From THE DEPARTMENT OF MEDICINE, HUDDINGE,
Karolinska Institutet, Stockholm, Sweden

IDENTIFICATION OF NOVEL FACTORS REGULATING HUMAN ADIPOCYTE FUNCTION AND THEIR LINK TO METABOLIC HEALTH

Paul Petrus

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Cover photo: Painting representing mature adipocytes surrounded by extracellular matrix and blood vessels.

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IDENTIFICATION OF NOVEL FACTORS REGULATING HUMAN ADIPOCYTE FUNCTION AND THEIR LINK TO METABOLIC HEALTH
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Paul Petrus

Principal Supervisor:
Professor Mikael Rydén
Karolinska Institutet
Department of Medicine
Unit of Endocrinology

Co-supervisor(s):
Dr. Hui Gao
Karolinska Institutet
Department of Biosciences and Nutrition

Professor Peter Arner
Karolinska Institutet
Department of Medicine
Division of Endocrinology

Opponent:
Professor Per-Anders Jansson
UNIVERSITY OF GOTHENBURG
Institute of Medicine
Department of Molecular and Clinical Medicine

Examination Board:
Professor Tore Bengtsson
Stockholm University
Department of Molecular Biosciences
Division of Wenner-Gren Institute

Professor Maria Eriksson
Karolinska Institutet
Department of Biosciences and Nutrition

Professor Sam Okret
Karolinska Institutet
Department of Biosciences and Nutrition

“Education is not the learning of facts, but the training of the mind to think”

-Albert Einstein

Till Meri och Lenox

ABSTRACT

The white adipose tissue (WAT) regulates energy homeostasis by storing and releasing energy in the form of fat as well as functioning as an endocrine organ secreting a myriad of different peptides. The heterogeneous WAT consists of various cell types including the lipid-storing cells termed adipocytes. The energy-storing capacity of WAT is challenged by the rapid worldwide changes in diet and physical activity. This thesis aimed to identify novel factors regulating adipocyte function and to assess their impact on metabolic health.

The transcription factor Early B Cell Factor 1 (EBF1) has previously been shown to regulate WAT morphology (adipocyte size and number). Low expression/activity is associated with WAT hypertrophy (few but large adipocytes), a metabolically detrimental phenotype. **Study I** aimed to determine if the expression and activity of EBF1 associated with metabolic risk markers. Results suggested that EBF1 expression and activity associated with parameters of the metabolic syndrome.

Adipocytes in WAT is continuously renewed however, the turnover is irreversibly increased when a person gains weight. In fact, fat cell number is increased during weight gain and kept constant after weight loss. **Study II** aimed to identify the factor/s contributing to the maintenance of the high adipocyte number. Prospective analyses in clinical cohorts identified a set of growth factors that were highly expressed in obese compared to never-obese and were kept high after weight loss. Among these, transforming growth factor beta 3 (TGFB3) induced immature adipocyte proliferation. The WAT was studied in a mouse model expressing half of the gene expression and the results displayed a reduced proliferative capacity of immature adipocytes, hypertrophic WAT and glucose intolerance.

The hypertrophic WAT is characterized by changes in several biological processes including inflammation. The chronic low grade inflammation in obesity is one of the processes believed to cause insulin resistance and type 2 diabetes mellitus. **Study III** focused on identifying upstream regulators of adipocyte inflammation. This led to the identification of *SLC19A1*, a gene encoding a cell membrane bound folate transporter. Folate is metabolized by the one-carbon-cycle, an important pathway for DNA-methylation. We linked the inflammatory effects of reduced *SLC19A1* expression to increased global DNA-methylation. In particular, methylation of a glucocorticoid receptor binding site in the promotor of the pro-inflammatory gene *CCL2* regulated its expression.

Altogether, this work contributes to the characterization of a dysfunctional WAT. Furthermore, the clinical relevance of the reported regulators of WAT function was evaluated. This knowledge confirms an emerging theory, that an important link between obesity and metabolic disease is limited WAT expansion. Therapies resolving WAT expansion exists and this knowledge could contribute in making these more effective. However, whether such therapies should replace interventions targeting behavior (nutrition and physical activity) warrants ethical appraisal and discussion.

