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AIMS AND SCOPE
The primary aim of World Journal of Diabetes (WJD, World J Diabetes) is to provide scholars and readers from various fields of diabetes with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJD mainly publishes articles reporting research results and findings obtained in the field of diabetes and covering a wide range of topics including risk factors for diabetes, diabetes complications, experimental diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, diabetic angiopathies, diabetic cardiomyopathies, diabetic coma, diabetic ketoacidosis, diabetic nephropathies, diabetic neuropathies, Donohue syndrome, fetal macrosomia, and prediabetic state.

INDEXING/ABSTRACTING
The WJD is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Current Contents/Clinical Medicine, Journal Citation Reports/Science Edition, PubMed, PubMed Central, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2024 Edition of Journal Citation Reports® cites the 2023 journal impact factor (JIF) for WJD as 4.2; JIF without journal self cites: 4.1; 5-year JIF: 4.2; JIF Rank: 40/186 in endocrinology and metabolism; JIF Quartile: Q1; and 5-year JIF Quartile: Q2.

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Basic Study

Interactions between myoblasts and macrophages under high glucose milieu result in inflammatory response and impaired insulin sensitivity

Wei Luo, Yue Zhou, Li-Ying Wang, Lei Ai

**Abstract**

**BACKGROUND**

Skeletal muscle handles about 80% of insulin-stimulated glucose uptake and become the major organ occurring insulin resistance (IR). Many studies have confirmed the interactions between macrophages and skeletal muscle regulated the inflammation and regeneration of skeletal muscle. However, despite of the decades of research, whether macrophages infiltration and polarization in skeletal muscle under high glucose (HG) milieu results in the development of IR is yet to be elucidated. C2C12 myoblasts are well-established and excellent model to study myogenic regulation and its responses to stimulation. Further exploration of macrophages’ role in myoblasts IR and the dynamics of their infiltration and polarization is warranted.

**AIM**

To evaluate interactions between myoblasts and macrophages under HG, and its effects on inflammation and IR in skeletal muscle.

**METHODS**

We detected the polarization status of macrophages infiltrated to skeletal muscles of IR mice by hematoxylin and eosin and immunohistochemical staining. Then, we developed an *in vitro* co-culture system to study the interactions between myoblasts and macrophages under HG milieu. The effects of myoblasts on macrophages were explored through morphological observation, CCK-8 assay,
Flow Cytometry, and enzyme-linked immunosorbent assay. The mediation of macrophages to myogenesis and insulin sensitivity were detected by morphological observation, CCK-8 assay, Immunofluorescence, and 2-NBDG assay.

RESULTS
The F4/80 and co-localization of F4/80 and CD86 increased, and the myofiber size decreased in IR group (P < 0.01, g = 6.26). Compared to Mc group, F4/80+CD86+CD206- cells, tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β) and IL-6 decreased, and IL-10 increased in McM group (P < 0.01, g > 0.8). In McM + HG group, F4/80+CD86+CD206- cells, monocyte chemoattractant protein 1, TNFα, IL-1β and IL-6 were increased, and F4/80+CD206+CD86- cells and IL-10 were decreased compared with Mc + HG group and McM group (P < 0.01, g > 0.8). Compared to M group, myotube area, myotube number and E-MHC were increased in MMc group (P < 0.01, g > 0.8). In McM + HG group, myotube area, myotube number, E-MHC, GLUT4 and glucose uptake were decreased compared with M + HG group and MMc group (P < 0.01, g > 0.8).

CONCLUSION
Interactions between myoblasts and macrophages under HG milieus result in inflammation and IR, which support that the macrophage may serve as a promising therapeutic target for skeletal muscle atrophy and IR.

Key Words: Macrophages phenotype; Myoblasts; Cross-talk; Glucose toxicity; Chronic inflammation; Insulin sensitivity

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Core Tip: This study demonstrated interactions between myoblasts and macrophages under high glucose (HG) milieus induced pro-inflammatory M1 polarization of macrophages to exacerbate inflammatory response. Subsequently, chronic inflammation induced by HG-related M1 macrophages damaged myogenesis and insulin sensitivity in myoblasts. Ultimately, interactions between myoblasts and macrophages resulted in skeletal muscle insulin resistance (IR), which supported macrophage may serve as a promising therapeutic target for skeletal muscle atrophy and IR. This is the first research about the mediation of macrophages to HG-related myogenic inhibition and IR in myoblasts, which provide new insights into the prevention and treatment of skeletal muscle atrophy and IR.

