DOI: 10.53660/CLM-2386-23S13

Irisin modulates bone marrow derived macrophage polarization towards M2 profile in high fat fed mice

Irisina modula a polarização de macrófagos derivados de medula óssea para perfil M2 em camundongos submetidos à dieta hiperlipídica

Received: 2023-10-18 | Accepted: 2023-11-21 | Published: 2023-11-27

Juliana Magalhães-Santos
ORCID: https://orcid.org/0000-0002-9732-5690
Instituto de Ensino Superior, País
E-mail: julianamsmagalhaes@gmail.com
Rafael Loureiro Simões
ORCID: https://orcid.org/0000-0002-0403-2048
Universidade do Estado do Rio de Janeiro, Brasil
E-mail: rlsimoes@gmail.com
Carla Ade Caldas
ORCID: https://orcid.org/0000-0002-9492-2000
Universidade Salgado de Oliveira, Brasil
E-mail: carlaade@hotmail.com
Silvio Rodrigues Marques-Neto
ORCID: https://orcid.org/0000-0001-5742-4646
Universidade Salgado de Oliveira, Brasil
E-mail: marquesilvio@gmail.com
Thereza Christina Barja-Fidalgo
ORCID: https://orcid.org/0000-0002-1917-4401
Universidade do Estado do Rio de Janeiro, Brasil
E-mail: cbarja.uerj@gmail.com
Eliete Bouskela
ORCID: http://orcid.org/0000-0002-9621-8252
State University of Rio de Janeiro, Brazil
E-mail: eliete.bouskela@gmail.com
Raquel Carvalho Castiglione
ORCID: https://orcid.org/0000-0002-7367-0238
Universidade do Estado do Rio de Janeiro, Brasil
E-mail: rccastiglione@gmail.com

ABSTRACT

Background and Aims: The chronic inflammatory process in the adipose tissue during obesity suggests constant polarization of classically activated macrophages (M1) and low polarization of alternately activated ones (M2). Since irisin has anti-inflammatory and antidiabetogenic properties our aim is to evaluate its effects on macrophage polarization in mice subjected to high fat diet. Methods and Results: C57Bl/6 mice were fed a high fat diet for 30 weeks. Bone marrow derived macrophages (BMDM) were isolated and incubated with 50 nM of irisin. Cellular phenotype, nitrite production, arginase-1 expression and cell viability were analyzed. Irisin was able to increase $F480^{+}CD206^{+}$ and $F480^{+}CD301^{+}$ cell number, and arginase1 expression. Irisin was not able to induce nitrite production and did not affect cell viability. Conclusion: Irisin was able to elicit an M2-like profile in macrophages in mice subjected to high fat diet, suggesting an anti-inflammatory action of this cytokine with potential therapeutic use in the control of imflammation during obesity.

Keywords: Obesity; Irisin; Macrophage.

RESUMO

Introdução e Objetivos: O processo inflamatório crônico no tecido adipose durante a obesidade sugere uma polarização constante de macrófagos classicamente ativados (M1) e baixa polarização dos alternativamente ativados (M2). Como a irisina possui um potencial antiinflamatório e propriedades antidiabetogênicas, nosso objetivo foi avaliar seus efeitos na polarização de macrófagos em camundongos submetidos à dieta hiperlipídica. Métodos: camundongos C57Bl/6 foram alimentados com dieta hiperlipídica por 30 semanas. Macrófagos derivados de medula (BMDM) foram isolados e incubados com 50 nM de irisina. O fenótipo celular, a produção de nitrito, a expressão de arginase-1 e a viabilidade celular foram analisados. A irisina foi capaz de aumentar o número de células $F480^{+}CD206^{+}$ e $F480^{+}CD301^{+}$ e a expressão de arginase-1. A irisina não induziu a produção de nitrito e não afetou a viabilidade celular. Conclusão: A irisina foi capaz de promover um perfil M2 em macrófagos de camundongos submetidos à dieta hiperlipídica, sugerindo uma ação antiinflamatória desta citocina com potencial terapêutico no controle da inflamação na obesidade.

Palavras-chave: Obesidade; Irisina; Macrófago.

INTRODUCTION

Obesity is described as a multifactorial disease characterized by expansion of adipose tissue involving genetic and environmental factors related to the imbalance between caloric intake and energy expenditure (Spiegelman & Flier, 2001). It is a major risk factor for other diseases, such as insulin resistance and type 2 diabetes mellitus, cardiovascular diseases and some types of cancer (Shimobayashi et al., 2018). It is considered a low-grade chronic inflammation (Liu et al., 2014) with the presence of crown-shaped aggregates of macrophages in the adipose tissue and higher levels of circulating inflammatory cytokines (De Heredia et al., 2012).

