The Effects of Obesity Interventions, Age, and Age of Obesity Onset on Adipose Tissue Characteristics and Markers of Cardiometabolic Health

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Abstract

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Laurent Turner

In recent years, the prevalence of overweight and obesity has risen dramatically worldwide affecting individuals of all ages. This increased prevalence has far-reaching consequences on health as individuals with obesity are more likely to develop cardiometabolic diseases, cancer, and other associated comorbidities. However, being extremely complex and heterogeneous, obesity does not present the same way in every individual as some develop diseases while others do not. As such, it is essential to further understand the mechanisms underlying the heterogeneity of obesity which may in part explain the ineffectiveness of obesity interventions in some individuals with obesity. This thesis aims to demonstrate how obesity interventions, age and age of obesity onset affect adipose tissue characteristics and ultimately disease risk. Chapter 1 discusses the impact of dietary fatty acids, exercise, and bariatric surgery on the mechanisms that affect adipose tissue macrophage presence and phenotype in obesity. We find robust evidence suggesting that with or without weight loss, exercise, dietary fatty acids, and bariatric surgery result in immunomodulation via the amelioration of adipose tissue characteristics. Chapter 2 aims to determine whether differences in regional adipose tissue characteristics vary with age and age of obesity onset, and whether these differences are associated with the markers of cardiometabolic health. We show that older adults with childhood-onset obesity are particularly vulnerable to the cardiometabolic effects associated with perturbations in adipose tissue characteristics. Collectively, this thesis demonstrates that indeed obesity interventions, age and age of obesity onset impact adipose tissue homeostasis and health making adipose tissue a potential therapeutic target. However, as age and age of onset should be recognized as risk

factors for obesity-associated comorbidities, their impact on the effectiveness of obesity interventions, whether it be exercise, dietary interventions, or bariatric surgery, are unknown. More research is needed to further explore the mechanisms underlying the heterogeneity of obesity and its effects on the efficacy of weight loss interventions.

Contribution of Authors

As the primary author, I was responsible for optimizing the research protocols, methodology, statistical analysis, interpreting results, conceptualizing the literature review in chapter 1, and writing of both manuscripts presented in this thesis. As the principal investigator, Dr. Santosa was responsible for the conceptualization of the literature review in chapter 1, and study presented in chapter 2, funding acquisition, and reviewing and editing both manuscripts.

For chapter 2, co-authorship contribution is as follows:

- Marie-Frédérique Gauthier was responsible for optimizing all the research protocols used in this study and reviewing the manuscript.
- Dr. André Tchernof was responsible for the conceptualization of the study, methodology, funding acquisition and reviewing the manuscript.

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Table of Content

List of Figures

List of Tables

Abbreviations

AMPK: AMP-activated protein kinase, ANG: angiopoietin, ANOVA: analysis of variance, AO: old, adulthood-onset, ARG-1: arginase-1, ATM: adipose tissue macrophage, AY: young, adulthood-onset, BMI: body mass index, CCR: C-C chemokine receptor, CO: old, childhood-onset, CRP: C-reactive protein, CVD: cardiovascular disease, CXCR: C-X-C chemokine receptor, CY: young, childhood-onset, C/EBPα: CCAAT-enhancer-binding protein α, ECM: extracellular matrix, FFAR-4: free fatty acid receptor, G-CSF: granulocyte colony-stimulating factor, HbA1c: glycated hemoglobin, HDL-c: high-density lipoprotein, HFD: high-fat diet, HGF: hepatocyte growth factor, HIF-1α: hypoxia-inducible factor 1-α, IHC: immunohistochemistry, iNOS: inducible nitric oxide synthase, LDL-c: low-density lipoprotein, LSD: least significant different test, MCP-1: monocyte chemoattractant protein-1, MIP-1α: macrophage inflammatory protein-1α, MRC1: mannose receptor C-Type 1, PAI-1: plasminogen activator inhibitor-1, PECAM-1: platelet endothelial cell adhesion molecule-1, ROS: reactive oxygenic species, SAT: subcutaneous adipose tissue, SVF: stromal vascular fraction, Tie-2: tyrosine-protein kinase receptor Tie-2, TIMP1: tissue inhibitor matrix metalloproteinase 1, TLR: toll-like receptor and VEGF: vascular endothelial growth factor

General Introduction

Obesity, prolonged sedentary behaviour, and poor diet are among the most critical health issues facing our society. With overweight and obesity prevalence constantly reaching new heights, it has been predicted that by 2030, nearly 60% of the world's population will be overweight or obese (1). Obesity promotes the development of potentially life-threatening comorbidities such as type 2 diabetes, cardiovascular diseases, and cancer (2). These conditions arise in response to excessive adipose tissue accumulation causing perturbations in adipose tissue. Persistent positive caloric balance triggers the rapid and pathogenic expansion of adipose tissue, which may act as a catalyst for various stress responses including increased macrophage infiltration, adipose tissue fibrosis, angiogenesis, hypoxia, and adipocyte death (3, 4). Consequently, adipocyte dysfunction leads to metabolic and immune derangements with repercussions locally and systemically resulting in the development of obesity-associated comorbidities (3).

In obesity, the persistent and abundant infiltration of macrophages in adipose tissue contributes to the chronic milieu of low grade inflammation (5, 6). Indeed, macrophages can be viewed as central to tissue stress and may play a pivotal role in the development of obesity-associated comorbidities. Increased adipose tissue macrophage content has been associated with the metabolic syndrome, type 2 diabetes, heart disease and most notably insulin resistance (3, 7, 8). In addition, macrophage infiltration may also exacerbate adipocyte dysfunction by increasing the deposition of extracellular matrix proteins leading to adipose tissue fibrosis, thus furthering the development of diseases (9, 10). *Ex vivo* experiments indicate that macrophages may have fibrosis-inducing capabilities by secreting pro-fibrotic factors to heal injured tissue (11, 12). As adipose tissue play a key role in whole body metabolism and homeostasis, it is important that we

understand factors that affect adipose tissue function. Thus, the objective of this thesis is to examine the factors affecting adipose tissue characteristics and disease risk.

Chapter 1 reviews and describes some of the mechanisms underlying the anti-inflammatory properties of exercise, dietary fatty acids, and bariatric surgery. The purpose of this review is to discuss the impact of dietary fatty acids, exercise, and bariatric surgery on cellular characteristics affecting adipose tissue macrophage presence and phenotypes in obesity. The resulting improvement in adipose tissue macrophage infiltration and phenotypes in individuals with obesity are observed with or without weight loss. However, these interventions are not always successful in part because obesity is a heterogeneous condition and individuals of equal adiposity may not develop the same obesity-associated comorbidities (13, 14). We do not understand what explains the underlying heterogeneity in those with this disease, though it has been observed that comorbidities are more likely to develop in those who are older and those who have had obesity since childhood.

Rationale and Objective – Chapter 2: Individuals with persistent overweight and obesity from childhood into adulthood have been previously found to have an increased likelihood of developing cardiometabolic diseases and cancer (15-17). This increased prevalence for disease in those with childhood-onset obesity may result from differences in adipose tissue of different depots. Indeed, studies indicate distinct cellular differences in adipose tissue of individuals with childhood- vs adulthood-onset obesity and that VAT accumulation is more pathogenic than SAT (though each appears to have their own role in contributing to disease risk) (16, 17). Moreover, obesity tends to mirror ageing as the comorbidities associated with obesity parallel those of ageing (18). Therefore, it is plausible that both age and age of obesity onset have compounded effects on regional adipose tissue and potentially disease risk. Thus, the aim of this study is to

characterize regional adipose tissue characteristics in younger and older adults with either childhood- and adulthood-onset obesity and determine whether these differences are associated with the markers of cardiometabolic health.

For the study presented in chapter 2, we hypothesized that adipocyte size, macrophage infiltration and pericellular fibrosis will differ in subcutaneous and visceral adipose tissue and these differences will be distinct in younger and older adults with either childhood or adulthood-onset obesity. Lastly, the markers of cardiometabolic health will be associated with the regional adipose tissue characteristics with an independent and cumulative effect based on age and age of obesity onset.

Overall, adipose tissue dysfunction represents an important mechanism underlying obesity-associated comorbidities. However, the patterns of disease development in those with obesity are heterogeneous and unclear. As such, this thesis will provide novel insights and an important first step towards understanding the heterogeneity of obesity which may eventually lead to the development of targeted interventions to prevent or delay the progression of cardiometabolic disease.

Chapter 1

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Putting ATM to BED: How Adipose Tissue Macrophages are affected by Bariatric surgery, Exercise, and Dietary fatty acids

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Abstract

With adipose tissue expansion in obesity, adipose tissue macrophages contribute to adipose tissue malfunction and increased circulating pro-inflammatory cytokines. The chronic low-grade inflammation that occurs in obesity ultimately gives rise to a state of meta-inflammation that increases the risk of metabolic disease. To date, only lifestyle and surgical interventions have shown to be somewhat effective at reversing the negative consequences of obesity and restoring the adipose tissue homeostasis. Exercise, dietary interventions, and bariatric surgery result in immunomodulation, and for some individuals their effects are significant with or without weight loss. Robust evidence suggests that these interventions reduce chronic inflammation, in part, by affecting macrophage infiltration and promoting a phenotype switch from the M1 to M2-like macrophages. The purpose of this review is to discuss the impact of exercise, dietary fatty acids and bariatric surgery on cellular characteristics affecting adipose tissue macrophage presence and phenotypes in obesity.

Teaser text: Adipose tissue macrophages play an important role in metabolic disease risk. This review discusses the effects of dietary fatty acids, exercise, and bariatric surgery on modulating adipose tissue macrophages.

Keywords: physical activity, dietary fatty acids, bariatric surgery, macrophages, adipose tissue characteristics, meta-inflammation

1. Introduction

According to the World Health Organization, the number of individuals with obesity worldwide, adults and children, has nearly tripled since 1975 (19). This increased prevalence poses a significant threat to health as individuals with obesity are more likely to develop a myriad of related conditions such as type 2 diabetes, cardiovascular diseases (CVD) and some type of cancers (2, 20). These health consequences, in part, stem from the negative effects of excess adipose tissue accumulation leading to morphologic and functional abnormalities (20). Subsequent, endocrine, metabolic and immune derangements follow which contribute to the obesity-associated inflammation that is, in part, mediated by macrophages (21, 22). Indeed, macrophages can be viewed as central to tissue stress, contributing to adipose tissue malfunction and increased circulating pro-inflammatory cytokines as obesity progresses (3, 22). The resulting chronic inflammatory state leads to adipocyte maladaptation and subsequent increases in angiogenesis, production of extracellular matrix (ECM), macrophage infiltration and pro-inflammatory response (3, 4, 22); all the aforementioned local consequences feed in a positive feedback loop exacerbating one another.

In both human and animal models, lifestyle and surgical interventions resulting in weight loss decreased macrophage infiltration and lead to a phenotypic switch of the adipose tissue macrophages (ATM) (23-28). In the case of exercise, the beneficial effects were observed regardless of weight loss. Several mechanisms have been proposed to explain the anti-inflammatory properties of physical activity and the differential properties of dietary fatty acids culminating in beneficial quantitative and qualitative changes in ATM profiles (29). The aim of this review is to discuss the impacts of exercise, dietary fatty acids and bariatric surgery on mechanisms that affect ATM presence and phenotype in obesity.

2. Macrophages and Obesity

2.1 What are macrophages?

Macrophages are innate immune cells that are typically found in every tissue and have the unique ability to sense and respond to pathogens and other environmental cues. Macrophages are particularly important for: tissue repair after an injury, clearance of foreign invaders and cellular debris through phagocytosis, and normal tissue development; they are especially efficient at integrating endocrine and paracrine signals in order to respond to stimuli (30, 31). Additionally, these phagocytes are prolific communicators as they directly interact with the receptors of other tissue-resident cells, immune cells recruited during injury (e.g., T cells) and extracellular proteins (30, 31). Other noteworthy characteristic features of these monocyte-derived cells are that they are heterogeneous and exhibit high levels of plasticity.

Macrophages are able to acquire different molecular and functional phenotypes after being exposed to different bioactive molecules and environments (31, 32). Indeed, macrophages can differentiate to pro-inflammatory M1 cell or anti-inflammatory M2 cell phenotype, though they need to be activated or polarized for this process to occur (4, 33). However, how M1 and M2 macrophages come to be in the adipose tissue remains ambiguous. It has been suggested that shifts in the M1:M2 macrophage ratio occurs from the transformation of resident macrophages during the course of resolution of an injury or from the continuous recruitment of monocytes in response to tissue stress (30).

The polarization of macrophages to M1 cells is mediated by type 1 T helper cells that secrete IFN- γ or with bacterial products (e.g., LPS). M1 macrophages produce pro-inflammatory cytokines such as TNFα and IL-6 and they express inducible nitric oxide synthase (iNOS),

reactive oxygenic species (ROS) and nitrogen intermediates (4, 33). These pro-inflammatory molecules have been associated with the onset of numerous diseases such as CVD or type 2 diabetes (34-36). For example, TNFα knockout mice had improved insulin-sensitivity and lower levels of circulating free fatty acids (37). Conversely, the polarization of macrophages to M2 cells is mediated by type 2 T helper cells that secrete IL-4 and IL-13. M2 macrophages produce anti-inflammatory cytokines such as IL-4, IL-10 and TGFβ which blocks the activity of iNOS and downregulates the synthesis of pro-inflammatory cytokines (4, 33, 38). M2 macrophages are more often associated with wound healing, resolution of inflammation, clearing of cellular debris, regulating proliferation, precursors of angiogenesis, and remodeling of the ECM, whereas M1-like macrophages appear to promote the opposite (3, 8). It should be noted that the M1/M2 paradigm is often seen as an oversimplified dichotomous division and should rather be considered as a continuum (3, 8, 30, 39). The identification of M1 and M2 cells is also challenging as phenotype markers are not specific and may indicate other cell types. The literature therefore identifies macrophage cells as M1-like and M2-like.

2.2 Macrophages in obesity

A plethora of immune cells accumulates within the expanding adipose tissue (3), although the macrophage population remains the predominant one (6). Macrophages make up around 5-10% of the stromal vascular fractions (SVF) cells derived from adipose tissue of lean individuals whereas in individuals with obesity, the SVF can consist of up to 40-50% macrophages (5). To preserve adipose tissue homeostasis and functionality, there has to be a balance between both populations of M1 and M2-like macrophages (4, 6). However, the phenotypic heterogeneity of macrophages is environment dependent (4, 6, 33). In the lean state, the balance of the macrophage population tends to shift towards the anti-inflammatory M2-like subpopulation (6).

In comparison, in the obese state, the balance tilts toward the M1-like subpopulation, thus, creating a pro-inflammatory environment within the adipose tissue (6, 40-42). The accumulation of ATM in individuals with obesity has been linked with adipocyte and metabolic dysfunction (3, 7, 8).

3. Lifestyle and Surgical Interventions

3.1 Dietary fatty acids

The seminal work of Weisberg et al. (43) and Xu et al. (44) were the first to demonstrate that high-fat diets increase macrophage content and trafficking within the fatty depots that are associated with the development of obesity-induced insulin resistance. Indeed, fatty acids are thought to be immunomodulators of inflammatory pathways. However, not all fats are equal and different fats may have differential effects on macrophages and adipose tissue characteristics (45, 46).

3.1.1 Saturated Fatty Acids

Effects of saturated fatty acids on macrophage polarization and infiltration in rodents

Studies suggest that diets rich in saturated fatty acids (SFA) are associated with inflammation as they are considered as naturally occurring ligands for the toll-like receptors (TLR) which activate downstream inflammatory pathways, on both adipocytes and macrophages/monocytes (47-49). Obese rodents, fed with diets rich in SFAs (mainly from lard), have increased expression of TLRs and markers associated with macrophage infiltration (49-56) (see Table 1). Moreover, the activation of the TLR inflammatory pathways by an increased flux of SFAs are thought to contribute to the classical polarization of M1-like macrophages. For example, Enos et al. (53) examined the effects of three high-fat diets (HFD), differing in the percentage of total calories

from saturated fat (6%, 12%, and 24%) but identical in total fat (40%), on macrophages behaviour. All HFDs increased adipose tissue inflammation, but the 12% and 24% saturated fat diets increased TLR2 expression and led to the greatest increase in M1 and M2 like macrophages (53). Additionally, several murine studies reported that feeding of SFAs-rich diets worsened ROS production, the expression of adipose tissue remodelling markers (e.g. TGF-β, tissue inhibitor matrix metalloproteinase 1 (TIMP1), collagen VI, hypoxia-inducible factor 1-α (HIF-1α) and PPARγ), decreased capillary density, and increased adipocyte size, pro-inflammatory cytokines (e.g. IL-6, TNF-α, monocyte chemoattractant protein-1 (MCP-1) and C-reactive protein (CRP)) and the number of crown-like structures (50, 52, 53, 55-63). These changes may collectively prompt the aggregation of pro-inflammatory macrophages. Thus, it appears that a diet rich in SFA may trigger the development of pathogenic remodelling processes in rodents in response to the accumulation of M1-like macrophages.

Effects of saturated fatty acids on macrophage polarization and infiltration in humans

In human studies, SFAs also increased TLR genes and pro-inflammatory cytokines (e.g., IL-1β, IL-6, IL-8, TNF-α, RANTES) in lean subjects, those with obesity, and in those with diabetes (64-66) (see Table 1). Van Dijk et al. (65) conducted a parallel controlled-feeding trial in 20 abdominally overweight subjects randomized to a SFA-based diet or a MUFA-based diet for 8 weeks. Whole-genome microarray and histologic analysis of the adipose tissue showed that the consumption of SFAs increased pro-inflammatory obesity-linked gene expression including the downregulation of PPARγ and upregulation of the TLRs and macrophage marker genes (CD14 and CD163) (65). Of particular note, is that the participants' weights were not significantly different between the diet groups and did not change throughout the intervention, ruling out the cofounding factor of weight gain. The direct effects of SFA on macrophage infiltration and

polarization, cellular characteristics and adipose tissue remodelling in individuals with obesity are still poorly documented and require further investigation.

Other contributing factors to inflammation in obesity

A diet rich in SFAs may also represent a crucial first step in disturbing the gut microbiota. This disruption in the gut microbiota results in alterations in the epithelial cells of the intestinal barrier while promoting the translocation of bacteria and their cellular components into the circulation (67, 68). Consequently, a diet high in SFAs may contribute to a rise in the systemic concentration of LPS, which can act as a ligand to the TLRs on the surface of ATM and adipocytes (69-74). As such, it has been hypothesized that the translocation of LPS and bacterial metabolites may increase the release of pro-inflammatory cytokine, promote macrophage infiltration, and prompt a phenotype switch towards the M1-like cells (75, 76). However, a phenotypic switch has yet to be demonstrated in humans and the influence of the LPS-mediated inflammation on adipose tissue characteristics is rather unknown. Moreover, recent studies suggest that lifestyle and surgical interventions may also partially revert gut microbiota dysbiosis to improve gut health and possibly inflammation (77-79). Overall, the gut microbiota-related inflammation represents a promising alternate pathway to explain the chronic low-grade inflammation seen in individuals with obesity.

