HHS Public Access

Author manuscript

Obesity (Silver Spring). Author manuscript; available in PMC 2013 February 01.

Published in final edited form as:

Obesity (Silver Spring). 2012 July ; 20(7): 1530–1533. doi:10.1038/oby.2012.24.

Low macrophage accumulation in skeletal muscle of obese type 2 diabetics and elderly subjects

Charmaine S Tam, PhD, **Lauren M Sparks, PhD**, **Darcy L Johannsen, PhD**, **Jeffrey D Covington, B.S**, **Timothy S Church, PhD, MD**, and **Eric Ravussin, PhD**

Pennington Biomedical Research Center, Baton Rouge, LA

Abstract

In addition to adipose tissue, recent studies suggest that skeletal muscle may also be a source of low grade inflammation, particularly in inactive and/or overweight individuals. The aim of this study was to examine the presence of macrophages in skeletal muscle from obese subjects with type 2 diabetes (T2D) before and after a 9-month exercise program (vs. a non-exercising control group) (Study 1) and in young vs. elderly subjects (Study 2). In both studies, CD68+ macrophages in *vastus lateralis* biopsies were determined by immunohistochemistry and inflammation gene expression measured. Macrophage content (%) was calculated by the number of macrophages per 100 muscle fibers. In *Study 1*, we found relatively low numbers (2–3%) of CD68+ macrophages in skeletal muscle in obese T2D subjects (BMI= 37.3 ± 5.2kg/m^2), which were unchanged after a 9-month exercise program (P=0.42). Similarly, in *Study 2* (BMI=27.1 ± 2.5kg/m^2), CD68+ macrophages were relatively low in muscle (4–5%) and were not different between young and elderly individuals (P=0.42). However, elderly subjects had 2-fold higher CD68 and CD206 gene expression (both P<0.002) than young participants. In both studies, CD68+ muscle macrophages were not associated with BMI. In conclusion, we found little evidence of macrophage accumulation in skeletal muscle in obese T2D subjects or in elderly individuals. A 9-month exercise program was not associated with a decrease in macrophage content.

Keywords

macrophage; muscle; inflammation; obesity; type 2 diabetes

Introduction

Adipose tissue from obese humans and high-fat fed animal models is characterized by significant immune cell accumulation. In particular, macrophages located in crown-like structures secrete pro-inflammatory mediators into the circulation leading to a state of low-grade inflammation. Some studies suggest that macrophages also directly infiltrate skeletal muscle, potentially further contributing to local and systemic inflammation in obesity (1–5).

Corresponding author and reprint requests to: Eric Ravussin, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA, USA, 70808, Phone: 225-763-3186, Fax: 225-763-3030, eric.ravussin@pbrc.edu.

Disclosure: The authors have no conflicts of interest and nothing to disclose

Weisberg et al were the first to show a three-fold increase in F4/80+ macrophages in muscle tissue adipose depots in high-fat diet fed mice (2). Similarly, Nguyen et al used fluorescence-activated cell sorting analysis to show increased numbers of M1-activated (CD11c+) macrophages in muscle from mice fed a high-fat diet (5). In humans, Varma et al reported 2.5-fold higher CD68+ macrophage numbers in skeletal muscle from obese subjects compared to lean. Strikingly, they also show that macrophage content in muscle was strongly associated with BMI and inversely related with insulin sensitivity (1). In addition, CD68+ macrophages in skeletal muscle are higher in lean subjects with impaired glucose tolerance (4). In contrast, two studies have reported relatively low expression of macrophage genes, CD68, CD14 and MCP1 in skeletal muscle of obese individuals (6;7).

Aging has also been described as a state of chronic low-grade systemic inflammation, referred to as 'inflamm-aging' (8). The evidence for this is mainly derived from epidemiological studies that have correlated systemic markers of inflammation with low muscle mass, physical inactivity and frailty in the elderly (8). To our knowledge, the only study to directly examine macrophages in skeletal muscle in young versus elderly people in resting conditions showed that CD68+macrophages are lower and cytokine gene expression is higher (IL-1β, IL-IRA) in elderly subjects (9).

