Obesity and muscle-macrophage crosstalk in humans and mice: A systematic review

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Abstract

Obesity is associated with the production of inflammatory cytokines that are implicated in insulin resistance (IR), and if not addressed, can lead to type 2 diabetes (T2D). The role of the immune system in skeletal muscle (SM) inflammation and insulin sensitivity is not yet well characterized. As SM insulin resistance is an important determinant of glycemia, it is critical that the muscle-immune phenotype is mapped to design interventions to target T2D. This systematic review synthesized the evidence for SM macrophage content and phenotype in human and murine models of obesity, and the association of muscle macrophage content and phenotype with IR. Results were synthesized narratively, as we were unable to conduct a meta-analysis. We included 28 studies (n=10 human, n=18 murine), and all studies detected macrophage markers in SM. Macrophage content was positively associated with IR. In humans and mice, there was variability in muscle macrophage content and phenotype in obesity. Overall certainty in the evidence was low due to heterogeneity in detection methods, and incompleteness of data reporting. Humans and mice show a presence of macrophage in SM in obesity and a positive association between macrophage content and IR, however there are differences in muscle macrophage phenotype in obesity.

INTRODUCTION

One-in-three people around the world are affected by overweight or obesity, and this is associated with an extensive global burden of noncommunicable diseases including myocardial infarction, stroke, hypertension, and type 2 diabetes (T2D)¹⁻³. While the biopsychosocial drivers of obesity continue to be revealed⁴⁻⁷, one consistent phenomenon is the expansion of the adipose compartment^{2, 8}. This expansion is associated with altered adipose tissue (AT) autocrine, paracrine, and endocrine functions with disrupted cellular metabolic signalling and insulin resistance (IR). If untreated, IR may progress to type 2 diabetes (T2D), a major contributor to cardiovascular disease risk⁹⁻¹¹.

While adipocyte expansion was considered as one of the main contributors to inflammation, contemporary insights have highlighted the role of immune cells in AT dysregulation and local inflammation and IR in obesity^{9, 12-14}. One of the most prominent cells in this immune-metabolic crosstalk is the macrophage¹⁴. Macrophages are innate immune cells that exist on a spectrum of phenotypes that at its extremes include pro-inflammatory (M1) and anti-inflammatory (M2)

macrophages¹⁵. In obesity, adipocyte production of chemokines including Chemokine C-C motif Ligand 2 (CCL2) and others creates a chemotactic gradient that attracts bone marrow-derived monocytes to exit the circulation and enter the AT and muscle. Sensing the local AT microenvironment, these cells differentiate to M1 macrophages, and there is a similar process of monocyte attraction evident in muscle^{14,24}. Excess adipose tissue M1 macrophage content has been linked, although inconsistently, to local tissue inflammation^{13, 16, 17}. It is likely that intermediate macrophage phenotypes that harbor markers of both M1 and M2 cells are generated based on the local microenvironment, and these cells remain to be fully characterized^{18, 19}. As the AT is one of the main organs that expand in obesity, adipose tissue inflammation and adipocyte-macrophage crosstalk has been widely studied²⁰⁻²⁶. On the other hand, macrophage existence and actions in other metabolic organs including skeletal muscle (SM) has received less attention. There is a critical need to understand muscle-immune crosstalk in obesity, as SM accounts for the majority of postprandial glucose clearance, and its IR is a primary determinant of glucose dysmetabolism early in obesity²⁷⁻²⁹. Factors affecting SM insulin sensitivity, including inflammation, can contribute to increased risk for developing obesity-driven T2D, making it a potential target for intervention to mitigate the risk of diabetes^{1, 30}. Murine models have been used to provide critical insights into the pathogenesis of obesity³¹⁻³⁶. Comparative transcriptional profiling suggests significant similarities in gene expression and their transcriptional regulation in murine and human monocytes but also some differences³⁷. Whether murine muscle-immune crosstalk mimics that of humans in obesity is critical to clarify, so that

therapeutic research findings are more confidently generalized from mice to humans. This will enhance the chances of success of moving obesity therapies from the bench to the clinic.