LIST OF SCIENTIFIC PAPERS

I. **Petrus P**, Mejhert N, Gao H, Bäckdahl J, Arner E, Arner P, Rydén M. Low early B-cell factor 1 (EBF1) activity in human subcutaneous adipose tissue is linked to a pernicious metabolic profile. Diabetes Metab. 2015 Dec;41(6):509-12. PMID: 25791133

II. **Petrus P**, Mejhert N, Corrales P, Lecoutre S, Li Q, Maldonado E, Kulyté A, Lopez Y, Campbell M, Acosta JR, Laurencikiene J, Douagi I, Gao H, Martínez-Álvarez C, Hedén P, Spalding KL, Vidal-Puig A, Medina-Gomez G, Arner P, Rydén M. Transforming Growth Factor-β3 Regulates Adipocyte Number in Subcutaneous White Adipose Tissue. Cell Reports. 2018 Oct. PMID: 30332637

III. **Petrus P**, Bialesova L, Checa A, Kerr A, Naz S, Bäckdahl J, Gracia A, Toft S, Dahlman-Wright K, Hedén P, Dahlman I, Wheelock CE, Arner P, Mejhert N, Gao H, Rydén M. Adipocyte Expression of SLC19A1 Links DNA Hypermethylation to Adipose Tissue Inflammation and Insulin Resistance. J Clin Endocrinol Metab. 2018 Feb 1. PMID: 29121255

INNEHÅLL

LIST OF ABBREVIATIONS

BMI	Body Mass Index
CCL2	C-C Motif Chemokine Ligand 2
DNA	Deoxyribonucleic Acid
EBF1	Early B cell Factor 1
GLP-1	Glucagon-Like Peptide 1
Kcal	Kilo-calories
PPARγ	Peroxisome Proliferator-Activated Receptor Gamma
SLC19A1	Solute Carrier Family 19 Member 1
TGFB3	Transforming Growth Factor Beta 3
WAT	White Adipose Tissue

1 BACKGROUND

1.1 OBESITY

The relevance of this thesis is signified by the obesity pandemic[1]. It has never been more vital to understand the mechanisms driving obesity and its subsequent co-morbidities including type 2 diabetes, cardio vascular disease and cancer[2].

Obesity is defined as BMI>30 kg/m^2, *i.e.* a large body-weight in relation to the height. It is obvious that changes in the former variable drives the obesity pandemic. In the common population, the excess weight is constituted by fat stored in the WAT, which characterizes individuals with obesity. Why is obesity so prevalent today? Albert Einstein published the mass-energy equivalence formula 1905 which revealed the association between energy and mass. Hence, the chemical energy humans consume in form of food is stored in the WAT or converted to kinetic and thermal energy which manifest in body movement and heat, respectively. Today, it is common knowledge that body weight and fat mass can be reduced by eating less and moving more. Yet, understanding this concept has proven insufficient to reverse the increasing prevalence of obesity. In this section, I will discuss the origin of obesity, why it is a problem and how it can be prevented/treated.

1.1.1 Evolutionary aspects of obesity

A classical theory explaining the obesity pandemic has been the thrifty and/or the drifty genotype[3]. It suggests that the human body has evolved to store excess energy to survive times of famine (thrifty genotype) and/or the lack of exposure to predators (drifty genotype). Hence, human biology, which is viewed as static during the relatively short period of industrialization, is not suited to prosper in modern environments that promote an overbalance in energy[4]. Although genetics explain a fraction of the variations in BMI[5], emerging knowledge suggest that the thrifty/drifty genotype theory is an oversimplification[6]. In fact, human biology is continuously evolving and maternal as well as paternal environments influence their own- and their offspring phenotype which is evident already in the F1 generation[7]. Hence, to understand the evolutionary aspects of obesity we must first appreciate the complexity of evolution.