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INTRODUCTION
The prevalence of diabetes, especially type 2 diabetes mellitus (T2DM), has been dramatically increasing in China, from 10.9% in 2013 to 12.4% in 2018. Insulin resistance (IR) is considered as the pathogenic driver of T2DM and precedes non-physiologic elevated plasma glucose levels, which is the primary clinical symptom of T2DM. In the prediabetic condition, insulin levels increase to meet normal insulin requirements leading to chronic hyperinsulinemia, hyperglycemia-induced β-cell failure, and eventually leads to T2DM[1,2]. The IR is defined physiologically as a state of reduced responsiveness in insulin-targeting tissues to high physiological insulin levels[3], which essentially reduced the insulin-activated glucose transport and metabolism in its target organs[4]. Skeletal muscle is one of the most important target organs of insulin, which handles about 80% of insulin-stimulated glucose uptake[5,6]. Numerous research have demonstrated the vicious cycle between myoatrophy and IR in skeletal muscle that promotes the further development of IR[7,8]. Atrophy in skeletal muscle impairs its insulin sensitivity by inhibiting glycometabolism, whereas, skeletal muscle hypertrophy and remodeling promote its insulin sensitivity[9-12]. Although the pathogenesis for IR has not yet been fully elucidated, increasing evidence prove that the chronic inflammatory response in insulin-targeting tissues plays an important role in the occurrence and development of IR[13,14].

Macrophages are one of the most abundant immunocyte populations infiltrating into tissues and can be polarized towards either pro-inflammatory M1 phenotype or anti-inflammatory M2 phenotype to regulate inflammatory responses [15,16]. The adipose tissues in obesity-induced IR subjects is characterized by the infiltration of pro-inflammatory M1 macrophages[17,18]. Meanwhile, accumulation of pro-inflammatory M1 macrophages are also reported in liver[19] and pancreatic islets[20] of IR subjects, which indicates that the macrophages may mediate the occurrence of IR. Although macrophage infiltration associated with IR has not been clearly reported in skeletal muscle, many studies have confirmed the interactions between skeletal muscle and macrophages that regulates the inflammatory reaction and regeneration in skeletal muscle[21,22]. Macrophages are indispensable for muscle remodeling and repair after injury, where they play either a promoting role for regeneration or a phagocytic role for degeneration depending on their polarized phenotype [23,24]. However, whether the interactions between skeletal muscle and macrophages results in the development of IR is...
yet to be elucidated. Therefore, this study focuses on macrophages to investigate the potential mechanism behind the chronic inflammation induced IR in skeletal muscle.

Our previous research to establish IR mice model has observed that the fasting blood glucose in mice fed with high-fat diet was significantly elevated at the early stage of treatment (about 7th weeks)\[25\], which indicates that the high glucose (HG) milieu maybe an early factor to induce IR. In line with our previous finding, a large body of data supports the HG per se can induce desensitization of insulin action to induce IR\[26-28\]. Whereas, effective blood glucose control can improve insulin sensitivity in skeletal muscle\[29\] and adipocytes\[30\]. HG is generally thought of as a factor that induces tissue and cell harm and dysfunction, commonly known as “glucose toxicity”\[31\]. However, despite of the decades of research, the pathogenesis of IR induced by HG still remains unclear. Hence, in this study, we treated macrophages and myoblasts with HG medium to explore the pathogenesis of IR in skeletal muscle.

In the present study, we first detected cross-sectional area of myofiber and polarization states of macrophages in skeletal muscle of IR mice. Subsequently, to explore interactions between myoblasts and macrophages under HG milieu, we prepared a co-culture system of C2C12 myoblasts and RAW264.7 macrophages in vitro in the absence or presence of HG. Our results, for the first time, demonstrated the mediation of macrophages to HG-related myogenic inhibition and IR in myoblasts, which may help to understand the pathogenesis of IR in skeletal muscle and the involvement of macrophages. Our study may contribute to further explore the influences of HG milieus on inflammatory response and insulin sensitivity in skeletal muscle in vivo.

**MATERIALS AND METHODS**

**Animals and treatments**

Male C57BL/6H mice were purchased at 5–6 wk old from Charles River Laboratories (Beijing, China). They were free fed in chamber with comfortable room temperature between 22-25 °C with 12 h day-night cycle. After one week of adaptation, animals were randomly fed with one of two diets for 12 wk: Normal standard diet (Con group, 3.42 kcal/g, 12% of energy from fat) or high-fat diet (IR group, 5.24 kcal/g, 60% of energy from fat). The contents of mineral and vitamin in these two diets was identical. This study was approved by the Institutional Animal Care and Use Committee of Nanjing Sport Institute (Protocol No. GZRDW-2022-02).

**Glucose tolerance test and insulin tolerance test**

Mice were fasted for 12 h and received i.p. injection of glucose (2 g/kg)\[32,33\]. The blood samples were collected from the tail vein and serum glucose level was determined at time 0, 30, 60, 90, and 120 min using glucometer (ACCU-CHEK Performa Roche, Germany). For insulin tolerance test (ITT), mice were fasted for 4 h and received i.p. injection of insulin (0.75 U/kg)\[33-35\]. The blood samples were collected and glucose concentrations were measured at time 0, 15, 30, 60, and 120 min using glucometer.