The aim of this systematic review is to summarize and assess the quality of the current evidence base for macrophage content and phenotype in SM in overweight/obesity in humans and murine models, and determine the association between muscle macrophage content and phenotype with IR.

Objectives

The primary objectives of this review are to evaluate the presence of macrophages in SM in humans and mice with overweight/obesity and assess their association with IR. The secondary objective is to assess muscle macrophage phenotype in humans and mice with overweight/obesity.

METHODS

This systematic review has been registered with PROSPERO (registration no. CRD42016033035) and the methods are available in a published protocol³⁸. This systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, and the completed PRISMA checklist is available in the supplementary materials (Table S1). We briefly report on the systematic review methods below.

Data sources & search strategy

We implemented a search strategy that collated evidence from several databases including MEDLINE, Embase, Web of Science, the Cochrane Library and SPORTDiscus from inception to December 2017. ProQuest Dissertations and Theses, the National Institutes of Health and the World Health Organization International clinical trials registries were searched as sources of grey literature. Additionally, we hand-searched reference lists of included studies. A sample of the search strategy was included in the published protocol for this review³⁸.

Eligibility criteria

Multiple study designs were eligible for inclusion in this systematic review including cohort, cross-sectional and case-control studies that investigated macrophage infiltration into SM in humans and mice with overweight and obesity. We also aimed to include pilot and feasibility studies and conference abstracts and posters if applicable. We included studies reporting on the detection of macrophage markers in SM regardless of the technique used for detection.

Studies including patients with hypertension, chronic renal disease, autoimmune disease, cancer, pregnancy, smokers and athletes were excluded as these conditions are known to alter immune activation status. We also excluded studies with participants receiving parenteral nutrition, steroids, anti-inflammatory or immunomodulating therapies.

While we did set out initially to capture observational studies reporting macrophages in muscle in obesity, we did identify intervention-based studies for inclusion that reported pre-intervention data on macrophage markers, and also for a comprehensive picture of muscle-immune crosstalk in obesity and its modulation with interventions.

Outcome Measures

The primary outcomes measured were macrophage content in SM in humans and mice with overweight/obesity and their association with IR. The secondary outcome was the phenotype of muscle-infiltrating macrophages in humans and mice with overweight/obesity.

Study selection and data abstraction

Two authors working independently within three teams reviewed the articles during each phase of the article selection process. For title and abstract selections, the reviewers screened the titles and abstracts emanating from the search results applying the inclusion and exclusion criteria to select eligible titles and abstracts. For the selected abstracts, the full-text papers were reviewed to determine suitability for inclusion in the systematic review. Disagreements were resolved to reach consensus through discussions among team members, and a third reviewer was consulted if consensus could not be reached.

Quality of evidence assessment

For human studies, we used the Newcastle Ottawa Scale (NOS) for case-control and cohort studies to assess the risk of bias³⁹. We applied the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool to evaluate mice studies⁴⁰. We also used the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) framework tool to provide an overall assessment of the quality of evidence, as we noted that it can be applicable for the included studies, and this was not initially proposed in our protocol.

Data synthesis

Results from studies were synthesized narratively and reported in summary tables. Descriptive statistics were used to report the frequencies for measurements of macrophage content and the various macrophage markers present in SM. It was not possible to conduct a meta-analysis, as proposed in the *a priori* protocol due to the heterogeneity in experimental procedures and the lack of exact reported values. Furthermore, data were not comparable across studies, since presence of macrophage markers was reported as relative proportions within studies.

RESULTS

We screened 1090 unique citations and included data following the full text screening from 10 studies in adult human subjects⁴¹⁻⁵⁰ and 18 studies in mice^{26, 42, 43, 47, 51-64}. Figure 1 outlines the number of articles included at each phase in the screening process, including the reasons for exclusion. In reporting the data for humans and mice, we describe the muscle macrophage content first, followed by the macrophage phenotypes and their association with IR.

Human studies

The results from the human studies are summarized in Table 1. Ten studies were identified, including studies that reported interventions. Significant variation was noted in the reported study design, group comparisons, macrophage marker choice, method of macrophage marker detection, and statistical adjustments. There was a total of 228 participants in all studies included⁴¹⁻⁵⁰. The age range of participants was 20-61 years, and one study did not report on the age of participants⁴⁸. Nine studies (n=212, 93%) reported on participants' sex distribution²², with the majority of participants being female (n=148; 69.8%).