It is already known that there is an increase in the number of M1 macrophages (classically activated) in the adipose tissue. On the other hand, in lean healthy subjects, the number of M2 macrophages (alternatively activated) predominates.

M1 macrophages are generally stimulated by T-helper 1 (Th1) type of cytokine and have high levels of proinflammatory cytokines secretion and nitric oxide (NO) production. Meanwhile, M2 macrophages have an immunosuppressive role and are associated with tissue remodeling and inflammation resolution, involving high phagocytic capacity and arginase-1 (Arg1) and PPAR-γ expression (Biswas & Mantovani, 2010; Castoldi et al., 2016).

It is already known that physical activity can reduce inflammation in lean and obese subjects (Han et al., 2019; Phillips et al., 2017) and proposed mechanisms to achieve this effect are: 1) increased release of anti-inflammatory myokines by contracting muscles (Gleeson et al., 2011) and 2) switch from M1 to M2 macrophage profile, decreasing the levels of pro-inflammatory cytokines (Bobinski et al., 2018; Gleeson et al., 2011; Kawanishi et al., 2010; Leung et al., 2016).

In 2012, a protein named irisin was described, released through clivation of the myokine named FNDC5 (Fibronectin type III domain-containing protein 5) and stimulated by physical activity, PGC1α and PPAR-γ (Boström et al., 2012). It is a peptide able to increase UCP-1 and mitochondria levels in white adipose tissue, inducing thermogenesis and browning of adipose tissue (Boström et al., 2012; Moreno-Navarrete et al., 2013).

Irisin is also able to modulate chronic inflammatory processes in different systemic diseases (Mazur-Bialy, 2017; Shao et al., 2017), suggesting a potential anti-inflammatory role. One of the mechanisms involved in irisin's anti-inflammatory functions is inducing M2-type macrophage polarization (Slate-Romano et al., 2022).

It has already been described that irisin alleviates acute lung injury (ALI) induced by LPS via modulating M1/M2 polarization of macrophage (Tu et al., 2023).

Due to the anti-inflammatory and antidiabetogenic properties of irisin our aim is to evaluate its effects on macrophage polarization in animals subjected to high fat diet.

MATERIALS AND METHODS

Animal care

This study followed the Principles of Laboratory Animal Care published by US National Institute of Health (NIH publication, 1985) and was approved by the State University of Rio de Janeiro Committee for Animal Experimentation (number 23/2016). Recently weaned male C57BL/6 mice (n=6) were used in the experiments and housed in ventilated microisolator cages under controlled conditions of light (12:12 hours light/dark cycle) and temperature (21.0±1.0°C) with chow and water provided ad libitum.

Experimental design

Immediately after the weaning period animals were fed a high fat diet with 62% of energy derived from fat for 30 weeks. Body weight was measured weekly.

Bone marrow cells were then extracted, differentiated into macrophages and then incubated with 50 nM of irisin (IRI) (described below). Their phenotype, nitrite production, arginase-1 expression, and cell viability were analyzed.

Preparation of differentiation medium (LCM Differentiation Medium)

Cells of fibroblastic origin L929 were maintained in culture (5% CO_2 at 37ºC) in medium bottle (75 cm^2; Corning®) until they reached confluence. When confluent, the entire content of the bottle was passed to a large bottle (150 cm^2) with 25 ml Dulbeco's modified essential medium (DMEM) (Gibco Invitrogen Corporation®) containing 10% fetal bovine serum (FBS). Upon reaching confluence, the medium was exchanged for 50 mL DMEM + 10% FBS. After 7 days in culture, the cell supernatant was collected, filtered on 0.2 µM polyethersulfone membrane (GVS® Filter Technology) and stored in the freezer at -80ºC until use.

Preparation of Bone Marrow Derived Macrophages (BMDM)

After 30 weeks with high fat diet, animals were euthanized in a carbon dioxide (CO_2) chamber and had their hind legs removed for femur and tibia isolation. In a sterile

environment, skin and muscles surrounding the bones were removed with the help of tweezers. Bones were washed in sterile PBS, inserted into conic polystyrene tubes and centrifuged at 400 x g for 5 min to remove bone marrow cells. Cells were then resuspended and cultivated in DMEM medium containing 15% LCM (differentiation medium). All surgical materials were sterilized.

Cells were kept at 37ºC at 5% CO_2 atmosphere for 7 days for macrophage differentiation, when they were removed from the bottles, plated either in 96-well or 24-well plates and left in a CO2 incubator for 90 min for adhesion.

