2009.00855.x. PubMed PMID: 19627396; PubMed Central PMCID: PMCPMC3823002.
301. Haider DG, Schindler K, Schaller G, Prager G, Wolzt M, Ludvik B. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. The Journal of clinical endocrinology and metabolism. 2006;91(4):1578-81. Epub 2006/02/02. doi: 10.1210/jc.2005-2248. PubMed PMID: 16449335.
302. Heo YJ, Choi SE, Jeon JY, Han SJ, Kim DJ, Kang Y, et al. Visfatin Induces Inflammation and Insulin Resistance via the NF-κB and STAT3 Signaling Pathways in Hepatocytes. Journal of diabetes research. 2019;2019:4021623. Epub 2019/08/10. doi: 10.1155/2019/4021623. PubMed PMID: 31396538; PubMed Central PMCID: PMCPMC6664505.
303. Chen D, Wang Y, Liu M, Cheng J, Liu Z, Song Y, et al. Visfatin promotes angiogenesis of RF/6A cells through upregulation of VEGF/VEGFR-2 under high-glucose conditions. Experimental and therapeutic medicine. 2021;21(4):389. Epub 2021/03/09. doi: 10.3892/etm.2021.9820. PubMed PMID: 33680111; PubMed Central PMCID: PMCPMC7918108.
304. Kim SR, Bae SK, Choi KS, Park SY, Jun HO, Lee JY, et al. Visfatin promotes angiogenesis by activation of extracellular signal-regulated kinase 1/2. Biochemical and biophysical research communications. 2007;357(1):150-6. Epub 2007/04/06. doi: 10.1016/j.bbrc.2007.03.105. PubMed PMID: 17408594.
305. Romacho T, Valencia I, Ramos-González M, Vallejo S, López-Esteban M, Lorenzo O, et al. Visfatin/eNampt induces endothelial dysfunction in vivo: a role for Toll-Like Receptor 4 and NLRP3 inflammasome. Sci Rep. 2020;10(1):5386. Epub 2020/03/28. doi: 10.1038/s41598-020-62190-w. PubMed PMID: 32214150; PubMed Central PMCID: PMCPMC7096459.
306. Goralski KB, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, Parlee SD, et al. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. J Biol Chem. 2007;282(38):28175-88. Epub 2007/07/20. doi: 10.1074/jbc.M700793200. PubMed PMID: 17635925.

307. Helfer G, Wu QF. Chemerin: a multifaceted adipokine involved in metabolic disorders. The Journal of endocrinology. 2018;238(2):R79-r94. Epub 2018/06/01. doi: 10.1530/joe-18-0174. PubMed PMID: 29848608; PubMed Central PMCID: PMCPMC6026924.
308. Huang X, Fu C, Liu W, Liang Y, Li P, Liu Z, et al. Chemerin-induced angiogenesis and adipogenesis in 3 T3-L1 preadipocytes is mediated by lncRNA Meg3 through regulating Dickkopf-3 by sponging miR-217. Toxicology and applied pharmacology. 2019;385:114815. Epub 2019/11/13. doi: 10.1016/j.taap.2019.114815. PubMed PMID: 31715267.
309. Dimitriadis GK, Kaur J, Adya R, Miras AD, Mattu HS, Hattersley JG, et al. Chemerin induces endothelial cell inflammation: activation of nuclear factor-kappa beta and monocyte-endothelial adhesion. Oncotarget. 2018;9(24):16678-90. Epub 2018/04/24. doi: 10.18632/oncotarget.24659. PubMed PMID: 29682177; PubMed Central PMCID: PMCPMC5908278.
310. Nakamura N, Naruse K, Kobayashi Y, Miyabe M, Saiki T, Enomoto A, et al. Chemerin promotes angiogenesis in vivo. Physiological reports. 2018;6(24):e13962. Epub 2018/12/28. doi: 10.14814/phy2.13962. PubMed PMID: 30588761; PubMed Central PMCID: PMCPMC6306368.
311. Ben Dhaou C, Del Prete A, Sozzani S, Parmentier M. CCRL2 Modulates Physiological and Pathological Angiogenesis During Retinal Development. Frontiers in cell and developmental biology. 2021;9:808455. Epub 2022/01/11. doi: 10.3389/fcell.2021.808455. PubMed PMID: 35004698; PubMed Central PMCID: PMCPMC8733553.
312. Ben Dhaou C, Mandi K, Frye M, Acheampong A, Radi A, De Becker B, et al. Chemerin regulates normal angiogenesis and hypoxia-driven neovascularization. Angiogenesis. 2022;25(2):159-79. Epub 2021/09/16. doi: 10.1007/s10456-021-09818-1. PubMed PMID: 34524600; PubMed Central PMCID: PMCPMC9054887.
313. De Henau O, Degroot GN, Imbault V, Robert V, De Poorter C, McHeik S, et al. Signaling Properties of Chemerin Receptors CMKLR1, GPR1 and CCRL2. PloS one. 2016;11(10):e0164179. Epub 2016/10/08. doi: 10.1371/journal.pone.0164179. PubMed PMID: 27716822; PubMed Central PMCID: PMCPMC5055294.
314. Huang C, Dai XY, Cai JX, Chen J, Wang BB, Zhu W, et al. A Screened GPR1 Peptide Exerts Antitumor Effects on Triple-Negative Breast Cancer. Molecular therapy oncolytics. 2020;18:602-12. Epub 2020/10/03. doi: 10.1016/j.omto.2020.08.013. PubMed PMID: 33005727; PubMed Central PMCID: PMCPMC7508919.
315. Tan L, Chen Z, Sun F, Zhou Z, Zhang B, Wang B, et al. Placental trophoblast-specific overexpression of chemerin induces preeclampsia-like symptoms. Clinical science (London, England : 1979). 2022;136(4):257-72. Epub 2022/02/02. doi: 10.1042/cs20210989. PubMed PMID: 35103285; PubMed Central PMCID: PMCPMC8844909.
316. Nigro E, Scudiero O, Monaco ML, Palmieri A, Mazzarella G, Costagliola C, et al. New insight into adiponectin role in obesity and obesity-related diseases. BioMed research international. 2014;2014:658913. Epub 2014/08/12. doi: 10.1155/2014/658913. PubMed PMID: 25110685; PubMed Central PMCID: PMCPMC4109424.
317. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nature medicine. 2001;7(8):941-6. Epub 2001/08/02. doi: 10.1038/90984. PubMed PMID: 11479627.
318. Huang CC, Law YY, Liu SC, Hu SL, Lin JA, Chen CJ, et al. Adiponectin Promotes VEGF Expression in Rheumatoid Arthritis Synovial Fibroblasts and Induces Endothelial Progenitor Cell Angiogenesis by Inhibiting miR-106a-5p. Cells. 2021;10(10). Epub 2021/10/24. doi: 10.3390/cells10102627. PubMed PMID: 34685605; PubMed Central PMCID: PMCPMC8534315.
319. Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, et al. Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and

Akt signaling in endothelial cells. J Biol Chem. 2004;279(2):1304-9. Epub 2003/10/15. doi: 10.1074/jbc.M310389200. PubMed PMID: 14557259; PubMed Central PMCID: PMCPMC4374490.
320. Wang S, Miao J, Qu M, Yang GY, Shen L. Adiponectin modulates the function of endothelial progenitor cells via AMPK/eNOS signaling pathway. Biochemical and biophysical research communications. 2017;493(1):64-70. Epub 2017/09/19. doi: 10.1016/j.bbrc.2017.09.073. PubMed PMID: 28919424.
321. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation. 2000;102(11):1296-301. Epub 2000/09/12. doi: 10.1161/01.cir.102.11.1296. PubMed PMID: 10982546.
322. Okui H, Hamasaki S, Ishida S, Kataoka T, Orihara K, Fukudome T, et al. Adiponectin is a better predictor of endothelial function of the coronary artery than HOMA-R, body mass index, immunoreactive insulin, or triglycerides. International journal of cardiology. 2008;126(1):53-61. Epub 2007/05/05. doi: 10.1016/j.ijcard.2007.03.116. PubMed PMID: 17477992.
323. Shibata R, Ouchi N, Kihara S, Sato K, Funahashi T, Walsh K. Adiponectin stimulates angiogenesis in response to tissue ischemia through stimulation of amp-activated protein kinase signaling. J Biol Chem. 2004;279(27):28670-4. Epub 2004/05/05. doi: 10.1074/jbc.M402558200. PubMed PMID: 15123726.
324. Li R, Du J, Yao Y, Yao G, Wang X. Adiponectin inhibits high glucose-induced angiogenesis via inhibiting autophagy in RF/6A cells. Journal of cellular physiology. 2019;234(11):20566-76. Epub 2019/04/16. doi: 10.1002/jcp.28659. PubMed PMID: 30982980.
325. Palanisamy K, Nareshkumar RN, Sivagurunathan S, Raman R, Sulochana KN, Chidambaram S. Anti-angiogenic effect of adiponectin in human primary microvascular and macrovascular endothelial cells. Microvascular research. 2019;122:136-45. Epub 2018/08/26. doi: 10.1016/j.mvr.2018.08.002. PubMed PMID: 30144414.
326. Bråkenhielm E, Veitonmäki N, Cao R, Kihara S, Matsuzawa Y, Zhivotovsky B, et al. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(8):2476-81. Epub 2004/02/26. doi: 10.1073/pnas.0308671100. PubMed PMID: 14983034; PubMed Central PMCID: PMCPMC356975.
327. Fasshauer M, Paschke R, Stumvoll M. Adiponectin, obesity, and cardiovascular disease. Biochimie. 2004;86(11):779-84. Epub 2004/12/14. doi: 10.1016/j.biochi.2004.09.016. PubMed PMID: 15589686.
328. Adya R, Tan BK, Chen J, Randeva HS. Protective actions of globular and full-length adiponectin on human endothelial cells: novel insights into adiponectin-induced angiogenesis. Journal of vascular research. 2012;49(6):534-43. Epub 2012/09/12. doi: 10.1159/000338279. PubMed PMID: 22964477.
329. Okada-Iwabu M, Yamauchi T, Iwabu M, Honma T, Hamagami K, Matsuda K, et al. A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. Nature. 2013;503(7477):493-9. Epub 2013/11/01. doi: 10.1038/nature12656. PubMed PMID: 24172895.
330. Wang SJ, Lu WY, Liu KY. Adiponectin receptor agonist AdipoRon suppresses adipogenesis in C3H10T1/2 cells through the adenosine monophosphate-activated protein kinase signaling pathway. Molecular medicine reports. 2017;16(5):7163-9. Epub 2017/09/14. doi: 10.3892/mmr.2017.7450. PubMed PMID: 28901521.
331. Kupchak BR, Garitaonandia I, Villa NY, Smith JL, Lyons TJ. Antagonism of human adiponectin receptors and their membrane progesterone receptor paralogs by TNFalpha and

a ceramidase inhibitor. Biochemistry. 2009;48(24):5504-6. Epub 2009/05/21. doi: 10.1021/bi9006258. PubMed PMID: 19453184; PubMed Central PMCID: PMCPMC2789275.
332. Augustin HG, Koh GY, Thurston G, Alitalo K. Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. Nature reviews Molecular cell biology. 2009;10(3):165-77. Epub 2009/02/24. doi: 10.1038/nrm2639. PubMed PMID: 19234476.
333. Saharinen P, Eklund L, Miettinen J, Wirkkala R, Anisimov A, Winderlich M, et al. Angiopoietins assemble distinct Tie2 signalling complexes in endothelial cell-cell and cell-matrix contacts. Nature cell biology. 2008;10(5):527-37. Epub 2008/04/22. doi: 10.1038/ncb1715. PubMed PMID: 18425119.
334. Girling JE, Rogers PA. Regulation of endometrial vascular remodelling: role of the vascular endothelial growth factor family and the angiopoietin-TIE signalling system. Reproduction (Cambridge, England). 2009;138(6):883-93. Epub 2009/09/17. doi: 10.1530/rep-09-0147. PubMed PMID: 19755482.
335. An YA, Sun K, Joffin N, Zhang F, Deng Y, Donzé O, et al. Angiopoietin-2 in white adipose tissue improves metabolic homeostasis through enhanced angiogenesis. eLife. 2017;6. Epub 2017/03/30. doi: 10.7554/eLife.24071. PubMed PMID: 28355132; PubMed Central PMCID: PMCPMC5391203.
336. Bae H, Hong KY, Lee CK, Jang C, Lee SJ, Choe K, et al. Angiopoietin-2-integrin α5β1 signaling enhances vascular fatty acid transport and prevents ectopic lipid-induced insulin resistance. Nature communications. 2020;11(1):2980. Epub 2020/06/14. doi: 10.1038/s41467-020-16795-4. PubMed PMID: 32532986; PubMed Central PMCID: PMCPMC7293240.
337. Xue Y, Cao R, Nilsson D, Chen S, Westergren R, Hedlund EM, et al. FOXC2 controls Ang-2 expression and modulates angiogenesis, vascular patterning, remodeling, and functions in adipose tissue. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(29):10167-72. Epub 2008/07/16. doi: 10.1073/pnas.0802486105. PubMed PMID: 18621714; PubMed Central PMCID: PMCPMC2481379.
338. Good DJ, Polverini PJ, Rastinejad F, Le Beau MM, Lemons RS, Frazier WA, et al. A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. Proceedings of the National Academy of Sciences of the United States of America. 1990;87(17):6624-8. Epub 1990/09/01. doi: 10.1073/pnas.87.17.6624. PubMed PMID: 1697685; PubMed Central PMCID: PMCPMC54589.
339. Bornstein P. Thrombospondins function as regulators of angiogenesis. Journal of cell communication and signaling. 2009;3(3-4):189-200. Epub 2009/10/03. doi: 10.1007/s12079-009-0060-8. PubMed PMID: 19798599; PubMed Central PMCID: PMCPMC2778581.
340. Matsuo Y, Tanaka M, Yamakage H, Sasaki Y, Muranaka K, Hata H, et al. Thrombospondin 1 as a novel biological marker of obesity and metabolic syndrome. Metabolism: clinical and experimental. 2015;64(11):1490-9. Epub 2015/08/25. doi: 10.1016/j.metabol.2015.07.016. PubMed PMID: 26298466; PubMed Central PMCID: PMCPMC4936918.
341. Varma V, Yao-Borengasser A, Bodles AM, Rasouli N, Phanavanh B, Nolen GT, et al. Thrombospondin-1 is an adipokine associated with obesity, adipose inflammation, and insulin resistance. Diabetes. 2008;57(2):432-9. Epub 2007/12/07. doi: 10.2337/db07-0840. PubMed PMID: 18057090; PubMed Central PMCID: PMCPMC2877915.
342. Voros G, Lijnen HR. Deficiency of thrombospondin-1 in mice does not affect adipose tissue development. Journal of thrombosis and haemostasis : JTH. 2006;4(1):277-8. Epub 2006/01/18. doi: 10.1111/j.1538-7836.2005.01696.x. PubMed PMID: 16409488.
343. Lee MJ. Transforming growth factor beta superfamily regulation of adipose tissue biology in obesity. Biochimica et biophysica acta Molecular basis of disease. 2018;1864(4 Pt A):1160-71. Epub 2018/02/08. doi: 10.1016/j.bbadis.2018.01.025. PubMed PMID: 29409985.

344. ten Dijke P, Arthur HM. Extracellular control of TGFbeta signalling in vascular development and disease. Nature reviews Molecular cell biology. 2007;8(11):857-69. Epub 2007/09/27. doi: 10.1038/nrm2262. PubMed PMID: 17895899.
345. Wang Z, Han Z, Tao J, Wang J, Liu X, Zhou W, et al. Role of endothelial-to-mesenchymal transition induced by TGF-β1 in transplant kidney interstitial fibrosis. Journal of cellular and molecular medicine. 2017;21(10):2359-69. Epub 2017/04/05. doi: 10.1111/jcmm.13157. PubMed PMID: 28374926; PubMed Central PMCID: PMCPMC5618680.
346. Liu J, Liao S, Diop-Frimpong B, Chen W, Goel S, Naxerova K, et al. TGF-β blockade improves the distribution and efficacy of therapeutics in breast carcinoma by normalizing the tumor stroma. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(41):16618-23. Epub 2012/09/22. doi: 10.1073/pnas.1117610109. PubMed PMID: 22996328; PubMed Central PMCID: PMCPMC3478596 MedImmune, and Roche; received consultant fees from Dyax, Enlight, Noxxon, and SynDevRx; owns equity in Enlight, SynDevRx, and XTuit; and serves on the Board of Directors of XTuit and Board of Trustees of H&Q Healthcare Investors and H&Q Life Sciences Investors. Y.B. received consultant fees from XTuit. No reagents or funding from these companies were used in these studies.
347. Penafuerte C, Bautista-Lopez N, Bouchentouf M, Birman E, Forner K, Galipeau J. Novel TGF-beta antagonist inhibits tumor growth and angiogenesis by inducing IL-2 receptor-driven STAT1 activation. Journal of immunology (Baltimore, Md : 1950). 2011;186(12):6933-44. Epub 2011/05/10. doi: 10.4049/jimmunol.1003816. PubMed PMID: 21551368.
348. Yadav H, Quijano C, Kamaraju AK, Gavrilova O, Malek R, Chen W, et al. Protection from obesity and diabetes by blockade of TGF-β/Smad3 signaling. Cell metabolism. 2011;14(1):67-79. Epub 2011/07/05. doi: 10.1016/j.cmet.2011.04.013. PubMed PMID: 21723505; PubMed Central PMCID: PMCPMC3169298.
349. Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. Biochemical pharmacology. 2009;78(6):539-52. Epub 2009/05/06. doi: 10.1016/j.bcp.2009.04.029. PubMed PMID: 19413999; PubMed Central PMCID: PMCPMC2730638.
350. Tsai HC, Cheng SP, Han CK, Huang YL, Wang SW, Lee JJ, et al. Resistin enhances angiogenesis in osteosarcoma via the MAPK signaling pathway. Aging. 2019;11(21):9767-77. Epub 2019/11/14. doi: 10.18632/aging.102423. PubMed PMID: 31719210; PubMed Central PMCID: PMCPMC6874472.
351. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nature medicine. 2002;8(7):731-7. Epub 2002/06/18. doi: 10.1038/nm724. PubMed PMID: 12068289.
352. DeBari MK, Abbott RD. Adipose Tissue Fibrosis: Mechanisms, Models, and Importance. International journal of molecular sciences. 2020;21(17). Epub 2020/08/23. doi: 10.3390/ijms21176030. PubMed PMID: 32825788; PubMed Central PMCID: PMCPMC7503256.
353. Chudek J, Wiecek A. Adipose tissue, inflammation and endothelial dysfunction. Pharmacological reports : PR. 2006;58 Suppl:81-8. Epub 2007/03/03. PubMed PMID: 17332676.
354. Freedman BR, Mooney DJ. Biomaterials to Mimic and Heal Connective Tissues. Advanced materials (Deerfield Beach, Fla). 2019;31(19):e1806695. Epub 2019/03/26. doi: 10.1002/adma.201806695. PubMed PMID: 30908806; PubMed Central PMCID: PMCPMC6504615.
355. O'Halloran NA, Dolan EB, Kerin MJ, Lowery AJ, Duffy GP. Hydrogels in adipose tissue engineering-Potential application in post-mastectomy breast regeneration. Journal of tissue engineering and regenerative medicine. 2018;12(12):2234-47. Epub 2018/10/20. doi: 10.1002/term.2753. PubMed PMID: 30334613.