Five studies compared adults who were lean (Body Mass Index (BMI) 18.5 to <25 kg/m²) to those with overweight (BMI ≥25-29.9 kg/m²) and obesity (BMI ≥30 kg/m²)^{41, 43-45, 47}. There were 100 participants (43.9%) with overweightedness or obesity across studies, and one study (n=14) did not specify the number of subjects who were lean or with obesity⁴⁸. While one study reported participants to be of normal weight, based on World Health Organization classification of BMI cutoffs of overweight and obesity in Asia⁶⁵, many participants were in the overweight category, and we did end up including the study in our analysis⁵⁰.

Of the 10 human studies included, heterogeneity was noted in study populations and comparison groups when it comes to body mass and glucose tolerance. Three studies reported on lean normal glucose tolerance (NGT) versus participants with obesity and NGT^{41, 45, 47}, and two studies recruited three groups including patients who were lean and had NGT, patients with obesity and NGT, and those with overweight/obesity and T2D^{43, 44}. One study included only patients with obesity, NGT and T2D⁴², two studies compared patients with NGT (n=28) to those with impaired glucose tolerance (IGT, n=34)^{49, 50}, and the remaining two studies included lifestyle interventions included patients with obesity only⁴⁶ and those with obesity and T2D⁴⁸ (n=21, 13 female). These studies investigated 9-month⁴⁶ and 15-week⁴⁸ exercise programs that we ended up including in our analysis.

The molecular techniques used to detect macrophages and their phenotype included the assessment of mRNA abundance (Real-Time Polymerase Chain Reaction (RT-PCR) and Quantitative RT-PCR (qRT-PCR)), and protein detection (western blot, Enzyme-Linked

Immunosorbent Assay (ELISA), and Immunohistochemistry (IHC)). One paper used Transmission Electron Microscopy to determine macrophage content³¹. Different measures were used to report on insulin action and its association with macrophages. The use of Insulin Sensitivity Index (ISI), the frequently sampled intravenous glucose tolerance test (FSIVGTT), oral glucose tolerance test (OGTT), euglycemic hyperinsulinemic clamp, fasting plasma insulin, fasting plasma glucose, from which the Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) and HOMA were calculated (Table 1).

Muscle macrophage content in human studies

All human studies identified macrophages in muscle⁴¹⁻⁵⁰. While we had proposed in our protocol that we are going to perform subgroup analyses based on age and sex, the reported data formats did not allow such analyses. In addition, we had proposed certain markers to characterize macrophages in muscle however we summarized findings on the markers reported in the studies.

The most common marker used to detect macrophages was the Cluster of Differentiation 68 (CD68)⁶⁶, a glycoprotein that is mainly localized to lysosomes and is expressed primarily in macrophages, and another marker used was CD40 (Table 1).

Muscle macrophage phenotype in human studies

Markers used to detect and broadly classify macrophage subtypes included those of inflammatory macrophages (CD14, CD16, CD11b, and Toll-Like Receptors (TLR) 2 and 4), anti-inflammatory or resident macrophages (CD14, CD16, CD163, CD206, CD11c the galactose-binding lectin (MGL) and Transforming Growth Factor β 1 (TGF- β 1)). The level of

expression of some markers denotes whether the cell is an inflammatory or resident macrophage e.g. CD14 and CD16. In addition, the most important macrophage chemoattractant to muscle (Chemokine C-C motif Ligand 2 (CCL2)) was also measured. Several cytokines produced by the muscle cells themselves or by the immune cells within muscle were detected including mainly inflammatory cytokines (Tumor necrosis factor α (TNF α), Interleukins (IL; IL-1 β , IL-6)) and anti-inflammatory cytokines (IL-10, IL-1receptor antagonist a (IL-1Ra)) (Table 1).