3.1.2 n-3 Polyunsaturated Fatty Acids

Conversely to SFAs, n-3 polyunsaturated fatty acids (n-3 PUFAs) have the capacity to induce anti-inflammatory and insulin-sensitizing effects on adipocytes and their resident macrophages. These metabolic improvements have been predominantly observed with n-3 PUFA supplementation from fish oil (i.e., EPA and DHA). The anti-inflammatory n-3 PUFAs are

known endogenous ligands to PPARγ and free fatty acid receptor 4 (FFAR4), have the ability to preferentially inhibit TLR-induced pathways, and reduce the expression of pro-inflammatory transcription factors (80-83).

Numerous studies conducted on both humans and rodents alike demonstrated the potential advantageous effects of n-3 PUFAs on macrophage infiltration and phenotypic shifts, culminating in the amelioration of adipose tissue homeostasis. Indeed, following a dietary regimen enriched in n-3 PUFAs, the number of macrophages and specific markers of macrophage polarization for the M1 and M2-like cells fluctuated favoring a M2-domiant ratio (26, 84-103) (see Table 2). Itariu et al. (86) conducted an 8-week randomized trial on 55 non-diabetic individuals with class III obesity who received either 3.36 g EPA/DHA or the equivalent amount of butterfat each day. They found that despite no changes in M2 macrophage markers (mannose receptor C type 1 (MRC1) and CD163), pan macrophage marker (CD68) as well as the total number of macrophages, the expression of CD40, a M1 marker, was downregulated by n-3 PUFA treatment. Another study demonstrated that after participants with class I obesity consumed 4 g of fish oil (~3.6 g EPA and DHA) per day for 12 weeks, significant decreases in total macrophage number and CD68 mRNA levels were observed (90). Further in vitro experiments showed that the addition of DHA to M1 macrophage cultures and co-cultures with adipocytes markedly reduced the expression of MCP-1 (90). Therefore, fish oils may not only reduce macrophage abundance in adipose tissue, but also decrease the migration and infiltration of monocytes into adipose tissue (90). More recently, three other *in vitro* studies also supported these findings through similar observations and conclusions (85, 87, 88). On the other hand, in another study where individuals with overweight to class I obesity consumed 3.5% of their diet as fish oil, no differences in ATM gene expression (CD14 and CD206) were observed (104). The

discrepancies in the findings may be explained by the differences in the n-3 PUFAs dose administered, the composition of the n-3 PUFAs used or the weight status of the participants. The studies by Itariu and Spencer (86, 90) recruited individuals with more severe cases of obesity, which may suggest that the anti-inflammatory properties of n-3 PUFAs are more significant in individuals with greater obesity severity.

Beneficial shifts in the M1:M2-like macrophage ratio following n-3 PUFA supplementation may be due to several underlying mechanisms. Supplementation resulted in improvements in cellular stress (56, 77, 96, 105-109), metabolic profile (110), synthesis and release of anti-inflammatory mediators (i.e. IL-10, IL-4, arginase-1 (ARG-1) and adiponectin), while decreasing the secretion of pro-inflammatory mediators (i.e. IL-1β, IL-6, TNF-α and MCP-1)(26, 84-86, 88, 90-92, 94-96, 111-117), adipocyte enlargement (26, 27, 84, 88, 95, 106, 111, 118, 119), and the deposition of ECM and the expression of its associated markers (27, 86, 91, 96). Increased capillary density (90), and adipogenesis (84, 106, 118, 120, 121) have also been shown with supplementation. Moreover, supplementation of n-3 PUFAs downregulated the expression of important inflammatory transcription factors and receptors such as NF-κB and TLR4 concomitant with an upregulation in adipogenic regulators (PPARγ and CCAAT-enhancer-binding protein α (C/EBPα)) (56, 84, 88, 91, 96, 101, 113, 114, 118, 121, 122). Additionally, some murine studies also observed changes in weight and fat mass loss with the previously mentioned improvements (84), whereas studies in humans showed the downregulation of inflammatory factors associated with PUFA consumption in absence of changes in weight or body composition (84, 86, 104).

Overall, n-3 PUFA supplementation may represent a potential therapeutic avenue to improve macrophage-mediated inflammation and adipose tissue characteristics, although the effects were less potent *in vivo* (84, 86, 104). The inconsistent results in human studies are likely to be

attributed to variability in study design, weight status of participants, and adherence as most trials are outpatient studies and rely on self-reporting. Other factors may include differences in the amount of dosage administered or methods of calculating n-3 PUFA intake (82, 84, 123). Nonetheless, further studies should continue to explore the role of n-3 PUFAs in mediating ATM infiltration and phenotype.

3.2 Physical activity

Lack of exercise and prolonged sedentary behaviours are important catalysts for a cluster of metabolic and chronic diseases, whereas regular exercise may prevent or delay the progression of insulin resistance, hypertension, CVD, and diabetes (124, 125). Although, numerous studies have denoted that the salutary effects of exercise are independent of weight loss (125-128); significant weight loss may amplify the exercise-induced benefits and have a greater impact on the inflammatory markers in humans (24, 129, 130). In fact, physical activity was shown to induce cellular and molecular changes in the adipose tissue in a way that alleviates the low-grade chronic inflammation that accompanies obesity (23-25, 131-133). The underlying mechanisms that contribute to the exercise-induced anti-inflammatory responses have not been completely elucidated. A major contributor to the reduction in inflammation accompanying exercise may reside in the mediation of ATM (29, 125, 128, 134, 135).

3.2.1 The effects of exercise on macrophage infiltration and phenotypes

Exercise, with or without weight loss, may decrease inflammation via promoting a phenotypic switching from M1 to M2 macrophages while simultaneously diminishing the trafficking of the macrophages within the adipose tissue. The early work of Kawanishi et al. (58) demonstrated that 16 weeks of cardiovascular exercise training (12-20 m/min, 60 min/day and 5 times/week) in

mice with obesity reduced M1-like and increased M2-like macrophage mRNA expression in adipose tissue such that the M1:M2-like ratio was ~50% lower with the exercise intervention relative to control. Quantification of macrophages in adipose tissue by flow cytometry also showed that exercise decreased both the proportion and absolute number of M1-like (CD11c+) macrophages (136). Another study found that in comparison to continuous training (steady state running at 20m/min), aerobic interval training (3-min bouts at 40 m/min, interspersed by 3-min active recovery at 20 m/min on a treadmill with 15% incline, repeated six times per session) has been shown to result in greater increases in the number of M2-like macrophages (181% vs 122%) in mesenteric adipose tissue (59). More recent murine studies have demonstrated diminished infiltration and phenotypic shifts in macrophages (59, 137-142) (see Table 3).

In humans, few studies have looked at the direct impact of exercise on the polarization of the macrophage populations (24, 131, 143-145) (see Table 3). These studies have found exercise-induced shifts towards a predominant M2-like phenotype. An 8 week low-intensity exercise intervention (walking 10 000 steps 3 times/week) in adults with overweight and class I obesity showed that exercise was associated with a ~2.1 fold upregulation of M2 markers and a downregulation of M1 markers independent of weight loss (145). Additionally, Auerbach et al. (131) and Bruun et al. (24) corroborated the previous findings through an exercise-induced weight-loss protocol suggesting that pronounced weight-loss may also further affect macrophage infiltration and phenotype resulting in an anti-inflammatory milieu within adipose tissue.

3.2.2 Mechanisms altering macrophage infiltration and phenotypes in exercise

Exercise decreases expression of pro-inflammatory and chemotactic signals

There is a growing body of evidence suggesting that exercise decreases the expression of pro-inflammatory and chemotactic cytokines involved in the recruitment of macrophages and monocytes (29, 125, 128). Among all the cytokines known to potentially contribute to inflammation within the adipose tissue and the chemotaxis of macrophages, TNF-α, IL-6 and MCP-1 appear to be the best studied cytokines and were consistently shown to have lower levels of expression following exercise treatment in humans, mice and rats (23-25, 129, 132, 133, 136, 138, 139, 145-157). Baturcam et al. (146) found that 3-month supervised exercise (combination of moderate intensity (50-80% of max HR) aerobic exercise and resistance training using either treadmill or cycling 3-5 times/week) significantly reduced the expression of both RANTES and C-C chemokine receptor type 5 (CCR5) in the adipose tissue of individuals with class I to class II obesity with decreases in the levels of the pro-inflammatory markers TNF-α, IL-6, and P-JNK. Complementing these findings, Barry et al. (23) demonstrated that both high intensity interval training (at 90% of HR_{peak}, for 1 min interspersed with 1 min of low-intensity recovery periods, progressing from 4 to 10 intervals) and moderate-intensity continuous training (at 65% of HR_{peak}, progressing from 20 to 50 min) in humans, in absence of weight and fat mass loss, altered leukocytes trafficking through the downregulation of inflammatory chemokine receptors such as CCR2, CCR5 and C-X-C chemokine receptor type 2 (CXCR2). In humans and rodents exercise has also been associated with an increase in the expression and release of anti-inflammatory signals such as IL-10, IL-6, ARG-1, and adiponectin (24, 29, 127, 129, 138, 149, 150, 158). Even though the positive effects of exercise have been observed in absence of weight loss, weight loss may compound the benefits. An exercise study (aerobic training, 60-75min/session

and 3 times/week) found that compared with subjects in the lowest tertile (-3%) of weight loss, those in the highest tertile of weight loss (-14.5% weight loss) had larger decreases in macrophage inflammatory protein-1α (MIP-1α) and IL-15 and greater increases in adiponectin (129). Aside from TNF-α, IL-6 and MCP-1, several other cytokines associated with inflammation were shown to have a reduced expression following an exercise intervention such as RANTES, plasminogen activator inhibitor-1 (PAI-1), MIP-1α, CRP, chemerin, IFN-γ, IL-1, IL-8, IL-15 and IL-18 which may also further improve the chronic low-grade inflammation seen in adipose tissue (24, 129, 132, 133, 139, 146, 147, 149, 153).

Exercise affects adipose tissue characteristics

In addition to decreasing pro-inflammatory and chemotactic signals associated with macrophage recruitment, exercise also directly affect adipose tissue characteristics that are associated with the recruitment and phenotypic changes of ATM. A recent murine study by Kolahdouzi et al. (59) found that aerobic interval training improved adipose tissue dysfunction induced by a HFD through increasing the number of M2-like cells and capillary density while decreasing the total number of crown-like structures and mean adipocyte size (59). Multiple murine studies have also demonstrated beneficial changes in cellularity that may be associated with decreased ATMs. Moreover, several key features of dysfunctional adipose tissue are improved such as lipid and glucose metabolism (147, 148, 159), improved mitochondrial activity and biogenesis (159-162), decreased expression of apoptotic signals (163), decreased expression of angiogenesis precursors (164-169), increased capillary density (59), reduced accumulation of fibrotic depots (25, 57), and reduced adipocyte size (59, 147, 160, 170, 171). Similar findings were also made in human studies where lipid metabolism, mean adipocyte size, adipose tissue fibrosis and pro-angiogenic

responses were improved following exercise training with or without weight loss (152, 172, 173).

Exercise modifies gene expression patterns

Underlying the mechanisms of the potent anti-inflammatory properties of exercise are tremendous gene expression alterations that may have a direct impact on chronic low-grade inflammation as well as the ubiquitous pro-inflammatory macrophage infiltration seen in adipose tissue of individuals with obesity (160, 174). Aside from the gene expression variations of the pro and anti-inflammatory cytokines, angiogenic regulators, ECM precursors, markers of mitochondrial activity, and lipid and glucose metabolism (24, 57, 132, 159, 165), physical activity also affects the gene expression of key adipogenic regulators such as PPARs and well-characterized immune receptors that modulate inflammatory pathways like the TLRs (29).

Several studies highlight the crucial immunomodulating role of PPARs, more specifically PPARγ, in regulating adipose tissue inflammation by promoting the infiltration of M2 macrophages in humans and mice (175-177). Macrophage-specific deletion of the PPARγ gene (175) and upregulation of PPARγ by rosiglitazone (176) in mice demonstrated the role of PPARγ in M2-like macrophage activation. Exercise upregulates PPARγ expression and its related signalling events in adipose tissue and monocytes/macrophages of humans, mice, and rats (144, 178-183), favoring a phenotypic shift towards the M2-like macrophages. In a human study, Yakeu et al. (145) found that low-intensity exercise (walking 10 000 steps 3 times/week) shares similar effects to the pharmacological activation of PPARγ and that a ~4-5 fold increase in PPARγ activity and expression coincided with a ~2.1 fold increase in the M2-like macrophage marker (CD14).

TLRs are a class of membrane proteins that play an important role in the innate immune system by initiating key downstream inflammatory pathways through recognition of exogenous and endogenous ligands (184). TLRs, especially TLR2 and TLR4, are present on the cell surface of adipocytes and macrophages (especially M1-like macrophages (185)) and play a pivotal role in obesity-related pathogenesis, including in the development of insulin resistance, and the metabolic syndrome (29, 184). The TLR family are activated by a vast array of ligands, many of which are higher in obesity such as LPS (a marker of gut permeability), oxidized low-density lipoproteins, and SFAs. The binding of a bioactive molecule to TLRs, results in the activation of NF-κB and the release of pro-inflammatory cytokines (3, 125, 184). The pivotal role of TLR4 in obesity-associated pathogenesis was demonstrated from the observations that TLR4 knockout mice were protected from the adverse effects of high-fat feeding with attenuated inflammation and macrophage infiltration (125, 184). In parallel with TLR4 knockout mice, exercise training resulted in similar metabolic improvements by decreasing the expression of TLR4 on the cell surface of monocytes and macrophages (151, 186-190); in some cases, TLR4 expression and activity was reduced by up to 35% following exercise interventions (190). In mice and rats, the reduced expression of TLR4 on the surface of the adipocytes and/or SVF cells following exercise correlates with the phenotypic shift in ATM from the M1 to the M2-like phenotype, and reduced macrophage infiltration (58, 139, 189, 191, 192). However, despite the decreased expression of TLR4 activity following exercise training in humans (149, 190, 193-199), more questions remain to be explored regarding the role of TLR4 in macrophages polarization and infiltration. To our knowledge, most human studies on TLR4 expression following exercise examined monocytes rather than ATM directly and results were sometimes inconclusive (200). Given that monocytes are precursor cells of macrophages, it is plausible that the decreased TLR4 expression may also

coincide with changes in the phenotype of macrophages as seen in rodents. Further investigations examining the effects of exercise on TLR4 expression in humans is required.

Overall, what remains unknown is which form of exercise training is best to mitigate ATM infiltration and phenotypes in obesity. Several studies indicate that higher intensities and combined training (e.g., combined aerobic and resistance training vs aerobic or resistance training alone) better improved obesity-associated inflammation (128, 201). However, the comparison of these training modalities has not been investigated in ATM infiltration. Future studies should further explore the mechanisms driving macrophage infiltration and polarization in response to exercise and focus on the training modalities (duration, type, volume, and intensity) that are best at mitigating ATM inflammation.

3.3 Bariatric surgery

Often times when first line treatment options such as dietary interventions and exercise programs are not enough to induce significant weight loss or metabolic improvements in individuals with obesity, many turn to bariatric surgery. Indeed, bariatric surgery is one of the most powerful tools to induce weight loss; a worldwide study from 31 countries found that the surgeries induced an overall 1 year-weight loss of about 30.5% (202). Aside from the effectiveness for weight loss, bariatric surgery is often accompanied with weight-loss-dependent metabolic improvements including the mitigation of ATM inflammation.

3.3.1 Effects of surgery on macrophage populations

Several studies observed significant reductions in macrophage number up to a year after surgery using the CD68 marker (42, 203-206) (see Table 4). Cancello et al. (42) found a ~12% reduction in the number of ATM after surgery which is likely due to the decreased expression of

chemotactic genes. Bariatric surgery was also found to alter the phenotype of macrophages favouring a shift towards M2-like macrophages (207-213) (see Table 4). Aron-Wisnewsky et al. (207) found that in pre-menopausal women without diabetes, the ratio of M1:M2-like (CD40+:CD206+) macrophages was 2-fold lower in subcutaneous adipose tissue (SAT) after 3 months than before surgery due to a simultaneous decrease of CD40+ and an increase of CD206+ macrophages. Similarly, others have found an increased presence of M2 over M1-like macrophages in the adipose tissue up to 12 to 24 months after surgery (210, 211). Altogether, these studies suggest that it is likely that the immune and inflammatory profile of bariatric surgery patients may take years to reach new baseline levels. Overall, robust evidence indicates quantitative and qualitative changes in ATM populations following weight loss surgery.

3.3.2 Mechanisms affecting macrophage infiltration and polarization after bariatric surgery

Weight loss by bariatric surgery decreases expression of pro-inflammatory cytokines and chemotactic signals

An extensive amount of research has studied how bariatric surgery affects cytokine-related macrophage chemotaxis and polarization. Although unclear, current literature suggests that bariatric surgery may improve the inflammatory status of individuals with obesity. Two recent reviews (28, 214) listed several cytokines and their variations at different time points after bariatric surgery. An example being that CRP and leptin unanimously decreased, adiponectin constantly increased and TNF-α remained unchanged at all time points after surgery. As for the other highly expressed cytokines during obesity such as IL-1β, IL-6, IL-10 and MCP-1, results are inconsistent even up to two years post-operation. For instance, 3 months after surgery, Xu et al. (215) observed improved insulin sensitivity, increased AMP-activated protein kinase (AMPK) expression and decreased oxidative stress with no changes of IL-1β, TNF-α, and IL-10 in

patients. Such discrepancies were hypothesized to be the result of the pre-surgical presence of diabetes and the baseline level of insulin-sensitivity (216-218). For example, a greater CRP reduction was observed after surgery in ex-obese diabetic patients compared to those who were not diabetic (218). Moreover, the inflammatory state of visceral adipose tissue and the patients' nutritional status pre-surgery were also suggested to influence the postsurgical inflammation state (214). Overall, the inconsistent effects of surgically induced weight loss on cytokine fluctuation remain unexplained due to a lack of convincing results. The long-term effects of cytokine secretion on health of individuals with obesity after surgery are unknown.

Effects of surgery on adipocyte morphology

Appreciable weight loss after the bariatric surgery results in extensive adipose tissue remodelling on multiple levels, implicating mechanisms underlying adipose tissue plasticity. The architecture and homeostasis of the adipose tissue and the cells composing the SVF are tightly regulated by the equilibrium between hypertrophy and hyperplasia which may be improved following weight loss resulting in diminished macrophage-mediated inflammation. Several studies analyzing either the volume or the area of the adipocytes found that post-surgery fat cells were smaller, ultimately approaching measurements similar to lean controls (203, 206, 219-221). For example, Casmatra et al. (222) and Löfgren et al. (223) reported a post-surgical reduction of fat cell area by 50% and volume by 43%, respectively. Additionally, Andersson et al. (224) reported significant adipocyte volume loss after surgery, but with no changes in cell number. Thus, suggesting that adipocyte atrophy is the main plastic event taking place during weight loss induced by surgery.