For MTT assay, BMDM were plated in 96-well plates at a density of $2x10^5$ cells per well. For flow cytometry and nitrite production, BMDM were plated in 24-well plates at a density of $1x10^6$ cells per well.

Bone Marrow Derived Macrophage Polarization

For BMDM polarization, half of the differentiation medium was removed and replace by specific polarization medium. For M1 differentiation, BMDM were stimulated with LPS (1 µg/ml) and IFN-γ (1000 ng/ml) for 72 hours. For M2 differentiation, BMDM were stimulated with IL-4 (20 ng/ml) for 72 hours.

For irisin treatment, BMDM were maintained for 48 h with DMEM medium containing 15% LCM. Then, half of the differentiation medium was removed and replaced by DMEM supplemented with 10% SFB and 50 nM of irisin for the next 24 h.

Macrophages without any stimulation were used as control (M0).

Flow Cytometry

BMDM cells ($1x10^6$ cells/mL) were resuspended with flow cytometry buffer 2% bovine serum albumin, 5% fetal bovine serum and 0.1% sodium azide), incubated for 30 min and then labeled with anti-F4/80-APC and anti-CD206-PE or anti-CD301-PE antibodies for 30 min in the dark. An isotype control antibody was used as negative control.

Nitrite Measurement (NO production)

Nitrite release was determined by accumulation of a stable nitrite end-product in cell-free culture supernatants using a modified Griess reaction method. After 8-h incubation at 37°C, samples were made to react with Griess reagent (1% sulphanylamide in 5% phosphoric acid, 0.1% naphtylethylenediamine) at room temperature for 10 min.

Thereafter, nitrite concentration was measured by absorbance at 346 nm, referring to the standard curve of sodium nitrite (dissolved in culture medium solution) within a concentration range.

BMDM Protein Extraction

Cells were detached from 24-weel plates with a cell scraper in 300 µl of RIPA buffer, respectively [1M Tris-HCl (pH 7.4); 1M NaCl; 0.5M Na4P2O7; 0.5M NaVO3; 200mM PMSF; 1% SDS; protease inhibitor (PI 11836170001; Sigma-Aldrich, St. Louis, MO, USA); Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA). Cells suspension was then centrifuged at 3000 x g for 10 min at 4°C. Total protein content was determined by Pierce BCA Protein Assay Kit (ThermoScientific, Rockford, IL, USA).

Immunoblotting assay and arginase-1 expression

50 µg of protein were subjected to electrophoresis (10% SDS-PAGE) and transferred to PVDF membranes that were blocked with 5% non-fat dry milk and probed with mouse monoclonal anti-arginase-1 (1:1000, R&D Systems, Minneapolis, MN, USA) or mouse monoclonal anti-beta-actin (1:10000; R&D Systems, Minneapolis, MN, USA) overnight at 4°C. After extensive washings in TBS-Tween, PVDF membranes were incubated for 2 h at room temperature with horseradish peroxidase-conjugated secondary antibody at concentration 1:10,000 and developed with ECL system.

Immunoreactive proteins were visualized using ECL system (BIO-RAD, California, USA) in ChemiDoc XRS+ image acquisition system (BIO-RAD, California, USA). Densitometric quantification of detected bands was performed with Image Lab Software version 6.1 (BIO-RAD, California, USA).

Cellular Viability

A modified MTT [3–(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide] assay was used to quantify the effect of irisin on macrophage viability. This method measures mitochondrial function as described previously (Simões et al., 2010). Briefly, macrophages ($2x10^5$ cells/well) were incubated with or without irisin for 48 h. Cell culture supernatant was removed and cells washed with PBS.

Each well had the addition of 50 mg of MTT diluted in PBS. After 4 h incubation at 37°C in the dark, the supernatant was removed, and the formazan crystals formed by reduction of MTT were solubilized in isopropyl alcohol for 30 min at 37°C. The reading

was made in the ENVISION® reader, at 540nm. Cell viability was assessed and expressed as percentages of absorbance with respect to control (M0).

Statistical Analysis

For statistical analysis, GraphPad Prism 8.4.2 was used. All data are presented as mean ± SEM. One-way analysis of variance (ANOVA) followed by Tukey post hoc test was used for all analysis. The significance level was set at $p < 0.05$.