It is not controversial that the modern environment is a major contributor to the obesity pandemic by encouraging high caloric intake and sedentary behavior[8]. However, not everybody becomes obese in this environment. This spurs the inevitable “nature vs nurture” question; how much of the phenotype is hardwired in human nature and how much can be modified by the environment? In reality, all life forms are constantly adapting to their environments and the acquired adaptation can be transferred transgenerationally[6,7,9]. The epigenome, defined as modifications of the chromatin that are not changes in the nucleotide sequence, function as a bridge between the environment and biological function, including human nature [10–13]. For instance maternal and/or paternal diet pre-conception influence the body weight and WAT biology in the offspring[6,9]. Thus, the influence from human nature on a trait, such as obesity, cannot be separated from the environment and *vice versa* because both

are variables, and they are interwoven. Just to give an example of this interplay; an individual with a "thrifty genotype" is more likely to become obese but being obese also influence the environment of the person via exposures to stimuli such as social stress[14] and reduced healthcare quality [15] which may contribute to drive the weight gain further[16].

Finally, phenotypic heterogeneity in body weight is observed in genetically identical organisms raised in the same environment suggesting that there is a stochastic aspect to obesity[17,18]. Thus, obesity may be acquired just by chance independent of the genes or environment.

1.1.2 The link between obesity and disease

Obesity is associated with a myriad of non-communicable diseases such as type 2 diabetes and cancer [2,19–21]. Several processes have been linked to the pathophysiology of obesity including inflammation and ectopic lipid accumulation[22,23]. Both processes are associated with a hypertrophic WAT (few but large adipocytes in relation to fat mass)[24,25]. These observations in combination with observations linking WAT expandability and adipocyte size to metabolic disease[25–29] indicate that obesity causes disease by exceeding the fat storage capacity. This theory suggests that the problem is not a large fat mass *per se* but rather the inability to further expand[30]. Hence, therapies treating obesity associated metabolic disease should aim at allowing further adipose tissue expansion via mechanisms such as adipogenesis[29]. This will be discussed in more detail in section 1.2.

1.1.3 Treatment/prevention of obesity and its comorbidities

As previously discussed, obesity becomes a problem when the body is not able to handle further energy supply. There are three solutions to the problem. The first is to reduce the energy supply which could be achieved by reducing food intake[31]. Several drugs has been used for this purpose but the most effective ones are the GLP-1 analogues[32] which has several other positive antidiabetic effects as well. Another effective method to limit caloric intake is by changing the gastrointestinal anatomy via bariatric surgery[33]. A second therapeutic method is to increase energy expenditure[31]. Physical exercise and browning of adipose tissue are potential therapies that could be used to convert the chemical energy to movement and heat, respectively[24,34]. The third therapeutic target, which also is the most relevant to the work in this thesis, is to expand the storage capacity of the WAT to avoid ectopic lipid deposition and disease[19,24,29]. An effective way to do so is by activating the master regulator of adipocyte development (adipogenesis) PPARγ which has indeed been used as an antidiabetic therapy[35]. However, metabolic disease is not the only factor that reduces the quality of life in individuals with obesity. Excess body fat affects other features of life quality such as social acceptance[14–16].

The feasibility, cost effectiveness and the ethical consideration of these therapies warrants further discussion. In my opinion, the most reasonable strategy is to change the initial problem which is how modern societies are structured[8]. It is not at all necessary to live like hunter gatherers in order to reverse and prevent the increasing prevalence of obesity. In fact,

the oversupply of energy that accumulates and result in excess body fat over time could be prevented by walking as little as 2000 to 2500 extra steps per day[8] or eating about 10-25 less kcal of energy per day[36] which is equivalent to 1-2 cubes of sugar (~3-6g). Nevertheless, the WAT storage capacity needs to be enhanced if interventions aiming at behavioral changes are unsuccessful. Such strategies, in combination with elimination of the weight-stigma, could lead to a healthy obese world population.