**Euthanasia**

After 12 wk of intervention, animals from Con and IR group (n = 6) were deeply anesthetized after deprived from food for 12 h (sodium amytal, i.p., 150 mg/kg). The left and right gastrocnemius muscles were then removed for further biochemical and molecular analyses.

**Determination of serum lipid and insulin level**

After a 30 min standing of mice blood, serum samples were obtained by 3500xg centrifugation for 15 min at 4 °C. Biochemical parameters, including triglycerides (TG), total cholesterol (TC), high density lipoprotein-cholesterol, and low density lipoprotein-cholesterol (LDL-C), were measured with kits from SEKISUI MEDICAL (Tokyo, Japan) on an automatic biochemical analyzer from Beckman (AU5800, CA, United States). Fasting insulin (FINS) concentration was measured by enzyme-linked immunosorbent assay (ELISA) by using a kit from Millipore (EZRMI-13K, MA, United States) according to the manufacturer’s instructions. Homeostasis model assessment of IR (HOMA-IR) is determined using the equations: HOMA-IR = [FINS (mU/L) × FPG (mmol/L)]/22.5.

**Hematoxylin and eosin and immunohistochemistry staining for muscle sample**

Muscle samples were collected and fixed in isopentane for cryopreservation at -80 °C. The muscle tissue was sliced to a thickness of 8 μm in frozen microtome by cross cutting and rip cutting. The sections were subjected to the hematoxylin and eosin staining and immunohistochemical staining. For H&E staining, muscle sections by cross cutting were used and stained with hematoxylin for 10 min, then washed and stained with eosin for 5 min. Lastly, sections were observed under light microscope (Nikon, Japan). For immunohistochemical staining, muscle sections by cross cutting were incubated with antibody mixture of F4/80 (ab111101, Abcam, United States) and CD86 (ab213044, Abcam, United States), or F4/80 and CD206 (ab8918, Abcam, United States) at 4 °C overnight. Then slides were protected from light and incubated with secondary Alexa Fluor 488- or Alexa Fluor 555-labeled goat anti-rabbit or mouse antibodies (A-11034, A-11035, Life Technologies, United States), or F4/80 and CD206 (ab8918, Abcam, United States) at 4 °C overnight. Then slides were protected from light and incubated with secondary Alexa Fluor 488- or Alexa Fluor 555-labeled goat anti-rabbit or mouse antibodies (A-11034, A-11035, Life Technologies, United States). After 12 wk of intervention, animals from Con and IR group (n = 6) were deeply anesthetized after deprived from food for 12 h (sodium amytal, i.p., 150 mg/kg). The left and right gastrocnemius muscles were then removed for further biochemical and molecular analyses.

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**Performa Roche, Germany**

**ACCU-CHEK Performa Roche, Germany**

**HOMA-IR = [FINS (mU/L) × FPG (mmol/L)]/22.5**

**Hematoxylin and eosin staining and immunohistochemical staining**

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macrophage infiltration. Co-localization of F4/80 with CD86 or CD206 were used to quantify macrophages M1 or M2 polarizations respectively.

**Co-cultures RAW264.7 macrophages and C2C12 myoblasts**

RAW264.7 macrophages and C2C12 myoblasts were cultured in Dulbecco’s modified Eagle’s Medium (DMEM; 25 mmol/L glucose; Gibco, United States) supplemented with 10% fetal bovine serum (Gibco, United States) under humidified atmosphere with 5% CO₂ at 37 °C. The 6-well and 24-well transwell co-culture system (Corning, United States) were employed for non-contactable co-culture of RAW264.7 macrophages and C2C12 myoblasts. For co-culture, 2 × 10⁶ and 4 × 10⁶ RAW264.7 macrophages were plated on upper chamber of 6-well and 24-well transwell plates, respectively. Then, 6.6 × 10⁵ and 13.3 × 10⁵ C2C12 myoblasts were plated on lower chamber of 6-well and 24-well transwell plates, respectively. Subsequently, the macrophages were plated at about 30% of the numbers of myoblasts, comparable to the abundance of macrophages observed in skeletal muscle from IR mice. The RAW264.7 macrophages and C2C12 myoblasts were allowed to adhere for 24 h before the co-culture was started. All co-culture treatments were performed for 5 d under DMEM containing 2% horse serum (Gibco, United States) to induce myogenic differentiation of myoblasts. As a control for the co-culture, the same cells were plated to the upper and lower chamber of the transwell co-culture system.

