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Metabolic dysfunction and adipose tissue macrophages: is there more to glean from studying the lean?:

Comment on "Adipose tissue infiltration in normal-weight subjects and its impact on metabolic function" by Moreno-Indias et al.

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Metabolic syndrome is a serious health condition that affects approximately 34% of adults. Hallmarks of this syndrome include abdominal obesity, elevated triglycerides, reduced high-density lipoprotein (HDL) cholesterol levels, hypertension, hyperglycemia, and insulin resistance, and those affected are at heightened risk for the development of type 2 diabetes, cardiovascular disease, and stroke. Over the past 20 years, an increasing role for low grade, sterile inflammation in insulin resistance and metabolic syndrome has been appreciated. Studies have shown that obesity induces an inflammatory state that is clinically manifested as altered concentrations of acute-phase response proteins (eg, increased C-reactive protein), proinflammatory cytokines (eg, increased interleukin [IL]-6 and tumor necrosis factor [TNF]α), adipokines (eg, reduced adiponectin and elevated resistin and leptin), coagulation factors (eg, increased plasminogen activator inhibitor-1), and increased white blood cell counts in the circulation. Data from rodent models and clinical samples support the idea that the vast majority of these proinflammatory mediators primarily originate from expanding adipose tissue depots. A variety of leukocytes (eg, macrophages, monocytes, dendritic cells, eosinophils, neutrophils, T cells, B cells, and natural killer T [NKT] cells) are now known to reside in adipose tissue, and these cells are either activated or recruited to fat in response to obesity. Infiltrating immune cells, and the proinflammatory cytokines/chemokines they produce, interact with metabolically stressed cells to generate a proinflammatory milieu that exacerbates insulin resistance not only in fat but in multiple metabolic tissues to promote metabolic syndrome.

Adipose tissue macrophages (ATMs) are the most abundant immune cell population in fat and were the first tissue leukocyte population linked to obesity-induced metabolic disease and inflammation in both mouse models and humans.[1,2] Tissue macrophages adapt a range

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of functional activation states, with "classically activated," proinflammatory M1 macrophages and "alternatively activated," anti-inflammatory M2 macrophages representing extreme ends of a continuum.[3] In lean mice and humans, ATMs in visceral fat are "M2-like," reside between adipocytes, produce anti-inflammatory cytokines (eg, IL-10), and contribute to tissue homeostasis.[4–6] In response to obesity, a second phenotypically and functionally distinct population of macrophages accumulates in fat. These ATMs express markers of proinflammatory M1 macrophages and produce proinflammatory cytokines (eg, TNFα, IL-1β, and IL-6) known to impair insulin sensitivity in adipose tissue.[4,7–12] In mice, "M1-like" ATMs are recruited in response to lipolysis[13] and aggregate around dead and hypoxic adipocytes,[14,15] suggesting that metabolic stress is a primary driving force for macrophage recruitment in adipose tissue. Collectively, these and other findings led to the "phenotypic switch" model, which proposes that obesity induces a collective shift in the activation state of ATMs from an anti-inflammatory "M2-like" state that protects adipocytes to a proinflammatory "M1-like" state that contributes to insulin resistance.[4] More recently, this linear paradigm has been challenged by proteomic studies demonstrating that human macrophages in fat have a "metabolically activated" phenotype that differs from classical M1 polarization.[16] Nonetheless, it is clear that macrophages accumulate in fat depots with obesity, and these cells can alter the inflammatory milieu to promote local and systemic dysfunction.

Even as being overweight (body mass index [BMI] = 25–29.9 kg/m^2) and obese (BMI 30 kg/m^2) is strongly associated with the likelihood of developing adipose tissue inflammation and insulin resistance, a fraction of obese people never fully manifest metabolic syndrome, insulin resistance, or diabetes.[17,18] These "metabolically healthy (MH) obese" individuals provide unique insight into pathogenesis of metabolic disease. Paradoxically, epidemiologic studies indicate that a subset of individuals with "normal" body weight (BMI <24.9 kg/m^2) may also develop insulin resistance and metabolic syndrome during their lifetime. Although the latter are more rarely studied, "metabolically unhealthy (MU) lean" subjects may hold equally important new clues into the origins of metabolic syndrome and its sequela.