In this study, we used immunohistochemistry to examine CD68+ macrophages in *vastus lateralis* biopsies from two studies: 1) obese type 2 diabetics randomized to either a 9-month exercise program (aerobic/resistance training) or no exercise and 2) young and elderly individuals. We also performed gene analysis for a range of macrophage and inflammation markers.. We hypothesized that a) A 9-month exercise program would decrease skeletal muscle inflammation in obese type 2 diabetics, and b) compared to young subjects, elderly subjects would have lower muscle inflammation.

Methods

Both studies were approved by the Pennington Biomedical Research Center Institutional Review Board and all participants gave written informed consent. *Study 1* was an ancillary study to a randomized control trial in which 262 men and women with T2D and HbA_{1c} levels of 6.5% or higher were enrolled in one of four 9-month exercise programs (aerobic training, resistance training, combination of aerobic and resistance training and a non-exercise control group) (10). A subset of 20 participants per group had skeletal muscle biopsies taken at baseline and post-intervention. Given that the overall finding of the parent study was that only the combination of aerobic and resistance training improved HbA_{1c} levels compared to a non-exercise control group (10), we only examined skeletal muscle macrophages in participants that had both baseline and month 9 muscle biopsies in these 2 groups. This consisted of 7 non-exercisers (1 male, 6 females; age= 56.0 ± 5.5y; BMI= 35.2 ± 4.4kg/m^2) versus 7 subjects who underwent 9 months of aerobic and resistance training (1 male, 6 females; age= 54.8 ± 5.5y; BMI= 39.4 ± 5.4kg/m^2). *Study 2* consisted of 13 young (7 males, 6 females; 21 to 33y; BMI= 25.2 ± 2.8kg/m^2) and 12 elderly (6 males, 6 females; 70 to 81y; BMI= 26.9 ± 2.5kg/m^2) who were part of a cross-sectional study of age-associated alterations in skeletal muscle mitochondrial activity and had muscle biopsies

taken (11). Elderly subjects (33.3 ± 9.2%) had significantly higher %body fat compared to young subjects (24.9 ± 7.9%, P=0.03).

Immunohistochemistry

Macrophages were quantified with an antibody to CD68 (MO718, DAKO, Carpinteria, CA). To quantify macrophage number, three non-consecutive sections per sample were examined at ×20 magnification. The total area and the number of CD68+ macrophages and skeletal muscle fibers in the whole section were determined. Partial myofibers located on section borders were excluded as were CD68+ cells associated with the vasculature. Particular care was taken to exclude areas which did not contain muscle fibers (connective tissue or gaps due to sectioning artifacts) or had longitudinal fibers (sectioning artifacts), and to avoid areas where adipose tissue was present. Macrophage presence is expressed in two ways: macrophage content in the skeletal muscle biopsy (number of macrophages per number of cross sectional fibers × 100%) and the number of macrophages per cross-sectional area of skeletal muscle. Data were analyzed using SPSS.V.19 and presented as mean ±SD or median (interquartile range).

Gene expression

Absolute quantification of mRNA expression was analyzed using ABI Prism 7900 (Applied Biosystems, Branchburg, NJ) and custom Taqman gene expression measured against a standard curve for CD68 (Hs00154355_m1), CCL2 (Hs00234140_m1), CD40 (Hs00374176_m1), CD206 (Hs00267207_m1), CD11c (HS01015070_m1) and Arginase 1 (Hs00968979_m1). Samples were run in duplicate and expression levels normalized to RPLPO (Hs99999902_m1). For *study 2*, we only had RNA for 10 young and 8 elderly subjects. Arginase 1 was not detected in 2/3 of the samples.

Results

Muscle macrophage content and inflammation was unchanged after a 9-month exercise program in obese type 2 diabetics

In *Study 1*, controls had 2.2 (2.0–3.1%) skeletal muscle macrophages at baseline which was not different after 9 months [1.7 (0.9–6.95%); P= 0.85]. Contrary to our hypothesis, subjects in the exercise group had 2.7 (2.0–3.2%) skeletal muscle macrophages at baseline which was unchanged after 9 months of the exercise program [4.0 (3.2–6.8%), P= 0.41] (Figure 1; panel A and B). Similarly, inflammation gene expression levels were unchanged in either group (data not shown). Using a repeated measures ANOVA, there was no significant effect of time (P=0.44) or group (P=0.74) on % muscle macrophages, and no significant interaction between time*group (P=0.90). Similar non-significant results were seen for inflammation gene expression (data not shown). There were no associations between baseline BMI and %macrophages and inflammation genes. Similar findings were seen when the number of macrophages per surface area was used in the above analyses (data not shown). Change in %macrophages between baseline and month 9 was not associated with changes in HbA_{1c}, C-reactive protein, BMI, %fat, %fat-free mass or HOMA (data not shown).