To observe the influence of HG on the interactions between myoblasts and macrophages, cells were treated with 60 mmol/L D-glucose in differentiation medium for 5 d from the start of co-culture[17,36,37]. A 60 mmol/L L-glucose was used in control group (Con group) for the HG as an osmotic control for 60 mmol/L D-glucose[38]. To detect the insulin sensitivity of myotube, C2C12 cells were incubated in the absence or presence of insulin (100 nmol/L) for 30 min at 37 °C before gathered[39,40]. After co-culture, the cells from the upper and lower chamber of the transwell system were collected separately for further biochemical and molecular analyses. Medium from these co-cultures were gathered for the assay of cytokines.

**Cell viability assay**

Cell viability was assessed by CCK-8 assay (APEXBio, United States). After 5 d of treatments, 10 μL CCK-8 solution were added to RAW264.7 macrophages or C2C12 myoblasts and cultured for another 1 h. Then the absorbance was measured at a wavelength of 450 nm using Microplate Reader (Bio-Rad, Hercules, CA, United States), and the cell viability was calculated using the formula: Cell viability (%) = (Aexperimental group - Ablank)/(Acontrol group - Ablank) × 100%. A indicates absorbance at the wavelength of 450 nm.

**Morphological analysis for myotube**

Cells were observed daily using light microscope to detect the effect of co-culture and HG on myoblasts morphology. After the intervention above, myotube area and number were calculated with Image Pro Plus 6.0 program (Media Cybernetics, United States). Six dishes for each group were used, and more than eight visual fields were randomly chosen and analyzed for each dish.

**Flow cytometry for macrophages**

After the intervention above, RAW264.7 macrophages were dissociated with trypsin-EDTA into single cell suspension. They were washed and stained with anti-CD16/32 (553142, BD Biosciences, United States) for 20 min at room temperature to block Fc receptors before incubation with PE/Cy7-labelled anti-F4/80 (ab111101, Abcam, United States), FITC-labelled anti-CD86 (ab213044, Abcam, United States), and PE-labelled anti-CD206 (ab8918, Abcam, United States) monoclonal antibodies in a dark room for 1 h. Subsequently, the cells were examined with flow cytometry (Beckman, United States). The expressions of cell markers were compared with an isotype control. The acquired flow cytometric images were analyzed with FlowJo program (version 10.0.7). The ratio of F4/80+ cells were used to quantify activated macrophage; the ratio of F4/80+CD86+CD206- cells were used to quantify M1 macrophage; the ratio of F4/80+CD206+CD86- cells were used to quantify M2 macrophage.

**ELISA for culture medium**

The protein levels of monocyte chemoattractant protein 1 (MCP1, MJ600C, R&D, United States), tumor necrosis factor-α (TNFα, MTA00B, R&D, United States), sensitivity: 0.1 pg/mL, specificity: Mouse), Inerleukin-6 (IL-6, M6000B, R&D, United States), sensitivity: 0.1 pg/mL, specificity: Mouse), and 13.3 × 10⁵ C2C12 myoblasts were plated on lower chamber of 6-well and 24-well transwell plates, respectively. Then, 6.6 × 10⁵ and 13.3 × 10⁵ C2C12 myoblasts were plated on lower chamber of 6-well and 24-well transwell plates, respectively. Subsequently, the macrophages were plated at about 30% of the numbers of myoblasts, comparable to the abundance of macrophages observed in skeletal muscle from IR mice. The RAW264.7 macrophages and C2C12 myoblasts were allowed to adhere for 24 h before the co-culture was started. All co-culture treatments were performed for 5 d under DMEM containing 2% horse serum (Gibco, United States) to induce myogenic differentiation of myoblasts. As a control for the co-culture, the same cells were plated to the upper and lower chamber of the transwell co-culture system.

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**ELISA for culture medium**

The protein levels of monocyte chemoattractant protein 1 (MCP1, MJ600C, R&D, United States), sensitivity: 0.66 pg/mL, specificity: Mouse), tumor necrosis factor-α (TNFα, MTA00B, R&D, United States), sensitivity: 7.21 pg/mL, specificity: Mouse), Inerleukin-6 (IL-6, M6000B, R&D, United States), sensitivity: 4.8 pg/mL, specificity: Mouse), and IL-10 (M1000B, R&D, United States), sensitivity: 5.22 pg/mL, specificity: Mouse) secreted into medium was quantified with ELISA kit according to the manufacturer’s instructions.