Effects of surgery on angiogenesis in adipose tissue

Adipose tissue expansion is intricately dependent on vasculature which is increased during obesity. Indeed, angiogenesis is a response to adipose tissue hypoxia that results from its expansion and poor blood supply. As such, angiogenic markers like vascular endothelial growth factor (VEGF), angiopoietin-1 (ANG-1), ANG2, tyrosine-protein kinase receptor Tie-2 (Tie-2) and HIF-1α are overexpressed in obesity which potentiate pro-angiogenic responses to improve tissue blood supply, inflammation, and ultimately adipocyte dysfunction (225). Bariatric surgery may induce significant reduction of these angiogenic markers while concomitantly decreasing the recruitment of M1-like macrophages. Weiwiora et al. (226) studied the levels of circulating angiogenesis biomarkers (ANG-2, granulocyte colony-stimulating factor (G-CSF), hepatocyte growth factor (HGF), platelet endothelial cell adhesion molecule-1 (PECAM-1), VEGF and follistatin) preoperatively and 12 months after surgery in 24 patients with class III obesity. The expression levels of these angiogenic markers were all downregulated post-surgery and their changes were dependent upon the amount of weight loss. Similarly, Figueroa-Vega et al. (225) found that before surgery the levels of pro-angiogenic markers (ANG-1, ANG-2, Tie-2 and HIF-1α) were overexpressed (both in serum and adipose tissue), which correlated with an increased number of infiltrating M1-like macrophages expressing angiogenic receptor Tie-2 especially in SAT (225). At 6 months after surgery, the expression of these markers was significantly reduced and correlated with a diminished number of infiltrating M1-like macrophages (225). Therefore, angiogenesis may not only be important for adipose tissue expansion, but it may also represent another pathway to explain the chronic inflammation observed in obesity, which is alleviated by weight loss surgery. However, the knowledge on angiogenic mechanisms and its impact on

adipose tissue dysfunction and health post-surgery is still rudimentary and requires more research.

Effects of surgery on adipose tissue fibrosis

Fibrosis is a hallmark feature of adipose tissue inflammation as it is triggered and exacerbated by macrophage infiltration (9, 227). However, the reversibility of adipose tissue fibrosis after surgery-induced weight loss is unclear. To our knowledge, only two studies have directly examined fibrosis pre and post bariatric surgery and both have concluded that levels of fibrosis remained unchanged and persisted despite the significant weight loss in most participants from 6 months up to 2 years after surgery (206, 228). In contrast, Liu et al. (210) and Reggio et al. (229) observed a downregulation of expression levels of genes encoding markers of adipose tissue fibrosis from 6 months to a year post-surgery (210, 229). Moreover, Liu et al. (210) observed a positive relationship between collagen accumulation and the number of M2-like (CD163+) cells prior to surgery, indicating a role in the generation of fibrosis in obese SAT. However, this M2-to-pericellular collagen accumulation relationship became a negative correlation at the 1 year-follow up despite the moderate increase in the number of CD163+ cells. Overall, the evolution of fibrosis post-surgery, the role played by ECM proteins and their link with ATM during weight loss are poorly documented.

4. Conclusion and Future Prospects

In this review, we discussed the impact of exercise, dietary fatty acids and bariatric surgery on cellular characteristics affecting ATM presence and phenotypes in obesity. We have shown that exercise training, dietary fatty acids and bariatric surgery decrease ATM and induce a phenotypic switch to M2-like macrophages through modifying a number of potential mechanisms. In the case of exercise and type of fat ingested, improvements in ATM occurred regardless of weight loss. These interventions modify ATM by affecting key adipose tissue characteristics such as adipocyte size, adipose tissue fibrosis, angiogenesis, and cytokine and adipokine secretion. Future studies should focus on gaining a better understanding of the underlying mechanisms and consequences of the reduction in macrophage presence and phenotypes, especially in humans. Understanding the series of events characterizing obesity pathogenesis may allow for the development of potential new therapies against obesity and its associated diseases.

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Table 1.1. The effect of saturated fatty acid-rich diets on macrophage infiltration and polarization in rodent and human studies

Reference	N	Rodents or participants	Diet	Weight change	Macrophage/phenotype change
Coenen et al. (49, 50)	48 and 58	C57BL/6 mice	• Western diet (42% fat + 0.15% cholesterol) vs. Control diet • 12 weeks.	↑	↑ infiltration of macrophages
Davis et al. (52)	75	control male C57BL/10J mice and male Tlr-4-deficient C57BL/10ScN mice	• 3 experimental diets: Low-fat control (LFC) vs. High-fat control (HFC) vs. High-fat palmitate (HFP). • 16 weeks	↑	↑ % of macrophages
Enos et al. (53)	45	Male C57BL/6 mice	• 5 treatment diets: 2 control diets vs. 3 HFDs (6%-SF, 12%-SF, and 24%-SF). • 16 weeks	↑ to a greater extent in the 12%-SF group	↑ M1 ↑ M2 ↑ infiltration of macrophages
Prieur et al. (55)	36	wild type C57BL/6 male mice and *ob/ob* mice	• HFD (45% fat) vs. Control diet (11.5% fat) • 12 weeks.	Ø	↑ M1 ↓ M2
Cullberg et al. (51)	Ø	Cell culture	• *In vitro*: 3T3-L1 adipocytes and THP-1 macrophages were incubated for 24 h with FFAs (oleic, palmitic and elaidic acid)	Ø	↑ 1.8-fold M1
Nguyen et al. (54)	40	• wild type male C57BL/mice and *ob/obJ* male mice • Cell culture	• HFD (40% fat) vs. Control diet (12% fat) • For 1, 12, or 20 weeks • In vitro: RAW264.7 cells were cultured and treated with FFA (arachidonic, lauric, linoleic, oleic, and myristic acids)	Ø	↑ M1 ↑ infiltration of macrophages

Van Dijk et al. (65)	20	Abdominally overweight middle-aged adults (10 male and 10 female)	• 2 experimental diets: • SFA-rich diet (19% SFAs and 11% MUFAs) vs. MUFA-rich diet (11% SFAs and 20% MUFAs) • 8 weeks	Ø	↑ M1 ↑ M2

FFA: free fatty acids; HF: High fat; HFD: High-fat diet; SF: Saturated fat; lean (BMI ≤ 24.9); overweight (BMI 25– 29.9); class I (BMI 30– 34.9); class II (BMI 35– 39.9); class III (BMI ≥ 40) ↓: significant decrease; ↑: significant increase; Ø: no significant change or not applicable

Table 1.2. The effects of n-3 PUFAs on macrophage infiltration and polarization in rodent and human studies.

Reference	N	Rodents or participants	Diet	Weight change	Macrophage/phenotype change
Bashir et al. (91)	25	• Male C57BL/6J mice	• 3 experimental diets: Control diet vs. HFD group (60% fat) vs. HFD + flaxseed oil (4, 8 or 16 mg/kg b.w.) • 18 weeks	↓	↓ M1 ↑ M2
Fan et al. (95)	47	• Male C57BL/6J mice	• 3 experimental diets: HFD with ALA-enriched butter vs. HFD with butter lacking ALA and LA vs. HFD with ALA and LA-enriched margarine • 10 weeks	Ø	↓ M1 ↑ M2 ↓ infiltration of macrophages
Lopez-Vicario et al. (97)	46	• wild type male mice and male hemizygous fat-1 mice	• 3 experimental diets: Control diet (13% fat) vs. HFD + placebo (60% fat) vs. HFD + sEH inhibitor • 16 weeks	↑	↑ M2 ↓ infiltration of macrophages
Titos et al. (98)	37	• male C57BL/6J mice	• Control diet (13% fat) vs. HFD (60% fat) • Animals then received a placebo or DHA (4 µg/g b.w.) every day for 10 days • 12 weeks	Ø	↓ M1 ↑ M2 Ø total ATM
Todoric et al. (99)	49	• Male C57BL/KsJ-lepr^{db}/lepr^{db} diabetic (*db/db*) mice and non-diabetic mice (*db/+*)	• 4 experimental diets: Control diet vs. HFD + SFAs + MUFAs vs. HFD + n-6 PUFAs vs. HFD + marine n-3 PUFAs • 6 weeks	↑	↓ M1 ↓ infiltration of macrophages

White et al. (100)	Ø	• Not specified	• Control diet vs. HFD (55% fat) • 8 weeks *Transgenic expression of *fat-1* n-3 fatty acid desaturase was used to endogenously produce n-3 fatty acids in HF-fed mice.	Ø	↓ infiltration of macrophages ↓ number of crown-like structures
Chan et al. (92)	Ø	• Cell culture	• low fat diet (10% fat) vs. HFD (60% fat) • 18 weeks • *In vitro*: bone marrow-derived macrophages were cultured with palmitate or palmitoleate.	Ø	↑ M2 ↓ M1
Chang et al. (93)	Ø	• Cell culture	• *In vitro*: Murine macrophage and human T lymphocyte were co-cultured and treated with DHA.	Ø	↑ M2 ↓ M1
Colson et al. (94)	24	• male C57BL/6J mice • Cell culture	• n-6-enriched control diet (12% fat) vs. n-3-enriched control diet (12% fat). • 12 weeks • In vitro: THP-1 cells were cultured for differentiation experiments	Ø	↑ M2 Ø M1
De Boer et al. (102)	32	• male and female C57BL/6 mice • Cell culture	• 4 experimental diets: HF control diet (34% fat) vs. HFD + FO (34% fat + 7.6% FO) vs. Low fat control diet (10% fat) vs. Low fat + FO (10% fat + 3% FO) • 12 weeks. • In vitro: macrophages were co-cultured with adipocytes	↑	↓ M1

De Boer et al. (103)	10	• male C57BL/6 mice • Cell culture	• Control diet (10% SO) vs. LC n-3 PUFA-enriched diet (3% FO + 7% SO) • 4 weeks • *In vitro*: Visceral adipose tissue were collected to create adipose tissue conditioned media and challenged with LPS to mimic acute and chronic conditions.	↑	↓ M1 ↓ M2
Liddle et al. (96)	30	• male and female C57BL/6 mice • Cell culture	• Control diet (10% SO) vs. Treatment diet (7% SO + 3% FO) • 4 weeks • *In vitro*: RAW264.7 macrophages were co-cultured with LPS-stimulated CD8+ T cells/adipocytes	Ø	↓ M1 ↑ M2
Baranowski et al. (26)	21	• male *fa/fa* Zucker rats and 7 lean Zucker rats	• Control diet vs. ALA-rich flaxseed oil diet • 8 weeks	Ø	Ø macrophage infiltration among groups
Itariu et al. (86)	55	• nondiabetic adults with class III obesity	• 3.36g long-chain n-3 PUFAs/day vs. 5g of butter/day in addition to an isocaloric diet (55% carbohydrates, 15% protein, and 30% fat) • 8 weeks	Ø	↓ M1 Ø M2 Ø total ATM and infiltration
Kratz et al. (104)	24	• individuals with overweight to class I obesity (8 males and 16 females)	• Control diet (0.5% n-3 PUFAs) vs. n-3 PUFA-rich diet (3.5%) • 14 weeks	↓ (minor and similar weight loss in both groups)	Ø macrophage phenotypes and infiltration

Spencer et al. (90)	33	• adults with class I obesity (11 males and 22 females) • Cell culture	• 4g of n-3 fatty acid ethyl esters vs. placebo (corn oil) • 12 weeks. • *In vitro*: M1 macrophage culture and M1 macrophage co-cultured with adipocytes were treated with DHA	Ø	↓ total ATM ↓ crown-like structures DHA decreased MCP-1 expression in cultured M1 macrophages and in co-cultures of macrophages and adipocytes.
Ferguson et al. (85)	Ø	• Cell culture	• *In vitro*: Human SAT from lean and obese subjects were treated with EPA and/or DHA throughout differentiation or for 72 h post-differentiation • THP-1 monocytes were added to adipocyte co-cultures	Ø	During monocyte to macrophage differentiation, EPA, and DHA: ↑ M2 ↓ M1
Pandurangan et al. (88)	Ø	• Cell culture	• *In vitro*: Human adipocytes and macrophages were co-cultured and treated with chia seed fatty acid (0–6.4µg/mL)	Ø	↓ M1 ↓ macrophage recruitment
Montserrat-de la Paz et al. (87)	6	• healthy adult males • Cell culture	• Participants were all given 3 times a meal rich in SFAs, in MUFAs or in MUFAs + omega-3 LC PUFAs with or without niacin. • *In vitro*: Monocytes were isolated to be differentiated into naïve macrophages; TLRs were also isolated.	Ø	↓ M1 ↑ M2

ALA: α-linoleic acid; FO: Fish oil; LA: Linoleic acid; LC: Long chain; SAT: Subcutaneous adipose tissue; sEH: Soluble epoxide hydrolase; SO: Safflower oil; TLR: Toll-like receptor; %fat expressed based on total energy; lean (BMI ≤ 24.9); overweight (BMI 25– 29.9); class I (BMI 30– 34.9); class II (BMI 35– 39.9); class III (BMI ≥ 40); ↓: significant decrease; ↑: significant increase; Ø: no significant change or not applicable

Table 1.3. The effects of exercise on macrophage infiltration and polarization in rodent and human studies

Reference	N	Rodents or participants	Exercise intervention	Weight change	Macrophage/phenotype change
Kawanishi et al. (58, 136)	40	Male C57BL/6 mice	• Treadmill running • 12-20m/min x 60 min/day • 16 weeks	Ø	↓ M1 ↑ M2 ↓ number of macrophages
Macpherson et al. (138)	27	Male C57BL/6 mice	• Treadmill running • 3-day x 15 min/day at 15m/min acclimation • 2h at 15m/min with 5% incline	Ø	↓ M1 ↑ M2 ↓ infiltration of M1-like macrophages
Linden et al. (137)	113	Male C57Bl6/J mice	• Treadmill running • 40 min/day at 12m/min with 8% incline • 4, 8 or 12 weeks.	Ø	↓ M1 ↑ M2 ↓ infiltration of macrophages
Luo et al. (140)	54	Male C57BL/6H mice	• Treadmill running • 45% of peak running speed, with 5% incline, 1h/day, 6 days/week • 8 weeks	↓	↓ M1 ↑ M2
Baek et al. (141)	49	Male C57BL/6 J mice	• Treadmill running at 10m/min for 60 min. • Mice ran at different intensities from week 2 to week 8	↓	↓ M1 ↑ M2
Oliveira et al. (139)	24	Male Wistar rats	• Swimming • 2-day swimming x 10 min/day acclimation • 3h of exercise with a 45 min rest period	Ø	↓ M1 ↑ M2 Ø in infiltration
Kolahdouzi et al. (59)	48	Male Wistar rats	• Treadmill running • 5 days/week • 3 groups: Sedentary vs. CT vs. AIT • 10 weeks	CT: ↓ 30% weight loss AIT: ↓ 40% weight loss	↓ M1 ↑ M2 ↓ number of macrophages

Shanaki et al. (142)	45	Male Wistar rats	• Treadmill running (HIIT or CT) • 5 days/week • 10 weeks	↓	↓ M1 ↑ M2
Bruun et al. (24)	27	Individuals with class III obesity (15 females and 12 males)	• 2–3 h of exercise • 5 days/week • 15 weeks • Included a diet	↓ ~ 14% weight loss	↓ ~55% M1 ↓ ~40% number of macrophages
Auerbach et al. (131)	60	Healthy and overweight adult men	• Endurance training for 7 days/week • 4 groups: Training-induced weight loss (T) vs. Diet-induced weight loss (D) vs. Training and increased diet without weight loss (T-iD) vs. Control (C). • 12 weeks	↓ 6% weight loss in the endurance training group (T)	↑ 2.5-fold M2 Ø macrophage number
Yakeu et al. (145)	17	Healthy overweight adults (9 males and 8 females)	• Walking on treadmill • 10,000 steps three times/week for 75 min • 8 weeks	Ø	↓ M1 ↑ M2
Ruffino et al. (144)	19	Overweight adult women	• Walking on treadmill • 3 times/week for 45 min • 8 weeks	↓	↓ M1 ↑ M2
Lee et al. (143)	26	Sedentary lean or overweight men with or without dysglycemia	• 2 sessions of strength training and 2 sessions of spinning • 4h/week • 12 weeks	↓	Ø M1 ↓ M2 ↓ infiltration of macrophages

AIT: Aerobic-interval training; CT: Continuous training; HIIT: high-intensity interval training; lean (BMI ≤ 24.9); overweight (BMI 25– 29.9); class I (BMI 30– 34.9); class II (BMI 35– 39.9); class III (BMI ≥ 40); ↓: significant decrease; ↑: significant increase; Ø no significant change or not applicable

Table 1.4. The effects of bariatric surgery on macrophage infiltration and polarization at different time points after surgery in human studies

Reference	**N**	**Participants**	**Macrophage/phenotype change**				
			≤ 1 mo.	**3 mo.**	**6 mo.**	**12 mo.**	**24 mo.**
Cancello et al. (42)	24	17 women with class III obesity and 7 lean women		↓ 12% ATM			
Aghamohammadzadeh et al. (203)	22	15 adults with class III obesity and 7 lean individuals			↓ ATM		
Haluzikova et al. (204)	32	17 women with class III obesity and 15 lean women			↑ATM surpassing baseline levels (before surgery)	↓ ATM	↓ ATM (levels similar to control)
Trachta et al. (205)	31	13 non-diabetic women with class III obesity and 18 lean women			↑ ATM	↓ ATM	In SAT: ↓ ATM (levels similar to baseline)
Liu et al. (210)	118	Individuals with class III obesity				↓ ATM ↑ M2	
Aron-Wisnewsky et al. (207)	26	16 women with class III obesity and 10 lean women		↓ M1 ↑ M2 ↓ 2-fold M1/M2			
Garcia-Rubio et al. (209)	71	43 individuals with class III obesity and 28 ex-morbidly obese individuals with class I obesity				↓ M1-like cells in SAT and VAT	

Cinkajzlova et al. (208)	83	32 non-diabetic individuals with class III obesity and 32 individuals with class III obesity and diabetes and 19 lean controls	In plasma: ↓ M2 In SVF of SAT: ↓ M2		In plasma: ↓ M2 In SVF of SAT: ↓ M2		
Moreno-Navarrete et al. (211)	6	Women with class III obesity and normal glucose metabolism					↑ M2
Hagman et al. (212)	17	Men and women with obesity class II and III	↑ M1 ↑ M2			↑ M1 ↑ M2	
Hess et al. (213)	40	20 individuals with class III obesity and 20 lean controls		↓ ~1.1% M1 ↑ ~10.1% M2			

ATM: Adipose tissue macrophages; SAT: Subcutaneous adipose tissue; SVF: Stromal vascular fraction; VAT: Visceral adipose tissue; lean (BMI ≤ 24.9); class I (BMI 30– 34.9); class II (BMI 35– 39.9); class III (BMI ≥ 40); ↓: significant decrease; ↑: significant increase; Ø: no significant change or not applicable

Bridge

In the previous chapter we found that exercise training, dietary fatty acids and bariatric surgery decrease ATM and induce a phenotypic switch to M2-like macrophages through modifying various mechanisms. These interventions affected ATM by shifting adipose tissue characteristics including adipocyte size, and adipose tissue fibrosis. As age and age of obesity onset influence the development of obesity-associated comorbidities, and as the underlying mechanisms are unclear, this next chapter is comprised of an original research article that further examines the effects of age vs age of obesity onset on ATM, adipocyte size, and fibrosis. Dysfunctional adipose tissue characterized by greater adipocyte hypertrophy, deposition of fibrotic depots and macrophage infiltration, have been shown to play a role in the development of cardiometabolic diseases. Moreover, research shows that SAT and VAT may contribute differently to disease risk due to differences in characteristics of these tissue beds. As such, it is plausible that age and age of obesity onset may each have their own effects on regional adipose tissue characteristics affecting the risk of obesity-related diseases. However, the relative contribution of age and age of obesity onset on adipocyte size, adipose tissue fibrosis and macrophage infiltration have never been examined. In addition, we also set out to investigate whether adipose tissue characteristics correlate with the markers of cardiometabolic health in each of our groups.