356. Di Somma M, Schaafsma W, Grillo E, Vliora M, Dakou E, Corsini M, et al. Natural Histogel-Based Bio-Scaffolds for Sustaining Angiogenesis in Beige Adipose Tissue. Cells. 2019;8(11). Epub 2019/11/23. doi: 10.3390/cells8111457. PubMed PMID: 31752157; PubMed Central PMCID: PMCPMC6912328.
357. Koduru SV, Leberfinger AN, Pasic D, Forghani A, Lince S, Hayes DJ, et al. Cellular Based Strategies for Microvascular Engineering. Stem cell reviews and reports. 2019;15(2):218-40. Epub 2019/02/11. doi: 10.1007/s12015-019-09877-4. PubMed PMID: 30739276.
358. Bharadwaz A, Jayasuriya AC. Recent trends in the application of widely used natural and synthetic polymer nanocomposites in bone tissue regeneration. Materials science & engineering C, Materials for biological applications. 2020;110:110698. Epub 2020/03/25. doi: 10.1016/j.msec.2020.110698. PubMed PMID: 32204012; PubMed Central PMCID: PMCPMC7433904.
359. Raghav PK, Mann Z, Ahlawat S, Mohanty S. Mesenchymal stem cell-based nanoparticles and scaffolds in regenerative medicine. European journal of pharmacology. 2022;918:174657. Epub 2021/12/07. doi: 10.1016/j.ejphar.2021.174657. PubMed PMID: 34871557.
360. Dharmadhikari S, Liu L, Shontz K, Wiet M, White A, Goins A, et al. Deconstructing tissue engineered trachea: Assessing the role of synthetic scaffolds, segmental replacement and cell seeding on graft performance. Acta biomaterialia. 2020;102:181-91. Epub 2019/11/11. doi: 10.1016/j.actbio.2019.11.008. PubMed PMID: 31707085; PubMed Central PMCID: PMCPMC7231426.
361. Nardo T, Chiono V, Carmagnola I, Fracchia L, Ceresa C, Tabrizian M, et al. Mussel-inspired antimicrobial coating on PTFE barrier membranes for guided tissue regeneration. Biomedical materials (Bristol, England). 2021;16(3). Epub 2021/03/27. doi: 10.1088/1748-605X/abf27e. PubMed PMID: 33770778.
362. Kral JG, Crandall DL. Development of a human adipocyte synthetic polymer scaffold. Plastic and reconstructive surgery. 1999;104(6):1732-8. Epub 1999/11/30. doi: 10.1097/00006534-199911000-00018. PubMed PMID: 10541176.
363. Li K, Tharwat M, Larson EL, Felgendreff P, Hosseiniasl SM, Rmilah AA, et al. Re-Endothelialization of Decellularized Liver Scaffolds: A Step for Bioengineered Liver Transplantation. Front Bioeng Biotechnol. 2022;10:833163. Epub 2022/04/02. doi: 10.3389/fbioe.2022.833163. PubMed PMID: 35360393; PubMed Central PMCID: PMCPMC8960611.
364. Liu C, Pei M, Li Q, Zhang Y. Decellularized extracellular matrix mediates tissue construction and regeneration. Frontiers of medicine. 2022;16(1):56-82. Epub 2021/12/29. doi: 10.1007/s11684-021-0900-3. PubMed PMID: 34962624; PubMed Central PMCID: PMCPMC8976706.
365. Nellinger S, Mrsic I, Keller S, Heine S, Southan A, Bach M, et al. Cell-derived and enzyme-based decellularized extracellular matrix exhibit compositional and structural differences that are relevant for its use as a biomaterial. Biotechnology and bioengineering. 2022;119(4):1142-56. Epub 2022/01/30. doi: 10.1002/bit.28047. PubMed PMID: 35092015.
366. Liu K, He Y, Yao Y, Zhang Y, Cai Z, Ru J, et al. Methoxy polyethylene glycol modification promotes adipogenesis by inducing the production of regulatory T cells in xenogeneic acellular adipose matrix. Materials today Bio. 2021;12:100161. Epub 2021/12/07. doi: 10.1016/j.mtbio.2021.100161. PubMed PMID: 34870140; PubMed Central PMCID: PMCPMC8626673.
367. Zhu Z, Yuan Z, Guo L, Nurzat Y, Xu H, Zhang Y. Construction of adipose tissue using a silica expander capsule and cell sheet-assembled of decellularized adipose tissue. Acta biomaterialia. 2022;141:89-101. Epub 2022/01/03. doi: 10.1016/j.actbio.2021.12.018. PubMed PMID: 34974176.

368. Cheung HK, Han TT, Marecak DM, Watkins JF, Amsden BG, Flynn LE. Composite hydrogel scaffolds incorporating decellularized adipose tissue for soft tissue engineering with adipose-derived stem cells. Biomaterials. 2014;35(6):1914-23. Epub 2013/12/18. doi: 10.1016/j.biomaterials.2013.11.067. PubMed PMID: 24331712.
369. Shridhar A, Amsden BG, Gillies ER, Flynn LE. Investigating the Effects of Tissue-Specific Extracellular Matrix on the Adipogenic and Osteogenic Differentiation of Human Adipose-Derived Stromal Cells Within Composite Hydrogel Scaffolds. Front Bioeng Biotechnol. 2019;7:402. Epub 2020/01/11. doi: 10.3389/fbioe.2019.00402. PubMed PMID: 31921807; PubMed Central PMCID: PMCPMC6917659.
370. James PT, Leach R, Kalamara E, Shayeghi M. The worldwide obesity epidemic. Obesity research. 2001;9 Suppl 4:228s-33s. Epub 2001/11/15. doi: 10.1038/oby.2001.123. PubMed PMID: 11707546.
371. Kang SI, Shin HS, Kim HM, Hong YS, Yoon SA, Kang SW, et al. Anti-obesity properties of a Sasa quelpaertensis extract in high-fat diet-induced obese mice. Bioscience, biotechnology, and biochemistry. 2012;76(4):755-61. Epub 2012/04/10. doi: 10.1271/bbb.110868. PubMed PMID: 22484945.
372. Cristina Oliveira de Lima V, Piuvezam G, Leal Lima Maciel B, Heloneida de Araújo Morais A. Trypsin inhibitors: promising candidate satietogenic proteins as complementary treatment for obesity and metabolic disorders? Journal of enzyme inhibition and medicinal chemistry. 2019;34(1):405-19. Epub 2019/02/09. doi: 10.1080/14756366.2018.1542387. PubMed PMID: 30734596; PubMed Central PMCID: PMCPMC6327991.
373. Komarnytsky S, Cook A, Raskin I. Potato protease inhibitors inhibit food intake and increase circulating cholecystokinin levels by a trypsin-dependent mechanism. International journal of obesity (2005). 2011;35(2):236-43. Epub 2010/09/08. doi: 10.1038/ijo.2010.192. PubMed PMID: 20820171; PubMed Central PMCID: PMCPMC3033477.
374. Panteliou E, Miras AD. What is the role of bariatric surgery in the management of obesity? Climacteric : the journal of the International Menopause Society. 2017;20(2):97-102. Epub 2017/01/05. doi: 10.1080/13697137.2017.1262638. PubMed PMID: 28051892.
375. Lee JH, Park A, Oh KJ, Lee SC, Kim WK, Bae KH. The Role of Adipose Tissue Mitochondria: Regulation of Mitochondrial Function for the Treatment of Metabolic Diseases. International journal of molecular sciences. 2019;20(19). Epub 2019/10/09. doi: 10.3390/ijms20194924. PubMed PMID: 31590292; PubMed Central PMCID: PMCPMC6801758.
376. Granneman JG, Li P, Zhu Z, Lu Y. Metabolic and cellular plasticity in white adipose tissue I: effects of beta3-adrenergic receptor activation. American journal of physiology Endocrinology and metabolism. 2005;289(4):E608-16. Epub 2005/06/09. doi: 10.1152/ajpendo.00009.2005. PubMed PMID: 15941787.
377. Park J, Morley TS, Kim M, Clegg DJ, Scherer PE. Obesity and cancer--mechanisms underlying tumour progression and recurrence. Nature reviews Endocrinology. 2014;10(8):455-65. Epub 2014/06/18. doi: 10.1038/nrendo.2014.94. PubMed PMID: 24935119; PubMed Central PMCID: PMCPMC4374431.
378. Zhou YZ, Cheng Z, Wu Y, Wu QY, Liao XB, Zhao Y, et al. Mesenchymal stem cell-derived conditioned medium attenuate angiotensin II-induced aortic aneurysm growth by modulating macrophage polarization. Journal of cellular and molecular medicine. 2019;23(12):8233-45. Epub 2019/10/05. doi: 10.1111/jcmm.14694. PubMed PMID: 31583844; PubMed Central PMCID: PMCPMC6850971.
379. Paulo Zambon J, Atala A, Yoo JJ. Methods to generate tissue-derived constructs for regenerative medicine applications. Methods (San Diego, Calif). 2020;171:3-10. Epub 2019/10/14. doi: 10.1016/j.ymeth.2019.09.016. PubMed PMID: 31606388.
380. Brown AC. Brown adipocytes from induced pluripotent stem cells-how far have we come? Annals of the New York Academy of Sciences. 2020;1463(1):9-22. Epub 2019/10/02.

doi: 10.1111/nyas.14257. PubMed PMID: 31573081; PubMed Central PMCID: PMCPMC7078043.
381. Srivastava S, Veech RL. Brown and Brite: The Fat Soldiers in the Anti-obesity Fight. Frontiers in physiology. 2019;10:38. Epub 2019/02/15. doi: 10.3389/fphys.2019.00038. PubMed PMID: 30761017; PubMed Central PMCID: PMCPMC6363669.
382. Dave K, Gomes VG. Interactions at scaffold interfaces: Effect of surface chemistry, structural attributes and bioaffinity. Materials science & engineering C, Materials for biological applications. 2019;105:110078. Epub 2019/09/25. doi: 10.1016/j.msec.2019.110078. PubMed PMID: 31546353.
383. Herrero-Mendez A, Palomares T, Castro B, Herrero J, Granado MH, Bejar JM, et al. HR007: a family of biomaterials based on glycosaminoglycans for tissue repair. Journal of tissue engineering and regenerative medicine. 2017;11(4):989-1001. Epub 2015/03/03. doi: 10.1002/term.1998. PubMed PMID: 25728195.
384. Luo H, Cha R, Li J, Hao W, Zhang Y, Zhou F. Advances in tissue engineering of nanocellulose-based scaffolds: A review. Carbohydrate polymers. 2019;224:115144. Epub 2019/09/02. doi: 10.1016/j.carbpol.2019.115144. PubMed PMID: 31472870.
385. Pandit AH, Mazumdar N, Ahmad S. Periodate oxidized hyaluronic acid-based hydrogel scaffolds for tissue engineering applications. International journal of biological macromolecules. 2019;137:853-69. Epub 2019/07/10. doi: 10.1016/j.ijbiomac.2019.07.014. PubMed PMID: 31284008.
386. Son YJ, Tse JW, Zhou Y, Mao W, Yim EKF, Yoo HS. Biomaterials and controlled release strategy for epithelial wound healing. Biomaterials science. 2019;7(11):4444-71. Epub 2019/08/23. doi: 10.1039/c9bm00456d. PubMed PMID: 31436261.
387. Shinoda K, Ohyama K, Hasegawa Y, Chang HY, Ogura M, Sato A, et al. Phosphoproteomics Identifies CK2 as a Negative Regulator of Beige Adipocyte Thermogenesis and Energy Expenditure. Cell metabolism. 2015;22(6):997-1008. Epub 2015/11/04. doi: 10.1016/j.cmet.2015.09.029. PubMed PMID: 26525534; PubMed Central PMCID: PMCPMC4670581.
388. Grillo E, Ravelli C, Corsini M, Ballmer-Hofer K, Zammataro L, Oreste P, et al. Monomeric gremlin is a novel vascular endothelial growth factor receptor-2 antagonist. Oncotarget. 2016;7(23):35353-68. Epub 2016/05/14. doi: 10.18632/oncotarget.9286. PubMed PMID: 27174917; PubMed Central PMCID: PMCPMC5085234.
389. Rezzola S, Di Somma M, Corsini M, Leali D, Ravelli C, Polli VAB, et al. VEGFR2 activation mediates the pro-angiogenic activity of BMP4. Angiogenesis. 2019;22(4):521-33. Epub 2019/08/01. doi: 10.1007/s10456-019-09676-y. PubMed PMID: 31363885.
390. Ravelli C, Mitola S, Corsini M, Presta M. Involvement of αvβ3 integrin in gremlin-induced angiogenesis. Angiogenesis. 2013;16(1):235-43. Epub 2012/10/12. doi: 10.1007/s10456-012-9309-6. PubMed PMID: 23053782.
391. Ling Y, Carayol J, Galusca B, Canto C, Montaurier C, Matone A, et al. Persistent low body weight in humans is associated with higher mitochondrial activity in white adipose tissue. The American journal of clinical nutrition. 2019;110(3):605-16. Epub 2019/08/03. doi: 10.1093/ajcn/nqz144. PubMed PMID: 31374571; PubMed Central PMCID: PMCPMC6736451.
392. Ferguson VL, Dodson RB. Bioengineering aspects of the umbilical cord. European journal of obstetrics, gynecology, and reproductive biology. 2009;144 Suppl 1:S108-13. Epub 2009/03/24. doi: 10.1016/j.ejogrb.2009.02.024. PubMed PMID: 19304367.
393. Jadalannagari S, Converse G, McFall C, Buse E, Filla M, Villar MT, et al. Decellularized Wharton's Jelly from human umbilical cord as a novel 3D scaffolding material for tissue engineering applications. PloS one. 2017;12(2):e0172098. Epub 2017/02/22. doi: 10.1371/journal.pone.0172098. PubMed PMID: 28222169; PubMed Central PMCID: PMCPMC5319682 Research and Epidemiology – BIPS, KT, BK, and TS have performed research studies sponsored by pharmaceutical companies (Bayer-Schering, Celgene, GSK,

Mundipharma, Novartis, Purdue, Sanofi-Aventis, Sanofi-Pasteur, Stada, and Takeda) unrelated to this study. This does not alter our adherence to PLOS ONE policies on sharing data and materials.
394. Kehtari M, Beiki B, Zeynali B, Hosseini FS, Soleimanifar F, Kaabi M, et al. Decellularized Wharton's jelly extracellular matrix as a promising scaffold for promoting hepatic differentiation of human induced pluripotent stem cells. Journal of cellular biochemistry. 2019;120(4):6683-97. Epub 2018/11/13. doi: 10.1002/jcb.27965. PubMed PMID: 30417406.
395. Beiki B, Zeynali B, Seyedjafari E. Fabrication of a three dimensional spongy scaffold using human Wharton's jelly derived extra cellular matrix for wound healing. Materials science & engineering C, Materials for biological applications. 2017;78:627-38. Epub 2017/06/04. doi: 10.1016/j.msec.2017.04.074. PubMed PMID: 28576031.
396. Augst AD, Kong HJ, Mooney DJ. Alginate hydrogels as biomaterials. Macromolecular bioscience. 2006;6(8):623-33. Epub 2006/08/02. doi: 10.1002/mabi.200600069. PubMed PMID: 16881042.
397. Pelletier S, Hubert P, Payan E, Marchal P, Choplin L, Dellacherie E. Amphiphilic derivatives of sodium alginate and hyaluronate for cartilage repair: rheological properties. Journal of biomedical materials research. 2001;54(1):102-8. Epub 2000/11/15. doi: 10.1002/1097-4636(200101)54:1<102::aid-jbm12>3.0.co;2-1. PubMed PMID: 11077408.
398. Lee KY, Mooney DJ. Alginate: properties and biomedical applications. Progress in polymer science. 2012;37(1):106-26. Epub 2011/11/30. doi: 10.1016/j.progpolymsci.2011.06.003. PubMed PMID: 22125349; PubMed Central PMCID: PMCPMC3223967.
399. Koo LY, Irvine DJ, Mayes AM, Lauffenburger DA, Griffith LG. Co-regulation of cell adhesion by nanoscale RGD organization and mechanical stimulus. Journal of cell science. 2002;115(Pt 7):1423-33. Epub 2002/03/16. doi: 10.1242/jcs.115.7.1423. PubMed PMID: 11896190.
400. Yang C, Hillas PJ, Báez JA, Nokelainen M, Balan J, Tang J, et al. The application of recombinant human collagen in tissue engineering. BioDrugs : clinical immunotherapeutics, biopharmaceuticals and gene therapy. 2004;18(2):103-19. Epub 2004/03/30. doi: 10.2165/00063030-200418020-00004. PubMed PMID: 15046526.
401. Schnabl K, Westermeier J, Li Y, Klingenspor M. Opposing Actions of Adrenocorticotropic Hormone and Glucocorticoids on UCP1-Mediated Respiration in Brown Adipocytes. Frontiers in physiology. 2018;9:1931. Epub 2019/02/02. doi: 10.3389/fphys.2018.01931. PubMed PMID: 30705635; PubMed Central PMCID: PMCPMC6344423.
402. del Mar Gonzalez-Barroso M, Pecqueur C, Gelly C, Sanchis D, Alves-Guerra MC, Bouillaud F, et al. Transcriptional activation of the human ucp1 gene in a rodent cell line. Synergism of retinoids, isoproterenol, and thiazolidinedione is mediated by a multipartite response element. J Biol Chem. 2000;275(41):31722-32. Epub 2000/08/02. doi: 10.1074/jbc.M001678200. PubMed PMID: 10921912.
403. (WHO) WHO. Global Health Estimates 2020: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2019. 2020.
404. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74. Epub 2011/03/08. doi: 10.1016/j.cell.2011.02.013. PubMed PMID: 21376230.
405. Zhang JM, An J. Cytokines, inflammation, and pain. Int Anesthesiol Clin. 2007;45(2):27-37. Epub 2007/04/12. doi: 10.1097/AIA.0b013e318034194e. PubMed PMID: 17426506; PubMed Central PMCID: PMCPMC2785020.
406. Tsiani E, Tsakiridis N, Kouvelioti R, Jaglanian A, Klentrou P. Current Evidence of the Role of the Myokine Irisin in Cancer. Cancers (Basel). 2021;13(11):2628. doi: 10.3390/cancers13112628. PubMed PMID: 34071869.

407. Mazur-Bialy AI, Bilski J, Pochec E, Brzozowski T. New insight into the direct anti-inflammatory activity of a myokine irisin against proinflammatory activation of adipocytes. Implication for exercise in obesity. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2017;68(2):243-51. Epub 2017/06/15. PubMed PMID: 28614774.
408. Mazur-Bialy AI, Bilski J, Wojcik D, Brzozowski B, Surmiak M, Hubalewska-Mazgaj M, et al. Beneficial Effect of Voluntary Exercise on Experimental Colitis in Mice Fed a High-Fat Diet: The Role of Irisin, Adiponectin and Proinflammatory Biomarkers. Nutrients. 2017;9(4). Epub 2017/04/21. doi: 10.3390/nu9040410. PubMed PMID: 28425943; PubMed Central PMCID: PMCPMC5409749.
409. Newton RU, Galvão DA. Exercise in Preventionand Management of Cancer. Current Treatment Options in Oncology. 2008;9(2):135-46. doi: 10.1007/s11864-008-0065-1.
410. Fatouros IG. Is irisin the new player in exercise-induced adaptations or not? A 2017 update. Clinical chemistry and laboratory medicine. 2018;56(4):525-48. Epub 2017/11/12. doi: 10.1515/cclm-2017-0674. PubMed PMID: 29127759.
411. Aydin S. Is irisin a decisive protein in cancer cachexia and death of cancer cells? European review for medical and pharmacological sciences. 2016;20(18):3727-9. Epub 2016/10/14. PubMed PMID: 27735050.
412. Gaggini M, Cabiati M, Del Turco S, Navarra T, De Simone P, Filipponi F, et al. Increased FNDC5/Irisin expression in human hepatocellular carcinoma. Peptides. 2017;88:62-6. Epub 2016/12/26. doi: 10.1016/j.peptides.2016.12.014. PubMed PMID: 28012856.
413. Kuloğlu T, Artaş G, Yardim M, Sahin I, Aydin Y, Beyoğlu N, et al. Immunostaining characteristics of irisin in benign and malignant renal cancers. Biotechnic & histochemistry : official publication of the Biological Stain Commission. 2019;94(6):435-41. Epub 2019/03/22. doi: 10.1080/10520295.2019.1586998. PubMed PMID: 30896263.
414. Kuloglu T, Celik O, Aydin S, Hanifi Ozercan I, Acet M, Aydin Y, et al. Irisin immunostaining characteristics of breast and ovarian cancer cells. Cellular and molecular biology (Noisy-le-Grand, France). 2016;62(8):40-4. Epub 2016/08/23. PubMed PMID: 27545213.
415. Kong G, Jiang Y, Sun X, Cao Z, Zhang G, Zhao Z, et al. Irisin reverses the IL-6 induced epithelial-mesenchymal transition in osteosarcoma cell migration and invasion through the STAT3/Snail signaling pathway. Oncology reports. 2017;38(5):2647-56. Epub 2017/10/20. doi: 10.3892/or.2017.5973. PubMed PMID: 29048621; PubMed Central PMCID: PMCPMC5780017.
416. Liu J, Song N, Huang Y, Chen Y. Irisin inhibits pancreatic cancer cell growth via the AMPK-mTOR pathway. Sci Rep. 2018;8(1):15247. Epub 2018/10/17. doi: 10.1038/s41598-018-33229-w. PubMed PMID: 30323244; PubMed Central PMCID: PMCPMC6189061.
417. Shi G, Tang N, Qiu J, Zhang D, Huang F, Cheng Y, et al. Irisin stimulates cell proliferation and invasion by targeting the PI3K/AKT pathway in human hepatocellular carcinoma. Biochemical and biophysical research communications. 2017;493(1):585-91. Epub 2017/09/05. doi: 10.1016/j.bbrc.2017.08.148. PubMed PMID: 28867187.
418. Arhire LI, Mihalache L, Covasa M. Irisin: A Hope in Understanding and Managing Obesity and Metabolic Syndrome. Frontiers in Endocrinology. 2019;10. doi: 10.3389/fendo.2019.00524.
419. Mayeux R. Biomarkers: potential uses and limitations. NeuroRx. 2004;1(2):182-8. doi: 10.1602/neurorx.1.2.182. PubMed PMID: 15717018.
420. (OHAT) OfHaAS. Tool for Risk of Bias. updated2020.
421. Mellow M, Crozier A, Dumuid D, Wade A, Goldsworthy M, Dorrian J, et al. How are combinations of physical activity, sedentary behaviour and sleep related to cognitive function in older adults? A systematic review2021.
422. Rohatgi A. WebPlotDigitizer. 4.5.