RESULTS

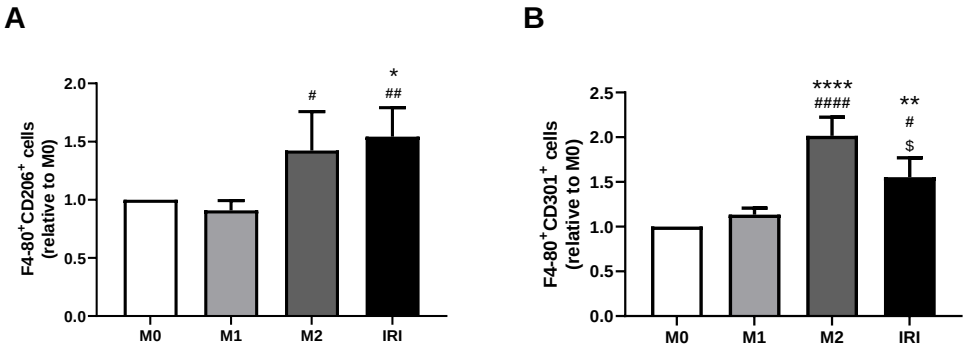
Irisin induces M2 polarization in HFD macrophages

As demonstrated in Figure 1, we observed that BMDM from mice feed with HFD shows an M1 profile, with low levels of F4-80$^+$CD206$^+$ and F4-80$^+$CD301$^+$ cells, both M2 markers. LPS + IFN-γ incubation was also able to polarize macrophages into M1 profile, as demonstrated by low levels of F4-80$^+$CD206$^+$ and F4-80$^+$CD301$^+$ cells.

Figure 1 shows that intereukin-4 (IL-4)-treated macrophage population express higher levels of F4-80$^+$CD206$^+$ and F4-80$^+$CD301$^+$ cells than in M0 and M1, suggesting that IL-4 treatment was able to promote polarization of M0 macrophages towards a M2 profile.

Interestingly, irisin treatment induces a higher amount of F4-80$^+$CD206$^+$ and F4-80$^+$CD301$^+$ cells in comparison to M0 and M1 groups, similarly to M2 group. However, F4-80$^+$CD301$^+$ cell number was lower than in M2 group, suggesting that irisin shifts M1 profile to a M2 phenotype in BMDM from HFD mice.

Figure 1. Irisin induces M2 polarization in HFD macrophages.



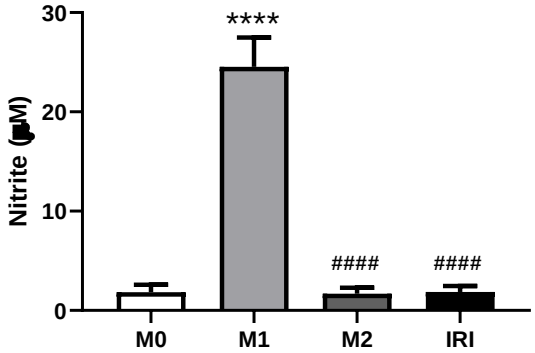
In A: F4-80$^+$CD206$^+$ cells relative to BMDM from HFD mice (M0). In B: F4-80$^+$CD301$^+$ cells relative to BMDM from HFD mice (M0). Values are presented as mean±SEM (n=6). * differs from M0 (*p<0.05;

**p<0.01; ****p<0.0001); # differs from M1 (#p<0.05; ##p<0.01; ####p<0.0001); $ differs from M2 ($p<0.05). Source: Authors (2023).

Irisin do not induces NO production by BMDM from HFD mice

In Figure 2 we can see that M1 macrophages, when compared to all other groups, presented higher production of nitrite, a marked characteristic of this phenotype. M2 macrophages and macrophages treated with irisin have low nitrite production, suggesting that irisin induces a M2 phenotype in BMDM from HFD mice.

Figure 2. Irisin do not induces NO production by BMDM from HFD mice.

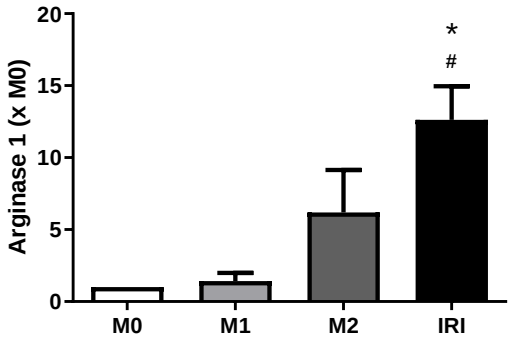


Values are presented as mean±SEM (n=6). * differs from M0 (****p<0.0001); # differs from M1 (####p<0.0001). Source: Authors (2023).

Irisin induces arginase-1 expression in BMDM from HFD mice

As observer in Figure 3, we show that M0 and M1 macrophages express low levels of arginase-1, an M2 marker. Interestingly, irisin induces arginase-1 overexpression on macrophages at levels statiscally similar to M2 macrophages, suggesting that irisin induces M2 profile in BMDM from HFD mice.