**Immunohistochemistry for C2C12 myoblasts**

After the intervention above, myoblasts were fixed in 4% paraformaldehyde for 15 min, and subjected to immunofluorescence staining. Myoblasts were incubated with antibody mixture of E-MHC (BF-G6, Hybridoma Bank, United States) or GLUT4 (ab654, Abcam, United States), at 4 °C overnight. Then, myoblasts were incubated with Alexa Fluor 488- or Alexa Fluor 555-labeled goat anti-rabbit or mouse antibodies (A-11034, A-21422, Molecular Probes, United States) for 2 h at room temperature. Finally, the stained cells were observed with fluorescence microscope (Leica, Germany). Six dishes for each group were used, and more than eight visual fields were randomly chosen and analyzed for each dish. The positive area of E-MHC and GLUT4 fluorescence were analyzed with Image-Pro plus 6.0 (Media Cybernetics, United States).
Glucose uptake assay for C2C12 myoblasts
After the intervention above, myotubes were starved in DMEM containing 2% fatty acid-free bovine serum albumin for 3 h, then incubated in the absence or presence of insulin (100 nM) for 30 min. Subsequently, they were incubated with 2-NBDG (Cayman, United States) (150 μg/mL) for 40 min. Fluorescence was measured using microplate reader (Bio-Rad, Hercules, CA, United States) at an excitation wavelength of 485 nm and emission wavelength of 535 nm. The negative control and blank control were set, and then standard curve generated to calculate the glucose uptake of myotube in the absence or presence of insulin, which is an indicator to evaluate the insulin sensitivity of myotube.

Statistical analysis
The data are representative of three separate experiments performed with 6 replicates. The data are reported as mean ± SD. The Shapiro-Wilk test was used to test for normality within each data set. According to the Shapiro-Wilk test, the data in each group followed a normal distribution. The two-tailed Student’s t-test was used for comparison between two groups. The multi-group comparisons were performed with two-way ANOVA test followed by Bonferroni post hoc test to analyze the interaction between co-culture and HG. SPSS statistical software (version 20.0) was used for the statistical analyses, and the P < 0.05 was considered as statistically significant. Hedges’ g effect sizes were calculated, and effect sizes were interpreted as: g < 0.2 very small, g = 0.2-0.5 small, g = 0.5-0.8 medium, g > 0.8 large.

RESULTS

High-fat diet fed for 12 wk induces IR in mice
Throughout the 12 wk of high-fat diet, the levels of body weight and fasting blood glucose in mice of IR group were significantly higher than Con group (Figure 1A and B; P < 0.01, g > 0.8). The levels of FINS, HOMA-IR, TG, TC, LDL-C in mice of IR group were also significantly higher than Con group (Figure 1C-E; P < 0.01, g > 0.8). To separate the response to glucose or insulin challenge from glucose homeostasis, we assessed the area under the curve of both glucose tolerance test and ITT. Glucose tolerance and insulin tolerance was impaired significantly in IR group compared with Con group (Figure 1F and G; P < 0.01, g > 0.8).

M1 macrophages increasingly infiltrated into atrophic skeletal muscle in IR mice
To detect whether IR is relevant with macrophage infiltration and M1 phenotype polarization in skeletal muscle, H&E and immunohistochemistry staining was carried on skeletal muscle. As demonstrated by H&E, skeletal muscle from IR mice displayed more inflammatory infiltration (Figure 2A, indicated with black arrows). Furthermore, by immunohistochemical staining, the positive area of F4/80 (a surface marker of macrophages) and co-localization of F4/80 and CD86 (a surface marker of M1 phenotype macrophages) was significantly increased in skeletal muscle from IR mice (Figure 2B; P < 0.01, g > 0.8). In addition, to detect the effect of IR on skeletal muscle morphology, we analyzed the cross-sectional area of myofiber. As shown in Figure 2A, myofiber size in IR group was significantly decreased than that in Con group (P < 0.01, g = 6.26).

Myoblasts in the presence of HG cooperatively induced macrophages polarizing to pro-inflammatory M1 phenotype
To understand the effect of myoblasts on macrophage polarization under HG surroundings, we researched the state of macrophages polarization in the absence or presence of myoblasts and HG. Firstly, cytotoxicity of myoblasts and HG to macrophages were tested by CCK-8 assay. As shown in Figure 3A, treatment of myoblasts and HG had no cytotoxicity on macrophages (P > 0.05, g < 0.2). By comparing the morphology of macrophages after treated without or with HG for 5 d, spindle cells and pseudopodia were observed in cells treated alone with HG (MC + HG group) (Figure 3B, indicated with red arrows), which indicated inflammatory phenotype of macrophages in morphology. We next observed whether myoblasts also cause morphological changes of macrophages. To address this, macrophages were co-cultured with myoblasts in the presence or absence of HG. The morphology was unchanged in macrophages co-cultured with myoblasts under physiological environment (McM group), however, even more spindle cells and pseudopodia were observed in cells co-cultured with myoblasts under HG environment (MCm + HG group) (Figure 3B, indicated with red arrows). Further, we examined surface markers of macrophages polarization by Flow Cytometry and expressions of related proteins in macrophages by Western Blot. Similarly, compared to Con group (MC group), F4/80+CD86+CD206-cells were decreased and F4/80+CD206+CD86- cells were increased in McM group (P < 0.01, g > 0.8), but F4/80+CD86+CD206- cells increased and F4/80+CD206+CD86- cells were decreased in MC + HG group (P < 0.01, g > 0.8; Figure 3C). In McM + HG group, F4/80+CD86+CD206-cells were significantly increased, and F4/80+CD206+CD86- cells were significantly decreased, compared both with MC + HG group and McM group (P < 0.01, g > 0.8; Figure 3C).