423. Moon HS, Dincer F, Mantzoros CS. Pharmacological concentrations of irisin increase cell proliferation without influencing markers of neurite outgrowth and synaptogenesis in mouse H19-7 hippocampal cell lines. Metabolism: clinical and experimental. 2013;62(8):1131-6. Epub 2013/05/15. doi: 10.1016/j.metabol.2013.04.007. PubMed PMID: 23664146; PubMed Central PMCID: PMCPMC4370428.
424. Aydin S, Kuloglu T, Ozercan MR, Albayrak S, Aydin S, Bakal U, et al. Irisin immunohistochemistry in gastrointestinal system cancers. Biotechnic & histochemistry : official publication of the Biological Stain Commission. 2016;91(4):242-50. Epub 2016/03/11. doi: 10.3109/10520295.2015.1136988. PubMed PMID: 26963139.
425. Altay DU, Keha EE, Karagüzel E, Menteşe A, Yaman SO, Alver A. The Diagnostic Value of FNDC5/Irisin in Renal Cell Cancer. International braz j urol : official journal of the Brazilian Society of Urology. 2018;44(4):734-9. Epub 2018/03/10. doi: 10.1590/s1677-5538.ibju.2017.0404. PubMed PMID: 29522296; PubMed Central PMCID: PMCPMC6092672.
426. Aslan R, Alp HH, Eryılmaz R, Huyut Z, Sevim M, Araz Ş, et al. Can the Irisin be a Biomarker for Prostate Cancer? A Case Control Study. Asian Pacific journal of cancer prevention : APJCP. 2020;21(2):505-9. Epub 2020/02/28. doi: 10.31557/apjcp.2020.21.2.505. PubMed PMID: 32102531; PubMed Central PMCID: PMCPMC7332134.
427. Cheng G, Xu D, Chu K, Cao Z, Sun X, Yang Y. The Effects of MiR-214-3p and Irisin/FNDC5 on the Biological Behavior of Osteosarcoma Cells. Cancer biotherapy & radiopharmaceuticals. 2020;35(2):92-100. Epub 2020/02/20. doi: 10.1089/cbr.2019.2933. PubMed PMID: 32073886.
428. Coletta AM, Agha NH, Baker FL, Niemiro GM, Mylabathula PL, Brewster AM, et al. The impact of high-intensity interval exercise training on NK-cell function and circulating myokines for breast cancer prevention among women at high risk for breast cancer. Breast cancer research and treatment. 2021;187(2):407-16. Epub 2021/02/09. doi: 10.1007/s10549-021-06111-z. PubMed PMID: 33555464; PubMed Central PMCID: PMCPMC8189992.
429. de Castro GS, Correia-Lima J, Simoes E, Orsso CE, Xiao J, Gama LR, et al. Myokines in treatment-naïve patients with cancer-associated cachexia. Clinical nutrition (Edinburgh, Scotland). 2021;40(4):2443-55. Epub 2020/11/17. doi: 10.1016/j.clnu.2020.10.050. PubMed PMID: 33190987.
430. Esawy MM, Abdel-Samd KM. The diagnostic and prognostic roles of serum irisin in bladder cancer. Current problems in cancer. 2020;44(4):100529. Epub 2020/03/07. doi: 10.1016/j.currproblcancer.2019.100529. PubMed PMID: 32139156.
431. Kim SH, Kim JW, Hwang IG, Jang JS, Hong S, Kim TY, et al. Serum frailty biomarkers to predict overall survival in older patients with metastatic solid tumors: A substudy of prospective multi-center cohort study (KCSG PC13-09). Journal of Clinical Oncology. 2017;35(15_suppl):10041-. doi: 10.1200/JCO.2017.35.15_suppl.10041.
432. Nowinska K, Jablonska K, Pawelczyk K, Piotrowska A, Partynska A, Gomulkiewicz A, et al. Expression of Irisin/FNDC5 in Cancer Cells and Stromal Fibroblasts of Non-small Cell Lung Cancer. Cancers. 2019;11(10). Epub 2019/10/17. doi: 10.3390/cancers11101538. PubMed PMID: 31614634; PubMed Central PMCID: PMCPMC6826442.
433. Panagiotou G, Pazaitou-Panayiotou K, Paschou SA, Komninou D, Kalogeris N, Vryonidou A, et al. Changes in Thyroid Hormone Levels Within the Normal and/or Subclinical Hyper- or Hypothyroid Range Do Not Affect Circulating Irisin Levels in Humans. Thyroid : official journal of the American Thyroid Association. 2016;26(8):1039-45. Epub 2016/06/09. doi: 10.1089/thy.2016.0098. PubMed PMID: 27267080.
434. Pazgan-Simon M, Zuwala-Jagiello J, Menzyk T, Bator M, Derra A, Lekstan A, et al. Serum betatrophin and irisin levels in hepatocellular carcinoma. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2020;71(1). Epub 2020/06/20. doi: 10.26402/jpp.2020.1.11. PubMed PMID: 32554846.
435. Provatopoulou X, Georgiou GP, Kalogera E, Kalles V, Matiatou MA, Papapanagiotou I, et al. Serum irisin levels are lower in patients with breast cancer: association with disease

diagnosis and tumor characteristics. BMC cancer. 2015;15:898. Epub 2015/11/13. doi: 10.1186/s12885-015-1898-1. PubMed PMID: 26560078; PubMed Central PMCID: PMCPMC4642638.
436. Sadim M, Xu Y, Selig K, Paulus J, Uthe R, Agarwl S, et al. A prospective evaluation of clinical and genetic predictors of weight changes in breast cancer survivors. Cancer. 2017;123(13):2413-21. Epub 2017/02/15. doi: 10.1002/cncr.30628. PubMed PMID: 28195643.
437. Shahidi S, Hejazi J, Moghimi M, Borji S, Zabihian S, Fathi M. Circulating Irisin Levels and Redox Status Markers in Patients with Gastric Cancer: A Case-Control Study. Asian Pacific journal of cancer prevention : APJCP. 2020;21(10):2847-51. Epub 2020/10/29. doi: 10.31557/apjcp.2020.21.10.2847. PubMed PMID: 33112539; PubMed Central PMCID: PMCPMC7798161.
438. Ugur K, Aydin S, Kuloglu T, Artas G, Kocdor MA, Sahin İ, et al. Comparison of irisin hormone expression between thyroid cancer tissues and oncocytic variant cells. Cancer management and research. 2019;11:2595-603. Epub 2019/05/23. doi: 10.2147/cmar.s201979. PubMed PMID: 31114326; PubMed Central PMCID: PMCPMC6497896.
439. Us Altay D, Keha EE, Ozer Yaman S, Ince I, Alver A, Erdogan B, et al. Investigation of the expression of irisin and some cachectic factors in mice with experimentally induced gastric cancer. QJM : monthly journal of the Association of Physicians. 2016;109(12):785-90. Epub 2016/06/04. doi: 10.1093/qjmed/hcw074. PubMed PMID: 27256459.
440. Zhang J, Ke M, Ren Y, Bi J, Du Z, Zhang M, et al. Serum Irisin Predicts Posthepatectomy Complications in Patients with Hepatocellular Carcinoma. Disease markers. 2019;2019:9850191. Epub 2020/01/25. doi: 10.1155/2019/9850191. PubMed PMID: 31976024; PubMed Central PMCID: PMCPMC6955133.
441. Zhang ZP, Zhang XF, Li H, Liu TJ, Zhao QP, Huang LH, et al. Serum irisin associates with breast cancer to spinal metastasis. Medicine. 2018;97(17):e0524. Epub 2018/04/29. doi: 10.1097/md.0000000000010524. PubMed PMID: 29703023; PubMed Central PMCID: PMCPMC5944558 declare that this manuscript has not been submitted or is not simultaneously being submitted elsewhere, and that no portion of the data has been or will be published in proceedings or transactions of meetings or symposium volumes. The authors report no conflicts of interest.
442. Zhu H, Liu M, Zhang N, Pan H, Lin G, Li N, et al. Serum and Adipose Tissue mRNA Levels of ATF3 and FNDC5/Irisin in Colorectal Cancer Patients With or Without Obesity. Frontiers in physiology. 2018;9:1125. Epub 2018/09/25. doi: 10.3389/fphys.2018.01125. PubMed PMID: 30246803; PubMed Central PMCID: PMCPMC6140752.
443. Zybek-Kocik A, Sawicka-Gutaj N, Szczepanek-Parulska E, Andrusiewicz M, Waligórska-Stachura J, Białas P, et al. The association between irisin and muscle metabolism in different thyroid disorders. Clinical endocrinology. 2018;88(3):460-7. Epub 2017/12/03. doi: 10.1111/cen.13527. PubMed PMID: 29197093.
444. Fan X, Du J, Wang MH, Li JM, Yang B, Chen Y, et al. Irisin Contributes to the Hepatoprotection of Dexmedetomidine during Intestinal Ischemia/Reperfusion. Oxidative medicine and cellular longevity. 2019;2019:7857082. Epub 2019/06/14. doi: 10.1155/2019/7857082. PubMed PMID: 31191804; PubMed Central PMCID: PMCPMC6525857.
445. Gannon NP, Vaughan RA, Garcia-Smith R, Bisoffi M, Trujillo KA. Effects of the exercise-inducible myokine irisin on malignant and non-malignant breast epithelial cell behavior in vitro. International journal of cancer. 2015;136(4):E197-202. Epub 2014/08/16. doi: 10.1002/ijc.29142. PubMed PMID: 25124080.
446. Huang CW, Chang YH, Lee HH, Wu JY, Huang JX, Chung YH, et al. Irisin, an exercise myokine, potently suppresses tumor proliferation, invasion, and growth in glioma. FASEB

journal : official publication of the Federation of American Societies for Experimental Biology. 2020;34(7):9678-93. Epub 2020/05/30. doi: 10.1096/fj.202000573RR. PubMed PMID: 32469121.
447. Liu J, Huang Y, Liu Y, Chen Y. Irisin Enhances Doxorubicin-Induced Cell Apoptosis in Pancreatic Cancer by Inhibiting the PI3K/AKT/NF-κB Pathway. Medical science monitor : international medical journal of experimental and clinical research. 2019;25:6085-96. Epub 2019/08/15. doi: 10.12659/msm.917625. PubMed PMID: 31412018; PubMed Central PMCID: PMCPMC6705179.
448. Moon HS, Mantzoros CS. Regulation of cell proliferation and malignant potential by irisin in endometrial, colon, thyroid and esophageal cancer cell lines. Metabolism: clinical and experimental. 2014;63(2):188-93. Epub 2013/11/26. doi: 10.1016/j.metabol.2013.10.005. PubMed PMID: 24268368.
449. Shao L, Li H, Chen J, Song H, Zhang Y, Wu F, et al. Irisin suppresses the migration, proliferation, and invasion of lung cancer cells via inhibition of epithelial-to-mesenchymal transition. Biochemical and biophysical research communications. 2017;485(3):598-605. Epub 2016/12/18. doi: 10.1016/j.bbrc.2016.12.084. PubMed PMID: 27986567.
450. Zhang D, Zhang P, Li L, Tang N, Huang F, Kong X, et al. Irisin functions to inhibit malignant growth of human pancreatic cancer cells via downregulation of the PI3K/AKT signaling pathway. OncoTargets and therapy. 2019;12:7243-9. Epub 2019/10/01. doi: 10.2147/ott.s214260. PubMed PMID: 31564907; PubMed Central PMCID: PMCPMC6732507.
451. Dirat B, Bochet L, Dabek M, Daviaud D, Dauvillier S, Majed B, et al. Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. Cancer research. 2011;71(7):2455-65. Epub 2011/04/05. doi: 10.1158/0008-5472.can-10-3323. PubMed PMID: 21459803.
452. Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. Nature reviews Cancer. 2011;11(12):886-95. Epub 2011/11/25. doi: 10.1038/nrc3174. PubMed PMID: 22113164.
453. Hudis CA, Jones L. Promoting exercise after a cancer diagnosis: easier said than done. British journal of cancer. 2014;110(4):829-30. Epub 2014/02/20. doi: 10.1038/bjc.2014.12. PubMed PMID: 24548883; PubMed Central PMCID: PMCPMC3929897.
454. Hojman P, Gehl J, Christensen JF, Pedersen BK. Molecular Mechanisms Linking Exercise to Cancer Prevention and Treatment. Cell metabolism. 2018;27(1):10-21. Epub 2017/10/24. doi: 10.1016/j.cmet.2017.09.015. PubMed PMID: 29056514.
455. Kim JS, Galvão DA, Newton RU, Gray E, Taaffe DR. Exercise-induced myokines and their effect on prostate cancer. Nature reviews Urology. 2021;18(9):519-42. Epub 2021/06/24. doi: 10.1038/s41585-021-00476-y. PubMed PMID: 34158658.
456. Zhang D, Tan X, Tang N, Huang F, Chen Z, Shi G. Review of Research on the Role of Irisin in Tumors. OncoTargets and therapy. 2020;13:4423-30. Epub 2020/06/18. doi: 10.2147/ott.s245178. PubMed PMID: 32547073; PubMed Central PMCID: PMCPMC7245464.
457. Dhanapal R, Saraswathi T, Govind RN. Cancer cachexia. Journal of oral and maxillofacial pathology : JOMFP. 2011;15(3):257-60. Epub 2011/12/07. doi: 10.4103/0973-029x.86670. PubMed PMID: 22144825; PubMed Central PMCID: PMCPMC3227249.
458. Pin F, Bonewald LF, Bonetto A. Role of myokines and osteokines in cancer cachexia. Experimental biology and medicine (Maywood, NJ). 2021;246(19):2118-27. Epub 2021/04/27. doi: 10.1177/15353702211009213. PubMed PMID: 33899538; PubMed Central PMCID: PMCPMC8524772.
459. Vliora M, Grillo E, Corsini M, Ravelli C, Nintou E, Karligiotou E, et al. Irisin regulates thermogenesis and lipolysis in 3T3-L1 adipocytes. Biochimica et Biophysica Acta (BBA) - General Subjects. 2022;1866(4):130085. doi: https://doi.org/10.1016/j.bbagen.2022.130085.
460. Huang M, Liu T, Ma P, Mitteer RA, Jr., Zhang Z, Kim HJ, et al. c-Met-mediated endothelial plasticity drives aberrant vascularization and chemoresistance in glioblastoma.

The Journal of clinical investigation. 2016;126(5):1801-14. Epub 2016/04/05. doi: 10.1172/jci84876. PubMed PMID: 27043280; PubMed Central PMCID: PMCPMC4855929.
461. Oladejo AO. Overview of the metabolic syndrome; an emerging pandemic of public health significance. Annals of Ibadan postgraduate medicine. 2011;9(2):78-82. Epub 2011/12/01. PubMed PMID: 25161488; PubMed Central PMCID: PMCPMC4111026.
462. Ordovas JM, Corella D. Metabolic syndrome pathophysiology: the role of adipose tissue. Kidney international Supplement. 2008;(111):S10-4. Epub 2008/11/27. doi: 10.1038/ki.2008.517. PubMed PMID: 19034318; PubMed Central PMCID: PMCPMC4426988.
463. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiological reviews. 2004;84(1):277-359. Epub 2004/01/13. doi: 10.1152/physrev.00015.2003. PubMed PMID: 14715917.
464. Langin D. In and out: adipose tissue lipid turnover in obesity and dyslipidemia. Cell metabolism. 2011;14(5):569-70. Epub 2011/11/08. doi: 10.1016/j.cmet.2011.10.003. PubMed PMID: 22055498.
465. Shook B, Rivera Gonzalez G, Ebmeier S, Grisotti G, Zwick R, Horsley V. The Role of Adipocytes in Tissue Regeneration and Stem Cell Niches. Annual review of cell and developmental biology. 2016;32:609-31. Epub 2016/05/06. doi: 10.1146/annurev-cellbio-111315-125426. PubMed PMID: 27146311; PubMed Central PMCID: PMCPMC5157158.
466. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Archives of medical science : AMS. 2013;9(2):191-200. Epub 2013/05/15. doi: 10.5114/aoms.2013.33181. PubMed PMID: 23671428; PubMed Central PMCID: PMCPMC3648822.
467. Grygiel-Górniak B, Puszczewicz M. A review on irisin, a new protagonist that mediates muscle-adipose-bone-neuron connectivity. European review for medical and pharmacological sciences. 2017;21(20):4687-93. Epub 2017/11/14. PubMed PMID: 29131244.
468. Dinas PC, Krase A, Nintou E, Georgakopoulos A, Granzotto M, Metaxas M, et al. Human white-fat thermogenesis: Experimental and meta-analytic findings. Temperature (Austin, Tex). 2020;8(1):39-52. Epub 2021/02/09. doi: 10.1080/23328940.2020.1769530. PubMed PMID: 33553504; PubMed Central PMCID: PMCPMC7849687.
469. Zhang Y, Xie C, Wang H, Foss RM, Clare M, George EV, et al. Irisin exerts dual effects on browning and adipogenesis of human white adipocytes. American journal of physiology Endocrinology and metabolism. 2016;311(2):E530-41. Epub 2016/07/21. doi: 10.1152/ajpendo.00094.2016. PubMed PMID: 27436609.
470. Korta P, Pocheć E, Mazur-Biały A. Irisin as a Multifunctional Protein: Implications for Health and Certain Diseases. Medicina (Kaunas, Lithuania). 2019;55(8). Epub 2019/08/25. doi: 10.3390/medicina55080485. PubMed PMID: 31443222; PubMed Central PMCID: PMCPMC6722973.
471. Rabiee F, Lachinani L, Ghaedi S, Nasr-Esfahani MH, Megraw TL, Ghaedi K. New insights into the cellular activities of Fndc5/Irisin and its signaling pathways. Cell & bioscience. 2020;10:51. Epub 2020/04/08. doi: 10.1186/s13578-020-00413-3. PubMed PMID: 32257109; PubMed Central PMCID: PMCPMC7106581.
472. Kim H, Wrann CD, Jedrychowski M, Vidoni S, Kitase Y, Nagano K, et al. Irisin Mediates Effects on Bone and Fat via αV Integrin Receptors. Cell. 2018;175(7):1756-68.e17. Epub 2018/12/15. doi: 10.1016/j.cell.2018.10.025. PubMed PMID: 30550785; PubMed Central PMCID: PMCPMC6298040.
473. Emanuele E, Minoretti P, Pareja-Galeano H, Sanchis-Gomar F, Garatachea N, Lucia A. Serum irisin levels, precocious myocardial infarction, and healthy exceptional longevity. The American journal of medicine. 2014;127(9):888-90. Epub 2014/05/13. doi: 10.1016/j.amjmed.2014.04.025. PubMed PMID: 24813865.
474. Qiao X, Nie Y, Ma Y, Chen Y, Cheng R, Yin W, et al. Irisin promotes osteoblast proliferation and differentiation via activating the MAP kinase signaling pathways. Sci Rep.