Chapter 2

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Adipocyte size, adipose tissue fibrosis, macrophage infiltration and disease risk are different in younger and older individuals with childhood and adulthood onset obesity

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Abstract

Background: Childhood- and adulthood-onset obesity and age have been shown to affect the risk of obesity-related comorbidities, but the underlying mechanisms are unclear.

Objective: The aim of this study is to determine whether differences in regional adipose tissue characteristics vary with age and age of obesity onset, and whether these differences are associated with the markers of cardiometabolic health.

Methods: Adipose tissue samples were from 80 female bariatric surgery candidates who were classified by age of obesity onset and age into 4 groups: 1) younger adults (<40 y) with childhood-onset obesity (<18 y) (CY), 2) younger adults with adulthood-onset obesity (>18 y) (AY), 3) older adults (>55 y) with childhood-onset obesity (CO) and 4) older adults with adulthood-onset obesity (AO). Adipocyte diameter, adipose tissue fibrosis and macrophage infiltration were determined in subcutaneous (SAT) and visceral adipose tissue (VAT). Clinical parameters were obtained from participants' medical records.

Results: Visceral adipocyte size in the CY group was the smallest of all the groups. Age affected visceral infiltration of M1-like cells in AO and CO, whereas onset, specifically childhood-onset, was more important for the visceral infiltration of M2-like cells in CO. Pericellular fibrosis accumulation in SAT and VAT varied with both age and onset, particularly in the CO groups having the lowest fibrosis levels. Markers of cardiometabolic health (fasted glucose, glycated hemoglobin, total, HDL- and LDL-cholesterol and triglycerides) were positively and well associated with adipose tissue characteristics of the CO group but not of the AY group.

Conclusion: Older adults with childhood onset obesity, who had the greatest duration of obesity exposure, were particularly vulnerable to the cardiometabolic effects associated with

perturbations in adipose tissue characteristics. These results, suggest that age and age of obesity onset have independent and cumulative effects on obesity pathology.

Keywords: Obesity, Age, Age of obesity onset, Adipose tissue characteristics, Cardiometabolic diseases

1. Introduction

In obesity pathology, the patterns of disease development in individuals of equal adiposity are heterogeneous and unclear. Research has shown that individuals with childhood-onset obesity are at greater risk for obesity-associated comorbidities (17, 230). Accordingly, those with persistent overweight and obesity from childhood into adulthood have a greater risk of developing type 2 diabetes, hypertension, dyslipidemia, cardiovascular diseases, cancer, and premature death (15-17, 231). For example, in comparison to those who only became obese as adults, the persistence of overweight from childhood results in more than twice the risk of developing diabetes and coronary heart disease (16). This increased risk of obesity-associated comorbidities may partially stem from fundamental differences in adipose tissue characteristics. Not only do those with childhood-onset obesity seem to have smaller adipocytes (232-234), we also have reported differences in adipose tissue markers of cellular metabolism compared to those with adulthood-onset obesity (235). Similar to obesity, as we age, we are more likely to develop chronic morbidities, such as cardiovascular diseases, diabetes, and cancer (236, 237). There are many parallels between obesity and aging from the whole body to the cellular levels (18). Both age and age of obesity onset could have independent or compounded effects in the development of obesity-related comorbidities. The relative contribution of age versus age of obesity onset has never been examined.

Obesity-associated comorbidities arise as a consequence of excess adipose tissue accumulation causing morphologic and functional abnormalities. However, fundamental differences exist between adipose tissue of different anatomical locations. Indeed, it is well established that the accumulation of visceral adipose tissue (VAT) mass is more strongly associated with obesity complications than subcutaneous adipose tissue (SAT) (238-240). Subcutaneous and visceral

adipose tissue have distinct cellular characteristics and inflammatory signatures that may affect disease risk differently (241). With adipose tissue expansion, adipocyte hypertrophy may act as a catalyst for cellular damage concomitant with pro-inflammatory responses including macrophage infiltration (4, 242). Consequently, the aggregation of adipose tissue macrophages exacerbates inflammation and is associated with adipose tissue maladaptation, particularly the production of extracellular matrix proteins leading to adipose tissue fibrosis (9, 243). Accordingly, several studies identified depot differences in adipocyte size, adipose tissue fibrosis and macrophage infiltration which may partially explain the differential pathogenic potential of SAT and VAT (241, 244). As no study has yet compared how age versus age of obesity onset affect these characteristics, the objective of this study is to determine how adipocyte size, macrophage infiltration and adipose tissue fibrosis in SAT and VAT vary with age and age of obesity onset. We will also examine whether these differences are associated with the markers of cardiometabolic health.

2. Methods

2.1 Study participants

Adipose tissue samples were obtained from 80 females undergoing bariatric surgery at the Quebec Heart and Lung Institute (IUCPQ; Université Laval, Quebec City, Canada), who had consented to be tissue donors for the IUCPQ Obesity Tissue Bank and who were selected to be in one of the following groups: 1) younger adults (<40 y) with childhood-onset obesity (<18 y) (CY), 2) younger adults with adulthood-onset obesity (>18 y) (AY), 3) older adults (>55 y) with childhood-onset obesity (CO) and 4) older adults with adulthood-onset obesity (AO). Participants across the 4 groups were matched for BMI and diabetes prevalence. Within each of the young and older aged groups participants were matched for age. The age of obesity onset was

determined via self-report. BMI, weight, fasted glucose, glycated hemoglobin (HbA1c), total cholesterol, HDL-C, LDL-C, and triglycerides were obtained from medical records. Written informed consent was obtained from all participants. The study was approved by the Human Research Ethics Committee of Concordia University and by the Comité d'éthique de la recherche de l'IUCPQ of Laval University.

2.2 Experimental procedures

Preparation and fixation of adipose tissue samples

Flash frozen visceral and abdominal subcutaneous adipose tissue samples collected via surgical excision were thawed and fixed in 10% formalin at 4°C, as previously described (245). Once fixed, adipose tissue were paraffin embedded, and histological slides were prepared for staining and immunohistochemistry protocols.

Adipocyte diameter measurements

Adipose tissue sections were stained with hematoxylin-eosin (H&E) for adipocyte diameter measurements. Photographs of samples were taken at 20X magnification using an inverted microscope (Motic AE2000 TRI, Xiamen, China), with Moticam 580 5.0MP camera and Motic Images Plus 3.0 software. The mean adipocyte diameter of at least 300 cells per tissue sample was acquired using ImageJ, as previously described (245).

Immunomorphological analysis of adipose tissue

Immunohistochemical detection of total macrophages, defined as CD68+ cells (1:50, Invitrogen, MA, USA), M1-like macrophages, as CD68+CD11c+ cells (1:100, Invitrogen, MA, USA) and M2-like macrophages, as CD68+CD163+ cells (1:100, Ebioscience, CA, USA) were performed

using the dual immunostaining method as previously described (246). The photographs of 15 randomly chosen areas were taken at 20X magnification using a fully automated inverted fluorescence microscope (Leica DMI6000 B, Wetzlar, Germany) with the Leica Application Suite software (V3.8, Leica Microsystem, Switzerland). The total count of CD68+, CD68+CD11c+ and CD68+CD163+ cells and adipocytes were acquired using ImageJ until at least 300 adipocytes were counted. To facilitate macrophage counting, a 10% threshold was set in place within ImageJ to reduce the noise, background staining and auto-fluorescence. The mean number of positive macrophages was normalized for the number adipocytes and expressed as a percentage of macrophages.

Adipose tissue fibrosis measurements

Tissue sections were stained using picrosirius red for adipose tissue fibrosis quantification, as previously described (247). Pictures of 10 randomly chosen areas were taken using a phase light contrast microscope (Leica DM2000, Wetzlar, Germany) at 20X magnification. The quantification of pericellular fibrosis was performed using ImageJ software and expressed as a percentage of the area stained in red (fibrosis) in each field.

2.3 Statistical analyses

Statistical analyses were performed using JMP 16.0.0 (SAS Institute, Carry, NC, USA). All data were expressed as means ± standard error of the mean (SEM). Shapiro-Wilks's test was used to check for normality. One-way analysis of variance and Kruskal-Wallis test (for non-normal data) with Tukey-Kramer HSD and Wilcoxon post-hoc tests were used to examine the differences in metabolic parameters between groups. If data were not normally distributed, they were log10-transformed prior to using mixed model analysis of variance with least significant difference

(LSD) post-hoc test to compare adipose tissue characteristics between depot and groups. Pearson correlation coefficients were used to explore the relationships between adipose tissue characteristics and the markers of cardiometabolic health and between characteristics. Statistical significance was defined as $p<0.05$.

3. Results:

3.1 Participants characteristics

Participants' anthropometric measurements and metabolic characteristics are shown in **Table 2.1**. Per study design, groups were matched for BMI and diabetes prevalence, average age between the two younger (AY and CY) and older groups (AO and CO) was different. Between the two younger and older groups age was not different. Average fasted blood glucose between the two older groups and younger groups was different ($p=0.014$). Though average HbA1c was lower ($p=0.004$) in the AY than in the 3 other groups, there were no differences in HbA1c between the CY, CO, and AO groups. Average LDL-C was greater ($p=0.004$) in the AY vs CO and AO groups. Total cholesterol, HDL-C and triglycerides concentrations were not different between the 4 groups.

3.2 Adipocyte size

Representative images of adipocyte size for SAT and VAT are shown in **Figure 2.1A** and **2.1B**. On average, visceral adipocytes were 17% smaller ($p<0.001$) compared to subcutaneous adipocytes. Adipocyte diameter in SAT was greater ($p<0.001$) than in VAT in every group (**Figure 2.1C**). Additionally, a main effect of group ($p=0.034$) was also observed, with post hoc tests further revealing that the diameter of visceral adipocytes from the CY group was smaller than those in the AY and AO groups ($p=0.004$ and 0.032, respectively). There was also a trend

(p=0.069) for visceral adipocytes in the CY group to be smaller than those in the CO group. Though not significant, there was a trend (p=0.099) for the subcutaneous adipocytes from CY to be smaller than those from the AY group.

Table 2.1: Clinical characteristics in young and older subjects with childhood- or adulthood-onset obesity

	Childhood onset - young adults (CY) (n = 20)	**Childhood onset - old adults (CO) (n = 20)**	**Adulthood onset - young adults (AY) (n = 19)**	**Adulthood onset - old adults (AO) (n = 20)**
Age	34.9 ± 0.67^{bd}	59.6 ± 0.76	34.7 ± 1.00^{bd}	59.3 ± 0.68
BMI (kg/m2)	48.2 ± 1.25	48.2 ± 1.26	48.7 ± 1.03	48.5 ± 1.25
Fasted blood glucose (mmol/L)	6.46 ± 0.46	7.15 ± 0.47^{a}	6.56 ± 0.57^{b}	6.54 ± 0.20^{ac}
HbA1c (%)	6.09 ± 0.16	6.43 ± 0.29	5.83 ± 0.17^{ab}	6.19 ± 0.14^{c}
Total cholesterol (mmol/L)	4.53 ± 0.14	4.51 ± 0.23	4.80 ± 0.15	4.45 ± 1.83
HDL-C (mmol/L)	1.31 ± 0.071	1.39 ± 0.058	1.25 ± 0.082	1.32 ± 0.080
LDL-C (mmol/L)	2.53 ± 0.12	2.34 ± 0.22^{c}	2.90 ± 0.12	2.40 ± 0.17^{c}
Triglycerides (mmol/L)	1.52 ± 0.14	1.72 ± 0.14	1.44 ± 0.13	1.61 ± 0.16

Abbreviation: BMI: body mass index; HbA1c: glycated hemoglobin; HDL: High-density lipoprotein; LDL: low-density lipoprotein; Data are represented as mean ± SEM; Differences between groups were assessed with Tukey-Kramer HSD and Wilcoxon post-hoc tests; a vs. CY; b vs. CO; c vs. AY; d vs. AO, ($p < 0.05$)

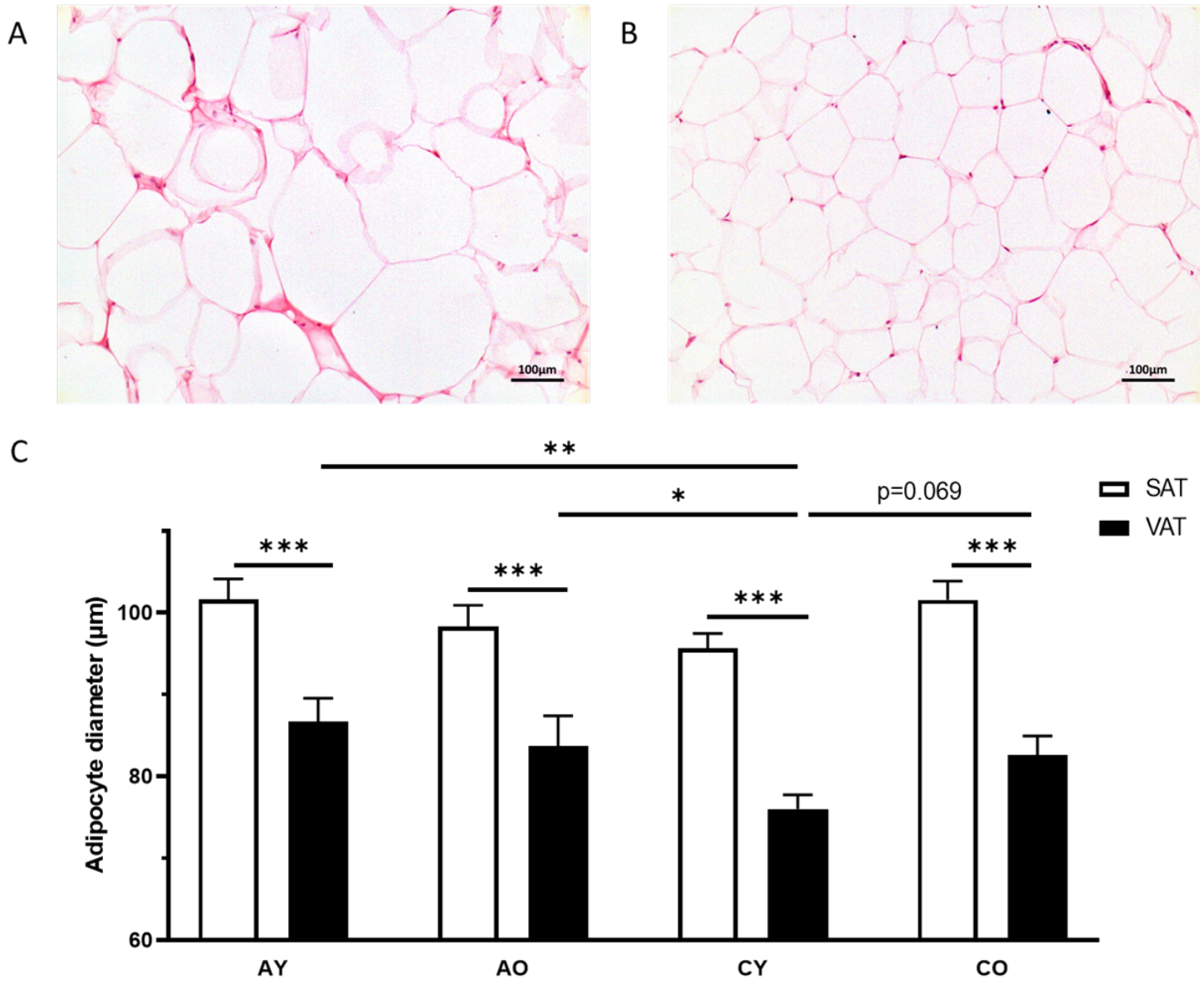

Figure 2.1: Representative images of depot-dependent hypertrophy of adipocytes in SAT **(A)** and VAT **(B)** at 20X magnification. Comparison of adipocyte diameter in subcutaneous (SAT) and visceral adipose tissue (VAT) depots from young and old individuals with either childhood- or adulthood-onset obesity **(C)**. Comparisons between groups were made using mixed model ANOVA followed by post hoc tests (n = 20/depot). Data are presented as ± SEM. Statistical significance was assessed by LSD post hoc test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

3.3 Regional adipose tissue macrophages

Figure 2.2A shows a representative image for the staining of CD68+, CD11c+ and CD68+CD11c+ cells. In line with the literature, overall VAT contained more macrophages of all phenotypes than SAT (CD68+: 14.0 ± 0.48% vs 15.8 ± 0.5%, $p<0.001$; CD68+CD11c+: 8.3 ± 0.34% vs 10.0 ± 0.41%, $p<0.001$; CD68+CD163+: 5.2 ± 0.28% vs 6.2 ± 0.33%, $p=0.001$). Percent CD68+ cells were greater in VAT vs SAT in the AY and AO ($p=0.042$ and 0.004, respectively) groups but not in the CY and CO groups (**Fig. 2.2B**). Additionally, there was a trend where there were more ($p=0.084$) %CD68+ cells in VAT than in SAT in the CO group. In both the older adult groups, %CD68+CD11c+ cells (M1-like) in VAT were greater than in SAT ($p=0.007$ and 0.012, AO and CO, respectively, **Fig. 2.2C**). Percent CD68+CD163+ cells (M2-like) was higher ($p=0.044$) in VAT than SAT of the CO group only (**Fig. 2.2D**). There was also a trend where there were more %CD68+CD163+ cells in VAT than in SAT in the CY group ($p=0.093$). Collectively these results indicate that both age and age of onset may factor into differences in adipose tissue macrophages between depots.