Association of muscle macrophages with obesity and glucose tolerance in human studies

Comparison of lean versus obesity in subjects with NGT:

In the three studies comparing individuals with NGT and lean status to those with NGT and obesity, there was conflicting evidence regarding the direction of the association between obesity and muscle macrophage content and variability in the markers used to ascertain muscle macrophage content and phenotype^{41, 45, 47}. One study reported increased CD68 expression in subjects with obesity compared to those who were lean⁴¹, while another study reported significantly higher CD11b and a non-significant trend for higher CD11c in individuals with obesity compared to those who were lean, yet no difference in CCL2 expression, a major macrophage chemoattractant⁴⁷. Macrophage content correlated negatively with insulin sensitivity (FSIVGTT)⁴¹. In contrast, one study reported lower macrophage content, reduced macrophage gene expression (CD68, CD11b, CD16, CD40, CD163, CD206,), with lower CCL2 and TNF α in SM of subjects with obesity compared to those who were lean, even after age and sex

adjustments. IL-6 and IL-10 were not detectable in most patients⁴⁵. After controlling for BMI or the fat mass only CD40, CD206, and CD163 were positively correlated with ISI⁴⁵.

Taken together, there is inconclusive evidence as to whether muscle macrophage markers change with obesity in NGT, and the exact phenotype of these macrophages is unclear. The increased macrophage marker expression is associated with IR.

Comparison of subjects with NGT versus IGT:

Between the two studies that compared subjects with NGT and IGT (without distinction of BMI)^{49, 50}, there was conflicting evidence on the association of glucose tolerance with SM macrophage content. One study reported that CD68 and CCL2 mRNA is detectable in muscle at similar levels in subjects with NGT and IGT⁴⁹. The other study found higher macrophage content on transmission electron microscopy and increased expression of CD68, TLR-4, TNF- α and IL-6 in subjects with IGT compared to those with NGT, and similar TLR-2 expression⁵⁰. Therefore, there was similar or higher muscle macrophage content in subjects with IGT compared to those with NGT, and cells had an inflammatory phenotype in IGT when a higher macrophage content was detected in muscle in the second study. There was no correlation between CD68 and insulin resistance.

Comparison of subjects with NGT, lean and obesity versus subjects with type 2 diabetes subjects:

In the two studies that compared subjects with NGT, lean/obesity to those with overweight/obesity and T2D, there was a trend for increased macrophages in T2D^{43, 44}. One

study found greater expression of macrophage markers CD11c and CD11b mRNA in subjects with obesity and T2D compared to those with obesity and NGT, yet this correlation was lost after adjustment of age. However CD206, CD163 and MGL mRNA abundance was similar between these two groups and TGF- β 1 mRNA abundance was lower in patients with obesity and T2D compared to those with obesity and NGT⁴⁴. MGL and TGF- β 1 negatively correlated with HbA1c and fasting glucose levels. The other study reported greater CD68 and TNF- α mRNA abundance in subjects with obesity and T2D compared to those with NGT, lean and obesity, and CD68 correlated positively with HOMA-IR⁴³. One study compared individuals with obesity and NGT and those with obesity and T2D⁴², and found a non-significant trend of higher CD68 macrophages in subjects with T2D compared to those with NGT, and CD68 positively correlated with BMI⁴². CD68 and CD11c correlated positively with fasting plasma insulin and negatively with glucose disposal rate measured by hyperinsulinemic-euglycemic clamp, and CD68 also positively correlated with fasting plasma glucose⁴².

Taken together, these data indicate that having T2D is associated with increased macrophage markers in muscle that is correlated positively with body mass. The exact phenotype of these macrophages is less clear, with some evidence of increased inflammatory and equal or reduced anti-inflammatory macrophage markers. Macrophage content was associated with insulin resistance in this population.

Impact of interventions on muscle macrophage content and phenotype:

Three studies investigated the effect of interventions on muscle macrophages^{46, 48}.

In a nine-month exercise program in subjects with obesity and T2D, CD68 was detectable in muscle at baseline, with no change in macrophage content, HbA1c, or HOMA with the intervention⁴⁶.