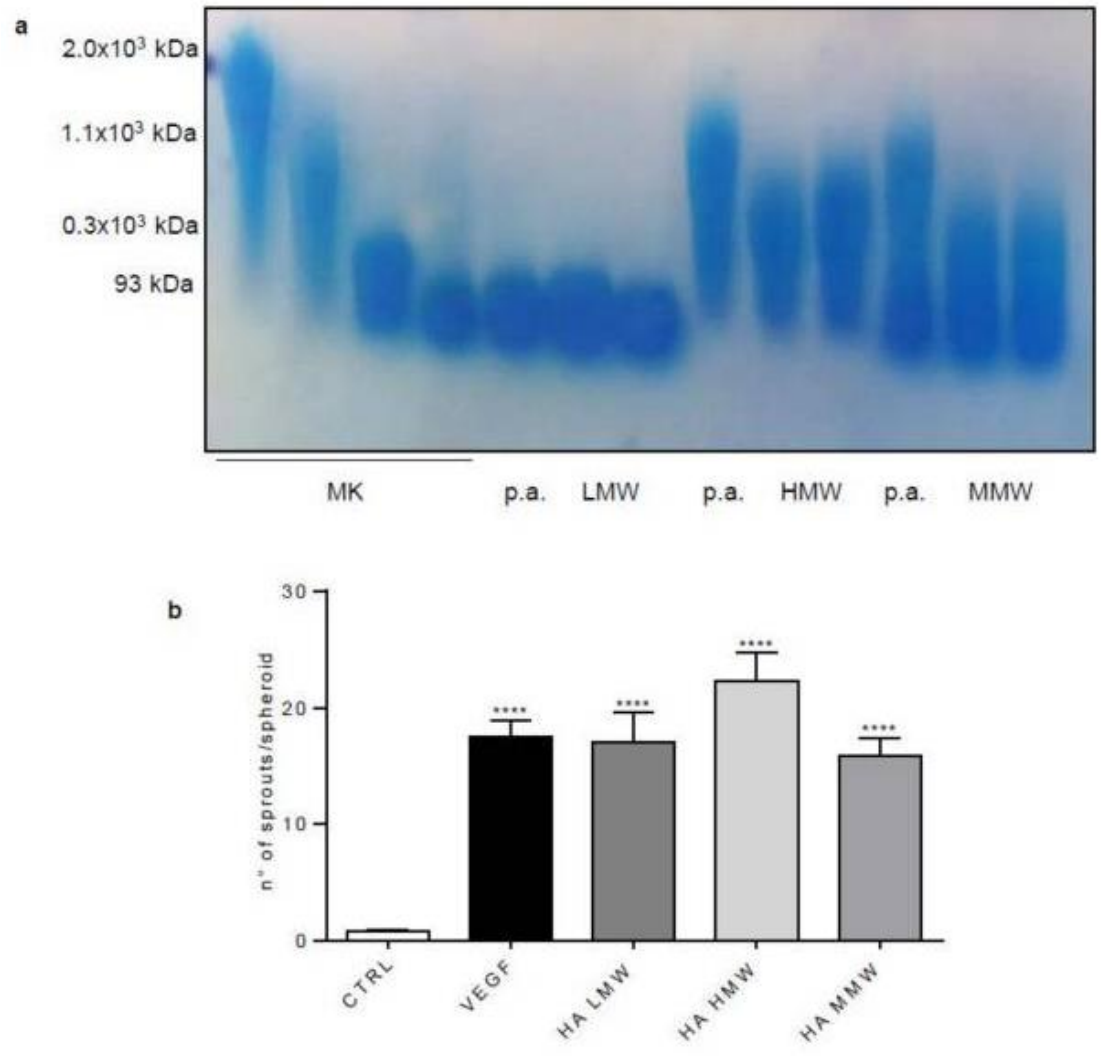
2016;6:18732. Epub 2016/01/08. doi: 10.1038/srep18732. PubMed PMID: 26738434; PubMed Central PMCID: PMCPMC4704023.
475. Raschke S, Eckel J. Adipo-myokines: two sides of the same coin--mediators of inflammation and mediators of exercise. Mediators of inflammation. 2013;2013:320724. Epub 2013/07/19. doi: 10.1155/2013/320724. PubMed PMID: 23861558; PubMed Central PMCID: PMCPMC3686148.
476. Valente A, Carrillo AE, Tzatzarakis MN, Vakonaki E, Tsatsakis AM, Kenny GP, et al. The absorption and metabolism of a single L-menthol oral versus skin administration: Effects on thermogenesis and metabolic rate. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2015;86:262-73. Epub 2015/10/03. doi: 10.1016/j.fct.2015.09.018. PubMed PMID: 26429629.
477. Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. The American journal of clinical nutrition. 2011;93(4):884s-90. Epub 2011/02/04. doi: 10.3945/ajcn.110.001917. PubMed PMID: 21289221; PubMed Central PMCID: PMCPMC3057551.
478. van Marken Lichtenbelt WD, Schrauwen P. Implications of nonshivering thermogenesis for energy balance regulation in humans. American journal of physiology Regulatory, integrative and comparative physiology. 2011;301(2):R285-96. Epub 2011/04/15. doi: 10.1152/ajpregu.00652.2010. PubMed PMID: 21490370.
479. Carrillo AE, Flouris AD. Caloric restriction and longevity: effects of reduced body temperature. Ageing research reviews. 2011;10(1):153-62. Epub 2010/10/26. doi: 10.1016/j.arr.2010.10.001. PubMed PMID: 20969980.
480. Ruiz-Ojeda FJ, Rupérez AI, Gomez-Llorente C, Gil A, Aguilera CM. Cell Models and Their Application for Studying Adipogenic Differentiation in Relation to Obesity: A Review. International journal of molecular sciences. 2016;17(7). Epub 2016/07/05. doi: 10.3390/ijms17071040. PubMed PMID: 27376273; PubMed Central PMCID: PMCPMC4964416.
481. Grillo E, Corsini M, Ravelli C, Zammataro L, Bacci M, Morandi A, et al. Expression of activated VEGFR2 by R1051Q mutation alters the energy metabolism of Sk-Mel-31 melanoma cells by increasing glutamine dependence. Cancer letters. 2021;507:80-8. Epub 2021/03/22. doi: 10.1016/j.canlet.2021.03.007. PubMed PMID: 33744390.
482. Castillo-Quan JI. From white to brown fat through the PGC-1α-dependent myokine irisin: implications for diabetes and obesity. Disease models & mechanisms. 2012;5(3):293-5. Epub 2012/05/09. doi: 10.1242/dmm.009894. PubMed PMID: 22566556; PubMed Central PMCID: PMCPMC3339822.
483. Boström PA, Fernández-Real JM, Mantzoros C. Irisin in humans: recent advances and questions for future research. Metabolism: clinical and experimental. 2014;63(2):178-80. Epub 2013/12/18. doi: 10.1016/j.metabol.2013.11.009. PubMed PMID: 24342075.
484. Shin H, Shi H, Xue B, Yu L. What activates thermogenesis when lipid droplet lipolysis is absent in brown adipocytes? Adipocyte. 2018;7(2):1-5. Epub 2018/04/06. doi: 10.1080/21623945.2018.1453769. PubMed PMID: 29620433; PubMed Central PMCID: PMCPMC6152517.
485. Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. Genes & development. 2000;14(11):1293-307. Epub 2000/06/03. PubMed PMID: 10837022.
486. Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, et al. PPARγ signaling and metabolism: the good, the bad and the future. Nature medicine. 2013;19(5):557-66. Epub 2013/05/09. doi: 10.1038/nm.3159. PubMed PMID: 23652116; PubMed Central PMCID: PMCPMC3870016.
487. Chung SW, Kang BY, Kim SH, Pak YK, Cho D, Trinchieri G, et al. Oxidized low density lipoprotein inhibits interleukin-12 production in lipopolysaccharide-activated mouse

macrophages via direct interactions between peroxisome proliferator-activated receptor-gamma and nuclear factor-kappa B. J Biol Chem. 2000;275(42):32681-7. Epub 2000/08/10. doi: 10.1074/jbc.M002577200. PubMed PMID: 10934192.
488. Mauro C, Leow SC, Anso E, Rocha S, Thotakura AK, Tornatore L, et al. NF-κB controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration. Nature cell biology. 2011;13(10):1272-9. Epub 2011/10/05. doi: 10.1038/ncb2324. PubMed PMID: 21968997; PubMed Central PMCID: PMCPMC3462316.
489. Riedlinger T, Dommerholt MB, Wijshake T, Kruit JK, Huijkman N, Dekker D, et al. NF-κB p65 serine 467 phosphorylation sensitizes mice to weight gain and TNFα-or diet-induced inflammation. Biochimica et biophysica acta Molecular cell research. 2017;1864(10):1785-98. Epub 2017/07/21. doi: 10.1016/j.bbamcr.2017.07.005. PubMed PMID: 28723419.
490. Ricquier D. Uncoupling protein 1 of brown adipocytes, the only uncoupler: a historical perspective. Front Endocrinol (Lausanne). 2011;2:85. Epub 2011/01/01. doi: 10.3389/fendo.2011.00085. PubMed PMID: 22649389; PubMed Central PMCID: PMCPMC3355862.
491. Adamson SE, Meher AK, Chiu YH, Sandilos JK, Oberholtzer NP, Walker NN, et al. Pannexin 1 is required for full activation of insulin-stimulated glucose uptake in adipocytes. Molecular metabolism. 2015;4(9):610-8. Epub 2015/09/29. doi: 10.1016/j.molmet.2015.06.009. PubMed PMID: 26413467; PubMed Central PMCID: PMCPMC4563021.
492. 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 (Basel, Switzerland). 2021;11(11). Epub 2021/11/28. doi: 10.3390/life11111227. PubMed PMID: 34833103; PubMed Central PMCID: PMCPMC8625343.
493. Altomare DA, Khaled AR. Homeostasis and the importance for a balance between AKT/mTOR activity and intracellular signaling. Current medicinal chemistry. 2012;19(22):3748-62. Epub 2012/06/12. doi: 10.2174/092986712801661130. PubMed PMID: 22680924; PubMed Central PMCID: PMCPMC3414727.
494. Nonogaki K. New insights into sympathetic regulation of glucose and fat metabolism. Diabetologia. 2000;43(5):533-49. Epub 2000/06/16. doi: 10.1007/s001250051341. PubMed PMID: 10855527.
495. Song NJ, Chang SH, Kim S, Panic V, Jang BH, Yun UJ, et al. PI3Ka-Akt1-mediated Prdm4 induction in adipose tissue increases energy expenditure, inhibits weight gain, and improves insulin resistance in diet-induced obese mice. Cell death & disease. 2018;9(9):876. Epub 2018/08/31. doi: 10.1038/s41419-018-0904-3. PubMed PMID: 30158592; PubMed Central PMCID: PMCPMC6115456.
496. Peltier J, O'Neill A, Schaffer DV. PI3K/Akt and CREB regulate adult neural hippocampal progenitor proliferation and differentiation. Developmental neurobiology. 2007;67(10):1348-61. Epub 2007/07/20. doi: 10.1002/dneu.20506. PubMed PMID: 17638387.
497. Cao W, Daniel KW, Robidoux J, Puigserver P, Medvedev AV, Bai X, et al. p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. Molecular and cellular biology. 2004;24(7):3057-67. Epub 2004/03/17. doi: 10.1128/mcb.24.7.3057-3067.2004. PubMed PMID: 15024092; PubMed Central PMCID: PMCPMC371122.
498. Valladares A, Roncero C, Benito M, Porras A. TNF-alpha inhibits UCP-1 expression in brown adipocytes via ERKs. Opposite effect of p38MAPK. FEBS letters. 2001;493(1):6-11. Epub 2001/03/30. doi: 10.1016/s0014-5793(01)02264-5. PubMed PMID: 11277995.
499. Li JJ, Xie D. Cleavage of focal adhesion kinase (FAK) is essential in adipocyte differentiation. Biochemical and biophysical research communications. 2007;357(3):648-54. Epub 2007/04/20. doi: 10.1016/j.bbrc.2007.03.184. PubMed PMID: 17442274.

500. Luk CT, Shi SY, Cai EP, Sivasubramaniyam T, Krishnamurthy M, Brunt JJ, et al. FAK signalling controls insulin sensitivity through regulation of adipocyte survival. Nature communications. 2017;8:14360. Epub 2017/02/07. doi: 10.1038/ncomms14360. PubMed PMID: 28165007; PubMed Central PMCID: PMCPMC5303880.
501. Sakellariou P, Valente A, Carrillo AE, Metsios GS, Nadolnik L, Jamurtas AZ, et al. Chronic l-menthol-induced browning of white adipose tissue hypothesis: A putative therapeutic regime for combating obesity and improving metabolic health. Medical Hypotheses. 2016;93:21-6. doi: https://doi.org/10.1016/j.mehy.2016.05.006.
502. Kida R, Noguchi T, Murakami M, Hashimoto O, Kawada T, Matsui T, et al. Supra-pharmacological concentration of capsaicin stimulates brown adipogenesis through induction of endoplasmic reticulum stress. Scientific Reports. 2018;8(1):845. doi: 10.1038/s41598-018-19223-2.
503. Carpentier AC, Blondin DP, Virtanen KA, Richard D, Haman F, Turcotte É E. Brown Adipose Tissue Energy Metabolism in Humans. Front Endocrinol (Lausanne). 2018;9:447. Epub 2018/08/23. doi: 10.3389/fendo.2018.00447. PubMed PMID: 30131768; PubMed Central PMCID: PMCPMC6090055.
504. Bielczyk-Maczynska E. White Adipocyte Plasticity in Physiology and Disease. Cells. 2019;8(12). Epub 2019/11/30. doi: 10.3390/cells8121507. PubMed PMID: 31775295; PubMed Central PMCID: PMCPMC6953026.
505. Ma RC, Kong AP, Chan N, Tong PC, Chan JC. Drug-induced endocrine and metabolic disorders. Drug safety. 2007;30(3):215-45. Epub 2007/03/09. doi: 10.2165/00002018-200730030-00005. PubMed PMID: 17343430.
506. Lippman SM, Hawk ET. Cancer prevention: from 1727 to milestones of the past 100 years. Cancer research. 2009;69(13):5269-84. Epub 2009/06/06. doi: 10.1158/0008-5472.can-09-1750. PubMed PMID: 19491253.
507. Krstic MN, Mijac DD, Popovic DD, Pavlovic Markovic A, Milosavljević T. General Aspects of Primary Cancer Prevention. Digestive diseases (Basel, Switzerland). 2019;37(5):406-15. Epub 2019/02/21. doi: 10.1159/000497191. PubMed PMID: 30783074.
508. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, et al. Risk Factors and Preventions of Breast Cancer. International journal of biological sciences. 2017;13(11):1387-97. Epub 2017/12/07. doi: 10.7150/ijbs.21635. PubMed PMID: 29209143; PubMed Central PMCID: PMCPMC5715522.
509. Jedrychowski Mark P, Wrann Christiane D, Paulo Joao A, Gerber Kaitlyn K, Szpyt J, Robinson Matthew M, et al. Detection and Quantitation of Circulating Human Irisin by Tandem Mass Spectrometry. Cell metabolism. 2015;22(4):734-40. doi: https://doi.org/10.1016/j.cmet.2015.08.001.
510. Health NIo. Biological Sciences Curriculum Study. NIH Curriculum Supplement Series . Bethesda (MD): National Institutes of Health (US); 2007. Understanding Cancer. . 2007.
511. Heni M, Kullmann S, Preissl H, Fritsche A, Häring H-U. Impaired insulin action in the human brain: causes and metabolic consequences. Nature Reviews Endocrinology. 2015;11(12):701-11. doi: 10.1038/nrendo.2015.173.
512. Ki NK, Lee HK, Cho JH, Kim SC, Kim NS. Factors affecting metabolic syndrome by lifestyle. Journal of physical therapy science. 2016;28(1):38-45. Epub 2016/03/10. doi: 10.1589/jpts.28.38. PubMed PMID: 26957725; PubMed Central PMCID: PMCPMC4755971.
513. Garg T, Singh O, Arora S, Murthy R. Scaffold: a novel carrier for cell and drug delivery. Critical reviews in therapeutic drug carrier systems. 2012;29(1):1-63. Epub 2012/02/24. doi: 10.1615/critrevtherdrugcarriersyst.v29.i1.10. PubMed PMID: 22356721.
514. Salih V. 8 - Biodegradable scaffolds for tissue engineering. In: Di Silvio L, editor. Cellular Response to Biomaterials: Woodhead Publishing; 2009. p. 185-211.
515. Kim SH, Kim JW, Hwang IG, Jang JS, Hong S, Kim TY, et al. Serum biomarkers for predicting overall survival and early mortality in older patients with metastatic solid tumors.

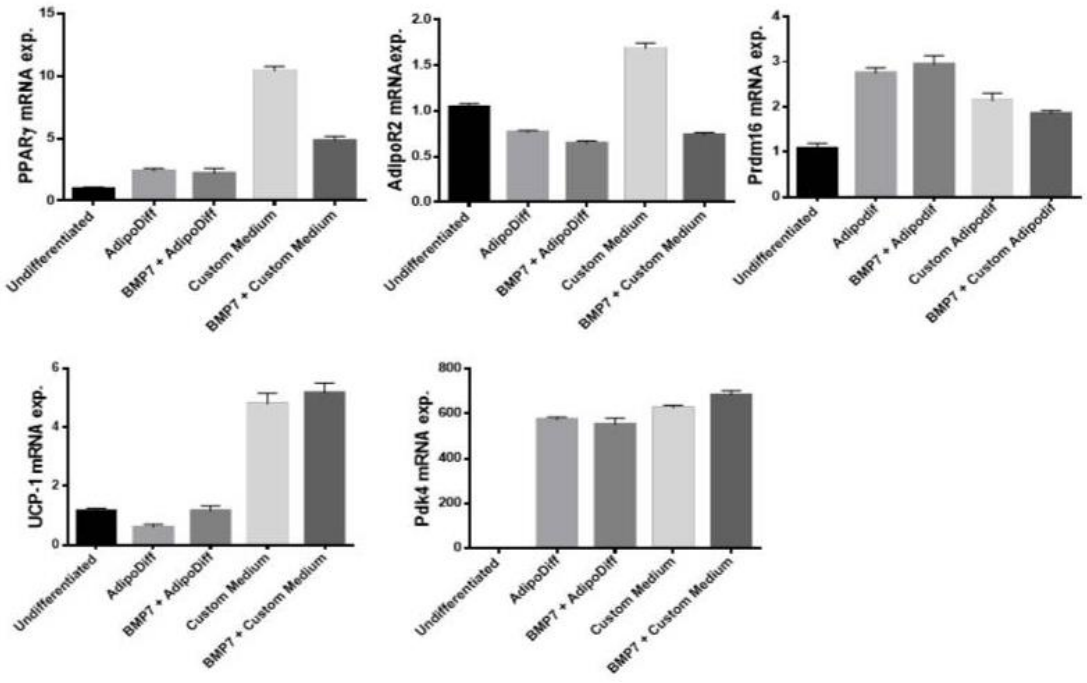
Journal of geriatric oncology. 2019;10(5):749-56. Epub 2019/04/07. doi: 10.1016/j.jgo.2019.03.015. PubMed PMID: 30952517.
516. Fan GH, Zhu TY, Huang J. FNDC5 promotes paclitaxel sensitivity of non-small cell lung cancers via inhibiting MDR1. Cellular signalling. 2020;72:109665. Epub 2020/05/01. doi: 10.1016/j.cellsig.2020.109665. PubMed PMID: 32353410.
517. Suat Tekin YE, Suleyman Sandal , Bayram Yilmaz. Is Irisin an Anticarcinogenic Peptide? Medicine Science. Dec,2015;4(2):2172-80.

Supplement (Chapter 4)



(a)Commercial low, high molecular weight HA was electrophoretically analyzed on agarose gel and stained with Stains-All solution. p.a. means pre-autoclaved. Three different standard molecules (2 × 103, 1.1 × 103, 300 kDa) were used as markers of molecular weight (MK). (b) HUVEC spheroids embedded in collagen gel were stimulated with commercial HA. The formation of radially growing sprouts was evaluated after 24 h of incubation. Data are the mean ± SEM of

three independent experiments (** $p < 0.001$, one-way ANOVA followed by Bonferroni's test versus the control).



Confluent ADSCs were differentiated in different conditions, i.e., DMEM 10% FCS, AdipoDiff (Milteny Biotech), DMEM complemented with 10% FCS and 3.3 nM of BMP7, DMEM 10% complemented with 20 nM insulin (Sigma), 5 μM dexamethasone (Sigma), 125 μM indomethacin (Sigma), 1 nM triiodothyronine (T3), and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) in the absence or in the presence of 3.3 nM of hBMP7 for 15 days. Expression of adipose markers was analyzed by RT-PCR. ADSC derived beige cells were analyzed on Day 15 of differentiation. PPARγ, AdipoR, Prdm16, UCP-1, and Pdk4 mRNA expression levels were measured by RT-qPCR analysis. Data are the mean ± SEM (n = 5) and are expressed as relative expression ratios (ΔΔCt – fold increase).

Supplement 1 (Chapter 5)

Implication of irisin in different types of cancer: A Systematic Review and Meta-analysis.

1. Materials and Methods

1.1. Searching algorithm used in PubMed

The following algorithm was applied to PubMed, EMBASE, and Cochrane library modified accordingly for each database:

Search ((((irisin[Title/Abstract]) OR fndc5/irisin[Title/Abstract]) OR fibronectin type III domain containing 5/irisin[Title/Abstract])) AND ((((((((((cancer[Title/Abstract]) OR tumor[Title/Abstract]) OR tumour[Title/Abstract]) OR tumor growth[Title/Abstract]) OR tumour growth[Title/Abstract]) OR tumor progression[Title/Abstract]) OR tumour progression[Title/Abstract]) OR cancer cachexia[Title/Abstract]) OR metastasis[Title/Abstract]) OR malignant growth[Title/Abstract]) OR malignancy[Title/Abstract]) Sort by: Best Match

1.2. Participants in *in vivo* studies

Table S1 Number of participants that were assessed in the systematic review

Author and year	Healthy	Diseased	Total
Altay et al 2016 [439]	12	48	60
Altay et al 2018 [425]	25	23	48
Aslan et al 2020 [426]	30	50	80
Castro G et al, 2020 [429]	-	94	94
Cheng et al., 2020 [427]	30	30	60
Coletta et al., 2021 [428]	33	-	33
Gaggini et al 2017 [412]	18	18	36
Esawy et al 2020 [430]	75	75	150
Kim H. et al., 2019[515]	-	138	138
Provatopoulou X et al [435]	51	101	152
Pazgan-Simon et al [434]	20	69	89
Panagiotou et al 2016 [433]	80	16	96
Sadim M.et al., 2017 [436]	-	393	393
Shi G. et al. [417]	20	20	40
Zhu et al. 2018 [442]	40	42	82
Zhang J et al. 2019 [440]	102	117	219
Zhang Z. et. al, 2018 [441]	-	148	148
Zybek-Kocik et al.[443]	12	23	55
Shahidi et al.[437]	29	22	51
Ugur et al 2019 [438]	20	140	160
Nowinska et al [432]	240	729	969
Total	**837**	**2296**	**3133**

2. Results

2.1. Risk of Bias Assessment

Table S2. Office for Human and Animal Studies (OHAT) tool. Risk of bias Assessment.