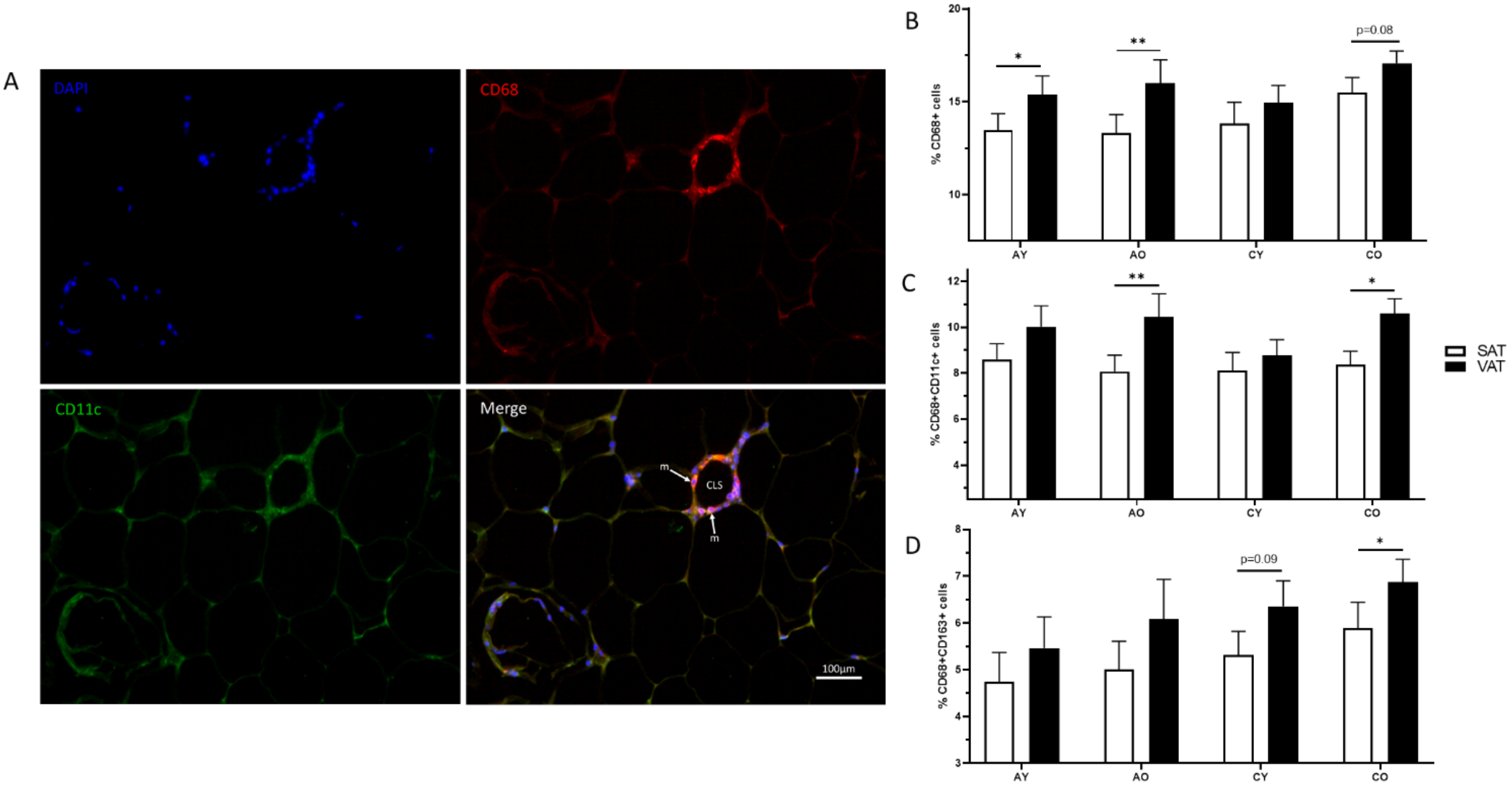

Figure 2.2: Representative detection of immunofluorescence staining of CD68+, CD11c+ and CD68+CD11c+ macrophages in VAT at 20X magnification. The arrows point towards several examples of CD68+CD11c+ macrophages, "m" indicates macrophage, and "CLS" indicates a crown-like structure **(A)**. Comparison of CD68+ **(B)**, CD68+CD11c+ **(C)** and CD68+CD163+ **(D)** macrophages in subcutaneous (SAT) and visceral adipose tissue (VAT) depots from young and old individuals with either childhood- or adulthood-onset obesity. Comparisons between groups were made using mixed model ANOVA followed by post hoc tests (n = 20/depot). Data are presented as ± SEM. Statistical significance was assessed by LSD post hoc test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

3.3 Adipose tissue fibrosis

Figure 2.3A and **2.3B** show representative images for the staining of pericellular fibrosis in SAT and VAT. Overall, pericellular fibrosis accumulation was on average 33% higher in SAT than in VAT ($p<0.001$). There was more ($p<0.01$) pericellular fibrosis in SAT than in VAT in all groups with the exception of the CO group (**Fig. 2.3C**). There was also a group*depot interaction ($p=0.032$, **Fig. 2.3C**) with post hoc test further indicating that the greater accumulation of fibrosis in SAT vs VAT in the AY, AO, and CY groups was different to the accumulation of fibrosis in the CO group, where no significant depot differences were observed.

3.4 Relationships between adipose tissue characteristics

In the AY group, subcutaneous pericellular fibrosis was associated with %CD68+ cells ($r=0.53$, $p=0.020$). In SAT of the AO group, greater fibrosis accumulation was associated with increased infiltration of CD68+ cells and larger adipocyte size ($r=0.48$, $p=0.034$ and $r=0.47$, $p=0.035$, respectively). We found in both the AO and CO groups that larger subcutaneous adipocyte size was related with more %CD68+ cells ($r=0.57$, $p=0.008$ and $r=0.45$, $p=0.045$, respectively). In VAT, larger adipocyte size was positively related to %CD68+CD163+ cells ($r=0.47$, $p=0.038$) in the AO group and negatively with %CD68+CD11c+ cells ($r=-0.47$, $p=0.036$) in the CO group.

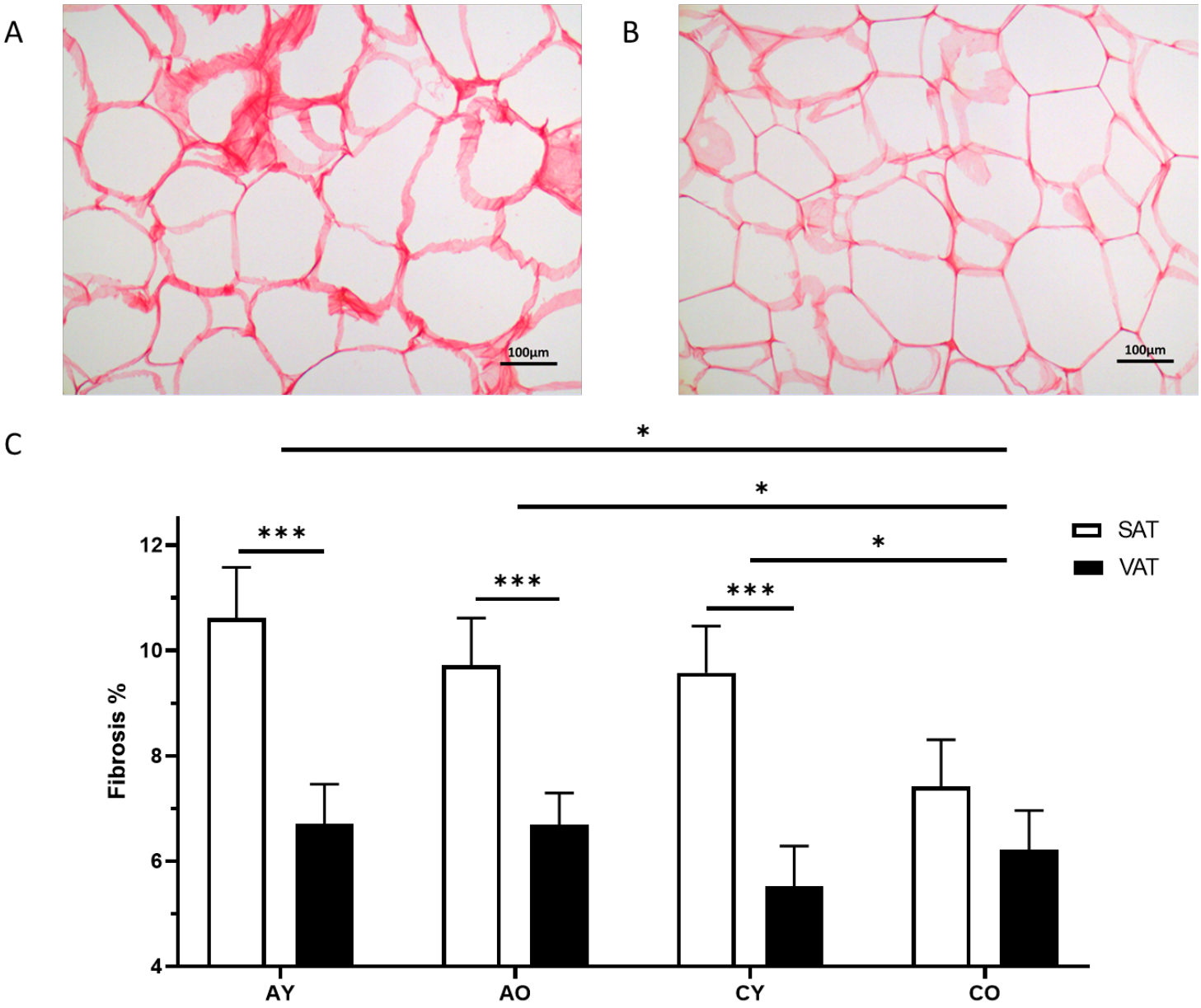

Figure 2.3: Representative images of pericellular fibrosis staining at 20X magnification in obese SAT **(A)** and VAT **(B)** Comparison of pericellular fibrosis in subcutaneous (SAT) and visceral adipose tissue (VAT) depots from young and old individuals with either childhood- or adulthood-onset obesity **(C)**. Comparisons between groups were made using mixed model ANOVA followed by post hoc tests (n = 20/depot). Data are presented as ± SEM. Statistical significance was assessed by LSD post hoc test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

3.5 Adipose tissue characteristics in relation with the markers of cardiometabolic health

The only correlation present (r=-0.46, p=0.048) in the AY group was between M2-like macrophages in VAT and HbA1c.

In the AO group, greater VAT cell size was accompanied (r=0.56, p=0.011) by higher HbA1c values. Both CD68+ cells and M2-like macrophages in SAT and VAT were associated with triglycerides concentrations (CD68+ cells: r=0.58, p=0.007 in SAT and r=0.57, p=0.086 in VAT; CD68+CD163+ cells: r=0.47, p=0.037 SAT and r=0.50, p=0.028 in VAT).

In the CY group, larger VAT adipocyte size was related with higher triglycerides concentrations (r=0.59, p=0.006). VAT %CD68+ cells were positively associated with HDL-C (r=0.53, p=0.016) and fasting glucose (r=0.53, p=0.015). More M1-like cells in VAT were related with greater HDL-C and fasting glucose (r=0.51, p=0.020 and r=0.55, p=0.013, respectively).

Overall, the CO group had the most associations between the examined adipose tissue characteristics and the metabolic markers. In the CO group, as VAT cells size increased, so did fasting glucose (r=0.47, p=0.036) and HbA1c (trend: r=0.44, p=0.055). Complementary, positive trends were observed between SAT adipocyte size and HbA1c (r=0.44, p=0.055). M1-like cells in SAT were associated with total and LDL-cholesterol (r=0.65, p=0.002 and r=0.58, p=0.007, respectively). Greater fibrosis in SAT was accompanied with higher fasting glucose and HbA1c (r=0.58, p=0.008 and r=0.48, p=0.034, respectively). Greater fibrosis in VAT was also associated with higher fasting glucose (r=0.54, p=0.014).

4. Discussion:

Our results indicate for the first time that in females both age and age of obesity onset affect adipose tissue characteristics and that these factors may have a cumulative effect. Specifically, we found that, despite groups being BMI-matched, visceral adipocyte size was smaller in the CY than in the other groups. Adipose tissue macrophage infiltration was also greater in VAT than SAT in all but the CY group. Age seemed to play a factor in regional M1-like macrophage infiltration, whereas onset appeared to be more important with regards to regional M2-like macrophage infiltration. Additionally, both age and age of obesity onset affected VAT and SAT fibrosis. We find here that though these characteristics were not well associated with metabolic health parameters in AY, they were especially well associated in the CO group.

Norris et al. (248) showed that individuals with increased duration of obesity exposure had worse values for all cardiometabolic disease risk factors. Though age of obesity onset was considered, their study found a strong correlation between age of obesity onset and duration and thus, were unable to tease out the effects of age of obesity onset vs duration of obesity. Another longitudinal study (249), found that both age of onset and duration of obesity increased risk of type 2 diabetes. Our study extended these findings through examining younger vs older adults with either childhood or adulthood onset obesity. Having only developed obesity in adulthood, those in the AY group had the shortest duration of obesity exposure. In examining the relationships between our measured adipose tissue parameters and cardiometabolic markers, a single correlation was observed in the AY group. On the other hand, older adults with persistent obesity from childhood had the greatest duration of obesity exposure of all the groups. Interestingly, we found that adipose tissue characteristics were positively and well associated with several markers of cardiometabolic health in AO, CY and particularly CO. These correlations, as well as those of

the AY group, indicate that adipose tissue may become increasingly more pathogenic with greater lifetime exposure to obesity based on age and age of onset.

Adipocyte hypertrophy can be viewed as a pivotal event in adipose tissue dysfunction with both local and systemic consequences on health (20). Previous studies which examined adipocyte size in the context of obesity-onset showed that adipose tissue expansion in those with adulthood-onset was characterized by hypertrophy, whereas in those with childhood-onset, hyperplasia was the dominant mechanism underlying adipose tissue growth (232-234). These studies based their conclusions on measurements in SAT alone and in those who had an average age of 40. As for age, previous studies observed that adipocytes were shown to peak in size in middle-aged subjects with or without obesity compared to their younger counterparts (250-254). In our groups, subcutaneous adipocyte size was not significantly different between the older and the younger groups. However, we found that visceral adipocytes were especially sensitive to the effects of both age and age of onset. Visceral adipocytes of the CY group were the smallest compared to those with adulthood-onset obesity regardless of age groups and a similar trend was also observed with CO. Therefore, these results suggest that both age and age of obesity onset affect adipocyte size differently in different depots.

Aside from age and onset, adipocyte size was also associated positively with HbA1c, fasted glucose and triglycerides concentrations mainly in VAT of AO, CO, and CY groups. Such associations of visceral fat cell size with the cardiometabolic risk factors corroborate previous findings suggesting that adipocyte hypertrophy of VAT is more strongly associated with adverse metabolic changes than hypertrophy of SAT (20, 242, 244, 255-257). Therefore, these correlations support visceral adipocyte size as a potential biomarker for cardiometabolic diseases in individuals with obesity.

This is the first study to have examined both the effects of age of obesity onset and age on regional adipose tissue macrophage profiles. In obesity, adipose tissue macrophages are central to tissue stress and have wide ranging effects on health (258). We have previously reviewed several studies which show abundant adipose tissue macrophage infiltration in obesity, the extent of which varies according to depot anatomical location (259). Consistent with the current literature (207, 260-264), CD68+, CD68+CD11c+ and CD68+CD163+ cells were more abundant in VAT than SAT in individuals with obesity, which highlights the unique inflammatory signature of VAT. Our findings further indicate that adipose tissue macrophage infiltration (CD68+ cells) was greater in VAT than SAT in all but the CY group, and they were higher in the adulthood- than childhood-onset groups. Therefore, these results suggest that both age and age of onset may contribute to the infiltration of CD68+ cells.

Our results also show that age is a stronger indicator of regional M1-like macrophage infiltration than onset, whereas in M2-like macrophage accumulation, age and onset may be contributing factors. We found greater M1-like cell accumulation in VAT relative to SAT in the older groups only (AO and CO). To our knowledge, no prior studies have investigated macrophage content in both SAT and VAT of younger and older adults with obesity. In non-obese older mice, M1 macrophage content was found to be greater in SAT than VAT (265). In contrast, another group observed no regional differences even though the infiltration of CD11b+ cells was greater in comparison to younger animals (266). As for M2-like macrophages, ageing resulted in decreases in CD206+ cells in older vs younger mice in absence of obesity (254). Oppositely, we observed that M2 macrophages tended to be higher in CO in comparison to the other groups. In fact, though not significant, there appeared to be a stepwise increase in M2-like macrophages in SAT and VAT with AY having the least then AO, CY, and CO. Thus, indicating that ageing and onset

may contribute to increased infiltration of M2 macrophages in older adults with obesity despite being anti-inflammatory in nature. More research needs to be conducted to determine whether there are indeed differences in M2-like macrophage accumulation in these groups and the implications these differences may have in overall metabolism. Altogether, these findings suggest that both age and onset influence regional macrophage infiltration.

Adipose tissue fibrosis is a newly appreciated feature of adipose tissue dysfunction and potentially an important contributor to obesity-associated comorbidities (9). While it is generally accepted the deposition of extracellular matrix proteins leads to adipose tissue fibrosis which increases in obesity, depot-specific accumulation of fibrosis varies greatly between studies (220, 247, 267-269). While some studies have shown greater levels of pericellular fibrosis or markers of fibrosis accumulation in VAT (220, 247, 268), others have shown no differences (247) or the opposite (267, 269). Overall, our results indicate that pericellular fibrosis accumulation is greater in SAT than VAT.

We show for the first time that both age and age of obesity onset may play a role in adipose tissue fibrosis accumulation. In particular, there were no differences between depots in the CO group, whereas the other groups had more than twice the level of fibrosis in SAT than VAT. While the pathogenic potential of adipose tissue fibrosis is unclear, several studies hypothesized that the accumulation of pericellular fibrosis may be beneficial by limiting adipocyte hypertrophy in omental adipose tissue with concomitant systemic improvements (220, 247, 268). In contrast, other studies show that more adipose tissue fibrosis correlates with insulin resistance, considering fibrosis to be a maladaptive mechanism contributing to obesity-associated comorbidities (11, 12, 270, 271). Similarly, in this study we have shown that pericellular fibrosis in SAT correlated positively with HbA1c and fasted glucose in CO highlighting the pathogenic

potential of adipose tissue fibrosis. Additionally, in the groups with the highest levels of fibrosis (AO and AY), subcutaneous fibrosis was linked with increased infiltration of CD68+ cells which corroborate previous data indicating a close relationship between adipose tissue fibrosis and macrophage infiltration (10, 12, 220, 272). Overall, SAT fibrosis may be a potential biomarker for macrophage infiltration and obesity-associated comorbidities, but further research is required to fully distinguish the effects of age and onset on the regional accumulation of pericellular fibrosis.

A major strength of our study was that participants across groups were individually matched for sex, BMI, diabetes prevalence, and age (in the older and younger groups). This matching is advantageous as it eliminates cofounding variables that would otherwise bias our results. As characteristics of adipose tissue are depot specific, examination of both SAT and VAT allowed us to delineate depot differences, thus providing a more thorough analysis of the effects of age and age of onset on adipose tissue characteristics.

Sex is known to influence adipose tissue characteristics and body fat distribution. Since men tend to accumulate more visceral fat (244), future investigations in a male only groups would provide a larger picture of how age and onset affect adipose tissue characteristics. Though childhood and adulthood onset obesity were self-reported, the biological differences in adipocyte size were consistent with previous studies showing smaller adipocytes in those with childhood vs adulthood onset obesity. In general, self-reported body weight has been previously shown to be accurate within 5 to 10% (273, 274).