1.2 WHITE ADIPOSE TISSUE

The WAT is the most plastic organ in the human body and a central regulator of energy homeostasis[24]. The tissue consists of several different cell-types including macrophages, T-cells, fibroblasts, endothelial cells and adipocytes. The latter is characterized by its energy storing ability. The energy is stored as triglycerides in a lipid droplet. The adipocyte fraction comprises the majority of the WAT volume. Furthermore, the WAT is an endocrine organ that secretes a number of hormones that may act in para- and/or endocrine fashion. Many of the secreted peptides are pro-inflammatory cyto- and chemokines. In metabolically healthy individuals, the WAT regulate whole body homeostasis by buffering energy and secreting hormones (adipokines) that modulate functions in other organs. However, in an metabolically unhealthy state, often observed in individuals with obesity, these functions becomes dysfunctional and the WAT is instead characterized by inefficient expansion, inefficient lipid mobilization and a low grade chronic release of pro-inflammatory adipokines[24].

1.2.1 White adipose tissue expansion

The plastic ability of the WAT is regulated by the energy balance in the body. Hyper-caloric conditions demand WAT expansion whereas hypo-caloric conditions induce the release of energy from WAT and thus, resulting in a reduction of its mass[24]. The expansion of WAT occurs via two processes, increased adipocyte size or increased adipocyte number[37]. The latter is constantly renewed with a 10% annual turnover-rate[37]. The inter-individual variation in the morphology for any given fat mass is large but on average, moving from low fat mass to higher is mainly associated with increased adipocyte size until a plateau of about 800 pL where the tissue starts to expand mainly via increased cell numbers[37]. A hyperplastic WAT (many small fat cells for a given fat mass) is associated with good metabolic health[24,26] probably because it is permissive for additional caloric over-supply. Several processes have been implicated in tissue expansion such as adipogenesis, angiogenesis, extracellular remodeling and inflammation[24,25,28,29]. In addition, proliferation of adipocyte progenitors (undifferentiated adipocytes) is also an important mechanism as the aforementioned processes are dependent of the availability of these cells. In contrast, weight loss is only associated with reduced fat cell size but no reduction in number, at least after rapid weight loss post bariatric surgery [37,38]. This phenomenon would suggest that a post obese state is metabolically beneficial in relation to a never obese state at a given fat mass. In fact, this notion has recently been confirmed[38]. The underlying factors determining WAT adipocyte cellularity are poorly understood and warrant further investigation. Stimuli during gestation and early life has been proposed to be of vital importance[6]. Other mechanisms involve

specific transcription factor activity[39] and disturbed circadian rhythms[40]. Increased understanding of the mechanisms regulating WAT expansion will allow us to develop strategies to maintain a high consumption of food combined with physical inactivity without disturbing metabolic health.

1.2.2 White adipose tissue inflammation

The intimate association between metabolism and the immune system is well established[41] and has set the foundation of a new term, namely immunometabolism [42]. It is well known that obesity is associated with an inflammatory state and that the WAT contribute to the systemic inflammation[22,43–46]. The pro-inflammatory state in WAT during obesity has been known in about two and a half decades[47] and its causal effect on insulin resistance is well studied[41,43,44,48]. Hence, it makes a lot of sense to treat metabolic disease by lowering inflammation. However, such treatments have not been successful[49] suggesting that the inflammation may have beneficial functions in maintaining metabolic homeostasis. In fact, it has been suggested that chronic insulin exposure and insulin resistance in adipocytes precedes the inflammation[46,50]. One could speculate that the insulin resistance is a result of energy oversupply and that the subsequent inflammation is activated to remodel the WAT to make room for the surplus energy. This model is supported by findings suggesting that transient activation of inflammation is essential for the WAT to expand and the lack of inflammation results in lipodystrophy[51]. Furthermore, inflammation is believed to cause insulin resistance through induced lipid mobilization from the WAT to ectopic deposition[52]. This may be true however; lipid mobilization is needed in some instances such as during physical exercise. Inhibition of inflammation during physical exercise limits visceral fat loss[53]. Taken together, inflammation has long been considered as a metabolically detrimental process, a view that is being challenged with emerging reports. Hence, the interplay between metabolism and the immune system seem to be more dynamic and context-dependent than previously thought. Understanding this complexity could help find strategies to fine-tune it and restore immunometabolic homeostasis.