	Selection Bias			Confounding bias	Performance bias		Attrition/ Exclusion bias	Detection Bias		Selective reporting bias	Other sources of bias	TOTAL SCORE
	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Did selection of study participants result in appropriate comparison groups?	Did the study design or analysis account for important confounding and modifying variables?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	
CASE-CONTROL STUDIES	**(min = 0 – max = 21)**											
Altay D. et. al, 2018	+			++			++	++	+	++	–	17
Aslan R. et al, 2020	++			++			++	++	+	++	++	20
Aydin S. et. al, 2016	+			++			++	–	–	++	–	14
Castro et al 2020	++			++			++	+	+	++	– –	16
Cheng et al 2020	+			++			++	++	+	++	NR	17
Esawy et al 2020	++			++			++	++	++	++	++	21
Gaggini M et. al, 2017	++			++			++	–	++	++	– –	16
Nowinska K et al 2019	++			++			++	++	++	++	++	21
Pazgan-Simon et al 2020	+			++			++	–	– –	++	– –	12

Provatopoulou X. et. al, 2015			+	++			++	++	++	++	++		20
Shahidi S et al 2020			++	++			++	++	–	++	–		17
Shi G. et. al, 2017			++	++			++	++	+	++	NR		18
Ugur et al, 2009			+	++			++	++	+	++	NR		17
Zhu H et. al, 2018			+	++			++	++	–	++	++		18
Zhang J. et al, 2019			++	++			++	++	++	++	+		20
Zybeck-Kocik et al, 2018			+	++			++	++	–	++	–		16
Kuloglu et al_2019			++	++			++	++	++	++	++		21
Kuloglu et. al, 2016			++	++			++	++	++	++	++		21
CROSS SECTIONAL STUDIES	**(min = 0 – max = 24)**												
Sadim_et_al-2017			++	++		++	++	++	+	++	++		23
EXPERIMENTAL ANIMAL	**(min = 0 – max = 27)**												
Altay D. et al, 2016	++	++			++	NR	++	++	++	++	++		25
HRCT	**(min = 0 – max = 27)**												
Coletta et al 2021	++	+		++		NR	++	++	++	++	++		24
panagiotou et al 2016	++	+		++		NR	++	++	NR	++	++		23
COHORT STUDIES	**(min = 0 – max = 21)**												
Zhang Z. et. al, 2018	++			++			++	++	+	++	–		18
Kim et al, 2019	++			++			++	NR	++	++	+		18
panagiotou et al 2016	++			++			++	NR	++	++	++		19

Table S3. Office for Human and Animal Studies (OHAT) modified tool for *in vitro* studies. Risk of bias Assessment.

	Selection Bias			Confounding bias	Performance bias	Attrition/ Exclusion bias	Detection Bias		Selective reporting bias	Other sources of bias	TOTAL SCORE
	Were appropriate negative and positive controls applied in the study design?	Were the chosen cell lines appropriate and from reliable source?	Is there sufficient number of biological/ technical replicates?	Did the study design or analysis account for important confounding and modifying variables?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure/treatment characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	
***IN VITRO* STUDIES**	**(min = 0 – max = 30)**										
Fan G. et al, 2020	++	++	+	++	++	++	++	++	++	++	29
Gannon et al, 2015	++	++	++	++	++	++	++	++	++	++	30
Huang C.W. et al, 2020	++	++	++	++	++	++	++	++	++	++	30
Kong G. et. Al, 2017	++	++	+	++	++	++	++	++	++	++	29
Liu J et al 2019	+	++	++	++	++	++	++	++	++	++	29
Liu J. et. al, 2018	+	++	+	++	++	++	++	++	++	++	28
Moon et. al, 2014	++	++	++	++	++	++	++	++	++	++	30
Nowinska K. et al, 2019	++	++	++	++	++	++	++	++	++	++	30
Shi G. et. al, 2017	++	–	++	++	++	++	++	++	++	++	28
Tekin S. et al, 2015	++	–	+	++	++	++	++	+	++	++	26
Zhang D et al., 2019	++	++	++	++	++	++	++	++	++	++	30
Shao L. et. al, 2017	++	++	+	++	++	++	++	++	++	++	29

2.2. Data extraction

The data that were extracted from the eligible publications have been divided in three tables: in vivo studies that have assessed irisin protein levels in blood samples, in vivo studies that assessed irisin protein levels on tissues, and in vitro studies.

Table S4. Data extraction table. *In vivo* studies that assessed serum irisin levels in blood samples.

Author and year	Type of cancer	Assay/ Antibody	General Conclusion
Altay et al 2016 [439]	gastric cancer	ELISA kit/ Sunred Biological Technology Co., Shanghai	Factors released from gastric tumor tissue activate a number of signaling pathways, stimulating FNDC5 and its coactivator PGC1-a in white and brown adipose tissue, and thus an increase in irisin in the circulation. No indication of how irisin is increased in the circulation though.
Altay et al 2018 [425]	renal cancer	ELISA kit/ Sunred Biological Technology Co., Shanghai	Increased irisin serum protein expression in renal cancer group. Increased irisin in the circulation leads to weight loss of mice with experimentally induced gastric cancer.
Aslan et al 2020 [426]	prostate cancer	ELISA kit/YL biont biotech Co. Shanghai	Decreased serum protein concentration of irisin in prostate cancer patients. Irisin can potentially be used as a biomarker for prostate cancer.
Castro G et al, 2020 [429]	Cancer associated cachexia	Myokine quantification kit/ HMYOMAG-56 K, Merck-Millipore, St. Charles, MO, USA	Different myokine content in skeletal muscle, plasma, and tumor from patients with cancer-associated cachexia may have a role in tumor evasion, inflammation, and tissue remodeling. These changes may be implicated in the decreased capacity for skeletal muscle regeneration, increased muscle breakdown and tumor aggressiveness associated with cachexia
Cheng et al., 2020 [427]	osteosarcoma (bone cancer)	ELISA kit/ Adipogen AG, Liestal, Switzerland	Decreased serum protein concentration of irisin in osteosarcoma patients.Irisin/FNDC5 and miR-214-3p might be used for the treatment of osteosarcoma patients in the future.
Coletta et al., 2021 [428]	breast cancer	Luminex® Human Myokine Magnetic Bead Panel / HMYOMAG-56 K, Millipore Corporation, Billerica MA	Reduction of resting levels of irisin after moderate-intensity continuous-training. Exercise intensity does not impact the myokines levels in overweight postmenopausal women with increased risk for breast cancer.
Gaggini et al 2017 [412]	hepatocellular carcinoma	ELISA kit/ Adipogen AG, Liestal, Switzerland	No change in irisin protein level. Increased mRNA expression in HCC patients. Irisin could be used a s a

			future therapeutic agent for treatment of carcinogenesis.
Esawy et al 2020 [430]	bladder cancer	ELISA kits/ BioVendor Laboratory Medicine, Brno, Czech Republic	Decreased serum irisin levels in cancer patients. Serum irisin can be a good biomarker for prognosis and diagnosis of bladder cancer
Kim H. et al., 2019[515]	Metastatic solid tumors	Luminex multiplex assay / Human Myokine magnetic bead panel kit, Merck Millipore	Irisin does not predict overall survival for patients with metastatic solid tumors.
Provatopoulou X et al [435]	breast cancer	ELISA kit/ AdipoGen International, Liestal, SW	Decreased serum levels of irisin breast cancer patients. Irisin is implicated in breast cancer development and can potentially serve as biomarker for the presence of the disease.
Pazgan-Simon et al [434]	hepatocellular carcinoma	Elisa Kit / BioVendor-Laboratorni Medicina	Decreased serum levels of irisin in HCC patients. Lower irisin levels favor faster fibrosis progression and facilitate cancer progression. No clear effect of irisin on carcinogenesis
Panagiotou et al 2016 [433]	thyroid cancer	ELISA kit/ Phoenix Pharmaceuticals, Burlingame, CA	Decreased serum irisin levels in patients. subclinical or interventional changes of thyroid state do not affect the levels of irisin in humans
Sadim M.et al., 2017 [436]	breast cancer	Radio-immunoassay kit /Phoenix Pharmaceuticals, Burlingame,Calif)	Irisin/ FNDC-5 SNP, rs726344 was significantly associated with weight change at 18 months in univariate analysis.
Shi G. et al. [417]	hepatocellular carcinoma	ELISA kit/ USCN Life Science, Wuhan, China	Serum irisin level unaltered in healthy and diseased participants. Irisin mRNA expression increased in patients, may have protective roles in liver cancer cells through activation of the PI3K/AKT pathway, which may induce liver cancer progression and decreased sensitivity to chemotherapy.
Zhu et al. 2018 [442]	colorectal cancer	ELISA kit/ USCN Life Science, Wuhan, China	Individuals with high ATF3 and low irisin levels were more likely to have CRC. Irisin may represent diagnostic biomarkers for CRC patients together with ATF3.
Zhang J et al. 2019 [440]	hepatocellular carcinoma	ELISA kit/ USCN Life Science, Wuhan, China	Decreased Irisin serum level in patients. Irisin may be a novel serum biomarker in the diagnosis of HCC and a predictor of complications after hepatectomy.
Zhang Z. et. al, 2018 [441]	breast cancer to spinal metastasis	ELISA kit/ Aviscera Biosciences, Santa Clara, CA	The serum irisin was higher in patients without spinal metastasis. Higher serum irisin can be a protective factor of spinal metastasis in patients with breast cancer.
Zybek-Kocik et al.[443]	thyroid cancer	ELISA kit/ Phoenix Pharmaceuticals, Burlingame, CA	Irisin concentration changes are associated with prolonged hypothyroidism and might primarily be the result of prolonged myopathy.
Shahidi et al.[437]	gastric cancer	ELISA kit/ Bioassay Technology Laboratory, China	Decreased irisin serum level could be a prognostic factor for gastric cancers

Table S5. Data extraction table. *In vivo* studies that assessed irisin protein levels on various tissues.

Author and year	Type of cancer	Antibody	General Conclusion
Aydin et al 2016 [424]	Hepatocellular carcinoma, brain cancer, colon cancer, stomach cancer, esophageal cancer, pancreatic cancer	anti-irisin, Phoenix Pharmaceuticals, Inc., Burlingame, CA	Increased irisin immunoreactivity on tissues of gastrointestinal system cancers but not on liver cancer. Additional investigation required for the use of irisin treatment of cancer by induction of hyperthermia.
Kuloglu et al 2016 [414]	Breast cancer, ovarian cancer	Not reported	Increased immunoreactivity of irisin in breast and ovarian cancers. Accordingly, irisin may regulate the thermal activity inside breast and ovarian tumors and then prohibit the proliferation of cancer cells.
Kuloglu et al 2019 [413]	Renal cancers	anti-irisin, Phoenix Pharmaceuticals, Inc., Burlingame, CA	Decreased irisin expression was detected in chromophobe RCC, while strong immunostaining was detected in oncocytomas. Irisin immunoreactivity may be a useful test for differentiating benign lesions from renal cancer.
Ugur et al 2019 [438]	Thyroid cancer	anti-irisin, Phoenix Pharmaceuticals, Inc., Burlingame, CA	Irisin immunoreactivity can be used as a biomarker for differentiating oncocytic variants of thyroid carcinomas from other types of thyroid cancer. Irisin is involved in carcinogenesis in the thyroid gland.
Nowinska et al [432]	Lung cancer	anti-irisin/FNDC5; Novus Biologicals, Littleton, CO, USA	Irisin was expressed in NSCLC tumors. Significant difference in the levels of irisin expression in cancer cells of SCC compared to the AC subtype. Expression of irisin in stromal fibroblasts may be associated with an increased proliferation of cancer cells and may also be an independent prognostic factor for survival in patients with NSCLC.

Table S6. Data extraction table. *In vitro* studies.

Author and year	Type of cancer/ cell lines	Type of irisin	General conclusions
Fan G. et al. 2020 [516]	Lung cancer/ A549, H358, H1299, H1650 HBE and BEAS-2	Human recombinant irisin, non-modified, Sigma-Aldrich (St. Louis, MO, USA).	Lower irisin expression in cancer cell lines. Irisin inhibits cell proliferation in cancer cell lines. Irisin a promising therapeutic agent for lung cancer.
Gannon_et_al-2015 [445]	Breast cancer/MCF-10a, MCF-7, MDA-MB-231	Human recombinant non-modified irisin (INM), Cayman Chemical (Ann Arbor, MI)	Irisin may allow for reduced doses of common antineoplastic agents (Increased tumor sensitivity) thereby improving patient tolerance and prognosis. Irisin may be a future therapeutic agent.
Huang C.W. et al. 2020 [446]	Glioblastoma/ U-87 MG, T98G, LN-18 and 3T3-L1 cell line	radioactive 68Ga-DOTA-irisin (no further info)	Consistent irisin administration can effectively inhibit cell proliferation, reduce protease secretion, impede cell invasion and restrict tumor growth. Irisin may be useful as a prognostic biomarker.
Kong G. et. al 2017 [415]	Osteosarcoma/ U2OS and MG-63	Irisin (modified) Phoenix Pharmaceuticals (Burlingame, CA, USA)	Irisin suppressed the migration and invasion of osteosarcoma Cells reversed the EMT induced by IL-6 in osteosarcoma cells. It has an inhibitory role in IL-6-induced EMT modulated via the STAT3/Snail pathway. Irisin is a promising agent in osteosarcoma treatment.
Liu J et al 2019 [447]	Pancreatic cancer/ MIA PaCa-2, BxPC-3, and H9c2	Non modified irisin by Sangon Biotech (Shanghai, China)	Irisin increases the chemosensitivity of PC cells to DOX or GEM and enhance DOX-induced apoptosis in PC cancer cells through upregulation of PARP and caspase-3 and downregulation Bcl-2, BCL-xL, and PI3K/AKT/NF-kB signaling pathway. irisin could be used as an adjunctive agent combined with chemotherapy for the treating PC cells.
Liu J. et. al, 2018 [416]	Pancreatic cancer/ MIA PaCa-2 and Panc03.27	Anti-Irisin (Human, Rat, Mouse, Canine specific) antibody, Phoenix Pharmaceuticals (CA, USA).	Irisin inhibits migration and invasion of PC cells via inhibition of epithelial-to-mesenchymal transition pathway. Irisin activates the AMPK-mTOR signaling pathway, which may play a critical role in irisin-induced inhibition of pancreatic cancer cell growth. Irisin may be employed as a therapeutic candidate for the

			treatment of pancreatic cancer in clinical practices.
Moon et. al, 2014 [448]	Endometrial, colon, thyroid and esophageal cancer/ KLE and RL95-2, HT29, MCA38, SW579, BHP7, OE13 and OE33	Human recombinant irisin made by Aviscera Bioscience (Santa Clara, CA) by Phoenix Pharmaceuticals (Burlingame, CA).	Irisin, in physiological and high physiological/ pharmacological concentrations, has no in vitro effect on cell proliferation and malignant potential of obesity-related cancer cell lines.
Nowinska K et al 2019 [432]	Lung cancer/ NCI-H1703 (SCC) and NCI-H522 (AC)	polyclonal rabbit anti-irisin/FNDC5, (Novus Biologicals, Littleton, CO, USA)	Irisin expression in stromal fibroblasts may influence cancer cell proliferation and may be a prognostic factor for survival in Lung cancer.
Shi G. et. al, 2017 [417]	Hepatocellular carcinoma/ SMMC7721 cells	human recombinant modified (glycosylated) irisin (IM) from PlexBio (San Francisco, CA, USA) and human recombinant nonmodified irisin (INM; Cayman Chemical, Ann Arbor, MI, USA)	Increased irisin levels may have protective roles in liver cancer cells through partial activation of the PI3K/AKT pathway, which may facilitate liver cancer progression and decrease the sensitivity to chemotherapy.
Tekin S. et al, 2015 [517]	Prostate cancer/ LNCaP, DU-145, PC3	Irisin (modified) Phoenix Pharmaceuticals (Burlingame, CA, USA)	Our study results exerted that treatment with the physiological and pharmacological concentrations of irisin decreased cell viability in androgen receptor positive and negative prostate cancer cell lines in a dose dependent manner. Cytotoxic effects of irisin emerge via an androgen receptor independent mechanism.
Zhang D et al., 2019 [450]	Pancreatic cancer/ PANC-1 and BxPC-3	Irisin human recombinant (modified), (Cayman Chemicals, MI, USA)	Irisin could inhibit cell proliferation and induce the apoptosis of pancreatic cancer cells. In addition, irisin was able to downregulate the activation of the PI3K/AKT signaling pathway in pancreatic cancer cells. Irisin could be used as a therapeutic agent for pancreatic cancer.
Shao L. et. al, 2017 [449]	Lung cancer/ A549 cells and NCIH446 cells	Not reported	Irisin inhibits EMT and reduces the invasion of lung cancer cells via mediating the PI3K/AKT/Snail signaling pathway. The migration and invasive ability of lung cancer cells may be controlled by irisin, by inhibiting the PI3K/AKT/Snail signaling pathway and EMT.

2.4. Metanalyses results

Figure S1. Standardized mean differences between serum irisin level in healthy and diseased participants

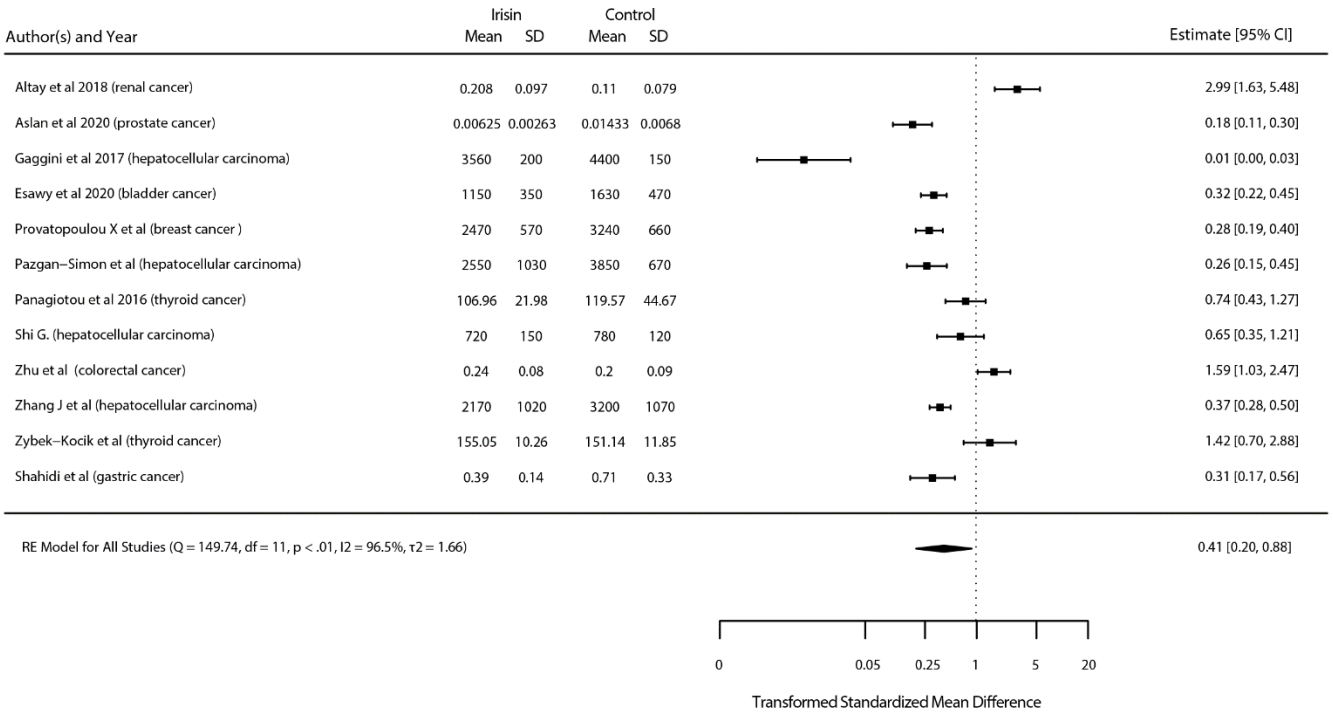


Figure S2. Standardized mean differences between irisin protein level on healthy and diseased tissues.