Interactions between myoblasts and macrophages in the presence of HG concordently increased secretion of pro-inflammatory cytokines
To verify whether the increased pro-inflammatory M1 phenotype in macrophages is complemented by increasing the secretion of cytokines, to result in chronic inflammation in muscle, we tested the protein levels of MCP1, TNFα, IL-1β, IL-6 and IL-10 in culture medium treated as above. As shown in Figure 4, compared to MC group, levels of pro-inflammatory TNFα, IL-1β and IL-6 were decreased, and level of anti-inflammatory IL-10 was increased in McM group (P < 0.01, g > 0.8); but, levels of MCP1, TNFα, IL-1β and IL-6 were increased, and level of IL-10 was decreased in MC + HG group (P < 0.01, g > 0.8). In McM + HG group, levels of MCP1, TNFα, IL-1β and IL-6 were significantly increased, and the expressions
of IL-10 was significantly decreased, compared with both Mc + HG group and McM group \( (P < 0.05, g > 0.8) \). In brief, results showed that the state of macrophages polarization described above were also reflected in secretions of cytokines in the culture medium, which resulted in chronic inflammation environment for myoblasts.

**Chronic inflammation induced by HG-related M1 macrophages efficiently inhibited myogenesis and induced myotube atrophy**

To explore the mediation of HG-related M1 phenotype macrophages to myogenesis, we researched the differentiation state of myoblast in the absence or presence of macrophages and HG. Firstly, we observed the morphology of myoblasts induced for differentiation in the absence or presence of HG and macrophages for 5 d. As shown in Figure 5A, myoblasts in Con group (M group) differentiated into multinucleated myotube after 5 d of differentiation (indicated with red arrows), and more myotube were observed when myoblasts co-cultured with macrophages under physiological environment (MMc group, \( P < 0.05, g > 0.8 \)); Whereas, area of myotube in myoblasts treated alone with HG (M + HG group) decreased compared to M group (\( P < 0.01, g = 3.37 \)). Both number and area of myotube in myoblasts co-cultured with macrophages under HG environment (MMc + HG group) significantly decreased when compared with M + HG group and McM group \( (P < 0.01, g > 0.8) \), which indicated inhibition effects of HG-related M1 phenotype macrophages on myogenesis in morphology. Then, treatments of macrophages and HG had no cytotoxicity on myoblasts by CCK-8 assay \( (P > 0.05, g < 0.2; \text{Figure 5B}) \). Further, we examined expression of E-MHC (marker of myotube formation) in myotube. Compared to M group, E-MHC positive area was increased in MMC group \( (P < 0.01, g = 2.44) \); but E-MHC positive area was decreased in M + HG group \( (P < 0.01, g = 5.35) \). In McM + HG group, E-MHC positive area was significantly decreased compared both with M + HG group and McM group \( (P < 0.01, g = 4.19; \text{Figure 5C}) \).
Figure 2 Insulin resistance induced myofiber atrophy and pro-inflammatory M1 phenotype macrophages infiltration in skeletal muscle. A: Hematoxylin eosin (H&E) staining of skeletal muscle and the areas of Myofiber (Scale bar: 50 μm); B: The expression of F4/80, and the co-localization of F4/80 with CD86 and CD206 respectively in skeletal muscle detected by Immunofluorescence (Scale bar: 50 μm). Data are means ± SD, n = 6 per group. *P < 0.01 vs Con group. P values were calculated by two-tailed Student’s t-test. Con: Control group; IR: Insulin resistance group.

Chronic inflammation induced by HG-related M1 macrophages hindered insulin sensitivity in myotube

To further probe the mediation of HG-related M1 phenotype macrophages to IR in myotube, we studied the insulin action to stimulate glucose uptake and expressions of related proteins of myoblast in the absence or presence of macrophages and HG. As shown in Figure 6, insulin-stimulated GLUT4 fluorescence (Figure 6A) and glucose uptake (Figure 6B) were increased significantly in the M and MMc groups (P < 0.01, g > 0.8), which displayed fair insulin
Macrophages mediate insulin resistance in myoblasts

Figure 3 Myoblast under high glucose milieus resulted in macrophages polarizing to pro-inflammatory M1 phenotype. A: The viability of macrophages by CCK-8 assay; B: Representative image of macrophages by Light Microscopy, inflammatory phenotype in morphology were indicated with orange arrows (Scale bar: 50 μm); C: The ratios of F4/80+ cells, F4/80+CD86+ cells, and F4/80+CD206+ cells in macrophages by Flow Cytometry. Data are means ± SD, n = 6 per group. aP < 0.01 main effect of high glucose; bP < 0.01 main effect of co-cultured with myoblasts. P values were calculated by two-way analysis of variances. Mc: Macrophages; Mc + HG: Macrophages cultured in high glucose medium; McM: Macrophages co-cultured with myoblasts; McM + HG: Macrophages co-cultured with myoblasts in high glucose medium; HG: High glucose.