Figure 3. Irisin induces arginase-1 expression in BMDM from HFD mice.

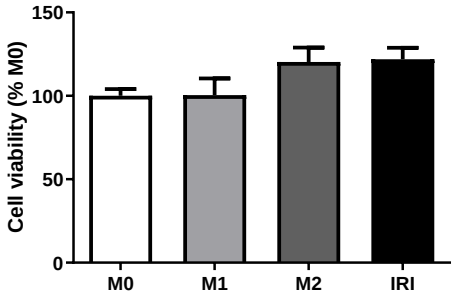


Values are presented as mean±SEM (n=6). * differs from M0 (*p<0.05); # differs from M1 (#p<0.05). Source: Authors (2023).

Irisin doest not alters BMDM viability

Although there is an increase of approximately 20% in cell viability of M2 and IRI related to M0 and M1, we did not oberve significant differences between groups (Figure 4), suggesting that irisin can modulate BMDM phenotype without alters cell viability.

Figure 4. Cell viability after irisin treatment.



Values are presented as mean±SEM (n=6). Source: Authors (2023).

DISCUSSION

It is already known that exercise has anti-inflammatory effects and can protect against development of chronic diseases if practiced in a regular manner. Possible mechanisms to explain these findings are: 1) reduction of visceral fat mass; 2) increased production and release of anti-inflammatory cytokines from contracting skeletal muscle; and 3) reduced expression of Toll-like receptors (TLRs) on monocytes and macrophages (Gleeson et al., 2011).

Monocytes and macrophages are cell populations characterized by diversity and plasticity, modulated by local microenvironment. Macrophages residing in or migrating to different tissues have distinct phenotypes and cell surface markers. The most often-used terms in macrophage phenotyping based on genetic expression are M1 and M2 macrophages. These populations are stimulated by different conditions: TLR stimulation, bacterial infection, and IFN-γ are potent M1 stimulators, while IL-4 and IL-13 stimulate M2 macrophages (Murray & Wynn, 2011).

M1 macrophages are considered inflammatory, characterized by expression of high levels of proinflammatory cytokines, promotion of Th1 response and high production of reactive nitrogen intermediates to enhance antimicrobial activity. They have high expression of inducible nitric oxide synthase (iNOS), an enzyme that catalyzes the conversion of arginine and oxygen to NO and citrulline and its most potent inducer is a combination of TLR agonist, such as LPS and IFN-γ (Murray & Wynn, 2011).

In contrast, M2 macrophages are considered anti-inflammatory and are involved in promotion of tissue remodeling, immunoregulatory functions and parasite containment. M2 activation by IL-4 is associated with upregulation of mannose receptor CD206 (Bisgaard et al., 2016; Müller et al., 2017; Stein et al., 1992; Ye et al., 2020; Zhu et al., 2017), macrophage galactose-binding lectin CD301 (Huang et al., 2016; Van den Bossche et al., 2016) and arginase-1 (Sica & Mantovani, 2012; Ye et al., 2020).

Arginase-1 expression is often used to provide evidence for M2 polarization in BMDM after IL-4 stimulation (Murray & Wynn, 2011). It is responsible for the inhibition of iNOS and, thus, NO production. In M1 macrophages, NO production is highly activated, while in M2 macrophages, arginase-1 synthesis is induced (Maloney et al., 2015; Yang & Ming, 2014).

A switch from M1 to M2 profile in mice adipose tissue (Bobinski et al., 2018; Gleeson et al., 2011) and inhibition of monocyte and macrophage infiltration into adipose tissue (Gleeson et al., 2011), decreasing the levels of pro-inflammatory cytokines, can be promoted by physical exercise, showing an anti-inflammatory effect of this practice.

Exercise training is able to induce the release of several myokines, some of which are able to induce browning of white adipose tissue, a process that is correlated with an improvement of metabolic changes during obesity, such as insulin resistance (Niranjan et al., 2019). Among these myokines is irisin, a molecule induced not only by physical activity but also by cold exposure (Vitali et al., 2012). Browning of white adipose tissue stimulates lipolytic actions, reduction of preadipocyte synthesis and increase in

expression of mitochondrial uncoupling protein 1 (UCP-1), involved in dissipation of energy through generation of heat (Mazur-Bialy, 2017).

Irisin has also a role in reduction of NF-κB expression, a transcription factor highly associated to induction of several proinflammatory molecules, such as TNF-α, IL-6 and MCP-1 (Arhire et al., 2019), being proposed to be an immunomodulator cytokine. Therefore, the present study aimed to relate obesity and macrophage polarization by irisin as a tool to reduce inflammation through immunomodulation.