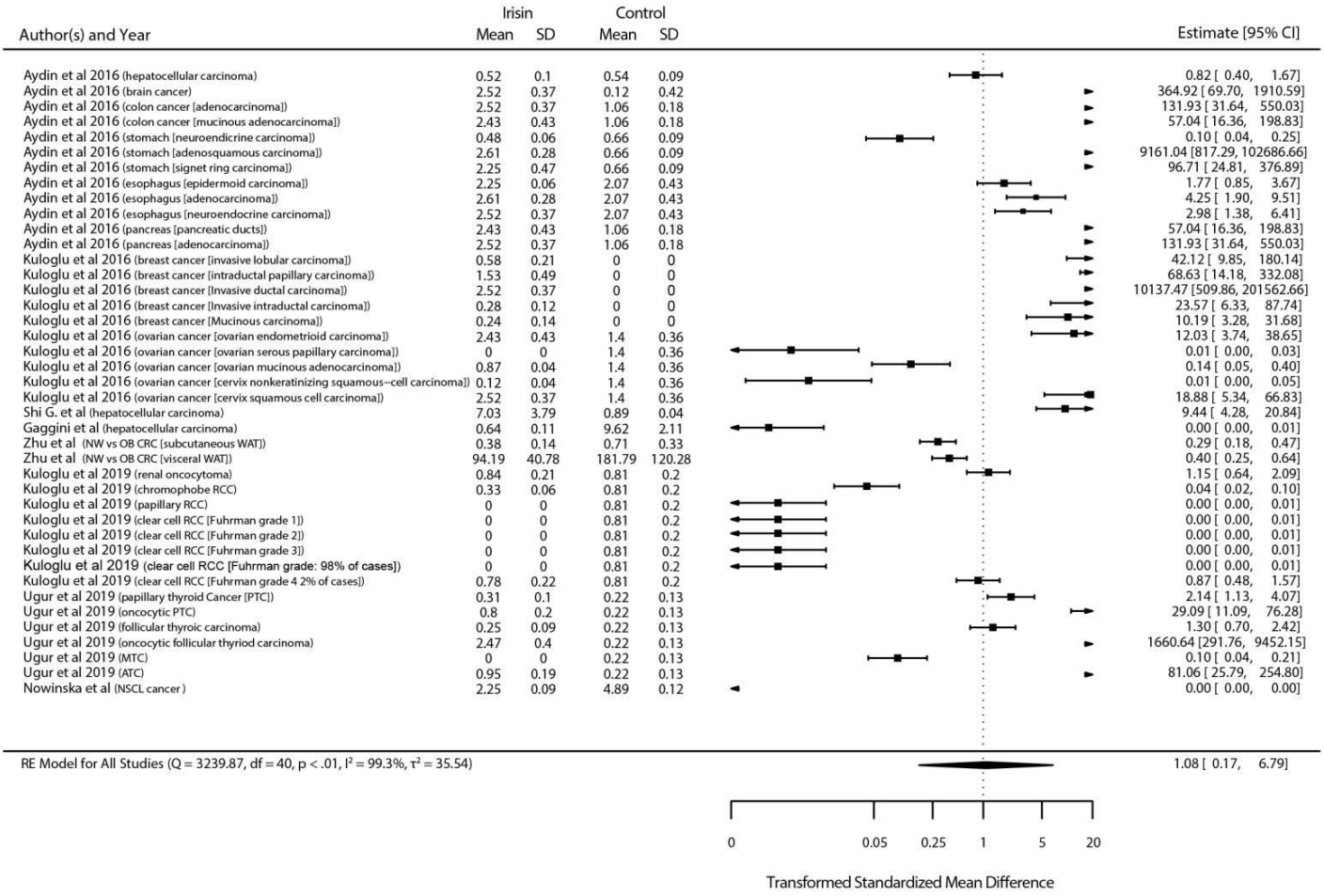


Figure S3. Standardized mean differences between treated and untreated cells with low dose of irisin after 12, 24 and 48 hours

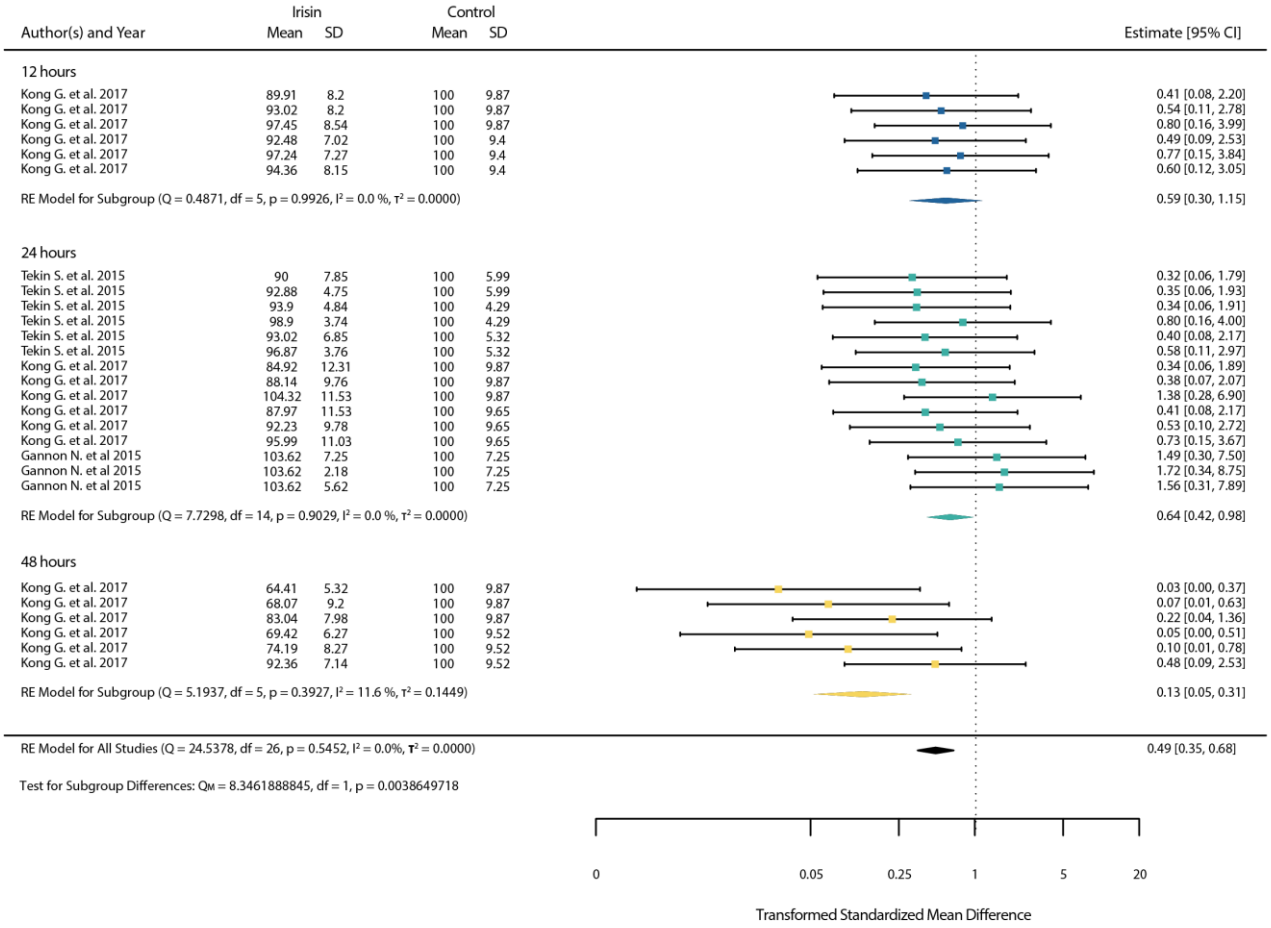


Figure S4. Standardized mean differences between treated and untreated cells with physiological dose of irisin after 12, 24,48 and 72 hours

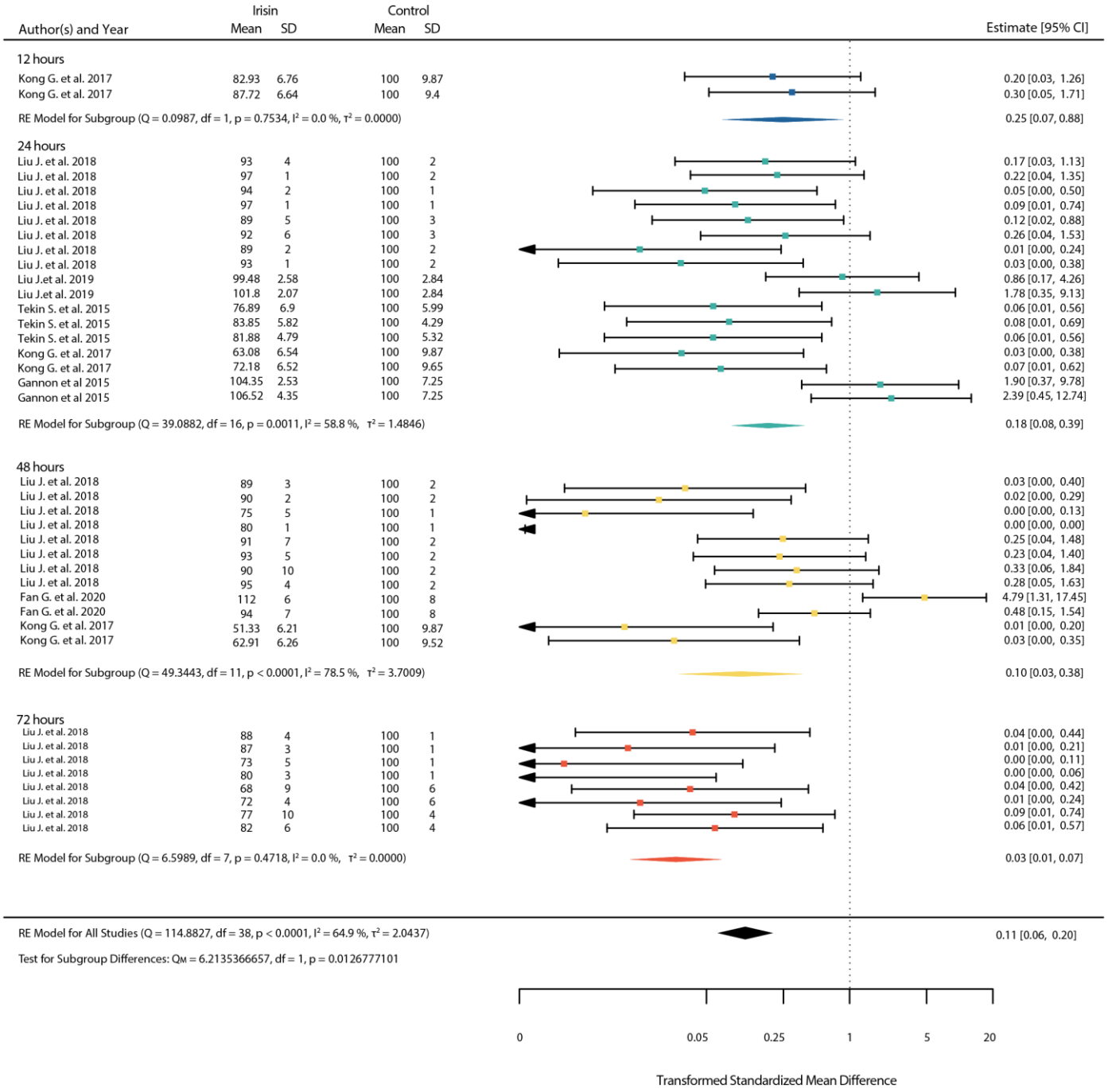
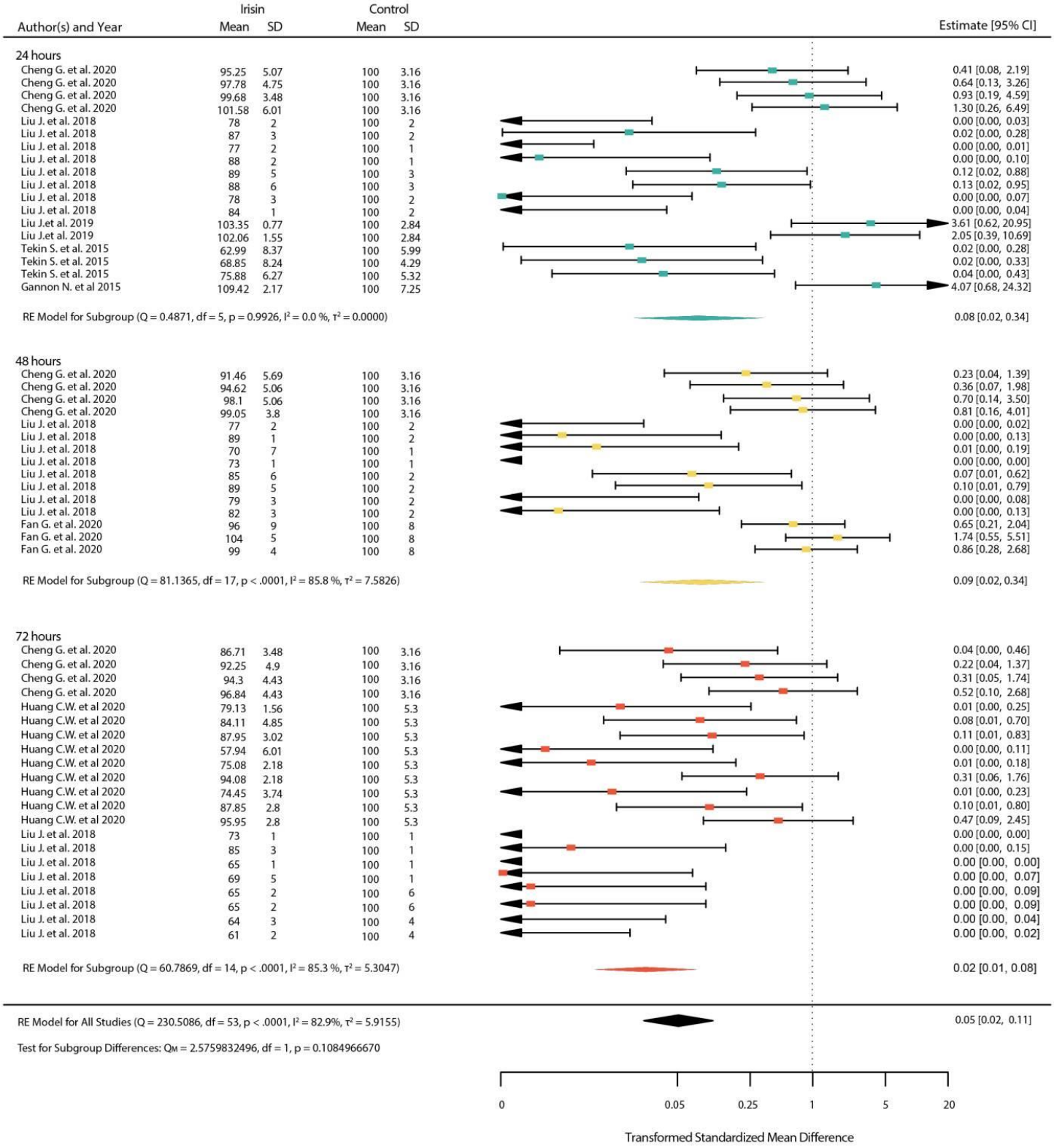


Figure S5. Standardized mean differences between treated and untreated cells with pharmacological dose of irisin after 24, 48 and 72 hours



Supplement 2 (Chapter 5)

Implication of irisin in different types of cancer: A Systematic Review and Meta-analysis.

Find below the list of the excluded papers for this systematic review

1. ORAL PRESENTATION. Respirology. 2016;21(S3):20-79. doi: https://doi.org/10.1111/resp.12939_14.

2. ESICON 2016 Abstracts. Indian J Endocrinol Metab. 2017;21(Suppl 1):S1-S90. PubMed PMID: PMC5381276.

3. Abd El-Mottaleb NA, Galal HM, El Maghraby KM, Gadallah AI. Serum irisin level in myocardial infarction patients with or without heart failure. Canadian journal of physiology and pharmacology. 2019;97(10):932-8. Epub 2019/04/09. doi: 10.1139/cjpp-2018-0736. PubMed PMID: 30958967.

4. Al-Hamad D, Raman V. Metabolic syndrome in children and adolescents. Transl Pediatr. 2017;6(4):397-407. doi: 10.21037/tp.2017.10.02. PubMed PMID: 29184820.

5. Anaszewicz M, Wawrzeńczyk A, Czerniak B, Banaś W, Socha E, Lis K, et al. Leptin, adiponectin, tumor necrosis factor α, and irisin concentrations as factors linking obesity with the risk of atrial fibrillation among inpatients with cardiovascular diseases. Kardiologia polska. 2019;77(11):1055-61. Epub 2019/09/26. doi: 10.33963/kp.14989. PubMed PMID: 31553329.

6. Asghari E, Rashidlamir A, Hosseini SRA, Moazzami M, Samarghandian S, Farkhondeh T. Synergism Effects of Ursolic Acid Supplementation on the Levels of Irisin, C-reactive Protein, IL-6, and TNF-α During High-intensity Resistance Training in Low Activity Men. Cardiovascular & hematological disorders drug targets. 2020;20(2):138-44. Epub 2019/09/20. doi: 10.2174/1871529x19666190918144727. PubMed PMID: 31533605.

7. Askari H, Rajani SF, Poorebrahim M, Haghi-Aminjan H, Raeis-Abdollahi E, Abdollahi M. A glance at the therapeutic potential of irisin against diseases involving inflammation, oxidative stress, and apoptosis: An introductory review. Pharmacological

research. 2018;129:44-55. Epub 2018/02/08. doi: 10.1016/j.phrs.2018.01.012. PubMed PMID: 29414191.

8. Aydin S. Is irisin a decisive protein in cancer cachexia and death of cancer cells? European review for medical and pharmacological sciences. 2016;20(18):3727-9. Epub 2016/10/14. PubMed PMID: 27735050.

9. Aydin S, Eren MN, Kuloglu T, Aydin S, Yilmaz M, Gul E, et al. Alteration of serum and cardiac tissue adropin, copeptin, irisin and TRPM2 expressions in DOX treated male rats. Biotechnic & histochemistry : official publication of the Biological Stain Commission. 2015;90(3):197-205. Epub 2014/12/02. doi: 10.3109/10520295.2014.977949. PubMed PMID: 25434395.

10. Bagheri R, Rashidlamir A, Ashtary-Larky D, Wong A, Alipour M, Motevalli MS, et al. Does green tea extract enhance the anti-inflammatory effects of exercise on fat loss? British journal of clinical pharmacology. 2020;86(4):753-62. Epub 2019/11/21. doi: 10.1111/bcp.14176. PubMed PMID: 31747468; PubMed Central PMCID: PMCPMC7098875.

11. Bagheri R, Rashidlamir A, Ashtary-Larky D, Wong A, Grubbs B, Motevalli MS, et al. Effects of green tea extract supplementation and endurance training on irisin, pro-inflammatory cytokines, and adiponectin concentrations in overweight middle-aged men. European journal of applied physiology. 2020;120(4):915-23. Epub 2020/02/26. doi: 10.1007/s00421-020-04332-6. PubMed PMID: 32095935.

12. Blüher S, Panagiotou G, Petroff D, Markert J, Wagner A, Klemm T, et al. Effects of a 1-year exercise and lifestyle intervention on irisin, adipokines, and inflammatory markers in obese children. Obesity (Silver Spring, Md). 2014;22(7):1701-8. Epub 2014/03/20. doi: 10.1002/oby.20739. PubMed PMID: 24644099.

13. Bonfante ILP, Chacon-Mikahil MPT, Brunelli DT, Gáspari AF, Duft RG, Oliveira AG, et al. Obese with higher FNDC5/Irisin levels have a better metabolic profile, lower lipopolysaccharide levels and type 2 diabetes risk. Archives of endocrinology and metabolism. 2017;61(6):524-33. Epub 2018/02/08. doi: 10.1590/2359-3997000000305. PubMed PMID: 29412381.

14. Bulut T, Yalcin A, Celik B, Genc GE, Kose S, Gocmen A, et al. Omalizumab treatment decreased IL-1β and irisin but increased chemerin without any impact on NK cells and APC cells in cases of severe asthma2016.

15. Buscemi S, Corleo D, Vasto S, Buscemi C, Barile AM, Rosafio G, et al. Serum Irisin Concentrations in Severely Inflamed Patients. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2020;52(4):246-50. Epub 2020/02/23. doi: 10.1055/a-1111-9249. PubMed PMID: 32079027.

16. Cheng CF, Ku HC, Lin H. PGC-1α as a Pivotal Factor in Lipid and Metabolic Regulation. International journal of molecular sciences. 2018;19(11). Epub 2018/11/08. doi: 10.3390/ijms19113447. PubMed PMID: 30400212; PubMed Central PMCID: PMCPMC6274980.

17. Christodoulatos GS, Spyrou N, Kadillari J, Psallida S, Dalamaga M. The Role of Adipokines in Breast Cancer: Current Evidence and Perspectives. Current obesity reports. 2019;8(4):413-33. Epub 2019/10/23. doi: 10.1007/s13679-019-00364-y. PubMed PMID: 31637624.