Higher CD68 and CD206 expression in elderly compared to young subjects, but no difference in macrophage content

In *Study 2*, elderly subjects had 2-fold higher CD68 (young= 0.4 ± 0.2; elderly= 0.8 ± 0.2AU; P=0.002) and CD206 mRNA levels (young= 0.4 ± 0.2; elderly= 0.9 ± 0.1AU; P<0.001) compared to young subjects. However, CD68+ macrophages were present in only 12 (6 young, 6 elderly) out of 25 skeletal muscle samples. In these 12 samples, %muscle macrophages were not different between young [4.7 (1.7–8.4%)] and elderly subjects [4.0 (0.6–6.1%); P=0.42] (Figure 1; panel C). Similar findings were seen when macrophages per cross sectional area was analyzed (data not shown). There were no significant correlations between BMI or %fat and macrophage content (% or number of macrophages/area) or inflammation genes. There were no differences in CD11c, CCL2 and CD40 mRNA levels between young and old subjects (data not shown).

Discussion

Recent studies in high-fat fed mouse models and humans have pointed to macrophage accumulation in obese skeletal muscle leading to the speculation that these macrophages may secrete additional pro-inflammatory mediators that contribute to the chronic-low grade inflammatory state seen in obesity (1;2;4;12). In contrast to these previous findings, our results show relatively low amounts (2–3%) of macrophages in skeletal muscle of obese type 2 diabetics, which were unchanged following a 9-month exercise program. In support of this, there were no significant changes in inflammation gene expression with exercise training. Varma et al previously reported 2.5 fold higher CD68+ muscle macrophages in 8 obese (25%) compared to 8 lean (10%) individuals (1). Subjects in Varma's study and our study had similar BMIs (Tam= 37.3kg/m^2; Varma= 36.9kg/m^2), and similar immunohistochemistry methods were used. Another study in subjects with impaired glucose tolerance (BMI=24.9kg/m^2) also showed increased CD68+ skeletal muscle macrophages and CD68, IL-6, TNF-α and TLR-4 protein levels, however that study did not describe the quantification of macrophages or report numbers of macrophages in the skeletal muscle (4). It may be considered a limitation of our study that we did not quantify macrophages in a lean control group, however the %macrophages (2–3%) in our study are considerably lower than both the lean (10%) and obese groups (25%) in Varma et al's study. It is possible that cross-contamination of adipose tissue in skeletal muscle leads to artificially higher macrophage numbers or inflammation levels, as was seen in some skeletal muscle samples from our study (see Figure; panel D). In agreement, the first study to report macrophages in skeletal muscle only showed them in the intermuscular adipose tissue region, and two other studies in morbidly obese subjects (BMI range= 37 to 45kg/m^2) reported low CD68 and MCP1 mRNA levels in skeletal muscle (6;7). Nevertheless, it is well-demonstrated *in-vitro* that an inflammatory response in muscle cells can lead to impaired insulin signaling (1;4;5;12). Further studies investigating macrophage accumulation and the association with lipid deposition in skeletal muscle *in vitro* and *in vivo* are necessary to explore the presence of inflammation in skeletal muscle in obesity and type 2 diabetes.

Our study also found that 2-fold higher CD68 and CD206 mRNA expression in elderly compared to young subjects. Despite this, skeletal muscle macrophages were not different

between the two groups (young =4.7%, elderly=4.0%). Aging has been suggested to be a state of low-grade inflammation (8) and elderly people have 2 to 3-fold higher circulating IL-6 (13). To our knowledge, the only study that has examined skeletal muscle macrophages in young and elderly patients showed that CD68+ macrophages are lower in *vastus lateralis* from elderly subjects (9). Notably, regardless of age, we were unable to demonstrate higher macrophage accumulation in skeletal muscle of mostly overweight subjects as has been reported in other studies (1).