In a 15-week lifestyle intervention in subjects with severe obesity, CD68, CD14, IL-8 and TNF α mRNA abundance were similar at baseline and post-intervention. However, IL-6 expression decreased significantly post-intervention⁴⁸. Another study reported minimal changes in macrophage markers with exercise intervention²⁶. In addition, there was no change in macrophage content when subjects with IGT were treated with the insulin sensitizer metformin and the anti-diabetic agent Pioglitazone⁴⁹. These data suggest that current interventions do not alter macrophage content in muscle in obesity or diabetes.

Risk of bias in human studies

Table 2 presents a summary of the risk of bias assessment for human studies. Risk of bias was *moderate to high* for nine out of the ten studies⁴¹⁻⁴⁹ and *low* for only one study⁵⁰. Sources of bias included lack of description of participant recruitment strategies^{41-43, 45-49} and unclear definition for the cases^{41, 45-47} and controls^{41, 42, 46-49}. Additionally, most studies did not conduct statistical analyses comparing cases and controls or adjust for confounders^{41-44, 46-49}.

Murine studies

The results from the 18 murine studies are presented in Table 3. Eight studies used C57BL/6 mice^{43, 51-56, 59}, nine used C57BL/6J mice^{26, 42, 47, 57, 58, 60-63, 67}, one study used FVB/N mice⁶⁴. Studies also included other mouse models such as the ob/ob leptin deficient mice^{43, 57} and the

RCS10 mice with obesity⁵³. In addition, IL-10 overexpression and CD11c knockout mice were also tested. All studies compared lean mice fed a standard chow diet (daily kcal from fat <20%) to diet-induced mice with obesity fed a high fat diet (HFD, daily kcal from fat >40%). The age range of mice at the start of the dietary intervention was 4-12 weeks, and one study did not report the initial age of the mice⁴³. The duration of high-fat feeding ranged from 2-24 weeks. The molecular techniques used to detect macrophages included gene expression (RT-PCR, qRT-PCR) and protein expression (western blot, ELISA, flow cytometry and IHC).

Muscle macrophage content in murine studies

All murine studies reported the presence of macrophages within skeletal muscle. The most frequent macrophage marker used was F4/80. In IHC analyses, macrophage markers were mostly localized to intermyocellular adipose depots^{26, 42, 43, 47, 52, 53, 56, 57, 59}, and in one study they were detected between myofibers as well as in the intermyocellular fat compartment⁴². While most studies reported higher macrophage marker expression in mice with obesity compared to those who were lean^{26, 42, 43, 47, 51-60, 62, 63, 68}, some others did not report this association^{61, 64}. One study reported lower mRNA abundance for IL-10 and Fractalkine receptor (CXCR3)⁵¹. Taken together, there data suggest that there is relatively consistent evidence for the increase in muscle macrophage marker expression in murine obesity.

Muscle macrophage phenotype in murine studies

Macrophage phenotyping used a broad set of macrophage markers. Pro-inflammatory (M1) macrophage markers used included CD11b, Toll-Like Receptors (TLR) 2 and 4, C-C chemokine

receptor type 2 (CCR2), and Galectin-3 (MAC2). The anti-inflammatory (M2) macrophage markers included CD11c (a dendritic cell marker), CD206, CD163, CD301b, Fractalkine receptor (CX3CR1), Found in inflammatory zone 1 (Fizz1), and Macrophage galactose lectin 1 (MGL1). Other chemokines or their receptors were also tested (Table 2).

Out of eight studies that tested the gene expression of CD11c^{42, 43, 47, 52, 56, 58, 62, 69}, six reported elevated CD11c among mice with obesity compared to those who were lean^{42, 43, 47, 52, 56, 69}. Higher CD206 and CD301b and lower CX3CR1 expression was noted in mice with obesity compared to lean ones^{42, 58, 70}. The evidence suggests that muscle macrophages in obesity trend towards an anti-inflammatory phenotype in mice.

Association of muscle macrophages with obesity and glucose tolerance in murine studies

Murine studies assessed glucose homeostasis and insulin action by measuring fasting blood glucose, glucose and insulin tolerance tests, HOMA-IR, 2-hour hyperinsulinemic-euglycemic clamp as well as protein kinase B (Akt) and insulin receptor substrate 1 (IRS-1) phosphorylations, which are markers of insulin signaling. These studies reported that mice with obesity had elevated fasting glucose and insulin levels, impaired glucose tolerance, and reduced insulin sensitivity compared to lean mice^{43, 47, 53, 57, 59, 62, 64}.