18. Clark IA, Vissel B. Neurodegenerative disease treatments by direct TNF reduction, SB623 cells, maraviroc and irisin and MCC950, from an inflammatory perspective - a Commentary. Expert review of neurotherapeutics. 2019;19(6):535-43. Epub 2019/05/17. doi: 10.1080/14737175.2019.1618710. PubMed PMID: 31092047.

19. Comassi M, Vitolo E, Pratali L, Del Turco S, Dellanoce C, Rossi C, et al. Acute effects of different degrees of ultra-endurance exercise on systemic inflammatory responses. Internal medicine journal. 2015;45(1):74-9. Epub 2014/11/06. doi: 10.1111/imj.12625. PubMed PMID: 25371101.

20. Dalamaga M. Interplay of adipokines and myokines in cancer pathophysiology: Emerging therapeutic implications. World J Exp Med. 2013;3(3):26-33. doi: 10.5493/wjem.v3.i3.26. PubMed PMID: 24520543.

21. Daneshi-Maskooni M, Keshavarz SA, Mansouri S, Qorbani M, Alavian SM, Badri-Fariman M, et al. The effects of green cardamom on blood glucose indices, lipids, inflammatory factors, paraxonase-1, sirtuin-1, and irisin in patients with nonalcoholic fatty liver disease and obesity: study protocol for a randomized controlled trial. Trials. 2017;18(1):260. Epub 2017/06/09. doi: 10.1186/s13063-017-1979-3. PubMed PMID: 28592311; PubMed Central PMCID: PMCPMC5463368.

22. Danielak A, Wojcik D, Mazur-Bialy A, Surmiak M, Bilski J, Targosz A, et al. Intestinal Alkaline Phosphatase Combined with Voluntary Physical Activity Alleviates Experimental Colitis in Obese Mice. Involvement of Oxidative Stress, Myokines, Adipokines and Proinflammatory Biomarkers. Antioxidants (Basel, Switzerland).

2021;10(2). Epub 2021/02/10. doi: 10.3390/antiox10020240. PubMed PMID: 33557311; PubMed Central PMCID: PMCPMC7914798.

23. Darkwah S, Park EJ, Myint PK, Ito A, Appiah MG, Obeng G, et al. Potential Roles of Muscle-Derived Extracellular Vesicles in Remodeling Cellular Microenvironment: Proposed Implications of the Exercise-Induced Myokine, Irisin. Frontiers in cell and developmental biology. 2021;9:634853. Epub 2021/02/23. doi: 10.3389/fcell.2021.634853. PubMed PMID: 33614663; PubMed Central PMCID: PMCPMC7892973.

24. de Castro GS, Correia-Lima J, Simoes E, Orsso CE, Xiao J, Gama LR, et al. Myokines in treatment-naïve patients with cancer-associated cachexia. Clinical Nutrition. 2021;40(4):2443-55. doi: https://doi.org/10.1016/j.clnu.2020.10.050.

25. Deng J, Zhang N, Chen F, Yang C, Ning H, Xiao C, et al. Irisin ameliorates high glucose-induced cardiomyocytes injury via AMPK/mTOR signal pathway. Cell biology international. 2020;44(11):2315-25. Epub 2020/08/10. doi: 10.1002/cbin.11441. PubMed PMID: 32770767.

26. Díaz BB, González DA, Gannar F, Pérez MCR, de León AC. Myokines, physical activity, insulin resistance and autoimmune diseases. Immunology letters. 2018;203:1-5. Epub 2018/09/09. doi: 10.1016/j.imlet.2018.09.002. PubMed PMID: 30194964.

27. Dobrodeeva LK, Samodova AV, Balashova SN. Cellular reactions in capillary and venous blood in northerners to a short-term period in a climatic chamber. Immunity, inflammation and disease. 2020;8(3):408-14. Epub 2020/06/20. doi: 10.1002/iid3.322. PubMed PMID: 32558359; PubMed Central PMCID: PMCPMC7416046.

28. Du J, Fan X, Yang B, Chen Y, Liu KX, Zhou J. Irisin pretreatment ameliorates intestinal ischemia/reperfusion injury in mice through activation of the Nrf2 pathway. International immunopharmacology. 2019;73:225-35. Epub 2019/05/21. doi: 10.1016/j.intimp.2019.05.011. PubMed PMID: 31108387.

29. Dupuis C, Berthon JY, Larue J, Rougé S, Filaire M, Filaire E. Effects of 6 weeks of betaine or C-phycocyanin supplementation associated or not with wheel running on redox status. Science & Sports. 2018;33(1):47-55. doi: https://doi.org/10.1016/j.scispo.2017.08.006.

30. Esawy MM, Abdel-Samd KM. The diagnostic and prognostic roles of serum irisin in bladder cancer. Current problems in cancer. 2020;44(4):100529. Epub 2020/03/07. doi: 10.1016/j.currproblcancer.2019.100529. PubMed PMID: 32139156.

31. Eskandari M, Hooshmand Moghadam B, Bagheri R, Ashtary-Larky D, Eskandari E, Nordvall M, et al. Effects of Interval Jump Rope Exercise Combined with Dark Chocolate Supplementation on Inflammatory Adipokine, Cytokine Concentrations, and Body Composition in Obese Adolescent Boys. Nutrients. 2020;12(10). doi: 10.3390/nu12103011.

32. Fan X, Du J, Wang MH, Li JM, Yang B, Chen Y, et al. Irisin Contributes to the Hepatoprotection of Dexmedetomidine during Intestinal Ischemia/Reperfusion. Oxidative medicine and cellular longevity. 2019;2019:7857082. Epub 2019/06/14. doi: 10.1155/2019/7857082. PubMed PMID: 31191804; PubMed Central PMCID: PMCPMC6525857.

33. Frille A, Kuhn H, Ebert T, Seyfarth HJ, Wirtz H. Brown adipose tissue impairs chemosensitivity in non-small cell lung cancer cells in the context of cancer cachexia. Pneumologie. 2019;73(S 01):P452.

34. Frille A, Kuhn H, Ebert T, Seyfarth HJ, Wirtz H. Non-small cell lung cancer cells induce the expression of adipokines in brown adipose tissue in the context of cancer cachexia2019.

35. Gaddam A, Galla C, Thummisetti S, Marikanty RK, Palanisamy UD, Rao PV. Role of Fenugreek in the prevention of type 2 diabetes mellitus in prediabetes. J Diabetes Metab Disord. 2015;14:74-. doi: 10.1186/s40200-015-0208-4. PubMed PMID: 26436069.

36. Gawlinska K, Gawlinski D, Przegalinski E, Filip M. Maternal high-fat diet during pregnancy and lactation provokes depressive-like behavior and influences the irisin/brain-derived neurotrophic factor axis and inflammatory factors in male and female offspring in rats. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2019;70(3). Epub 2019/09/21. doi: 10.26402/jpp.2019.3.07. PubMed PMID: 31539886.

37. Gehrke FS, Gouveia MC, Barbosa CGM, Murad N, Reis B, Fonseca FLA, et al. Irisin and troponin I expression in dialysis patients submitted to remote ischemic preconditioning: a pilot study. Jornal brasileiro de nefrologia : 'orgao oficial de Sociedades Brasileira e Latino-Americana de Nefrologia. 2020;42(1):47-52. Epub

2019/12/05. doi: 10.1590/2175-8239-jbn-2019-0051. PubMed PMID: 31799981; PubMed Central PMCID: PMCPMC7213940.

38. Gerhard GS, Styer AM, Strodel WE, Roesch SL, Yavorek A, Carey DJ, et al. Gene expression profiling in subcutaneous, visceral and epigastric adipose tissues of patients with extreme obesity. International journal of obesity (2005). 2014;38(3):371-8. Epub 2013/08/21. doi: 10.1038/ijo.2013.152. PubMed PMID: 23949615; PubMed Central PMCID: PMCPMC3925764.

39. Gouveia MC, Vella JP, Cafeo FR, Affonso Fonseca FL, Bacci MR. Association between irisin and major chronic diseases: a review. European review for medical and pharmacological sciences. 2016;20(19):4072-7. Epub 2016/10/25. PubMed PMID: 27775791.

40. Huang CW, Chang YH, Lee HH, Wu JY, Huang JX, Chung YH, et al. Irisin, an exercise myokine, potently suppresses tumor proliferation, invasion, and growth in glioma. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2020;34(7):9678-93. Epub 2020/05/30. doi: 10.1096/fj.202000573RR. PubMed PMID: 32469121.

41. Huang SH, Yang SM, Lo JJ, Wu SH, Tai MH. Irisin Gene Delivery Ameliorates Burn-Induced Sensory and Motor Neuropathy. International journal of molecular sciences. 2020;21(20). Epub 2020/10/25. doi: 10.3390/ijms21207798. PubMed PMID: 33096842; PubMed Central PMCID: PMCPMC7589574.

42. Jiang X, Shen Z, Chen J, Wang C, Gao Z, Yu S, et al. Irisin Protects Against Motor Dysfunction of Rats with Spinal Cord Injury via Adenosine 5'-Monophosphate (AMP)-Activated Protein Kinase-Nuclear Factor Kappa-B Pathway. Frontiers in pharmacology. 2020;11:582484. Epub 2020/12/15. doi: 10.3389/fphar.2020.582484. PubMed PMID: 33312127; PubMed Central PMCID: PMCPMC7701590.

43. Jürimäe J, Purge P. Irisin and inflammatory cytokines in elite male rowers: adaptation to volume-extended training period. The Journal of sports medicine and physical fitness. 2021;61(1):102-8. Epub 2020/09/17. doi: 10.23736/s0022-4707.20.11076-4. PubMed PMID: 32936569.

44. Jürimäe J, Purge P, Tillmann V. Serum sclerostin and cytokine responses to prolonged sculling exercise in highly-trained male rowers. Journal of sports sciences. 2021;39(5):591-7. Epub 2020/11/03. doi: 10.1080/02640414.2020.1837428. PubMed PMID: 33135583.

45. Karstoft K, Pedersen BK. Skeletal muscle as a gene regulatory endocrine organ. Current opinion in clinical nutrition and metabolic care. 2016;19(4):270-5. Epub 2016/04/22. doi: 10.1097/mco.0000000000000283. PubMed PMID: 27101470.

46. Khalighfard S, Rajbi H, Gharakhanlou R, Setoudeh V, Alizadeh AM. The Effect of 8 Weeks of Interval Aerobic Exercise before and after Induction of Breast Cancer on Serum Level of Irisin and Tumor Growth in Balb/c mice. Journal of Isfahan Medical School. 2018;35:1775-84. doi: 10.22122/jims.v35i459.9266.

47. Kim MK, Kim Y, Park S, Kim E, Kim Y, Kim Y, et al. Effects of Steady Low-Intensity Exercise on High-Fat Diet Stimulated Breast Cancer Progression Via the Alteration of Macrophage Polarization. Integr Cancer Ther. 2020;19:1534735420949678-. doi: 10.1177/1534735420949678. PubMed PMID: 32909498.

48. Kim SH, Kim JW, Hwang IG, Jang JS, Hong S, Kim TY, et al. Serum frailty biomarkers to predict overall survival in older patients with metastatic solid tumors: A substudy of prospective multi-center cohort study (KCSG PC13-09). Journal of Clinical Oncology. 2017;35(15_suppl):10041-. doi: 10.1200/JCO.2017.35.15_suppl.10041.

49. Korta P, Pocheć E, Mazur-Biały A. Irisin as a Multifunctional Protein: Implications for Health and Certain Diseases. Medicina (Kaunas, Lithuania). 2019;55(8). Epub 2019/08/25. doi: 10.3390/medicina55080485. PubMed PMID: 31443222; PubMed Central PMCID: PMCPMC6722973.

50. Kristóf E, Klusóczki Á, Veress R, Shaw A, Combi ZS, Varga K, et al. Interleukin-6 released from differentiating human beige adipocytes improves browning. Experimental cell research. 2019;377(1-2):47-55. Epub 2019/02/23. doi: 10.1016/j.yexcr.2019.02.015. PubMed PMID: 30794803.

51. Li DJ, Li YH, Yuan HB, Qu LF, Wang P. The novel exercise-induced hormone irisin protects against neuronal injury via activation of the Akt and ERK1/2 signaling pathways and contributes to the neuroprotection of physical exercise in cerebral ischemia. Metabolism: clinical and experimental. 2017;68:31-42. Epub 2017/02/12. doi: 10.1016/j.metabol.2016.12.003. PubMed PMID: 28183451.

52. Li F, Li Y, Duan Y, Hu CA, Tang Y, Yin Y. Myokines and adipokines: Involvement in the crosstalk between skeletal muscle and adipose tissue. Cytokine & growth factor reviews. 2017;33:73-82. Epub 2016/10/22. doi: 10.1016/j.cytogfr.2016.10.003. PubMed PMID: 27765498.

53. Li X, Jamal M, Guo P, Jin Z, Zheng F, Song X, et al. Irisin alleviates pulmonary epithelial barrier dysfunction in sepsis-induced acute lung injury via activation of AMPK/SIRT1 pathways. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2019;118:109363. Epub 2019/09/24. doi: 10.1016/j.biopha.2019.109363. PubMed PMID: 31545277.

54. Li X, Liu Y, Zheng Y, Wang P, Zhang Y. The Effect of Vitamin D Supplementation on Glycemic Control in Type 2 Diabetes Patients: A Systematic Review and Meta-Analysis. Nutrients. 2018;10(3). Epub 2018/03/23. doi: 10.3390/nu10030375. PubMed PMID: 29562681; PubMed Central PMCID: PMCPMC5872793.

55. Liu Y, Xu F, Jiang P. Effect of sitagliptin on expression of skeletal muscle peroxisome proliferator-activated receptor γ coactivator-1α and irisin in a rat model of type 2 diabetes mellitus. The Journal of international medical research. 2020;48(5):300060519885569. Epub 2020/05/05. doi: 10.1177/0300060519885569. PubMed PMID: 32364035; PubMed Central PMCID: PMCPMC7218978.

56. Lu Y, Li G. Auricular acupuncture induces FNDC5/irisin and attenuates obese inflammation in mice. Acupuncture in medicine : journal of the British Medical Acupuncture Society. 2020;38(4):264-71. Epub 2020/03/21. doi: 10.1136/acupmed-2017-011405. PubMed PMID: 32195595.

57. Maalouf GE, El Khoury D. Exercise-Induced Irisin, the Fat Browning Myokine, as a Potential Anticancer Agent. Journal of obesity. 2019;2019:6561726. Epub 2019/05/09. doi: 10.1155/2019/6561726. PubMed PMID: 31065382; PubMed Central PMCID: PMCPMC6466922.

58. Manole E, Ceafalan LC, Popescu BO, Dumitru C, Bastian AE. Myokines as Possible Therapeutic Targets in Cancer Cachexia. Journal of immunology research. 2018;2018:8260742. Epub 2018/11/15. doi: 10.1155/2018/8260742. PubMed PMID: 30426026; PubMed Central PMCID: PMCPMC6217752.

59. Matsuo Y, Gleitsmann K, Mangner N, Werner S, Fischer T, Bowen TS, et al. Fibronectin type III domain containing 5 expression in skeletal muscle in chronic heart failure-relevance of inflammatory cytokines. Journal of cachexia, sarcopenia and muscle. 2015;6(1):62-72. Epub 2015/07/03. doi: 10.1002/jcsm.12006. PubMed PMID: 26136413; PubMed Central PMCID: PMCPMC4435098.

60. Mazur-Bialy AI. Superiority of the Non-Glycosylated Form Over the Glycosylated Form of Irisin in the Attenuation of Adipocytic Meta-Inflammation: A

Potential Factor in the Fight Against Insulin Resistance. Biomolecules. 2019;9(9). Epub 2019/08/24. doi: 10.3390/biom9090394. PubMed PMID: 31438646; PubMed Central PMCID: PMCPMC6770638.

61. Mazur-Bialy AI, Bilski J, Pochec E, Brzozowski T. New insight into the direct anti-inflammatory activity of a myokine irisin against proinflammatory activation of adipocytes. Implication for exercise in obesity. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2017;68(2):243-51. Epub 2017/06/15. PubMed PMID: 28614774.

62. Mazur-Bialy AI, Bilski J, Wojcik D, Brzozowski B, Surmiak M, Hubalewska-Mazgaj M, et al. Beneficial Effect of Voluntary Exercise on Experimental Colitis in Mice Fed a High-Fat Diet: The Role of Irisin, Adiponectin and Proinflammatory Biomarkers. Nutrients. 2017;9(4). Epub 2017/04/21. doi: 10.3390/nu9040410. PubMed PMID: 28425943; PubMed Central PMCID: PMCPMC5409749.

63. Mazur-Bialy AI, Oplawski M, Wypasek E, Zarawski M. ID: 228: Irisin – A newly discovered adipomiokine – Impairs growth and progression of breast cancer MDA-MB-231 cell line. Cytokine. 2015;76(1):107. doi: https://doi.org/10.1016/j.cyto.2015.08.232.

64. Mazur-Bialy AI, Pocheć E, Zarawski M. Anti-Inflammatory Properties of Irisin, Mediator of Physical Activity, Are Connected with TLR4/MyD88 Signaling Pathway Activation. International journal of molecular sciences. 2017;18(4). Epub 2017/03/28. doi: 10.3390/ijms18040701. PubMed PMID: 28346354; PubMed Central PMCID: PMCPMC5412287.

65. Metzger CE, Anand Narayanan S, Phan PH, Bloomfield SA. Hindlimb unloading causes regional loading-dependent changes in osteocyte inflammatory cytokines that are modulated by exogenous irisin treatment. NPJ microgravity. 2020;6:28. Epub 2020/10/22. doi: 10.1038/s41526-020-00118-4. PubMed PMID: 33083525; PubMed Central PMCID: PMCPMC7542171.

66. Panagiotou G, Mu L, Na B, Mukamal KJ, Mantzoros CS. Circulating irisin, omentin-1, and lipoprotein subparticles in adults at higher cardiovascular risk. Metabolism: clinical and experimental. 2014;63(10):1265-71. Epub 2014/07/26. doi: 10.1016/j.metabol.2014.06.001. PubMed PMID: 25060690; PubMed Central PMCID: PMCPMC4175146.

67. Park EJ, Myint PK, Ito A, Appiah MG, Darkwah S, Kawamoto E, et al. Integrin-Ligand Interactions in Inflammation, Cancer, and Metabolic Disease: Insights Into the

Multifaceted Roles of an Emerging Ligand Irisin. Frontiers in cell and developmental biology. 2020;8:588066. Epub 2020/11/17. doi: 10.3389/fcell.2020.588066. PubMed PMID: 33195249; PubMed Central PMCID: PMCPMC7649757.

68. Park KS, Ganesh AB, Berry NT, Mobley YP, Karper WB, Labban JD, et al. The effect of physical activity on cognition relative to APOE genotype (PAAD-2): study protocol for a phase II randomized control trial. BMC neurology. 2020;20(1):231. Epub 2020/06/07. doi: 10.1186/s12883-020-01732-1. PubMed PMID: 32503473; PubMed Central PMCID: PMCPMC7274941.

69. Pazgan-Simon M. Serum irisin level a new possible marker of HCC progression. Conference abstrac.

70. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nature reviews Endocrinology. 2012;8(8):457-65. Epub 2012/04/05. doi: 10.1038/nrendo.2012.49. PubMed PMID: 22473333.

71. Rahmanabadi A, Mahboob S, Amirkhizi F, Hosseinpour-Arjmand S, Ebrahimi-Mameghani M. Oral α-lipoic acid supplementation in patients with non-alcoholic fatty liver disease: effects on adipokines and liver histology features. Food & function. 2019;10(8):4941-52. Epub 2019/07/26. doi: 10.1039/c9fo00449a. PubMed PMID: 31343010.

72. Reinehr T, Roth CL. Inflammation Markers in Type 2 Diabetes and the Metabolic Syndrome in the Pediatric Population. Current diabetes reports. 2018;18(12):131. Epub 2018/10/20. doi: 10.1007/s11892-018-1110-5. PubMed PMID: 30338401.

73. Ren YF, Wang MZ, Bi JB, Zhang J, Zhang L, Liu WM, et al. Irisin attenuates intestinal injury, oxidative and endoplasmic reticulum stress in mice with L-arginine-induced acute pancreatitis. World journal of gastroenterology. 2019;25(45):6653-67. Epub 2019/12/14. doi: 10.3748/wjg.v25.i45.6653. PubMed PMID: 31832004; PubMed Central PMCID: PMCPMC6906211.

74. Sanderson M, McKinlay BJ, Theocharidis A, Kouvelioti R, Falk B, Klentrou P. Changes in Inflammatory Cytokines and Irisin in Response to High Intensity Swimming in Adolescent versus Adult Male Swimmers. Sports (Basel, Switzerland). 2020;8(12). Epub 2020/12/05. doi: 10.3390/sports8120157. PubMed PMID: 33271764; PubMed Central PMCID: PMCPMC7760547.