In summary, our study is the first to provide evidence that age and age of obesity onset have different effects of adipocyte size, macrophage infiltration and adipose tissue fibrosis. Older adults with childhood onset obesity, who had the greatest duration of obesity exposure, were

particularly vulnerable to the cardiometabolic effects associated with perturbations in adipose tissue characteristics. These results, suggest that age and age of obesity onset have independent and cumulative effects on obesity pathology. Further investigation should delve into defining adipose tissue characteristics in these groups and examine the mechanisms by which these characteristics affect obesity-associated comorbidities. Collectively, these observations facilitate our understanding of the heterogeneity of obesity and adipose tissue biology.

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Author Contributions: LT was responsible for optimizing the research protocols, methodology, formal and statistical analysis, interpreting results and writing of the manuscript. MFG was responsible for optimizing the research protocols. AT was responsible for the conceptualization of the study, methodology, funding and reviewing the manuscript. SS, as the principal investigator, was responsible for the conceptualization of the study, funding, reviewing, and editing of the manuscript.

Discussion and Conclusion

Obesity and its associated comorbidities are among the leading causes of hospitalization, thus exerting immense pressure on the Canadian healthcare system (275). Estimates of the economic burden of obesity in Canada ranges from $4.6 billion to $7.1 billion annually (276). As the prevalence for obesity is ever increasing, enhanced research and treatment are essential for the treatment and prevention of obesity and obesity-associated comorbidities. However, obesity is a heterogeneous condition with numerous factors that may affect the risk of developing cardiometabolic diseases (13, 238) and possibly the efficacy of obesity interventions. Therefore, it is critical to understand the mechanisms underlying the heterogeneity of obesity in order to adapt treatments to each individual's need.

The main objective of this thesis was to determine how adipose tissue characteristics vary in relation to lifestyle and surgical interventions, and age and age of obesity onset. This thesis demonstrates the mechanisms by which dietary fatty acids, exercise and bariatric surgery exert their anti-inflammatory effects at both local and systemic levels. Indeed, these obesity interventions decrease ATM content and induce a phenotypic switch to M2-like macrophages through modifying adipocyte size, adipose tissue fibrosis, angiogenesis, pro-inflammatory cytokine secretion and patterns of gene expression. Although cellular and metabolic improvements were observed in absence of weight loss, significant weight loss may also compound the salutary effects of these treatments.

Several factors may influence the effectiveness of obesity interventions. Being a heterogeneous condition, obesity does not present the same way in all individuals of equal adiposity which may affect disease risk and weight loss interventions. In examining the heterogeneity of obesity, we show for the first time that both age and onset influence adipocyte size, adipose tissue fibrosis,

and macrophage infiltration. More precisely, visceral adipocyte size varied with childhood-onset obesity and age, pericellular fibrosis was affected by adulthood-onset obesity and age and regional macrophage infiltration was affected by age and both onsets. These results support the notion that childhood- and adulthood-onset obesity are two different types of obesity with potentially different effects on health. Furthermore, in older adults with childhood-onset obesity but not in younger adults with adulthood-onset obesity, adipose tissue characteristics were positively and well associated with markers of cardiometabolic health. Therefore, suggesting that age and age of obesity onset have independent and cumulative effects on obesity pathology.

Collectively, this thesis indicates age and age of obesity onset are risk factors for obesity-associated comorbidities and should possibly be considered when prescribing interventions. Further studies may want to examine whether different approaches should be used in treating younger or older individuals with either childhood- or adulthood-onset obesity. Lifestyle and surgical interventions have been shown to be effective in reducing adipocyte size, adipose tissue fibrosis, and macrophage infiltration with or without significant weight loss. However, the ideal treatment for different obesity subtypes and how they might impact the effectiveness of lifestyle interventions is unknown. Future studies should focus on the mechanisms underlying the differences between childhood- and adulthood-onset obesity in old and young subjects and how different obesity subtypes may impact the efficacy of weight loss interventions.

The findings presented in this thesis are particularly significant as they confirm that age and age of obesity onset both affect adipose tissue characteristics. More importantly, they provide an important first step towards understanding the heterogeneity of obesity and the development of its associated comorbidities. Characterizing the differences between childhood- and adulthood-

onset obesity in younger or older individuals is critical in developing individualized treatment approaches for obesity and obesity-associated comorbidities.

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Appendix A

December 20, 2021

To whom it may concern,

The purpose of this letter is to confirm that Sylvia Santosa, co-author of the below mentioned manuscripts, agrees to the inclusion of these manuscripts in the thesis of Laurent Turner, MSc candidate.

Manuscripts to be included:

- Turner L, Santosa S. Putting ATM to BED: How Adipose Tissue Macrophages are affected by Bariatric surgery, Exercise, and Dietary fatty acids
- Turner L, Gauthier MF, Tchernof A, Santosa S. Adipocyte size, adipose tissue fibrosis, macrophage infiltration and disease risk are different in younger and older individuals with childhood and adulthood onset obesity

The candidate's role in writing this thesis was in writing the sections on how obesity interventions affect adipose tissue macrophage infiltration and phenotype and the independent effects of age and onset on adipose tissue characteristics. The candidate also coordinated the revision of the manuscript between co-authors. The candidate was fully responsible for the analysis of adipose tissue samples and statistical analysis of data. The candidate wrote the manuscript with guidance and feedback of the co-authors.

I, the co-author, agree that the candidate, Laurent Turner, include the above-mentioned manuscripts in his MSc thesis.

Sylvia Santosa

Appendix B

Other work done in conjunction with master's studies

Turner L, and Santosa S, Putting ATM to BED: How Adipose Tissue Macrophages are affected by Bariatric surgery, Exercise, and Dietary fatty acids, Advances in Nutrition, 2021, Volume 12, Issue 5, Pages 1893‑1910.

REVIEW

Putting ATM to BED: How Adipose Tissue Macrophages Are Affected by Bariatric Surgery, Exercise, and Dietary Fatty Acids

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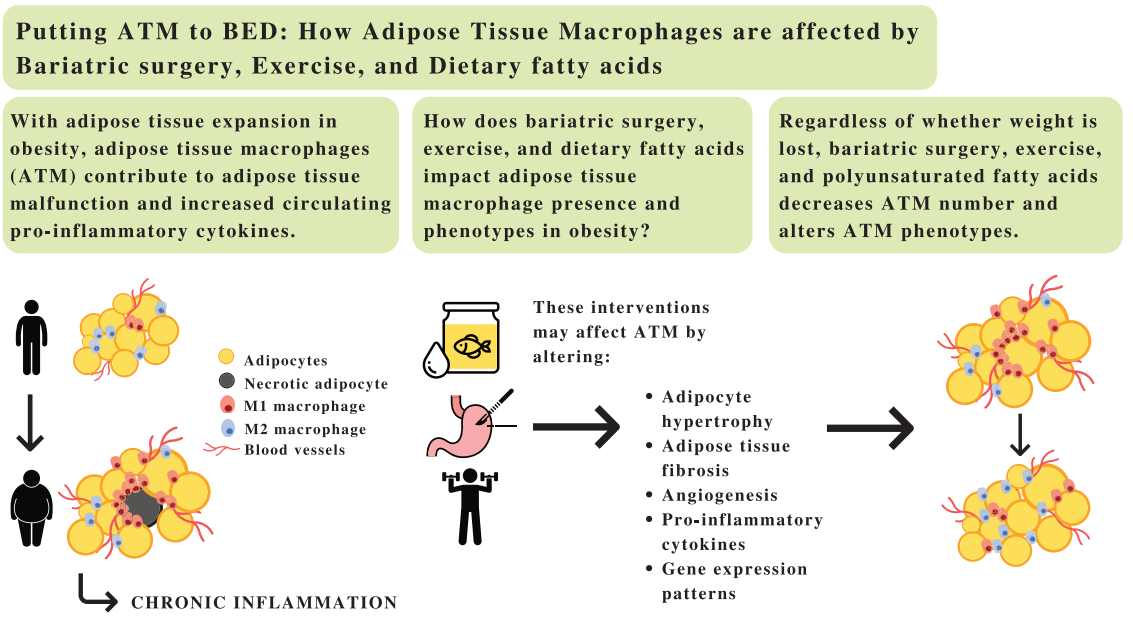
ABSTRACT

With increasing adiposity in obesity, adipose tissue macrophages contribute to adipose tissue malfunction and increased circulating proinflammatory cytokines. The chronic low-grade inflammation that occurs in obesity ultimately gives rise to a state of metainflammation that increases the risk of metabolic disease. To date, only lifestyle and surgical interventions have been shown to be somewhat effective at reversing the negative consequences of obesity and restoring adipose tissue homeostasis. Exercise, dietary interventions, and bariatric surgery result in immunomodulation, and for some individuals their effects are significant with or without weight loss. Robust evidence suggests that these interventions reduce chronic inflammation, in part, by affecting macrophage infiltration and promoting a phenotypic switch from the M1- to M2-like macrophages. The purpose of this review is to discuss the impact of dietary fatty acids, exercise, and bariatric surgery on cellular characteristics affecting adipose tissue macrophage presence and phenotypes in obesity. *Adv Nutr* 2021;00:1–18.

GRAPHICAL ABSTRACT



Keywords: physical activity, dietary fatty acids, bariatric surgery, macrophages, adipose tissue characteristics, meta-inflammation

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Introduction

According to the WHO, the number of individuals with obesity worldwide, adults and children, has nearly tripled since 1975 (1). This increased prevalence poses a significant threat to health as individuals with obesity are more likely to develop a myriad of related conditions such as type 2 diabetes, cardiovascular diseases (CVD), and certain types of cancers (2, 3). These health consequences, in part, stem from the negative effects of excess adipose tissue accumulation leading to morphologic and functional abnormalities (2). Subsequent, endocrine, metabolic, and immune derangements follow, which contribute to the obesity-associated inflammation that is, in part, mediated by macrophages (4, 5). Indeed, macrophages can be viewed as central to tissue stress, contributing to adipose tissue malfunction and increased circulating proinflammatory cytokines as obesity progresses (5, 6). The resulting chronic inflammatory state leads to adipocyte maladaptation and subsequent increases in angiogenesis, production of extracellular matrix (ECM), macrophage infiltration, and proinflammatory response (5–7); all of the aforementioned local consequences feed in a positive feedback loop exacerbating one another.

In both human and animal models, lifestyle and surgical interventions resulting in weight loss decreased macrophage infiltration and led to a phenotypic switch of the adipose tissue macrophages (ATM) (8–13). In the case of exercise, the beneficial effects were observed regardless of weight loss. Several mechanisms have been proposed to explain the anti-inflammatory properties of physical activity and the differential properties of dietary fatty acids culminating in beneficial quantitative and qualitative changes in ATM profiles (14). The aim of this review is to discuss the impact of dietary fatty acids, exercise, and bariatric surgery on the mechanisms that affect ATM presence and phenotype in obesity.

Macrophages and Obesity

What are macrophages?

Macrophages are innate immune cells that are typically found in every tissue and have the unique ability to sense and respond to pathogens and other environmental cues. Macrophages are particularly important for: tissue repair after an injury, clearance of foreign invaders and cellular debris through phagocytosis, and normal tissue development; they are especially efficient at integrating endocrine and paracrine signals in order to respond to stimuli (15, 16). Additionally, these phagocytes are prolific communicators as they interact directly with the receptors of other tissue-resident cells, immune cells recruited during injury (e.g. T cells), and extracellular proteins (15, 16). Other noteworthy characteristic features of these monocyte-derived cells are that they are heterogeneous and exhibit high levels of plasticity.

Macrophages are able to acquire different molecular and functional phenotypes after being exposed to different bioactive molecules and environments (16, 17). Indeed, macrophages can differentiate to proinflammatory M1 or anti-inflammatory M2 cell phenotypes, though for this process to occur macrophages need to be activated or polarized (7, 18). However, how M1 and M2 macrophages come to be in the adipose tissue remains ambiguous. It has been suggested that shifts in the M1: M2 macrophage ratio occurs from the transformation of resident macrophages during the course of resolution of an injury or from the continuous recruitment of monocytes in response to tissue stress (15).

The polarization of macrophages to M1 cells is mediated by type 1 T helper cells that secrete IFN-γ or with bacterial products (e.g. LPS). M1 macrophages produce proinflammatory cytokines such as TNF-α and IL-6 and they express inducible nitric oxide synthase (iNOS), reactive oxygen species (ROS), and nitrogen intermediates (7, 18). These proinflammatory molecules have been associated with the onset of numerous diseases such as CVD or type 2 diabetes (19–21). For example, TNF-α knockout mice had improved insulin sensitivity and lower concentrations of circulating free fatty acids (22). Conversely, the polarization of macrophages to M2 cells is mediated by type 2 T helper cells that secrete IL-4 and IL-13. M2 macrophages produce anti-inflammatory cytokines such as IL-4, IL-10, and TGF-β, which block the activity of iNOS and downregulate the synthesis of proinflammatory cytokines (7, 18, 23). M2 macrophages are more often associated with wound healing, resolution of inflammation, clearing of cellular debris, regulating proliferation, precursors of angiogenesis, and remodeling of the ECM, whereas M1-like macrophages appear to promote the opposite (6, 24). It should be noted that the M1/M2 paradigm is often seen as an oversimplified dichotomous division and should rather be considered as a continuum (6, 15, 24, 25). The identification of M1 and M2 cells is also challenging as phenotype markers are not specific and may indicate other cell types. The literature therefore identifies macrophage cells as M1-like and M2-like.

Macrophages in obesity

A plethora of immune cells accumulate within the expanding adipose tissue (6), although the macrophage population remains the predominant one (26). Macrophages make up ~5–10% of the stromal vascular fraction (SVF) cells derived from adipose tissue of lean individuals, whereas in individuals with obesity, the SVF can consist of ≤40–50% macrophages (27).

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Address correspondence to SS (e-mail: s.santosa@concordia.ca).
Abbreviations used: ANG, angiopoietin; ARG-1, arginase-1; ATM, adipose tissue macrophage; CCL5, chemokine (C-C motif) ligand 5; CCR, C-C chemokine receptor; CRP, C-reactive protein; CVD, cardiovascular disease; ECM, extracellular matrix; HFD, high-fat diet; HIF-1α, hypoxia-inducible factor 1-α; HR, heart rate; iNOS, inducible nitric oxide synthase; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; ROS, reactive oxygenic species; SAT, subcutaneous adipose tissue; SVF, stromal vascular fraction; Tie-2, tyrosine-protein kinase receptor Tie-2; TLR, toll-like receptor; VEGF, vascular endothelial growth factor.

To preserve adipose tissue homeostasis and functionality, there has to be a balance between both populations of M1- and M2-like macrophages (7, 26). However, the phenotypic heterogeneity of macrophages is environment dependent (7, 18, 26). In the lean state, the balance of the macrophage population tends to shift towards the anti-inflammatory M2-like subpopulation (26). In comparison, in the obese state, the balance tilts toward the M1-like subpopulation, thus, creating a proinflammatory environment within the adipose tissue (26, 28–30). The accumulation of ATM in individuals with obesity has been linked with adipocyte and metabolic dysfunction (6, 24, 31).

Lifestyle and Surgical Interventions

Dietary fatty acids

The seminal work of Weisberg et al. (32) and Xu et al. (33) were the first to demonstrate that high-fat diets (HFDs) increase macrophage content and trafficking within the fatty depots that are associated with the development of obesity-induced insulin resistance. Indeed, fatty acids are thought to be immunomodulators of inflammatory pathways. However, not all fats are equal and different fats may have differential effects on macrophages and adipose tissue characteristics (34, 35).

Saturated Fatty Acids.

Effects of saturated fatty acids on macrophage polarization and infiltration in rodents. Studies suggest that diets rich in saturated fatty acids (SFA) are associated with inflammation as they are considered naturally occurring ligands for the toll-like receptors (TLR), which activate downstream inflammatory pathways, on both adipocytes and macrophages/monocytes (36–38). Obese rodents, fed with diets rich in SFAs (mainly from lard), have increased expression of TLRs and markers associated with macrophage infiltration (38–45) (see **Table 1**). Moreover, the activation of the TLR inflammatory pathways by an increased flux of SFAs are thought to contribute to the classical polarization of M1-like macrophages. For example, Enos et al. (42) examined the effects of 3 HFDs, differing in the percentage of total calories from saturated fat (6%, 12%, and 24%) but identical in total fat (40%), on macrophage behavior. All HFDs increased adipose tissue inflammation, but the 12% and 24% saturated fat diets increased TLR2 expression, and led to the greatest increase in M1- and M2-like macrophages (42). Additionally, several murine studies reported that feeding of SFA-rich diets worsened ROS production, the expression of adipose tissue remodeling markers [e.g. TGF-β, tissue inhibitor matrix metalloproteinase 1 (TIMP1), collagen VI, hypoxia-inducible factor 1-α (HIF-1α), and PPARγ], decreased capillary density, and increased adipocyte size, proinflammatory cytokines [e.g. IL-6, TNF-α, monocyte chemoattractant protein-1 (MCP-1), C-reactive protein (CRP)] and the number of crown-like structures (39, 41, 42, 44–52). These changes may collectively prompt the aggregation of proinflammatory macrophages. Thus, it appears that a diet rich in SFAs may trigger the development of pathogenic remodeling processes in rodents in response to the accumulation of M1-like macrophages.

Effects of saturated fatty acids on macrophage polarization and infiltration in humans. In human studies, SFAs also increased TLR genes and proinflammatory cytokines [e.g. IL-1β, IL-6, IL-8, TNF-α, chemokine (C-C motif) ligand 5 (CCL5)] in lean subjects, those with obesity, and in those with diabetes (54, 55) (see Table 1). van Dijk et al. (53) conducted a parallel controlled-feeding trial in 20 subjects who were abdominally overweight randomized to a SFA-based diet or a MUFA-based diet for 8 wk. Whole-genome microarray and histologic analysis of the adipose tissue showed that the consumption of SFAs increased proinflammatory obesity-linked gene expression including the downregulation of PPARγ and upregulation of the TLRs and macrophage marker genes (CD14 and CD163) (53). Of particular note, is that the participants' weights were not significantly different between the diet groups and did not change throughout the intervention, ruling out the cofounding factor of weight gain. The direct effects of SFA on macrophage infiltration and polarization, cellular characteristics, and adipose tissue remodeling in individuals with obesity are still poorly documented and require further investigation.