In this issue of *Translational Research*, Moreno-Indias et al. have attempted to address this knowledge gap. The authors investigated the expression levels of human macrophage markers, including CD68, CD33, CD11c, CD163, MerTK, CD64, and CD206, in visceral and subcutaneous adipose tissue (SAT) depots in normal-weight (BMI <24.9 kg/m^2) subjects stratified into MH or MU populations, based on indices of metabolic syndrome.[19] Despite having comparable BMI, the MU group had significantly elevated fasting glucose, higher triglyceride levels, increased total and low-density lipoprotein (LDL) cholesterol levels, and lower HDL cholesterol levels than the MH group. As predicted, MU subjects had more relative insulin resistance, as indicated by increased HOMA-IR levels, as well as elevated serum insulin and c-peptide levels.

Despite marked differences in metabolic indices between MH and MU, there were no differences in the expression of macrophage markers in visceral adipose tissue between the two groups. This was surprising given the relationship between visceral/omental fat inflammation, ATM content, and insulin resistance/metabolic syndrome previously documented in obese adults. Furthermore, although serum C-reactive protein levels were

higher in MU subjects, the expression of proinflammatory cytokines (IL-1β, IL-6, and TNFα) and chemokines associated with leukocyte recruitment (CCL2 and CCL3) was not different between groups, irrespective of the adipose tissue depot surveyed. Rather, CD68, CD11c, CD163, CD33, MerTK, and CD206 messenger RNA levels were unexpectedly elevated in SAT from MU subjects. Notably, peroxisome proliferator-activated receptor gamma (PPARγ) expression was also lower in SAT from MU subjects, suggesting a relationship between adipose tissue dysfunction and macrophage infiltration in the subcutaneous depot.

These findings fall in line with other reports. For instance, Wentworth et al.[11] reported that CD11c 1 CD2061 ATMs accumulate in visceral as well as subcutaneous depots collected from obese women. Notably, the number of CD11c + CD206+ ATMs was even greater in obese subjects with metabolic syndrome. In a more recent study, the expression of CD163 in subcutaneous fat was previously shown to positively correlate with HOMA-IR in obese patients.[20] Although CD11c + CD206+ or CD163+ ATMs were not enumerated in the present study, the transcriptional profile (elevated macrophage markers with reduced PPARγ expression) supports a model in which ATMs accumulate in SAT to potentially promote insulin resistance and metabolic dysfunction in that depot. What sets the work of Moreno-Indias et al. apart is the documentation of these phenomena in normal-weight subjects with indices of metabolic syndrome, suggesting an important but largely unexplored link between ATMs and metabolic syndrome that arises independent of obesity and inflammation in visceral fat.

At the same time, the Moreno-Indias study reminds us of the "chicken-and-egg" question that continues to remain largely unanswered—Does recruitment of ATMs to fat depots cause insulin resistance and metabolic dysfunction, or does insulin resistance/metabolic dysfunction precipitate recruitment of ATMs to fat? Studies in mice models of obesity (both genetic and diet induced) largely favor the hypothesis that accumulating, proinflammatory ATMs promote chronic tissue inflammation and exaggerate insulin resistance.[1,2,4,21] Certainly, ablating macrophages in obese mice or preventing macrophage recruitment into adipose tissue improves glucose tolerance, insulin sensitivity, and metabolic function.[22–24] However, there are also studies that suggest metabolic dysfunction precedes ATM recruitment and activation. For example, induced lipolysis[13] and adipocyte apoptosis[25] result in massive infiltration of ATMs to visceral fat in the mouse. More recently, the Czech laboratory demonstrated that circulating cytokine profiles more closely associate with serum insulin levels rather than BMI in human subjects and that pharmacologically correcting hyperinsulinemia and hyperglycemia in obese mice limits ATM accumulation and adipose tissue inflammation.[26] The effects in mice may be explained, at least in part, by direct effects of obesity, hyperglycemia, hyperinsulinemia, or a combination thereof on the process of myelopoiesis,[27–29] which would give rise to pools of monocytes capable of infiltrating adipose tissue, ultimately giving rise to proinflammatory ATMs. Notably, one recent human study reported that monocyte and lymphocyte markers are increased in subcutaneous fat of obese women following 3-hour-long hyperglycemic clamps.[30] Although Tencerova et al. did not assess ATM content, their work suggests that hyperglycemia may provoke a coordinated immune response in adipose tissue.