Furthermore, four studies investigated the link between different muscle inflammation markers and IR^{43, 53, 57, 59}. The enhanced mRNA abundance of macrophage markers CD68 and CD11c negatively correlated with glucose tolerance measured via glucose tolerance tests (GTT) and insulin tolerance tests (ITT)²⁴. In addition, knockout of molecules associated with inflammation

including CD11c⁺ or the overexpression of the anti-inflammatory cytokine IL-10 along with exercise interventions led to protection against high fat diet-induced IR^{53, 57, 59}. Higher muscle macrophage content was inversely correlated with HOMA-IR, GTT, ITT, as well as Akt phosphorylation^{43, 59}.

The evidence from murine studies was supported by *in vitro* experiments to investigate the effect of lowering inflammation on glucose uptake and insulin signaling in myotubes. Inflammation neutralization via CD11c⁺ macrophage ablation⁵⁷ and atherosclerosis-prone apolipoprotein E (ApoE) knockout⁶⁰ were all shown to improve insulin-stimulated glucose uptake in myotubes, and co-cultures of myotubes with macrophages led to reduced insulin-stimulated glucose uptake⁵⁶. In summary, mice with obesity have insulin resistance, a finding that correlates positively with their muscle macrophage content.

Impact of interventions on murine muscle macrophages and insulin action

Several studies reported interventions to reduce obesity and inflammation in mice with studies reporting exercise regimens and pharmacotherapies. In the former, a six-week endurance exercise program consisting of 3 days per week of 15 minutes exercise at 10-15 meters/minute led to reduced macrophage F4/80 expression in active mice with obesity versus a control group of sedentary mice with obesity⁵⁹. The second exercise regimen consisted of a 12-week treadmill protocol of 40 minutes per day, 5 days per week at 5% grade at 12 meters/minute. Exercise reduced the F4/80 and TGF-β1 levels associated with HFD⁵⁸.

There were several studies examining the use of pharmacotherapy in modulating muscle macrophage content and inflammation with high fat diet-induced obesity. Treatment with the antidiabetic medication Rosiglitazone, a Thiozolidinedione, reduced the expression of CD68, CD11c, CCR2, and CCL2 that was increased by HFD. The antidiabetic agent Canagliflozin, a sodium glucose co-transporter 2 inhibitor, decreased mRNA abundance of F4/80 and TNF α in mice with obesity⁶³. The use of Quercetin, a plant-based flavonoid, inhibited the rise in gene and protein expression of CCL2 and TNF α ⁵⁴. Baricitinib, a Janus kinase (JAK) 1 and 2 inhibitor, reduced T-Lymphocyte but not macrophage markers in skeletal muscle of high fat-fed mice⁴⁷.

Genetic manipulation of murine models and its effects on muscle macrophages

Several murine models were used to study muscle inflammation in obesity. The overexpression of the anti-inflammatory cytokine IL-10 reduced macrophage levels in skeletal muscle and protected the mice against HFD-related IR⁵³. Ablation of CD11c in macrophages on an HFD resulted in preservation of insulin sensitivity and reduced expression of CD68, TNF α , and CCL2⁵⁷.

The blockade of 4-1BB and 4-1BBL, type 2 transmembrane glycoproteins found on T-cells and macrophages and are important in T-Lymphocyte activation, led to reduced obesity-induced skeletal muscle inflammation⁵⁵. In ApoE^{-/-} mice, a mouse model of atherosclerosis with systemic inflammation, Akt phosphorylation was preserved in mice with obesity⁶⁰.

One study examined muscle monocyte chemoattractant protein 1 (MCK-MCP1) transgenic mice, over expressing MCP-1(also known as CCL2), which is one of the most important chemokines

that recruit macrophages to sites of inflammation, and reported dysglycemia and systematic IR with elevated levels of CD68, CD11c, TNF- α , IL-1 β and Regulated on Activation Normal T-cell Expressed and Secreted (RANTES) compared to wild type mice⁴³.