Other contributing factors to inflammation in obesity. A diet rich in SFAs may also represent a crucial first step in disturbing the gut microbiota. This disruption in the gut microbiota results in alterations in the epithelial cells of the intestinal barrier while promoting the translocation of bacteria and their cellular components into the circulation (56, 57). Consequently, a diet high in SFAs may contribute to a rise in the systemic concentration of LPS, which can act as a ligand to the TLRs on the surface of ATM and adipocytes (58–63). As such, it has been hypothesized that the translocation of LPS and bacterial metabolites may increase the release of proinflammatory cytokines, promote macrophage infiltration, and prompt a phenotype switch towards the M1-like cells (64, 65). However, a phenotypic switch has yet to be demonstrated in humans, and the influence of LPS-mediated inflammation on adipose tissue characteristics is rather unknown. Moreover, recent studies suggest that lifestyle and surgical interventions may also partially revert gut microbiota dysbiosis to improve gut health and possibly inflammation (66–68). Overall, the gut microbiota-related inflammation represents a promising alternate pathway to explain the chronic low-grade inflammation seen in individuals with obesity.

n–3 Polyunsaturated Fatty Acids.

Conversely to SFAs, n–3 PUFAs have the capacity to induce anti-inflammatory and insulin-sensitizing effects on adipocytes and their resident macrophages. These metabolic improvements have been predominantly observed with n–3 PUFA supplementation from fish oil (i.e. EPA and DHA).

TABLE 1 The effect of SFA-rich diets on macrophage infiltration and polarization in rodent and human studies

Reference	*N*	Rodents or participants	Diet	Weight change	Macrophage/ phenotype change
Coenen et al. (38, 39)	48 and 58	C57BL/6 mice	Western diet (42% fat + 0.15% cholesterol) vs. control diet 12 wk	↑	↑ infiltration of macrophages
Davis et al. (41)	75	Control male C57BL/10J mice and male Tlr-4-deficient C57BL/10ScN mice	3 experimental diets: low-fat control (LFC) vs. high-fat control (HFC) vs. high-fat palmitate (HFP) 16 wk	↑	↑ % of macrophages
Enos et al. (42)	45	Male C57BL/6 mice	5 treatment diets: 2 control diets vs. 3 HFDs (6% SF, 12% SF, and 24% SF) 16 wk	↑	↑ M1 ↑ M2 ↑ infiltration of macrophages
Prieur et al. (44)	36	Wild-type C57BL/6 male mice and *ob/ob* mice	HFD (45% fat) vs. control diet (11.5% fat) 12 wk	Ø	↑ M1 ↓ M2
Cullberg et al. (40)	N/A	Cell culture	In vitro: 3T3-L1 adipocytes and THP-1 macrophages were incubated for 24 h with FFAs (oleic, palmitic, and elaidic acids)	Ø	↑ 1.8-fold M1
Nguyen et al. (43)	40	Wild-type male C57BL/mice and *ob/obJ* male mice Cell culture	HFD (40% fat) vs. control diet (12% fat) For 1, 12, or 20 wk In vitro: RAW264.7 cells were cultured and treated with FFA (arachidonic, lauric, linoleic, oleic, and myristic acids)	Ø	↑ M1 ↑ infiltration of macrophages
van Dijk et al. (53)	20	Abdominally overweight middle-aged adults (10 male and 10 female)	2 experimental diets: SFA-rich diet (19% SFAs and 11% MUFAs) vs. MUFA-rich diet (11% SFAs and 20% MUFAs) 8 wk	Ø	↑ M1 ↑ M2

FFA, free fatty acids; HFD: High-fat diet; SF, saturated fat; lean (BMI ≤24.9); overweight (BMI 25–29.9); class I (BMI 30–34.9); class II (BMI 35–39.9); class III (BMI ≥40) ↓ : significant decrease; ↑ : significant increase; Ø: no significant change; N/A: not applicable.

The anti-inflammatory n–3 PUFAs are known endogenous ligands to PPARγ and free fatty acid receptor 4 (FFAR4), have the ability to preferentially inhibit TLR-induced pathways, and reduce the expression of proinflammatory transcription factors (69, 70–72).

Numerous studies conducted on both humans and rodents alike have demonstrated the potential advantageous effects of n–3 PUFAs on macrophage infiltration and phenotypic shifts, culminating in the amelioration of adipose tissue homeostasis. Indeed, following a dietary regimen enriched in n–3 PUFAs, the number of macrophages and specific markers of macrophage polarization for the M1- and M2-like cells fluctuated favoring an M2-dominant ratio (11, 73–92) (see **Table 2**). Itariu et al. (75) conducted an 8-wk randomized trial on 55 nondiabetic individuals with class III obesity who received either 3.36 g EPA/DHA or the equivalent amount of butterfat each day. They found that, despite no changes in M2 macrophage markers [mannose receptor C type 1 (MRC1) and CD163], pan macrophage marker (CD68), and the total number of macrophages, the expression of CD40, an M1 marker, was downregulated by n–3 PUFA treatment. Another study demonstrated that after participants with class I obesity consumed 4 g of fish oil (~3.6 g EPA and DHA) per day for 12 wk, significant decreases in total macrophage number and CD68 mRNA concentrations were observed (79). Further in vitro experiments showed that the addition of DHA to M1 macrophage cultures and cocultures with adipocytes markedly reduced the expression of MCP-1 (79). Therefore, fish oils may not only reduce macrophage abundance in adipose tissue, but also decrease the migration and infiltration of monocytes into adipose tissue (79). More recently, 3 other in vitro studies also supported these findings through similar observations and conclusions (74, 76, 77). On the other hand, in another study where individuals with overweight to class I obesity consumed 3.5% of their diet as fish oil, no difference in ATM gene expression (CD14 and CD206) was observed (93). The discrepancies in the findings may be explained by the differences in the n–3 PUFAs dose administered, the composition of the n–3 PUFAs used, or the weight status of the participants. The studies by Itariu and Spencer (75, 79) included individuals with more severe cases of obesity, which may suggest that the anti-inflammatory properties of n–3 PUFAs are more significant in individuals with greater obesity severity.

TABLE 2 The effects of n–3 PUFAs on macrophage infiltration and polarization in rodent and human studies

Reference	*N*	Rodents or participants	Diet	Weight change	Macrophage/ phenotype change
Bashir et al. (80)	25	Male C57BL/6J mice	3 experimental diets: control diet vs. HFD group (60% fat) vs. HFD + flaxseed oil (4, 8 or 16 mg/kg b.w.) 18 wk	↓	↓ M1 ↑ M2
Fan et al. (84)	47	Male C57BL/6J mice	3 experimental diets: HFD with ALA-enriched butter vs. HFD with butter lacking ALA and LA vs. HFD with ALA and LA-enriched margarine 10 wk	Ø	↓ M1 ↑ M2 ↓ infiltration of macrophages
Lopez-Vicario et al. (86)	46	Wild-type male mice and male hemizygous fat-1 mice	3 experimental diets: control diet (13% fat) vs. HFD + placebo (60% fat) vs. HFD + sEH inhibitor 16 wk	↑	↑ M2 ↓ infiltration of macrophages
Titos et al. (87)	37	Male C57BL/6J mice	Control diet (13% fat) vs. HFD (60% fat) Animals then received a placebo or DHA (4 μg/g b.w.) every day for 10 d 12 wk	Ø	↓ M1 ↑ M2 Ø total ATM
Todoric et al. (88)	49	Male C57BL/KsJ-lepr^{db}/lepr^{db} diabetic (*db/db*) mice and nondiabetic mice (*db/+*)	4 experimental diets: control diet vs. HFD + SFA + MUFA vs. HFD + n–6 PUFA vs. HFD + marine n–3 PUFA 6 wk	↑	↓ M1 ↓ infiltration of macrophages
White et al. (89)	4–14 depending on measurement	Male hemizygous *fat-1* (+/−)	Control diet vs. HFD (55% fat) 8 wk *Transgenic expression of *fat-1* n–3 fatty acid desaturase was used to endogenously produce n–3 fatty acids in HF-fed mice	Ø	↓ infiltration of macrophages ↓ crown-like structures
Chan et al. (81)	Ø	Cell culture	Low-fat diet (10% fat) vs. HFD (60% fat) 18 wk In vitro: bone marrow-derived macrophages were cultured with palmitate or palmitoleate	Ø	↑ M2 ↓ M1
Chang et al. (82)	Ø	Cell culture	In vitro: murine macrophages and human T lymphocytes were cocultured and treated with DHA	Ø	↑ M2 ↓ M1
Colson et al. (83)	24	Male C57BL/6J mice Cell culture	n–6-enriched control diet (12% fat) vs. n–3-enriched control diet (12% fat) 12 wk In vitro: THP-1 cells were cultured for differentiation experiments	Ø	↑ M2 Ø M1
De Boer et al. (91)	32	Male and female C57BL/6 mice Cell culture	4 experimental diets: HF control diet (34% fat) vs. HFD + FO (34% fat + 7.6% FO) vs. low-fat control diet (10% fat) vs. low fat + FO (10% fat + 3% FO) 12 wk In vitro: macrophages were cocultured with adipocytes	↑	↓ M1
De Boer et al. (92)	10	Male C57BL/6 mice Cell culture	Control diet (10% SO) vs. LC n–3 PUFA-enriched diet (3% FO + 7% SO) 4 wk In vitro: visceral adipose tissue were collected to create adipose tissue conditioned media and challenged with LPS to mimic acute and chronic conditions	↑	↓ M1 ↓ M2

(*Continued*)

TABLE 2 (*Continued*)

Reference	*N*	Rodents or participants	Diet	Weight change	Macrophage/ phenotype change
Liddle et al. (85)	30	Male and female C57BL/6 mice Cell culture	Control diet (10% SO) vs. treatment diet (7% SO + 3% FO) 4 wk In vitro: RAW264.7 macrophages were cocultured with LPS-stimulated CD8+ T cells/adipocytes	Ø	↓ M1 ↑ M2
Baranowski et al. (11)	21	Male *fa/fa* Zucker rats and 7 lean Zucker rats	Control diet vs. ALA-rich flaxseed oil diet 8 wk	Ø	Ø macrophage infiltration among groups
Itariu et al. (75)	55	Nondiabetic adults with class III obesity	3.36 g long-chain n–3 PUFAs/d vs. 5 g of butter/d in addition to an isocaloric diet (55% carbohydrates, 15% protein, and 30% fat) 8 wk	Ø	↓ M1 Ø M2 Ø total ATM and infiltration
Kratz et al. (93)	24	Individuals with overweight to class I obesity (8 males and 16 females)	Control diet (0.5% n–3 PUFAs) vs. n–3 PUFA-rich diet (3.5%) 14 wk	↓	Ø macrophage phenotypes and infiltration
Spencer et al. (79)	33	Adults with class I obesity (11 males and 22 females) Cell culture	4 g of n–3 fatty acid ethyl esters vs. placebo (corn oil) 12 wk In vitro: M1 macrophage culture and M1 macrophage cocultured with adipocytes were treated with DHA	Ø	↓ total ATM ↓ crown-like structures DHA decreased MCP-1 expression in cultured M1 macrophages and in cocultures of macrophages and adipocytes
Ferguson et al. (74)	N/A	Cell culture	In vitro: human SAT from lean and obese subjects were treated with EPA and/or DHA throughout differentiation or for 72 h postdifferentiation. THP-1 monocytes were added to adipocyte cocultures	Ø	↑ M2 ↓ M1
Pandurangan et al. (77)	Ø	Cell culture	In vitro: human adipocytes and macrophages were cocultured and treated with chia seed fatty acid (0–6.4 μg/mL)	Ø	↓ M1 ↓ macrophage recruitment
Montserrat-de la Paz et al. (76)	6	Healthy adult males Cell culture	Participants were all given 3 times a meal rich in SFA, MUFA or MUFA + ω-3 LC PUFA with or without niacin. In vitro: monocytes were isolated to be differentiated into naïve macrophages; TLRs were also isolated	Ø	↓ M1 ↑ M2

ALA, α-linoleic acid; ATM, adipose tissue macrophage; b.w., body weight; FO, fish oil; HF, high-fat; HFD, high-fat diet; LA, linoleic acid; LC, long chain; SAT, subcutaneous adipose tissue; sHE, soluble epoxide hydrolase; SO, safflower oil; TLR, toll-like receptor; %fat expressed based on total energy; lean (BMI ≤24.9); overweight (BMI 25–29.9); class I (BMI 30–34.9); class II (BMI 35–39.9); class III (BMI ≥40); ↓ : significant decrease; ↑ : significant increase; Ø: no significant change; N/A: not applicable.

Beneficial shifts in the M1-:M2-like macrophage ratio following n–3 PUFA supplementation may be due to a number of underlying mechanisms. Supplementation resulted in improvements in cellular stress (45, 66, 85, 94–98), metabolic profile (99), synthesis and release of anti-inflammatory mediators [i.e. IL-10, IL-4, arginase-1 (ARG-1) and adiponectin], while decreasing the secretion of proinflammatory mediators (i.e. IL-1β, IL-6, TNF-α, and MCP-1) (11, 73–75, 77, 79–81, 83–85, 100–105, 100), adipocyte enlargement (11, 12, 73, 77, 84, 95, 100, 106, 107), and the deposition of ECM and the expression of its associated markers (12, 75, 80, 85). Increased capillary density (79) and adipogenesis (73, 95, 106, 108, 109) have also been shown with supplementation. Moreover, supplementation of n–3 PUFAs downregulated the expression of important inflammatory transcription factors and receptors, such as NF-κB and TLR4, concomitant with an upregulation in adipogenic regulators [PPARγ and CCAAT-enhancer-binding protein α (C/EBPα)] (45, 73, 77, 80, 85, 90, 102, 103, 106, 109, 110). Additionally, some murine studies also observed changes in weight and fat

mass loss with the previously mentioned improvements (73), whereas studies in humans showed the downregulation of inflammatory factors associated with PUFA consumption in the absence of changes in weight or body composition (73, 75, 93).

Overall, n–3 PUFA supplementation may represent a potential therapeutic avenue to improve macrophage-mediated inflammation and adipose tissue characteristics, although the effects were less potent in vivo (73, 75, 93). The inconsistent results in human studies are likely to be attributed to variability in study design, weight status of participants, and adherence as most trials are outpatient studies and rely on self-reporting. Other factors may include differences in the amount of dosage administered or methods of calculating n–3 PUFA intake (71, 73, 111). Nonetheless, further studies should continue to explore the role of n–3 PUFAs in mediating ATM infiltration and phenotype.

Physical activity

Lack of exercise and prolonged sedentary behaviors are important catalysts for a cluster of metabolic and chronic diseases, whereas regular exercise may prevent or delay the progression of insulin resistance, hypertension, CVD, and diabetes (112, 113). Although numerous studies have denoted that the salutary effects of exercise are independent of weight loss (113–116); significant weight loss may amplify the exercise-induced benefits and have a greater impact on the inflammatory markers in humans (9, 117, 118). In fact, physical activity was shown to induce cellular and molecular changes in the adipose tissue in a way that alleviates the low-grade chronic inflammation that accompanies obesity (8–10, 119–121). The underlying mechanisms that contribute to the exercise-induced anti-inflammatory responses have not been completely elucidated. A major contributor to the reduction in inflammation accompanying exercise may reside in the mediation of ATM (14, 113, 116, 122, 123).

The effects of exercise on macrophage infiltration and phenotypes.

Exercise, with or without weight loss, may decrease inflammation via promoting a phenotypic switching from M1 to M2 macrophages while simultaneously diminishing the trafficking of the macrophages within the adipose tissue. The early work of Kawanishi et al. (47) demonstrated that 16 wk of cardiovascular exercise training (12–20 m/min, 60 min/d, and 5 times/wk) in mice with obesity reduced M1-like and increased M2-like macrophage mRNA expression in adipose tissue such that the M1-:M2-like ratio was ∼50% lower with the exercise intervention relative to control. Quantification of macrophages in adipose tissue by flow cytometry also showed that exercise decreased both the proportion and absolute number of M1-like (CD11c+) macrophages (124). Another study found that in comparison to continuous training (steady state running at 20 m/min), aerobic interval training (3-min bouts at 40 m/min, interspersed by 3-min active recovery at 20 m/min on a treadmill with 15% incline, repeated 6 times per session) has been shown to result in greater increases in the number of M2-like macrophages (181% versus 122%) in mesenteric adipose tissue (48). More recent murine studies have demonstrated diminished infiltration and phenotypic shifts in macrophages (48, 125–130) (see **Table 3**).

In humans, few studies have looked at the direct impact of exercise on the polarization of the macrophage populations (9, 119, 131) (see Table 3). These studies have found exercise-induced shifts towards a predominant M2-like phenotype. An 8-wk low-intensity exercise intervention (walking 10,000 steps 3 times/wk) in adults with overweight and class I obesity showed that exercise was associated with a ∼2.1-fold upregulation of M2 markers and a downregulation of M1 markers independent of weight loss (132). Additionally, Auerbach et al. (119) and Bruun et al. (9) corroborated the previous findings through an exercise-induced weight-loss protocol suggesting that pronounced weight loss may also further affect macrophage infiltration and phenotype resulting in an anti-inflammatory milieu within adipose tissue.

Mechanisms altering macrophage infiltration and phenotypes in exercise.

Exercise decreases expression of proinflammatory and chemotactic signals. There is a growing body of evidence suggesting that exercise decreases the expression of proinflammatory and chemotactic cytokines involved in the recruitment of macrophages and monocytes (14, 113, 116). Among all the cytokines known to potentially contribute to inflammation within the adipose tissue and the chemotaxis of macrophages, TNF-α, IL-6, and MCP-1 appear to be the best studied and were consistently shown to have lower levels of expression following exercise treatment in humans, mice, and rats (8–10, 117, 120, 121, 124, 126, 127, 132, 133–144). Baturcam et al. (133) found that 3-mo supervised exercise {combination of moderate intensity [50–80% of max heart rate (HR)] aerobic exercise and resistance training using either a treadmill or cycling 3–5 times/wk} significantly reduced the expression of both CCL5 and C-C chemokine receptor type 5 (CCR5) in the adipose tissue of individuals with class I to class II obesity with decreases in the concentrations of the proinflammatory markers TNF-α, IL-6, and protein and c-jun NH2 terminal kinase (P-JNK). Complementing these findings, Barry et al. (8) demonstrated that both high-intensity interval training (at 90% of HR_{peak}, for 1 min interspersed with 1 min of low-intensity recovery periods, progressing from 4 to 10 intervals) and moderate-intensity continuous training (at 65% of HR_{peak}, progressing from 20 to 50 min) in humans, in the absence of weight and fat mass loss, altered leukocyte trafficking through the downregulation of inflammatory chemokine receptors such as CCR2, CCR5, and C-X-C chemokine receptor type 2 (CXCR2). In humans and rodents, exercise has also been associated with an increase in the expression and release of anti-inflammatory signals such as IL-10, IL-6, ARG-1, and adiponectin (9, 14, 115, 117, 126, 136, 137, 145).