A study of Mazur Bialy and co-workers (2017) have shown that RAW 264.7 cells exhibit M2 profile after irisin stimulation. The present work was also able to show that in high-fat fed animals irisin was able to induce a profile similar to M2 in naive BMDM, suggesting a role for this cytokine in the regulation of inflammation.

We could observe an approximate 6-fold increase in arginase 1 expression in macrophages stimulated with IL-4 (although not statistically significant) and a higher and statistically significant increase after irisin treatment. Our hypothesis is that irisin, as IL4, can stimulates M2 profile in macrophages from HF animals through an increase in arginase levels that probably inhibits NO production in these cells.

Reactive nitrogen intermediates are produced by M1 macrophages and are important molecules in their anti-tumor and anti-microbial activities (Padgett & Pruett, 1992). They are derived from L-arginine and produce nitrite and nitrate ions as stable and easily measured breakdown products. Therefore, nitrite may be used to indicate reactive nitrogen intermediates production (Padgett & Pruett, 1992). We have observed an increase in nitrite production by M1 macrophages, but not by M2 or irisin stimulated macrophages.

We were also able to show that irisin treatment did not reduce cell viability, as already show in the literature (Mazur-Bialy et al., 2018). However, there is a possibility that MTT reduction can have interference of metabolism during different cellular phases, altering its reliability (Pozzolini et al., 2003).

It was recently showed by Ye et al (2020) that irisin was able to increase cell viability in RAW264.7 murine lineage in a dose-dependent manner. Indeed, our results show that there is an increase of 20% in cell viability in after irisin treatment when compared to macrophages without stimuli (M0). However, we did not observe statistical difference between these groups.

Taken together, our results indicate that irisin is able to induce a M2-like profile in BMDM from high fat fed animals. We suggest that irisin is a potential

immunomodulator and its administration may be able to reduce the chronic inflammation observed during obesity.

REFERENCES

Arhire, L. I., Mihalache, L., & Covasa, M. (2019). Irisin: A Hope in Understanding and Managing Obesity and Metabolic Syndrome. *Frontiers in Endocrinology*, *10*(August), 1–12. https://doi.org/10.3389/fendo.2019.00524

Bisgaard, L. S., Mogensen, C. K., Rosendahl, A., Cucak, H., Nielsen, L. B., Rasmussen, S. E., & Pedersen, T. X. (2016). Bone marrow-derived and peritoneal macrophages have different inflammatory response to oxLDL and M1/M2 marker expression - Implications for atherosclerosis research. *Scientific Reports*, *6*(March), 1–10. https://doi.org/10.1038/srep35234

Biswas, S. K., & Mantovani, A. (2010). Macrophage plasticity and interaction with lymphocyte subsets: Cancer as a paradigm. *Nature Immunology*, *11*(10), 889–896. https://doi.org/10.1038/ni.1937

Bobinski, F., Teixeira, J. M., Sluka, K. A., & Santos, A. R. S. (2018). Interleukin-4 mediates the analgesia produced by low-intensity exercise in mice with neuropathic pain. *Pain*, *159*(3), 437–450. https://doi.org/10.1097/j.pain.0000000000001109

Boström, P., Wu, J., Jedrychowski, M. P., Korde, A., Ye, L., Lo, J. C., Rasbach, K. A., Boström, E. A., Choi, J. H., Long, J. Z., Kajimura, S., Zingaretti, M. C., Vind, B. F., Tu, H., Cinti, S., Højlund, K., Gygi, S. P., & Spiegelman, B. M. (2012). A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*, *481*(7382), 463–468. https://doi.org/10.1038/nature10777

Castoldi, A., De Souza, C. N., Saraiva Câmara, N. O., & Moraes-Vieira, P. M. (2016). The macrophage switch in obesity development. *Frontiers in Immunology*, *6*(JAN), 1–11. https://doi.org/10.3389/fimmu.2015.00637

De Heredia, F. P., Gómez-Martínez, S., & Marcos, A. (2012). Chronic and degenerative diseases: Obesity, inflammation and the immune system. *Proceedings of the Nutrition Society*, *71*(2), 332–338. https://doi.org/10.1017/S0029665112000092

Gleeson, M., Bishop, N. C., Stensel, D. J., Lindley, M. R., Mastana, S. S., & Nimmo, M. A. (2011). The anti-inflammatory effects of exercise: Mechanisms and implications for the prevention and treatment of disease. *Nature Reviews Immunology*, *11*(9), 607–610. https://doi.org/10.1038/nri3041