**DISCUSSION**

Skeletal muscle handles about 80% of insulin-stimulated glucose uptake and become the major organ occurring IR[4,5]. HG per se result in desensitization of insulin action and induce IR, which is known as “glucose toxicity”[26-28,31]. However, despite decades of research, whether macrophages infiltration and polarization in skeletal muscle under HG milieus results in the development of IR is yet to be elucidated. Herein, the present study demonstrated the interactions between myoblasts and macrophages induce pro-inflammatory M1 polarization of macrophages to aggravate the inflammatory state, resulting impaired myogenesis and insulin sensitivity in myoblasts under HG milieus. More concretely, we could show that: (1) Pro-Inflammatory M1 phenotype macrophages increasingly infiltrated into atrophic skeletal muscle in IR mice; (2) Myoblasts and HG cooperatively induced macrophages polarizing to pro-inflammatory M1 phenotype to aggravate inflammation; and (3) Chronic inflammation induced by HG-related M1 phenotype macrophages damage myogenesis and insulin sensitivity in myoblasts. As we have seen, this is the first report about the mediation of ma-
Although macrophages infiltration associated with IR has not been clearly reported in skeletal muscle, many studies showed evidently smaller myofiber in IR mice, which proves the muscular atrophy in IR mice. This results are in line with some of the previous studies[17,18], which propose that the chronic inflammation contributes to skeletal muscle atrophy and sarcopenia. Currently, several studies have indicated that macrophages may mediate the occurrence of IR[19], but infiltration and polarization of macrophages associated with IR has not been clearly reported in skeletal muscle. Results from this study suggest that macrophages (F4/80+CD206+CD86- cells), and then alleviated inflammation both in macrophages and surrounding environment.

Although macrophages infiltration associated with IR has not been clearly reported in skeletal muscle, many studies have confirmed the interactions between skeletal muscle and macrophages regulated the inflammatory reaction and regeneration in skeletal muscle[20,21]. Our results again confirmed the fact that macrophages are capable of promoting myogenesis in physiological environment, which have proposed in several recent studies[22,23]. However, when exposed to HG milieu, the effects of macrophages to promote myogenesis were reversed. Myogenic differentiation is crucial for muscle repair and quality maintenance, which were obviously suppressed in HG milieu. What’s more, the participation of macrophages in HG environment led to more seriously myogenic inhibition. These results suggested inflammatory reaction induced by HG-related pro-inflammatory macrophages efficiently inhibited myogenesis and induced myotube atrophy and sarcopenia. Currently, several studies have indicated that macrophages may mediate the occurrence of IR[19], but infiltration and polarization of macrophages associated with IR has not been clearly reported in skeletal muscle. Results from this study suggest that macrophages (F4/80+ CD206+CD86- cells), and then alleviated inflammation both in macrophages and surrounding environment.

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Figure 5 Inflammatory reaction induced by M1 macrophages inhibited myogenic differentiation and led to myotube atrophy. A: Representative image of myotube by light microscopy, myotube were indicated with orange arrows (scale bar: 100 μm); B: The viability of myoblasts by CCK-8 assay; C: The ratios of E-MHC positive area in myotube by Immunofluorescence (scale bar: 50 μm). Data are means ± SD, n = 6 per group. *P < 0.05, **P < 0.01 main effect of high glucose; *P < 0.05, **P < 0.01 main effect of co-cultured with macrophages. P values were calculated by two-way analysis of variances. M: Myoblasts; M + HG: Myoblasts cultured in high glucose medium; MMc: Myoblasts co-cultured with macrophages; MMc + HG: Myoblasts co-cultured with macrophages in high glucose medium; HG: High glucose.