In conclusion, we found little evidence of macrophage accumulation in skeletal muscle in obese type 2 diabetic and elderly subjects. Furthermore, macrophages in skeletal muscle were not influenced by 9-months of combined aerobic and resistance exercise despite an improvement in glucose control. Future studies need to carefully examine the presence of adipose tissue in skeletal muscle depots as contamination with adipose tissue may potentially lead to artificially higher numbers of skeletal muscle macrophages being reported.

Acknowledgments

Funding sources: This work was supported by NIH grant # DK068298. This work utilized the facilities of the Cell Biology and Bioimaging Core that are supported in part by NIH center grants NORC (P30-DK072476) and COBRE (P20-RR021945).

References

1. Varma V, Yao-Borengasser A, Rasouli N, et al. Muscle inflammatory response and insulin resistance: synergistic interaction between macrophages and fatty acids leads to impaired insulin action. Am J Physiol Endocrinol Metab. 2009; 296(6):E1300–E1310. [PubMed: 19336660]
2. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003; 112(12):1796–1808. [PubMed: 14679176]
3. Kewalramani G, Bilan PJ, Klip A. Muscle insulin resistance: assault by lipids, cytokines and local macrophages. Curr Opin Clin Nutr Metab Care. 2010; 13(4):382–390. [PubMed: 20495453]
4. Kim TH, Choi SE, Ha ES, et al. IL-6 induction of TLR-4 gene expression via STAT3 has an effect on insulin resistance in human skeletal muscle. Acta Diabetol. 2011
5. Nguyen MT, Favelyukis S, Nguyen AK, et al. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J Biol Chem. 2007; 282(48):35279–35292. [PubMed: 17916553]
6. Bruun JM, Helge JW, Richelsen B, Stallknecht B. Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. Am J Physiol Endocrinol Metab. 2006; 290(5):E961–E967. [PubMed: 16352667]
7. Di Gregorio GB, Yao-Borengasser A, Rasouli N, et al. Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. Diabetes. 2005; 54(8):2305–2313. [PubMed: 16046295]
8. Peake J, Della GP, Cameron-Smith D. Aging and its effects on inflammation in skeletal muscle at rest and following exercise-induced muscle injury. Am J Physiol Regul Integr Comp Physiol. 2010; 298(6):R1485–R1495. [PubMed: 20393160]
9. Przybyla B, Gurley C, Harvey JF, et al. Aging alters macrophage properties in human skeletal muscle both at rest and in response to acute resistance exercise. Exp Gerontol. 2006; 41(3):320–327. [PubMed: 16457979]

10. Church TS, Blair SN, Cocreham S, et al. Effects of aerobic and resistance training on hemoglobin A1c levels in patients with type 2 diabetes: a randomized controlled trial. JAMA. 2010; 304(20): 2253–2262. [PubMed: 21098771]

11. Johannsen DL, Conley KE, Bajpeyi S, et al. Ectopic Lipid Accumulation and Reduced Glucose Tolerance in Elderly Adults Are Accompanied by Altered Skeletal Muscle Mitochondrial Activity. J Clin Endocrinol Metab. 2011

12. Bilan PJ, Samokhvalov V, Koshkina A, Schertzer JD, Samaan MC, Klip A. Direct and macrophage-mediated actions of fatty acids causing insulin resistance in muscle cells. Arch Physiol Biochem. 2009; 115(4):176–190. [PubMed: 19671019]

13. Pedersen M, Bruunsgaard H, Weis N, et al. Circulating levels of TNF-alpha and IL-6-relation to truncal fat mass and muscle mass in healthy elderly individuals and in patients with type-2 diabetes. Mech Ageing Dev. 2003; 124(4):495–502. [PubMed: 12714258]