1.3 FROM ENVIRONMENT TO PHENOTYPE: THE ROLE OF THE EPIGENOME AND THE TRANSCRIPTOME

As discussed in section 1.1.1 of this thesis, biology cannot be viewed separate from its environment and they are not necessarily more or less plastic than each other. Charles Darwin's theory of natural selection may have contributed to a static view of biology but today we know that individual organisms constantly adapt to their environments, a phenomenon termed phenotypic plasticity[54]. Environmental stimuli alter the structure of the chromatin itself which results in an altered gene expression[12]. The metabolism functions as a bridge between the environment and the epigenome which result in an altered gene expression[11,12]. We know of thousands of metabolites that can function as substrates or co-substrates to regulate hundreds of epigenetic modification and/or other post translational modifications in the cell[13] whereas the most well-studied is DNA-methylation. This knowledge has helped us understand that the nucleotide order of the DNA-molecule is

insignificant without an environment. Altogether, it is important to study epigenetics and gene expression in order to fully understand the mechanisms regulating WAT dysfunction.

2 AIMS

2.1 GENERAL AIM

The overarching aim was to identify novel regulators of adipocytes and link them to traits of the metabolic syndrome. This was achieved by starting with WAT biopsies from well-phenotyped clinical cohorts and study associations between gene expression and various clinical measures or WAT phenotypes. Subsequent functional analyses of these factors were studied *in vitro* in various cell types and/or *in vivo* in a mouse model.

2.2 SPECIFIC AIMS

2.2.1 Study I

The aim was to characterize the clinical relevance of EBF1, a transcription factor previously described to regulate adipogenesis and WAT morphology.

2.2.2 Study II

The primary aim was to identify regulators of the induced and irreversible adipocyte cellularity in WAT of obese individuals.

2.2.3 Study III

The aim was to study the regulation of DNA-methylation in adipocytes and its link to adipocyte function and insulin resistance in individuals suffering from obesity.

3 METHODOLOGICAL CONSIDERATIONS

3.1 HUMAN COHORTS

All studies in this thesis include human cohorts. The study-individuals are grouped depending on the research question (such as comparisons between lean and obese or obese and post-obese etc.). Characteristics of the WAT, including the transcriptome, is mapped and correlated with clinical characterization of the study-subjects.

WAT biopsies are taken in the mornings after an overnight fast. This minimizes the influence of dietary and/or circadian factors on the results. However, the timing of the last meal and the misalignment between biological and astronomical circadian rhythms may still constitute as confounders[55]. In addition, differences between groups are context dependent and may be- or not be evident in certain settings such as in the presence of insulin[56].

The study-participants are mainly females and the data may not be extrapolated to males. It warrants particular consideration when studying WAT as there are remarkable differences in its expansion and distribution between genders[24,57]. Furthermore, the fat distribution is not taken into account when subdividing the BMI groups (*i.e.* lean, overweight or obese). Inter-individual differences in body-weight are influenced by muscle mass. However, the study participants are not athletes or body builders thus; fat mass is the main variable explaining the variations in BMI. The total fat mass is less important than the body-fat distribution as a predictor of metabolic health[23,57]. The subdivision according to BMI was still used as it is common practice in the clinic.

The transcriptome of WAT was compared between groups to identify new factors that may influence its function. The gene expression level *ex vivo* may not be a perfect representation of the state *in vivo*. The procedure in which the biopsy is taken may induce a stress response locally in the tissue as well as systemically if the individual experience fear/nervousness when being exposed to the needle. Extraction of the different cell fractions of the WAT requires collagenase treatment and centrifugation which most likely alter the transcriptome. A better representation of the WAT transcriptome/proteome may be assessed when novel techniques are developed and made available.

3.2 CELL CULTURES

Cell cultures were used in study I and II. Differentially expressed transcripts identified in the clinical cohorts were studied *in vitro* in primary human and murine WAT-derived cells or mouse embryonic fibroblasts. These systems allow us to study the cells in an isolated setting and manipulate gene expression or the micro-environment in the conditioned media. However, adipocytes crosstalk with other cell types to facilitate tissue function *in vivo* [42,58]. Thus, some factors may not have effects *in vitro* but still be relevant for WAT function.