Numerous researches have demonstrated the vicious cycle between myoatrophy and IR[7,8], which prompt us myogenic inhibition induced by HG-related classically activated pro-inflammatory macrophages may further hinder insulin sensitivity in myotube. The GLUT4 translocation is indispensable for glucose transport in skeletal muscle and myoblast, and also participated in adjusting to myogenesis[46,47]. In this research, the actions of insulin to stimulate GLUT4 translocation and glucose uptake in myotube were augmented in the presence of macrophages in physiological environment, which suggested increased insulin sensitivity of myotube. Whereas, when exposed to HG milieu, the effects of macrophages to increase insulin sensitivity in myotube were reversed. Both basal and insulin-stimulated GLUT4 translocation and glucose uptake down-regulated in HG milieu. Further, consistently with the effect on myogenic differentiation, the participation of macrophages in HG environment led to more seriously IR. These results suggested inflammatory reaction induced by HG-related classically activated pro-inflammatory macrophages efficiently hindered insulin sensitivity in myotube.
Figure 6 Inflammatory reaction induced by M1 macrophages impaired myotube insulin sensitivity. A: The GLUT4 fluorescence of myotube in the absence or presence of 100 nmoL insulin by Immunofluorescence (scale bar: 50 μm). B: The 2-Deoxyglucose transport of myotube in the absence or presence of 100 nmoL insulin by glucose uptake assay. Data are means ± SD, n = 6 per group. aP < 0.05, bP < 0.01 main effect of insulin; cP < 0.01 main effect of high glucose; dP < 0.01 main effect of co-cultured with macrophages. P values were calculated by two-way analysis of variances. M: Myoblasts; M + HG: Myoblasts cultured in high glucose medium; MMc: Myoblasts co-cultured with macrophages; MMc + HG: Myoblasts co-cultured with macrophages in high glucose medium; HG: High glucose.
In addition, 25 mmol/L glucose is recommended by the American Type Culture Collection for myoblasts culture, so DMEM with 25 mmol/L glucose should be regarded as the norm caloric conditions\[48\]. For the HG milieus, we originally treated macrophages and myoblasts with 40 mmol/L or 60 mmol/L glucose. By preliminary experiments, we found that 40 mmol/L glucose had a slight but non-significant effect on macrophages polarization and myoblasts differentiation, but 60 mmol/L glucose significantly induced M1 macrophages and inhibited myoblasts differentiation. Since the main aim of this study is to explore the potential mechanisms of HG inducing inflammatory response and myoblasts IR, the glucose dosage that significantly effected macrophages polarization and myoblasts differentiation should be used. So, in this study, we used 60 mmol/L glucose as the HG condition. However, this study has certain limitations. Firstly, in order to really demonstrate the effects on inflammation and IR in this study is just related to glucose per se and not to hyperosmolarity induced by 60 mmol/L glucose, we used L-glucose as an osmotic control for 60 mmol/L D-glucose. So, future research may be needed to analyze the damage of hyperosmolarity caused by HG to inflammation and insulin sensitivity. Further, the sample size (n = 6 per group) may be relatively small in this study, the study with higher sample size would be needed to reconfirm the results of this study.

In summary, the current study revealed that macrophages promoted myogenesis in physiological conditions, but interactions between myoblasts and macrophages under HG milieus induced pro-inflammatory M1 polarization of macrophages to exacerbate inflammatory response. Subsequently, chronic inflammation induced by HG-related M1 macrophages damaged myogenesis and insulin sensitivity in myoblasts, which ultimately resulted in skeletal muscle IR. This is the first research about the mediation of macrophages to HG-related myogenic inhibition and IR in myoblasts, which supported macrophage may serve as a promising therapeutic target for skeletal muscle atrophy and IR. This study provides new insights into the prevention and treatment of skeletal muscle atrophy and IR, which may contribute to further explore in vivo the pathogeny of IR and the involve of macrophages.

CONCLUSION

Overall, interactions between myoblasts and macrophages under HG milieus induced pro-inflammatory M1 polarization of macrophages to exacerbate inflammatory response, which ultimately resulted in skeletal muscle atrophy and IR. These findings provide new insights into the prevention and treatment of skeletal muscle atrophy and IR, which support macrophage may serve as a promising therapeutic target for skeletal muscle atrophy and IR.

FOOTNOTES

Author contributions: Ai L, Luo W and Zhou Y designed the research study; Luo W, Zhou Y, Wang LY and Ai L performed the research; Luo W, Ai L and Wang LY analyzed the data and wrote the manuscript; All authors have read and approve the final manuscript.

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REFERENCES


25 Johnson H, Stanfield E, Campbell GI, Eberl EE, Cooney GJ, Bell-Anderson KS. Glucose mediates insulin sensitivity via a hepatoportal
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Abdollahi A, Dowden BN, Buhrman KK, Zembroski AS, Henderson GC. Albumin knockout mice exhibit reduced plasma free fatty acid concentration and enhanced insulin sensitivity. *Physiol Rep 2022; 10:* e15161 [PMID: 35238481 DOI: 10.14814/phy2.15161]

Kravchenko IV, Furalev VA, Popov VO. The Influence of Myofibrils on the Proliferation and Differentiation of Myoblasts Cocultured with Macrophages. *Dokl Biochem Biophys* 2018; 479: 72-76 [PMID: 29779100 DOI: 10.1134/S1607672918020060]


Chazaud B. Inflammation during skeletal muscle regeneration and tissue remodeling: application to exercise-induced muscle damage management. *Immuno Cell Biol 2016; 94:* 140-145 [PMID: 26526620 DOI: 10.1038/icb.2015.97]