Han, Y., Liu, Y., Zhao, Z., Zhen, S., Chen, J., Ding, N., Ma, Y., & Wen, D. (2019). Does physical activity-based intervention improve systemic proinflammatory cytokine levels in overweight or obese children and adolescents? Insights from a meta-Analysis of randomized control trials. *Obesity Facts*, *12*(6), 653–668. https://doi.org/10.1159/000501970

Huang, S. C. C., Smith, A. M., Everts, B., Colonna, M., Pearce, E. L., Schilling, J. D., & Pearce, E. J. (2016). Metabolic Reprogramming Mediated by the mTORC2-IRF4 Signaling Axis Is Essential for Macrophage Alternative Activation. *Immunity*, *45*(4), 817–830. https://doi.org/10.1016/j.immuni.2016.09.016

Kawanishi, N., Yano, H., Yokogawa, Y., & Suzuki, K. (2010). Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. *Exercise Immunology Review*, *16*, 105–118.

Leung, A., Gregory, N. S., Allen, L.-A. H., & Sluka, K. A. (2016). Regular physical activity prevents chronic pain by altering resident muscle macrophage phenotype and increasing interleukin-10 in mice. *PAIN*, *157*(1), 70–79. https://doi.org/10.1097/j.pain.0000000000000312

Liu, Y.-C., Zou, X.-B., Chai, Y.-F., & Yao, Y.-M. (2014). Macrophage Polarization in Inflammatory Diseases. *International Journal of Biological Sciences*, *10*(5), 520–529. https://doi.org/10.7150/ijbs.8879

Maloney, J., Keselman, A., Li, E., & Singer, S. M. (2015). Macrophages expressing arginase 1 and nitric oxide synthase 2 accumulate in the small intestine during Giardia lamblia infection. *Microbes and Infection*, *17*(6), 462–467. https://doi.org/10.1016/j.micinf.2015.03.006

Mazur-Bialy, A. I. (2017). Irisin acts as a regulator of macrophages host defense. *Life Sciences*, *176*, 21–25. https://doi.org/10.1016/j.lfs.2017.03.011

Mazur-Bialy, A. I., Kozlowska, K., Pochec, E., Bilski, J., & Brzozowski, T. (2018). Myokine irisin-induced protection against oxidative stress in vitro. Involvement of heme oxygenase-1 and antioxidazing enzymes superoxide dismutase-2 and glutathione peroxidase. *Journal of Physiology and Pharmacology : An Official Journal of the Polish Physiological Society*, *69*(1), 117–125. https://doi.org/10.26402/jpp.2018.1.13

Moreno-Navarrete, J. M., Ortega, F., Serrano, M., Guerra, E., Pardo, G., Tinahones, F., Ricart, W., & Fernández-Real, J. M. (2013). Irisin Is Expressed and Produced by Human Muscle and Adipose Tissue in Association With Obesity and Insulin Resistance. *The Journal of Clinical Endocrinology & Metabolism*, *98*(4), E769–E778. https://doi.org/10.1210/jc.2012-2749

Müller, J., Von Bernstorff, W., Heidecke, C. D., & Schulze, T. (2017). Differential S1P Receptor Profiles on M1- and M2-Polarized Macrophages Affect Macrophage Cytokine Production and Migration. *BioMed Research International*, *2017*, 9–11. https://doi.org/10.1155/2017/7584621

Murray, P. J., & Wynn, T. A. (2011). Obstacles and opportunities for understanding macrophage polarization. *Journal of Leukocyte Biology*, *89*(4), 557–563. https://doi.org/10.1189/jlb.0710409

Niranjan, S. B., Belwalkar, S. V., Tambe, S., Venkataraman, K., & Mookhtiar, K. A. (2019). Recombinant irisin induces weight loss in high fat DIO mice through increase in

energy consumption and thermogenesis. *Biochemical and Biophysical Research Communications*, *519*(2), 422–429. https://doi.org/10.1016/j.bbrc.2019.08.112

Padgett, E. L., & Pruett, S. B. (1992). Evaluation of nitrite production by human monocyte-derived macrophages. *Biochemical and Biophysical Research Communications*, *186*(2), 775–781. https://doi.org/10.1016/0006-291X(92)90813-Z

Phillips, C. M., Dillon, C. B., & Perry, I. J. (2017). Does replacing sedentary behaviour with light or moderate to vigorous physical activity modulate inflammatory status in adults? *International Journal of Behavioral Nutrition and Physical Activity*, *14*(1), 1–12. https://doi.org/10.1186/s12966-017-0594-8