In summary, murine studies inconsistently demonstrated that HFD-induced obesity was associated with an increase in muscle macrophage content and that exercise and pharmacotherapeutic agents lowered macrophage markers in obesity.

Risk of bias across mouse studies

The risk of bias was assessed using the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) tool across mouse studies (Table 4) and was *unclear* or *high* for multiple study domains.

There was *unclear* or *high* risk of bias surrounding sequence generation, allocation concealment and random housing for 17 of the 18 studies. While the blinding of investigators may be difficult due to visible changes in the mice receiving high fat diet, blinding of outcome assessors was reported only in three studies. Random outcome assessment was not documented clearly in the included studies. We rated risk of bias to be *unclear* for incomplete outcome data because studies did not provide the number of mice randomized to intervention groups, and it was unclear whether all mice were included in the final data analysis. There was *high* risk of bias surrounding selective outcome reporting in five studies^{26, 43, 52, 56, 61}, such that data for pre-specified outcomes were not shown or not described in detail mostly when the findings were not significant.

GRADE assessment

We assessed overall certainty in the evidence using GRADE framework (Table S2)⁷¹. The assessment found a *very low* certainty of evidence in the included studies for the outcomes of macrophage content, phenotype, and the association between macrophages and insulin resistance. This was due to ratings of ‘serious’ to ‘very serious’ for risk of bias, inconsistency, indirectness, and imprecision. Indirectness was rated as ‘serious’ due to variability in recruitment, exposure measurement, outcome measurements, and populations. Imprecision was rated as serious due to the small sample sizes. For the mouse studies, we considered inconsistency to be ‘not serious’ for all outcomes. However, for the human studies, inconsistency was rated as ‘serious’ for all outcomes due to differences in study populations and measurement techniques for exposure and outcomes. Risk of bias was rated ‘serious’ among all outcomes for human and murine studies due to issues with participant selection and randomization, confounding bias, and selective reporting.

Due to the variability in macrophage markers and lack of exact results being reported, we were unable to determine the presence of publication bias through funnel plot or Egger’s test however we expect that there is publication bias with murine studies.

DISCUSSION

Skeletal muscle is the largest metabolic organ in humans and mice, and impaired insulin signaling and postprandial glucose disposal in obesity is a critical event that primes the risk for developing T2D, one of the most significant chronic diseases of the 21st century⁷².

On a mechanistic level, inflammation is one pathway through which obesity can drive muscle insulin resistance and beget T2D. This inflammatory response can result from the local inflammatory cytokines produced by myocytes or immune cells that infiltrate muscle pursuing a chemokine gradient⁴³. In addition, the intermyocellular fat depot expands with obesity and attracts monocytes that will contribute to the local inflammatory process. Muscle is also subjected to the influx of systemic inflammatory cytokines and fatty acids that are produced in remote organs including the adipose tissue and arrive to muscle through the circulation⁷³⁻⁷⁵. The identification of muscle-macrophage crosstalk pathways is critical to unlock strategic approaches to target inflammation and prevent, delay, and treat obesity and T2D. This may significantly improve the health outcomes of billions of people around the world by mitigating the effects of obesity and T2D on health. In this systematic review, we demonstrate that the evidence base for addressing the question of SM and macrophage crosstalk in humans and mice with obesity has limitations.

In human studies, macrophage content and phenotype were variable with obesity in NGT, while in subjects with obesity and IGT, macrophages were either equal or increased. T2D was associated with increased macrophage markers with either enhanced, equal, or reduced anti-inflammatory macrophage marker expression. Macrophage content in muscle was positively associated with insulin resistance in NGT and T2D but not in IGT. Rather surprisingly, interventions including exercise and pharmacotherapy did not alter muscle macrophage marker expression in subjects with obesity or T2D. This may be due to short-term nature of all

interventions, which limits the full understanding of their impact on muscle-immune crosstalk in obesity and diabetes. This is an area that requires further research.

In mice, there was some inconsistency in the reported macrophage expansion in obesity. Most studies reported an increase in total macrophage content and suggested an increase in one marker of anti-inflammatory macrophages/dendritic cells CD11c and increased anti-inflammatory macrophage markers CD206 and CD301b and a reduction in CX3CR1. More consistent evidence of the association of macrophage markers with insulin resistance was reported in mice, and exercise interventions and pharmacotherapy resulted in the reduction of muscle macrophage markers and inflammation.