75. Seo DY, Lee SR, Heo JW, No MH, Rhee BD, Ko KS, et al. Ursolic acid in health and disease. The Korean journal of physiology & pharmacology : official journal of the Korean Physiological Society and the Korean Society of Pharmacology. 2018;22(3):235-48. Epub 2018/05/03. doi: 10.4196/kjpp.2018.22.3.235. PubMed PMID: 29719446; PubMed Central PMCID: PMCPMC5928337.

76. Shimba Y, Togawa H, Senoo N, Ikeda M, Miyoshi N, Morita A, et al. Skeletal Muscle-specific PGC-1α Overexpression Suppresses Atherosclerosis in Apolipoprotein E-Knockout Mice. Scientific Reports. 2019;9(1):4077. doi: 10.1038/s41598-019-40643-1.

77. Śliwicka E, Cisoń T, Pilaczyńska-Szcześniak Ł, Ziemba A, Straburzyńska-Lupa A. Effects of marathon race on selected myokines and sclerostin in middle-aged male amateur runners. Sci Rep. 2021;11(1):2813. Epub 2021/02/04. doi: 10.1038/s41598-021-82288-z. PubMed PMID: 33531538; PubMed Central PMCID: PMCPMC7854637.

78. Spiegelman BM. Banting Lecture 2012: Regulation of adipogenesis: toward new therapeutics for metabolic disease. Diabetes. 2013;62(6):1774-82. Epub 2013/05/25. doi: 10.2337/db12-1665. PubMed PMID: 23704518; PubMed Central PMCID: PMCPMC3661621.

79. Sumsuzzman DM, Jin Y, Choi J, Yu JH, Lee TH, Hong Y. Pathophysiological role of endogenous irisin against tumorigenesis and metastasis: Is it a potential biomarker and therapeutic? Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2019;41(12):1010428319892790. Epub 2019/12/10. doi: 10.1177/1010428319892790. PubMed PMID: 31815594.

80. Tekin S, Erden Y, Etem E, Tektemur A, Kirbag S, Sandal S. Effect of intracerebroventricular irisin injection on the uncoupling protein expression in the rat brain. Acta Physiol. 2014;211.

81. Thomas RJ, Kenfield SA, Jimenez A. Exercise-induced biochemical changes and their potential influence on cancer: a scientific review. Br J Sports Med. 2017;51(8):640-4. Epub 12/19. doi: 10.1136/bjsports-2016-096343. PubMed PMID: 27993842.

82. Tsiani E, Tsakiridis N, Kouvelioti R, Jaglanian A, Klentrou P. Current Evidence of the Role of the Myokine Irisin in Cancer. Cancers (Basel). 2021;13(11):2628. doi: 10.3390/cancers13112628. PubMed PMID: 34071869.

83. Vaughan RA, Gannon NP, Mermier CM, Conn CA. Irisin, a unique non-inflammatory myokine in stimulating skeletal muscle metabolism. Journal of physiology and biochemistry. 2015;71(4):679-89. Epub 2015/09/25. doi: 10.1007/s13105-015-0433-9. PubMed PMID: 26399516.

84. Viitasalo A, Atalay M, Pihlajamäki J, Jääskeläinen J, Korkmaz A, Kaminska D, et al. The 148 M allele of the PNPLA3 is associated with plasma irisin levels in a population sample of Caucasian children: The PANIC Study. Metabolism: clinical and experimental. 2015;64(7):793-6. Epub 2015/02/14. doi: 10.1016/j.metabol.2015.01.010. PubMed PMID: 25676803.

85. Wuensch T, Wizenty J, Quint J, Spitz W, Bosma M, Becker O, et al. Expression Analysis of Fibronectin Type III Domain-Containing (FNDC) Genes in Inflammatory Bowel Disease and Colorectal Cancer. Gastroenterology Research and Practice. 2019;2019:3784172. doi: 10.1155/2019/3784172.

86. Xiao S, Zhou Y, Yi W, Luo G, Jiang B, Tian Q, et al. Fra-1 is downregulated in cervical cancer tissues and promotes cervical cancer cell apoptosis by p53 signaling pathway in vitro. Int J Oncol. 2015;46(4):1677-84. doi: 10.3892/ijo.2015.2873.

87. Xie T, Leung PS. Roles of FGF21 and irisin in obesity-related diabetes and pancreatic diseases. Journal of Pancreatology. 2020;3(1).

88. Xu L, Shen L, Yu X, Li P, Wang Q, Li C. Effects of irisin on osteoblast apoptosis and osteoporosis in postmenopausal osteoporosis rats through upregulating Nrf2 and inhibiting NLRP3 inflammasome. Experimental and therapeutic medicine. 2020;19(2):1084-90. Epub 2020/02/06. doi: 10.3892/etm.2019.8313. PubMed PMID: 32010273; PubMed Central PMCID: PMCPMC6966163.

89. Yamakage H, Tanaka M, Inoue T, Odori S, Kusakabe T, Satoh-Asahara N. Effects of dapagliflozin on the serum levels of fibroblast growth factor 21 and myokines and muscle mass in Japanese patients with type 2 diabetes: A randomized, controlled trial. Journal of diabetes investigation. 2020;11(3):653-61. Epub 2019/11/14. doi: 10.1111/jdi.13179. PubMed PMID: 31721467; PubMed Central PMCID: PMCPMC7232283.

90. Yang BC, Leung PS. Irisin Is a Positive Regulator for Ferroptosis in Pancreatic Cancer. Molecular therapy oncolytics. 2020;18:457-66. Epub 2020/09/22. doi: 10.1016/j.omto.2020.08.002. PubMed PMID: 32953980; PubMed Central PMCID: PMCPMC7475648.

91. Zhang D, Tan X, Tang N, Huang F, Chen Z, Shi G. Review of Research on the Role of Irisin in Tumors. OncoTargets and therapy. 2020;13:4423-30. Epub 2020/06/18. doi: 10.2147/ott.s245178. PubMed PMID: 32547073; PubMed Central PMCID: PMCPMC7245464.

92. Zsuga J, Tajti G, Papp C, Juhasz B, Gesztelyi R. FNDC5/irisin, a molecular target for boosting reward-related learning and motivation. Medical hypotheses. 2016;90:23-8. Epub 2016/04/12. doi: 10.1016/j.mehy.2016.02.020. PubMed PMID: 27063080.

93. Serum frailty biomarkers to predict overall survival in older patients with metastatic solid tumors: A substudy of prospective multi-center cohort study (KCSG PC13-09)., Journal of Clinical Oncology. Conference: 2017 Annual Meeting of the American Society of Clinical Oncology, ASCO. United States. 35 (15 Supplement 1) (no pagination), 2017. Date of Publication: 20 Jun 2017. Hwang I.G., Jang J.S., Hong S., Kim T.Y., Baek J.Y., Shin S.H., Sun D.S., Hong D.S., Kim H.J., Hong Y.S., Lee J.-H., Woo I.S., Kim J.H.

94. Fibronectin type III domain containing 5(FNDC5) gene expression level in colorectal cancer cell lines. European Journal of Human Genetics. Conference: 51st European Society of Human Genetics Conference. Italy. 27 (Supplement 1) (pp 1014), 2019. Date of Publication: 2019, Ghazvini Zadegan F., Seyed Frotan F., Ghaedi K., Esfahani M.

95. Physical activity and Alzheimer's disease-2: Clinical trial protocol. Journal of Prevention of Alzheimer's Disease. Conference: 12th Conference Clinical Trials Alzheimer's Disease. United States. 6 (Supplement 1) (pp S50), 2019. Date of Publication: 2019. Williams T.M., Mobley Y.P., Slutsky A.B., Park K.S., Berry N.T

96. Non-small cell lung cancer cells induce the expression of adipokines in brown adipose tissue in the context of cancer cachexia, Pneumologie. Conference: 60. Kongress der Deutschen Gesellschaft fur Pneumologie und Beatmungsmedizin e. V.. Germany. 73 (SUPPPL. 1) (no pagination), 2019. Date of Publication: March 2019., Frille A., Kuhn H., Ebert T., Seyfarth H.J., Wirtz H.

97. Brown adipose tissue impairs chemosensitivity in non-small cell lung cancer cells in the context of cancer cachexia. Pneumologie. Conference: 60. Kongress der Deutschen Gesellschaft fur Pneumologie und Beatmungsmedizin e. V.. Germany. 73 (SUPPPL. 1) (no pagination), 2019. Date of Publication: March 2019., Frille A., Kuhn H., Ebert T., Seyfarth H.J., Wirtz H.

98. Steady low intensity physical activity and healthy dietary habits are differently affect breast cancer progression. Breast. Conference: 16th St. Gallen International Breast Cancer Conference. Austria. 44 (Supplement 1) (pp S34-S35), 2019. Date of Publication: April 1 2018. Kim M.K., Ahn S.K., Kim J.-H

99. Brown adipose tissue derived media impairs chemosensitivity in non-small lung cancer cells in the context of cancer cachexia. Herbsttagung der Mitteldeutschen Gesellschaft fur Pneumologie und Thoraxchirurgie. Germany. 73 (3) (pp 189), 2019. Date of Publication: March 2019Frille A., Kuhn H., Ebert T., Seyfarth H.J., Wirtz H.

100. Serum irisin level a new possible marker of HCC progression.Clinical and Experimental Hepatology. Conference: 20th Conference of the Polish Association for the Study of the Liver. Poland. 4 (2) (pp 139), 2018. Date of Publication: 2018. Pazgan-Simon M., Kukla M., Simon K., Lekstan A., Gabrek E., Zuwala-Jagiello J.

101. Skeletal muscle-specific PGC-1a overexpression prevents atherosclerosis in apolipoprotein e-knockout mice. Atherosclerosis Supplements. Conference: 18th International Symposium on Atherosclerosis, ISA 2018. Canada. 32 (pp 17-18), 2018. Date of Publication: June 2018. Shimba Y., Togawa H., Senoo N., Ikeda M., Miyoshi N., Morita A., Miura S.

102. Irisin a candidate biomarker for distinguishing thyroid oncocytic variant carcinomas. Turkish Journal of Biochemistry. Conference: 2nd In-Vitro Diagnostic Symposium, IVD 2017. Turkey. 42 (Supplement 2) (pp 79), 2017. Date of Publication: December 2017., Aydin S., Kuloglu T., Artas G., Kocdor M.A., Sahin I., Yardim M., Ozercan I.H.

103. Irisin, an exercise-induced hormone, targets glioblastoma tumor in vivo PET/CT imaging may serve as a novel theranostic agent. European Journal of Nuclear Medicine and Molecular Imaging. Conference: 30th Annual Congress of the European Association of Nuclear Medicine, EANM 2017. Austria. 44 (2 Supplement 1) (pp S468), 2017. Date of Publication: 2017. Chiu H., Chang W., Lin Y., Chung Y., Yen T., Huang F., Huang C.

104. New method of Irisin levels identification in gastric cancer patients with cachexia. FEBS Journal. Conference: 42nd Congress of the Federation of European Biochemical Societies, FEBS 2017. Israel. 284 (Supplement 1) (pp 284), 2017. Date of Publication: September 2017., Drabik A., Mudlaff K., Silberring J., Kulig J., Sierzega M.

Supplement (Chapter 6)

Irisin regulates thermogenesis and browning in 3T3-L1 adipocytes.

Find below the supplementary figures that correspond to the main text.

Figure S1. UCP1 expression in undifferentiated, differentiated 3T3-L1 preadipocytes and treated for 2-h and 4-h with 20nM of human recombinant irisin. 50μg of protein was used to determine protein expression (A). UCP1 relative expression at the same timepoints (B). AU= arbitrary unit.

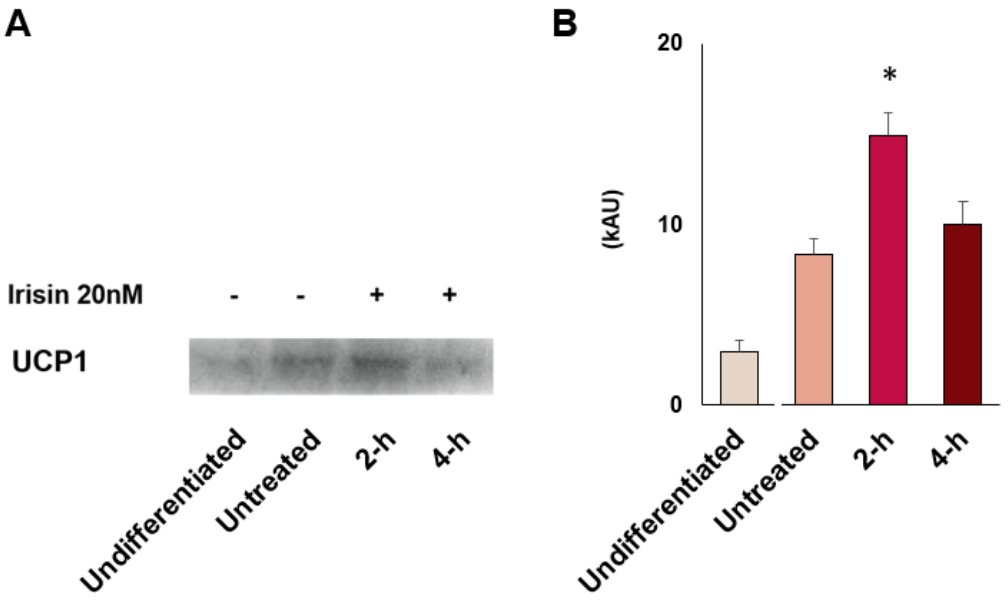
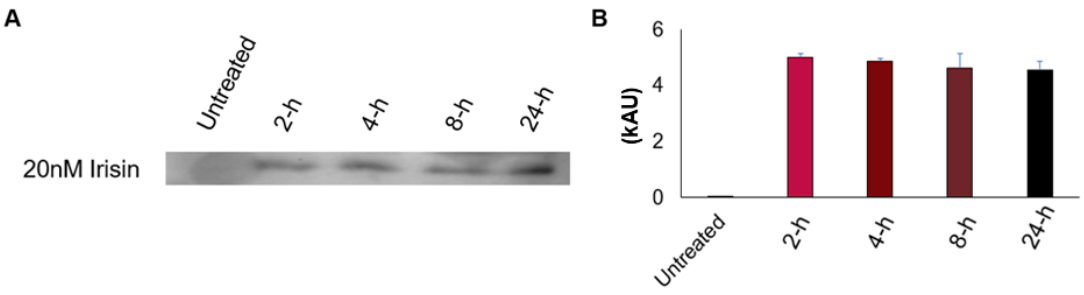


Figure S2. Irisin concentration was not affected throughout the assays. **A)** Western Blot of irisin and **B)** Quantification of irisin concentration at different time points in 24-h.



Annex 1

Student's Contribution in Paper

Natural Histogel-Based Bio-Scaffolds for Sustaining Angiogenesis in Beige Adipose Tissue

Margherita Di Somma, Wandert Schaafsma, Elisabetta Grillo, Maria Vliora, Eleni Dakou, Michela Corsini, Cosetta Ravelli, Roberto Ronca, Paraskevi Sakellariou, Jef Vanparijs, Begona Castro, and Stefania Mitola.

I hereby state that Maria Vliora had adequate contribution in the experimental work that was conducted and, in the reviewing of the manuscript, for the above paper to be completed.

Author name: MARGHERITA DI SOMMA

Signature: 

Natural Histogel-Based Bio-Scaffolds for Sustaining Angiogenesis in Beige Adipose Tissue

Margherita Di Somma, Wandert Schaafsma, Elisabetta Grillo, Maria Vliora, Eleni Dakou, Michela Corsini, Cosetta Ravelli, Roberto Ronca, Paraskevi Sakellariou, Jef Vanparijs, Begona Castro, and Stefania Mitola.

I hereby state that Maria Vliora had adequate contribution in the experimental work that was conducted and, in the reviewing of the manuscript, for the above paper to be completed.

Author name: ELISABETTA GRILLO

Signature: 

Natural Histogel-Based Bio-Scaffolds for Sustaining Angiogenesis in Beige Adipose Tissue

Margherita Di Somma, Wandert Schaafsma, Elisabetta Grillo, Maria Vliora, Eleni Dakou, Michela Corsini, Cosetta Ravelli, Roberto Ronca, Paraskevi Sakellariou, Jef Vanparijs, Begona Castro, and Stefania Mitola.

I hereby state that Maria Vliora had adequate contribution in the experimental work that was conducted and, in the reviewing of the manuscript, for the above paper to be completed.

Author name: MICHELA CORSINI

Signature:

Natural Histogel-Based Bio-Scaffolds for Sustaining Angiogenesis in Beige Adipose Tissue

Margherita Di Somma, Wandert Schaafsma, Elisabetta Grillo, Maria Vliora, Eleni Dakou, Michela Corsini, Cosetta Ravelli, Roberto Ronca, Paraskevi Sakellariou, Jef Vanparijs, Begona Castro, and Stefania Mitola.

I hereby state that Maria Vliora had adequate contribution in the experimental work that was conducted and, in the reviewing of the manuscript, for the above paper to be completed.

Author name: MITOLA STEFANIA

Signature:

Natural Histogel-Based Bio-Scaffolds for Sustaining Angiogenesis in Beige Adipose Tissue

Margherita Di Somma, Wandert Schaafsma, Elisabetta Grillo, Maria Vliora, Eleni Dakou, Michela Corsini, Cosetta Ravelli, Roberto Ronca, Paraskevi Sakellariou, Jef Vanparijs, Begona Castro, and Stefania Mitola.

I hereby state that Maria Vliora had adequate contribution in the experimental work that was conducted and, in the reviewing of the manuscript, for the above paper to be completed.

Author name: COSETTA RAVELLI

Signature:

Natural Histogel-Based Bio-Scaffolds for Sustaining Angiogenesis in Beige Adipose Tissue

Margherita Di Somma, Wandert Schaafsma, Elisabetta Grillo, Maria Vliora, Eleni Dakou, Michela Corsini, Cosetta Ravelli, Roberto Ronca, Paraskevi Sakellariou, Jef Vanparijs, Begona Castro, and Stefania Mitola.

I hereby state that Maria Vliora had adequate contribution in the experimental work that was conducted and, in the reviewing of the manuscript, for the above paper to be completed.

Author name: RONCA ROBERTO

Signature:

Natural Histogel-Based Bio-Scaffolds for Sustaining Angiogenesis in Beige Adipose Tissue

Margherita Di Somma, Wandert Schaafsma, Elisabetta Grillo, Maria Vliora, Eleni Dakou, Michela Corsini, Cosetta Ravelli, Roberto Ronca, Paraskevi Sakellariou, Jef Vanparijs, Begona Castro, and Stefania Mitola.

I hereby state that Maria Vliora had adequate contribution in the experimental work that was conducted and, in the reviewing of the manuscript, for the above paper to be completed.

Author name: Eleni Dakou

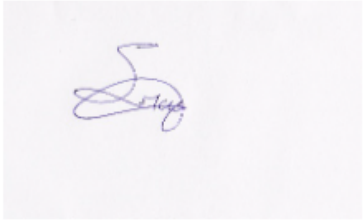
Signature: 

Natural Histogel-Based Bio-Scaffolds for Sustaining Angiogenesis in Beige Adipose Tissue

Margherita Di Somma, Wandert Schaafsma, Elisabetta Grillo, Maria Vliora, Eleni Dakou, Michela Corsini, Cosetta Ravelli, Roberto Ronca, Paraskevi Sakellariou, Jef Vanparijs, Begona Castro, and Stefania Mitola.

I hereby state that Maria Vliora had adequate contribution in the experimental work that was conducted and, in the reviewing of the manuscript, for the above paper to be completed.

Author name: Paraskevi Sakellariou

Signature: 

Annex 2

Authorization for the Use of Laboratory Animals

UNIVERSITÀ DEGLI STUDI DI BRESCIA

DIPARTIMENTO DI MEDICINA MOLECOLARE E TRASLAZIONALE
Organismo Preposto al Benessere degli Animali

Brescia, October 1th, 2014

To Whom it may concern:

The Animal Facility of the University of Brescia is authorized to use animals for experimental and other scientific purposes with Ministerial Decree no. 92/2009-A issued on 19/05/2009 and Decree No. 204/2010-A issued on 16/11/2010 by the "Ministry for Health. Department of Veterinary Public Health, Nutrition and food safety. General Directorate of Animal Health and Veterinary drug" of the Italian Republic complying with Art. 12 of Legislative Decree 116/92.
Supervisor for Animal Welfare and Care: Prof. Marco Presta
Assigned Veterinarian: Dr. Francesca Battioni
Scientific Board: Prof. Anna Maria Caroli, Prof. Bruno Mario Cesana, Prof. Marina Pizzi, Dr. William Vermi

This is to certify that the experiments described in project 645640SCAFFY, Work Package 2 concerning the use of rodents by the Unit coordinated by Dr. Stefania Mitola can be performed in the Animal House of the Brescia University after submission of an application form to the Animal Welfare Committee and the Ministry for Health. Department of Veterinary Public Health before the start of the experiments. The approval of such an application has a validity period of max 5 consecutive years.

Supervisor for Animal Welfare and Care
Prof. Marco Presta