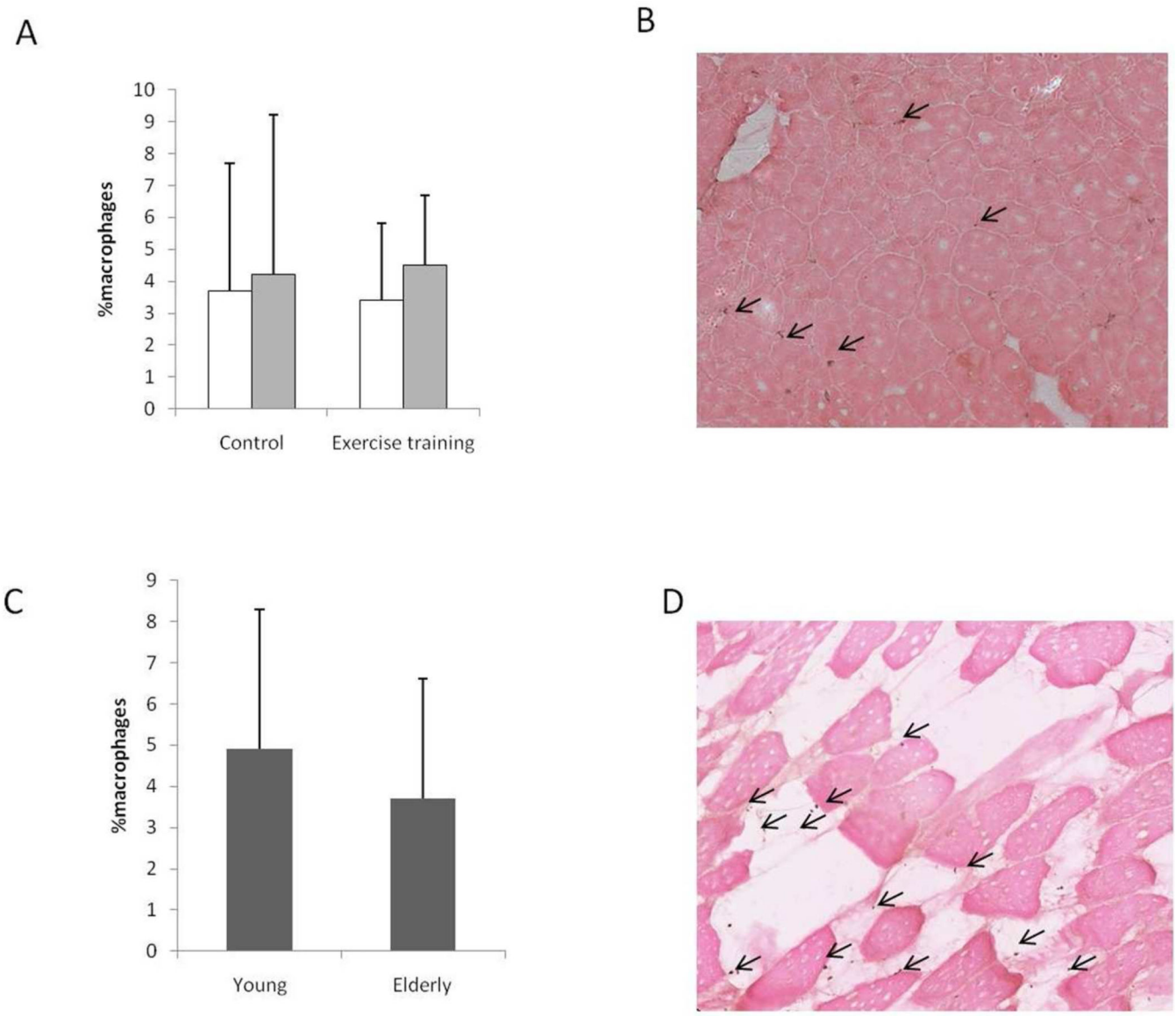

Figure 1.

CD68+ macrophages in *vastus lateralis* in obesity and aging. A) %Muscle macrophages (mean ± SD) in obese type 2 diabetics at baseline (unfilled bars) and 9 months after (filled bars) no exercise or an exercise program. B) %Muscle macrophages (mean ± SD) in young vs. elderly subjects. %muscle macrophages was calculated by the number of macrophages per number of cross sectional fibers X 100. C) CD68+ staining of skeletal muscle from a representative obese type 2 diabetic subject and D) example of adipose tissue located in the skeletal muscle depot, with greater CD68+ staining.