As mentioned in chapter 1, WAT dysfunction is characterized by *inter alia* chronic inflammation, reduced adipogenic ability and increased basal lipolysis. Hence, these readouts

are commonly used in the *in vitro* system to characterize the role of a gene/protein on WAT function. The processes are subdivided into "good" and "bad" *i.e.* a gene that is highly expressed in a metabolically unhealthy state is expected to induce "bad" pathways and *vice versa* for the opposite. As discussed previously, these processes are dynamic and context-dependent *in vivo* and the "good/bad" subdivision of tissue function may mislead and limit our understanding of biology.

3.3 ANIMAL MODELS

A transgenic mouse model was used in study II to test the causal role of *Tgfb3 in vivo*. It was of particular importance to use an animal model in this study as the data *in vitro* suggested that TGFB3 regulate progenitor proliferation. The effects on WAT cellularity is also dependent on adipogenesis and hence, induced proliferation may not necessarily translate into increased adipocyte cellularity.

The way humans and mice store fat is different. Mice store fat mainly in the visceral depot and humans in the subcutanous[59]. The differences between depots, at least in mice, seem to be dependent of the microenvironment of the depot and not intrinsic in the cells[60]. Although mice are not equal to humans, they constitute a model organism that can be used to identify qualitative effects on WAT function.

4 RESULTS AND DISCUSSION

4.1 STUDY I

In study I, we demonstrated that EBF1 levels and activity in WAT are associated with several measures of metabolic health. As EBF1 is a well-established regulator of WAT function (regulating adipogenesis, inflammation and lipolysis)[39,61,62], these data support the notion that WAT function is relevant for overall metabolic health. The causal link between EBF1 expression and adipose tissue function has been established prior to this study[39] but they did not report the association to clinical parameters in humans. This study in combination with the previous studies on EBF1 function in WAT suggests that therapies aiming at targeting its transcriptional activity could be used to treat metabolic disease.

How is EBF1 activity regulated and how can its activity be targeted? Specific ligands that activates EBF1 activity remain unknown however, inflammation (*i.e.* TNFα stimulation *in vitro*) reduce *EBF1* expression in adipocytes[39]. This is not likely mediated via direct mechanisms as TNFα stimulation perturbs the characteristics of adipocytes, including high *EBF1* expression. It is tempting to just imagine the DNA strand as a two-dimensional static molecule that EBF1 bind to, at specific motifs, after receiving a signal in form of a ligand or similar. Today we know that the chromatin is a three-dimensional structure and it is dynamic[63]. It can move specific regions of DNA into machineries of proteins involved in specific nuclear functions such as gene transcription. The three-dimensional chromatin structure is in large parts shaped by the cellular metabolic state via epigenetic mechanisms[11]. Thus, it is conceivable that EBF1 activity is regulated by specific metabolic states which shape the chromatin allowing EBF1-motifs to be accessible for gene transcription.

In mice and humans EBF1 activity is associated with adipocyte cellularity independent of fat mass[39] suggesting that interventions increasing EBF1 activity could increase adipocyte number without influencing total fat mass. However, in individuals with behavioral patterns resulting in a caloric oversupply, the WAT will continue to expand and a threshold for a hypertrophic phenotype will be reached at a larger fat mass. This suggests that potential therapies targeting EBF1 activity should be combined with intervention managing behavioral patterns.

4.2 STUDY II

Study II was performed to identify regulators of WAT adipocyte cellularity. A novel regulator of adipocyte cellularity, namely TGFB3 was identified. This factor is proposed to induce fat cell number in obesity by regulating pre-adipocyte proliferation. When obese individuals lose weight (termed post-obesity) *TGFB3* expression is unaltered indicating that it contributes and explains part of the maintenance of elevated and irreversible adipocyte turnover once an individual becomes obese. Mice expressing half the mRNA levels compared to wild-type littermates displayed perturbed glucose metabolism when fed with a high fat diet

but not on a regular chow diet. These findings support that WAT expandability is important for metabolic health.