Reporting of study methodology had limitations among both human and murine studies.

Observational studies in human subjects lacked several details including the reporting of the full profile of obesity and leanness and recruitment strategies. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement has been published for improving reporting of observational studies⁷⁶, and endorsement by publishing journals of future studies will likely improve the reporting of eligibility criteria for participants. Use of the STROBE extension for studies in molecular epidemiology (STROBE-ME) can also improve methodological reporting for laboratory-based experimentation among studies investigating biomarkers of inflammation and insulin action in obesity⁷⁷.

The majority of murine studies did not report on random sequence generation, allocation concealment, random housing and random outcome assessments. It may be that the methodology

across included studies was adequate, however it was not possible to judge this based on the data reported, resulting in an unclear risk of bias. Although inadequate reporting of methods may be due to seven mouse studies^{26, 52, 53, 56, 57, 61, 62} being published prior to the availability of the SYRCLE reporting statement for animal studies, a number of recent studies have similar methodological limitations. The Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, published in 2010, aimed to improve the quality of reporting of animal studies⁷⁸. These reporting guidelines are now endorsed by the Enhancing the QUality and Transparency Of health Research (EQUATOR) Network, which is an international initiative that aims to increase reliability of published health research by promoting transparency in the reporting of studies⁷⁹. It is critical that investigators use the ARRIVE guidelines to report their experimental work and results to allow the comparisons across studies using their detailed methods. Furthermore, mandated requirements from preclinical journals to use reporting guidelines and submit a completed checklist is important in enhancing the quality of published data and allow replication. This has already been shown to be valuable in clinical research with randomized controlled trials⁸⁰, and can be useful in bench-based research.

Strengths and limitations

The strengths of this review include the comprehensive literature search and *a priori* determined approach to investigate macrophage infiltration in SM during obesity in humans and mice to ensure the transparency of the search process.

Some of the limitations include the variability in reporting of the macrophage content results as relative gene and protein expression, such that we were unable to conduct a meta-analysis. In addition, the differences noted in human and murine muscle macrophage phenotype suggests that murine models are an important tool to study muscle-macrophage crosstalk, however this may not be the case for other immune cells and this requires further assessment. More focus on well-designed, adequately-powered human and murine studies with well-defined macrophage classification marker sets is critical to provide insights into the muscle immune phenotype in obesity and T2D. This will support the enhancement of the translational potential of murine studies moving forward.

CONCLUSION

Humans and mice with obesity appear to have a heterogenous muscle-macrophage crosstalk phenotype with certain similarities but some important differences. The adoption of tools including STROBE or STROBE-ME and the recently established ARRIVE guidelines when reporting preclinical and translational research studies will improve transparency and quality of reporting of the research findings. Ultimately, this will enhance the potential for knowledge translation to benefit patients.

Authors' contributions

MCS is the study guarantor. SR, MB, DP, LB, GRS, MT and MCS were responsible for the development of the study question. SR, MB, GRS, LT, LB, KWW, PWW, RB, DP, MT and MCS were responsible for the study design. SR, LB, MB, PWW, KWW, GRS, LT, DP, RB, and

MCS developed the search strategy. SR, LB, MB, E.D.S., W.E., PWW, KWW, RB, and MCS executed the search strategy.

SR, PWW, RB, EDS, WE, and MB conducted risk of bias assessments. SR, MB, GRS, KWW, EDS, WE, LT, DP, MT, and MCS were responsible for synthesis and interpretation of data. SR, MB, EDS, WE, and MCS developed the first draft of the manuscript. All authors provided critical revisions of the manuscript and approved the final version. SR & MB contributed equally to this work.

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Legends for tables and figures:

Figure 1. Flow diagram of study inclusion process

Table 1. Summary of human studies

Table 2. Risk of bias summary for human studies

Table 3. Summary of mouse studies

Table 4. Risk of bias summary for mouse studies

Supporting information:

Table S1. PRISMA Checklist

Table S2. GRADE Table

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Figure 1. Flow diagram of study inclusion process

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