Study limitations make it difficult to establish a clear causal relationship between adipose tissue leukocyte content, inflammation, and indices of metabolic syndrome in human subjects. In the future, the Precision Medicine Initiative could be leveraged to support studies aimed at assessing the contribution of various adipose tissue leukocytes to tissue inflammation longitudinally in metabolically defined populations. Given the sheer volume of bariatric procedures performed in this country annually, and the high retention rates in the National Institutes of Health Longitudinal Assessment of Bariatric Surgery consortium,[31] it seems reasonable that prospective, multi-site studies can address the relationship between surgery-induced weight loss, changes in adipose tissue immune cell composition, and insulin sensitivity over time. By contrast, determining whether leukocyte infiltration and proinflammatory activation leads to metabolic disease in humans will be a much more challenging endeavor, as this pathophysiology could take decades to develop. Nonetheless, the findings by Moreno-Indias et al. suggest that studying an often overlooked population—the MU lean—may also lead to advances. Clearly new prospective studies should include as many metabolic parameters from as many divergent phenotypic populations as possible, as it is becoming increasingly clear that BMI alone is not strongly correlated with adipose tissue leukocytosis, metabolic syndrome, and disease risk.

To be successful, new research initiatives also have to address another recurring limitation of human studies: getting the most information from a small bio-specimen. Most laboratories have the capacity to perform gene expression profiling or cytokine measurements from fat biopsies. Although this gives a general sense as to the relative state of inflammation or leukocyte infiltration within the fat depots, this approach fails to take into consideration the cellular complexity of adipose tissue and the wide heterogeneity of adipose tissue leukocytes. For example, CD206 is expressed by at least 2 distinct populations of macrophages in human fat[11,20,32]; therefore, CD206 messenger RNA levels can only serve as a surrogate for macrophage accumulation and cannot be used to infer which macrophage subset is amassing. To overcome this limitation, tissue dissociation and flow cytometry techniques have been developed that allow for not only the enumeration and immunophenotyping of adipose tissue stromal cells but also isolation of these cells for gene expression profiling and functional studies.[33–35] Nonetheless, our collective understanding of the immune cell repertoire in human adipose tissue depots is still in its infancy. Emerging "-omics" techniques, including single-cell RNA-seq and mass cytometry (eg, CyTOF), have the capacity to deeply interrogate the transcriptome and proteome (and underlying systems biology) of rare tissue leukocytes with single cell resolution.[36–38] Adopting these platforms, developing suitable workflows for limited adipose tissue samples (eg, needle biopsies) from a variety of depots, and implementing unbiased computational analysis of the resulting data sets promises to greatly accelerate our basic understanding of human adipose tissue leukocyte diversity in lean and obese subjects.

Given the prevalence of obesity and metabolic syndrome, understanding the underlying biology of human adipose tissue inflammation and its relationship with cardiovascular disease, type 2 diabetes, and stroke is a question of paramount importance. Specifically, findings presented by Moreno-Indias et al. in this issue suggest that adipose tissue leukocytes may also be key determinants of metabolic fitness even in normal-weight subjects. Clearly, their results suggest there is more to glean from studying the lean.

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