The factors inducing *TGFB3* expression during weight gain remain unknown but it is possible that, as discussed above, the metabolic changes induce its expression via epigenetic mechanisms. Another explanation could be that the progenitor pool is induced during hyper-caloric conditions and the accumulation of this cell type maintains a high *TGFB3* expression in the WAT. Nevertheless, instead of understanding the mechanisms regulating its expression, recombinant TGFβ3 can be injected in patients. In fact, this has already been tested for anti-scarring therapies under the trademark Avotermin[64]. However, the timing of TGFβ3 injections in relation to adipogenic signals need to be investigated in order to expand the tissue efficiently as a chronic induction of progenitor proliferation may inhibit adipocyte differentiation.

4.3 STUDY III

A novel regulator of inflammation, namely SLC19A1, was identified and characterized in Study III. We observed that the expression of several genes involved in folate and methionine metabolism (one carbon cycle) was altered in WAT of individuals with obesity. *SLC19A1* expression displayed the strongest association to pro-inflammatory pathways. We linked the reduced expression of this gene to an induced global DNA-methylation. In particular, we showed that DNA-methylation in the promoter of the pro-inflammatory gene *CCL2* induced its gene expression by modulating glucocorticoid receptor activity. These findings contribute to the understanding of the link between metabolism and adipocyte function.

An important controversy of this study is that knockdown of *SLC19A1 in vitro* resulted in increased DNA methylation even though folate and the universal methyl donor S-adenosylmethionine levels decreased. In fact, whole cellular metabolism is inter-woven and the one carbon cycle is not an isolated system[65]. In this study we reduced the expression of one gene encoding a folate transporter. The intracellular folate is connected to a myriad of metabolic pathways which involves several other intermediary metabolites as well as other enzymes and transporters[65]. Furthermore, S-adenosylmethionine and DNA methylation is just one out of hundreds of epigenetic modifications that may be influenced by the metabolome[13]. Understanding the complexity of such systems is made possible with omics tools and deeper understanding of these systems will probably be elucidated in the near future.

This study suggest that folate metabolism is important for adipocyte function and metabolic health which is supported by a randomized control trial in humans[66]. However, folate alone is not likely enough to treat insulin resistance but it may constitute an ingredient in a mixture of metabolites. In fact, several metabolites are altered in individuals with obesity and metabolic disease[67].

4.4 FUTURE PERSPECTIVE

The studies in this thesis report three regulators of adipocyte function and metabolic health. Where do we go from here? Before moving on discussing future perspectives we need to define what the end goal is. The answer should be obvious; to reduce human suffering and improve the quality of life for humankind. If this is the goal, future research should focus on identifying therapies that can regulate WAT function. These interventions could involve treatment with drugs targeting specific mechanisms in adipocyte or behavioral interventions. The latter would require understanding of what behavioral patterns that causes an unhealthy WAT phenotype (hypertrophy and inflammation).

The next step is to evaluate if therapies targeting WAT expansion and/or inflammation can be used to treat metabolic disease. If such therapies are successful, the impact on the subjective improvement in patient life-quality needs to be evaluated.

5 CONCLUSION

The structure of modern societies promotes an imbalance between energy intake and energy expenditure resulting in increased fat mass and obesity over time. This spurred scientists to map WAT function in health and disease in order to counteract obesity itself as well as its comorbidities. Collectively, decades of characterizations suggests that the problem with obesity is not that the WAT expands but rather that its expansion is limited and that once this limit is reached, individuals become metabolically sick. Chronic low grade inflammation is one characteristic of WAT that has reached its expansion. The work herein supports the existing theory and contributes to the understanding of the underlying mechanisms. The first two studies and the last study link metabolic health to tissue remodeling/expansion and inflammation, respectively. Taken together, this suggests that therapies to treat metabolic disease by mechanisms in WAT should aim at allowing it to further expand. The future will tell if humankind will solve the problem with obesity by modifying the fat storage capacity of the body, adjust the behavior to adapt to the environment or re-format the environment to adapt to the existing behavior.

"There are very few new things in this world, very few. That's why people that are young, if they're smart, try to profit from the experience of an older guy so they won't have to go through all the pain and suffering. But a certain amount of pain and suffer is good, because it makes a person think they've learned."

– Cus D'Amato

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