TABLE 3 The effects of exercise on macrophage infiltration and polarization in rodent and human studies

Reference	*N*	Rodents or participants	Exercise intervention	Weight change	Macrophage/ phenotype change
Kawanishi et al. (47, 124)	40	Male C57BL/6 mice	Treadmill running 12–20 m/min × 60 min/d 16 wk	Ø	↓ M1 ↑ M2 ↓ number of macrophages
Macpherson et al. (126)	27	Male C57BL/6 mice	Treadmill running 3 d × 15 min/d at 15 m/min acclimation 2 h at 15 m/min with 5% incline	Ø	↓ M1 ↑ M2 ↓ infiltration of M1-like macrophages
Linden et al. (125)	113	Male C57Bl6/J mice	Treadmill running 40 min/d at 12 m/min with 8% incline 4, 8, or 12 wk	Ø	↓ M1 ↑ M2 ↓ infiltration of macrophages
Luo et al. (128)	54	Male C57BL/6H mice	Treadmill running 45% of peak running speed, with 5% incline, 1 h/d, 6 d/wk 8 wk	↓	↓ M1 ↑ M2
Baek et al. (129)	49	Male C57BL/6 J mice	Treadmill running at 10 m/min for 60 min Mice ran at different intensities from week 2 to week 8	↓	↓ M1 ↑ M2
Oliveira et al. (127)	24	Male Wistar rats	Swimming 2-d swimming × 10 min/d acclimation 3 h of exercise with a 45-min rest period	Ø	↓ M1 ↑ M2 Ø in infiltration
Kolahdouzi et al. (48)	48	Male Wistar rats	Treadmill running 5 d/wk 3 groups: Sedentary vs. CT vs. AIT 10 wk	CT: ↓ 30% weight loss AIT: ↓ 40% weight loss	↓ M1 ↑ M2 ↓ number of macrophages
Shanaki et al. (130)	45	Male Wistar rats	Treadmill running (HIIT or CT) 5 d/wk 10 wk	↓	↓ M1 ↑ M2
Bruun et al. (9)	27	Individuals with class III obesity (15 females and 12 males)	2–3 h of exercise 5 d/wk 15 wk Included a diet	↓ ~ 14% weight loss	↓ ~55% M1 ↓ ~40% number of macrophages
Auerbach et al. (119)	60	Healthy and overweight adult men	Endurance training for 7 d/wk 4 groups: training-induced weight loss (T) vs. diet-induced weight loss (D) vs. training and increased diet without weight loss (T-iD) vs. control (C) 12 wk	↓ 6% weight loss in the endurance training group (T)	↑ 2.5-fold M2 Ø macrophage number
Yakeu et al. (132)	17	Healthy overweight adults (9 males and 8 females)	Walking on treadmill 10,000 steps 3 times/wk for 75 min 8 wk	Ø	↓ M1 ↑ M2
Ruffino et al. (146)	19	Overweight adult women	Walking on treadmill 3 times/wk for 45 min 8 wk	↓	↓ M1 ↑ M2
Lee et al. (131)	26	Sedentary lean or overweight men with or without dysglycemia	2 sessions of strength training and 2 sessions of spinning 4 h/wk 12 wk	↓	Ø M1 ↓ M2 ↓ infiltration of macrophages

AIT, aerobic-interval training; CT, continuous training; HIIT, high-intensity interval training; lean (BMI ≤24.9); overweight (BMI 25–29.9); class I (BMI 30–34.9); class II (BMI 35–39.9); class III (BMI ≥40); ↓ : significant decrease; ↑ : significant increase; Ø no significant change.

Despite the fact that the positive effects of exercise have been observed in the absence of weight loss, weight loss may compound the benefits. An exercise study (aerobic training, 60–75 min/session and 3 times/wk) found that compared with subjects in the lowest tertile (–3%) of weight loss, those in the highest tertile of weight loss (–14.5% weight loss) had larger decreases in macrophage inflammatory protein-1α (MIP-1α) and IL-15 and greater increases in adiponectin

(117). Aside from TNF-α, IL-6, and MCP-1, several other cytokines associated with inflammation were shown to have a reduced expression following an exercise intervention such as CCL5, plasminogen activator inhibitor-1 (PAI-1), MIP-1α, CRP, chemerin, IFN-γ, IL-1, IL-8, IL-15, and IL-18, which may also further improve the chronic low-grade inflammation seen in adipose tissue (9, 117, 120, 121, 127, 133, 134, 136, 140).

Exercise affects adipose tissue characteristics. In addition to decreasing proinflammatory and chemotactic signals associated with macrophage recruitment, exercise also directly affects adipose tissue characteristics that are associated with the recruitment and phenotypic changes of ATM. A recent murine study by Kolahdouzi et al. (48) found that aerobic interval training improved adipose tissue dysfunction induced by a HFD through increasing the number of M2-like cells and capillary density while decreasing the total number of crown-like structures and mean adipocyte size (48). Multiple murine studies have also demonstrated beneficial changes in cellularity that may be associated with decreased ATMs. Moreover, several key features of dysfunctional adipose tissue are improved such as lipid and glucose metabolism (134, 135, 147), improved mitochondrial activity and biogenesis (147–150), decreased expression of apoptotic signals (151), decreased expression of angiogenesis precursors (152–157), increased capillary density (48), reduced accumulation of fibrotic depots (10, 46), and reduced adipocyte size (48, 134, 148, 158, 159). Similar findings were also made in human studies where lipid metabolism, mean adipocyte size, adipose tissue fibrosis, and proangiogenic responses were improved following exercise training with or without weight loss (139, 160, 161).

Exercise modifies gene expression patterns. Underlying the mechanisms of the potent anti-inflammatory properties of exercise are tremendous gene expression alterations that may have a direct impact on chronic low-grade inflammation as well as the ubiquitous proinflammatory macrophage infiltration seen in adipose tissue of individuals with obesity (148, 162). Aside from varying the gene expression of pro- and anti-inflammatory cytokines, angiogenic regulators, ECM precursors, markers of mitochondrial activity, and lipid and glucose metabolism (9, 46, 120, 147, 153), physical activity also affects the gene expression of key adipogenic regulators (such as PPARs) and well-characterized immune receptors that modulate inflammatory pathways (like the TLRs) (14).

Several studies highlight the crucial immunomodulating role of PPARs, more specifically PPARγ, in regulating adipose tissue inflammation by promoting the infiltration of M2 macrophages in humans and mice (163–165). Macrophage-specific deletion of the PPARγ gene (163) and upregulation of PPARγ by rosiglitazone (164) in mice demonstrated the role of PPARγ in M2-like macrophage activation. Exercise upregulates PPARγ expression and its related signaling events in adipose tissue and monocytes/macrophages of humans, mice, and rats (146, 166–171), favoring a phenotypic shift towards the M2-like macrophages. In a human study, Yakeu et al. (132) found that low-intensity exercise (walking 10,000 steps 3 times/wk) shares similar effects to the pharmacological activation of PPARγ and that a ~4–5-fold increase in PPARγ activity and expression coincided with a ~2.1-fold increase in the M2-like macrophage marker (CD14).

TLRs are a class of membrane proteins that play an important role in the innate immune system by initiating key downstream inflammatory pathways through recognition of exogenous and endogenous ligands (172). TLRs, especially TLR2 and TLR4, are present on the cell surface of adipocytes and macrophages [especially M1-like macrophages (173)] and play a pivotal role in obesity-related pathogenesis, including in the development of insulin resistance, and the metabolic syndrome (14, 172). The TLR family are activated by a vast array of ligands, many of which are higher in obesity such as LPS (a marker of gut permeability), oxidized LDLs, and SFAs. The binding of a bioactive molecule to TLRs, results in the activation of NF-κB and the release of proinflammatory cytokines (6, 113, 172). The pivotal role of TLR4 in obesity-associated pathogenesis was demonstrated from the observations that TLR4 knockout mice were protected from the adverse effects of high-fat feeding with attenuated inflammation and macrophage infiltration (113, 172). In parallel with TLR4 knockout mice, exercise training resulted in similar metabolic improvements by decreasing the expression of TLR4 on the cell surface of monocytes and macrophages (138, 174–178); in some cases, TLR4 expression and activity was reduced by $\leq$35% following exercise interventions (178). In mice and rats, the reduced expression of TLR4 on the surface of the adipocytes and/or SVF cells following exercise correlates with the phenotypic shift in ATM from the M1- to the M2-like phenotype, and reduced macrophage infiltration (47, 127, 177, 179, 180). However, despite the decreased expression of TLR4 activity following exercise training in humans (136, 178, 181–187), more questions remain to be explored regarding the role of TLR4 in macrophage polarization and infiltration. To our knowledge, most human studies on TLR4 expression following exercise examined monocytes rather than ATM directly and results were sometimes inconclusive (188). Given that monocytes are precursor cells of macrophages, it is plausible that the decreased TLR4 expression may also coincide with changes in the phenotype of macrophages as seen in rodents. Further investigations examining the effects of exercise on TLR4 expression in humans is required.

Overall, what remains unknown is which form of exercise training is best to mitigate ATM infiltration and phenotypes in obesity. Several studies indicate that higher intensities and combined training (e.g. combined aerobic and resistance training versus aerobic or resistance training alone) better improved obesity-associated inflammation (116, 189). However, the comparison of these training modalities has not been investigated in ATM infiltration. Future studies should further explore the mechanisms driving macrophage infiltration and polarization in response to exercise and

focus on the training modalities (duration, type, volume, and intensity) that are best at mitigating ATM inflammation.

Bariatric surgery

Often, when first-line treatment options, such as dietary interventions and exercise programs, are not enough to induce significant weight loss or metabolic improvements in individuals with obesity, many turn to bariatric surgery. Indeed, bariatric surgery is one of the most powerful tools to induce weight loss; a worldwide study from 31 countries found that the surgeries induced an overall 1 y-weight loss of ∼30.5% (190). Aside from the effectiveness for weight loss, bariatric surgery is often accompanied with weight-loss-dependent metabolic improvements including the mitigation of ATM inflammation.

Effects of surgery on macrophage populations. Several studies observed significant reductions in macrophage number up to a year after surgery using the CD68 marker (30, 191–194) (see **Table 4**). Cancello et al. (30) found an ∼12% reduction in the number of ATM after surgery, which is likely due to the decreased expression of chemotactic genes. Bariatric surgery was also found to alter the phenotype of macrophages favoring a shift towards M2-like macrophages (195–201) (see Table 4). Aron-Wisnewsky et al. (195) found that in premenopausal women without diabetes, the ratio of M1-: M2-like (CD40+: CD206+) macrophages was 2-fold lower in subcutaneous adipose tissue (SAT) after 3 mo than before surgery due to a simultaneous decrease of CD40+ and an increase of CD206+ macrophages. Similarly, others have found an increased presence of M2- over M1-like macrophages in the adipose tissue ≤12 to 24 mo after surgery (198, 199). Altogether, these studies suggest that the immune and inflammatory profile of bariatric surgery patients may take years to reach new baseline levels. Overall, robust evidence indicates quantitative and qualitative changes in ATM populations following weight loss surgery.

Mechanisms affecting macrophage infiltration and polarization after bariatric surgery.

Weight loss by bariatric surgery decreases expression of proinflammatory cytokines and chemotactic signals. An extensive amount of research has studied how bariatric surgery affects cytokine-related macrophage chemotaxis and polarization. Although unclear, current literature suggests that bariatric surgery may improve the inflammatory status of individuals with obesity. Two previous reviews (13, 202) listed several cytokines and their variations at different time points after bariatric surgery. An example being that CRP and leptin unanimously decreased, adiponectin constantly increased, and TNF-α remained unchanged at all time points after surgery. As for the other highly expressed cytokines during obesity such as IL-1β, IL-6, IL-10, and MCP-1, results are inconsistent even ≤2 y postoperation. For instance, 3 mo after surgery, Xu et al. (203) observed improved insulin sensitivity, increased AMP-activated protein kinase (AMPK) expression, and decreased oxidative stress with no changes in IL-1β, TNF-α, and IL-10 levels in patients. Such discrepancies were hypothesized to be the result of the presurgical presence of diabetes and the baseline level of insulin sensitivity (204–206). For example, a greater CRP reduction was observed after surgery in ex-obese patients with diabetes compared with those who were not diabetic (206). Moreover, the inflammatory state of visceral adipose tissue and the patients' nutritional status presurgery were also suggested to influence the postsurgical inflammation state (202). Overall, the inconsistent effects of surgically induced weight loss on cytokine fluctuation remain unexplained due to a lack of convincing results. The long-term effects of cytokine secretion on health of individuals with obesity after surgery are unknown.

Effects of surgery on adipocyte morphology. Appreciable weight loss after bariatric surgery results in extensive adipose tissue remodeling on multiple levels, implicating mechanisms underlying adipose tissue plasticity. The architecture and homeostasis of the adipose tissue and the cells composing the SVF are tightly regulated by the equilibrium between hypertrophy and hyperplasia, which may be improved following weight loss resulting in diminished macrophage-mediated inflammation. Several studies analyzing either the volume or the area of the adipocytes found that postsurgery fat cells were smaller, ultimately approaching measurements similar to lean controls (191, 194, 207–209). For example, Casmatra et al. (210) and Löfgren et al. (211) reported a postsurgical reduction of fat cell area by 50% and volume by 43%, respectively. Additionally, Andersson et al. (212) reported significant adipocyte volume loss after surgery, but with no changes in cell number. Thus, suggesting that adipocyte atrophy is the main plastic event taking place during weight loss induced by surgery.

Effects of surgery on angiogenesis in adipose tissue. Adipose tissue expansion is intricately dependent on vasculature, which is increased during obesity. Indeed, angiogenesis is a response to adipose tissue hypoxia that results from its expansion and poor blood supply. As such, angiogenic markers like vascular endothelial growth factor (VEGF), angiopoietin-1 (ANG-1), ANG2, tyrosine-protein kinase receptor Tie-2 (Tie-2), and HIF-1α are overexpressed in obesity which potentiate proangiogenic responses to improve tissue blood supply, inflammation, and ultimately adipocyte dysfunction (213). Bariatric surgery may induce significant reduction of these angiogenic markers while concomitantly decreasing the recruitment of M1-like macrophages. Weiwiora et al. (214) studied the concentrations of circulating angiogenesis biomarkers [ANG-2, granulocyte colony-stimulating factor (G-CSF), hepatocyte growth factor (HGF), platelet endothelial cell adhesion molecule-1 (PECAM-1), VEGF, and follistatin] preoperatively and 12 mo after surgery in 24 patients with class III obesity. The expression levels of these angiogenic markers were all downregulated postsurgery and their changes were dependent upon the

TABLE 4 The effects of bariatric surgery on macrophage infiltration and polarization at different time points after surgery in human studies

Reference	*N*	Participants	Macrophage/phenotype change				
			≤1 mo	3 mo	6 mo	12 mo	24 mo
Cancello et al. (30)	24	17 women with class III obesity and 7 lean women		↓ 12% ATM			
Aghamohammadzadeh et al. (191)	22	15 adults with class III obesity and 7 lean individuals			↓ ATM		
Haluzikova et al. (192)	32	17 women with class III obesity and 15 lean women			↑ ATM surpassing baseline concentrations (before surgery)	↓ ATM	↓ ATM (concentration similar to control)
Trachta et al. (193)	31	13 nondiabetic women with class III obesity and 18 lean women			↑ ATM	↓ ATM	In SAT: ↓ ATM (concentrations similar to baseline)
Liu et al. (198)	118	Individuals with class III obesity				↓ ATM ↑ M2	
Aron-Wisnewsky et al. (195)	26	16 women with class III obesity and 10 lean women		↓ M1 ↑ M2 ↓ 2-fold M1/M2			
Garcia-Rubio et al. (197)	71	43 individuals with class III obesity and 28 exmorbidly obese individuals with class I obesity				↓ M1-like cells in SAT and VAT	
Cinkajzlova et al. (196)	83	32 nondiabetic individuals with class III obesity and 32 individuals with class III obesity and diabetes and 19 lean controls	In plasma: ↓ M2 In SVF of SAT: ↓ M2		In plasma: ↓ M2 In SVF of SAT: ↓ M2		
Moreno-Navarrete et al. (199)	6	Women with class III obesity and normal glucose metabolism					↑ M2
Hagman et al. (200)	17	Men and women with obesity class II and III	↑ M1 ↑ M2			↑ M1 ↑ M2	
Hess et al. (201)	40	20 individuals with class III obesity and 20 lean controls		↓ ∼1.1% M1 ↑ ∼10.1% M2			

ATM, adipose tissue macrophages; SAT, subcutaneous adipose tissue; SVF, stromal vascular fraction; VAT: visceral adipose tissue; lean (BMI ≤24.9); class I (BMI 30–34.9); class II (BMI 35–39.9); class III (BMI ≥40); ↓ : significant decrease; ↑ : significant increase; Ø: no significant change or not applicable.

amount of weight loss. Similarly, Figueroa-Vega et al. (213) found that before surgery the concentrations of proangiogenic markers (ANG-1, ANG-2, Tie-2, and HIF-1α) were overexpressed (both in serum and adipose tissue), which correlated with an increased number of infiltrating M1-like macrophages expressing angiogenic receptor Tie-2 especially in SAT (213). At 6 mo after surgery, the expression of these markers was significantly reduced and correlated with a diminished number of infiltrating M1-like macrophages (213). Therefore, angiogenesis may not only be important for adipose tissue expansion, but it may also represent another pathway to explain the chronic inflammation observed in obesity, which is alleviated by weight loss surgery. However, the knowledge of angiogenic mechanisms and its impact on adipose tissue dysfunction and health postsurgery is still rudimentary and requires more research.

Effects of surgery on adipose tissue fibrosis. Fibrosis is a hallmark feature of adipose tissue inflammation as it is triggered and exacerbated by macrophage infiltration (215, 216). However, the reversibility of adipose tissue fibrosis after surgery-induced weight loss is unclear. To our knowledge, only 2 studies have directly examined fibrosis pre- and post-bariatric surgery and both have concluded that levels of fibrosis remained unchanged and persisted despite the significant weight loss in most participants from 6 mo to $\leq$2 y after surgery (194, 217). In contrast, Liu et al. (198) and Reggio et al. (218) observed a downregulation in the expression levels of genes encoding markers of adipose tissue fibrosis from 6 mo to 1 y postsurgery (198, 218). Moreover, Liu et al. (198) observed a positive relationship between collagen accumulation and the number of M2-like (CD163+) cells prior to surgery, indicating a role in the generation of fibrosis in obese SAT. However, this M2-to-pericellular collagen accumulation relation became a negative correlation at the 1-y-follow-up despite the moderate increase in the number of CD163+ cells. Overall, the evolution of fibrosis postsurgery, the role played by ECM proteins, and their link with ATM during weight loss are poorly documented.

Conclusion and Future Prospects

In this review, we discussed the impact of dietary fatty acids, exercise, and bariatric surgery on cellular characteristics affecting ATM presence and phenotypes in obesity. We have shown that dietary fatty acids, exercise training, and bariatric surgery decrease ATM and induce a phenotypic switch to M2-like macrophages through modifying a number of potential mechanisms. In the case of the type of fat ingested and exercise, improvements in ATM occurred regardless of weight loss. These interventions modify ATM by affecting key adipose tissue characteristics such as adipocyte size, adipose tissue fibrosis, angiogenesis, and cytokine and adipokine secretion. Future studies should focus on gaining a better understanding of the underlying mechanisms and consequences of the reduction in macrophage presence and phenotypes, especially in humans. Understanding the events contributing to the pathogenesis of obesity may allow for the development of potential new therapies against obesity and its associated comorbidities.

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