Pozzolini, M., Scarfì, S., Benatti, U., & Giovine, M. (2003). Interference in MTT cell viability assay in activated macrophage cell line. *Analytical Biochemistry*, *313*(2), 338–341. https://doi.org/10.1016/S0003-2697(02)00631-0

Shao, L., Li, H., Chen, J., Song, H., Zhang, Y., Wu, F., Wang, W., Zhang, W., Wang, F., Li, H., & Tang, D. (2017). Irisin suppresses the migration, proliferation, and invasion of lung cancer cells via inhibition of epithelial-to-mesenchymal transition. *Biochemical and Biophysical Research Communications*, *485*(3), 598–605. https://doi.org/10.1016/j.bbrc.2016.12.084

Shimobayashi, M., Albert, V., Woelnerhanssen, B., Frei, I. C., Weissenberger, D., Meyer-Gerspach, A. C., Clement, N., Moes, S., Colombi, M., Meier, J. A., Swierczynska, M. M., Jenö, P., Beglinger, C., Peterli, R., & Hall, M. N. (2018). Insulin resistance causes inflammation in adipose tissue. *Journal of Clinical Investigation*, 1–13. https://doi.org/10.1172/JCI96139

Sica, A., & Mantovani, A. (2012). Plasticity and Polarization. *Journal of Clinical Investigation*, *122*(3), 787–795. https://doi.org/10.1172/JCI59643DS1

Simões, R. L., Niconi-de-Almeida, Y., da-Fé, A. R., Barja-Fidalgo, C., & Fierro, I. M. (2010). A synthetic analog of 15-epi-lipoxin A4 inhibits human monocyte apoptosis: Involvement of ERK-2 and PI3-kinase. *Prostaglandins and Other Lipid Mediators*, *91*(1–2), 10–17. https://doi.org/10.1016/j.prostaglandins.2009.12.001

Slate-Romano, J. J., Yano, N., & Zhao, T. C. (2022). "Irisin Reduces Inflammatory Signaling Pathways in Inflammation-Mediated Metabolic Syndrome ". *Molecular and Cellular Endocrinology*, *552*, 111676. https://doi.org/10.1016/J.MCE.2022.111676

Spiegelman, B. M., & Flier, J. S. (2001). Obesity and the regulation of energy balance. *Cell*, *104*(4), 531–543. https://doi.org/10.1016/S0092-8674(01)00240-9

Stein, B. M., Keshav, S., Harris, N., & Gordon, S. (1992). Interleukin 4 Potently Enhances Murine Macrophage Mannose Receptor Activity: A Marker of Alternative Immunologic Macrophage Activation By Michael Stein, Satish Keshav, Neil Harris,* and Siamon Gordon. *J Exp Med*, *176*(July), 287–292.

Tu, Y., Liu, J., Kong, D., Guo, X., Li, J., Long, Z., Peng, J., Wang, Z., Wu, H., Liu, P., Liu, R., Yu, W., & Li, W. (2023). Irisin drives macrophage anti-inflammatory

differentiation via JAK2-STAT6-dependent activation of PPARγ and Nrf2 signaling. *Free Radical Biology and Medicine*, *201*, 98–110. https://doi.org/10.1016/J.FREERADBIOMED.2023.03.014

Van den Bossche, J., Baardman, J., Otto, N. A., van der Velden, S., Neele, A. E., van den Berg, S. M., Luque-Martin, R., Chen, H. J., Boshuizen, M. C. S., Ahmed, M., Hoeksema, M. A., de Vos, A. F., & de Winther, M. P. J. (2016). Mitochondrial Dysfunction Prevents Repolarization of Inflammatory Macrophages. *Cell Reports*, *17*(3), 684–696. https://doi.org/10.1016/j.celrep.2016.09.008

Vitali, A., Murano, I., Zingaretti, M. C., Frontini, A., Ricquier, D., & Cinti, S. (2012). The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes. *Journal of Lipid Research*, *53*(4), 619–629. https://doi.org/10.1194/jlr.M018846

Yang, Z., & Ming, X. F. (2014). Functions of arginase isoforms in macrophage inflammatory responses: Impact on cardiovascular diseases and metabolic disorders. *Frontiers in Immunology*, *5*(OCT), 1–10. https://doi.org/10.3389/fimmu.2014.00533

Ye, W., Wang, J., Lin, D., & Ding, Z. (2020). The immunomodulatory role of irisin on osteogenesis via AMPK-mediated macrophage polarization. *International Journal of Biological Macromolecules*, *146*, 25–35. https://doi.org/10.1016/j.ijbiomac.2019.12.028

Zhu, Y., Zhang, L., Lu, Q., Gao, Y., Cai, Y., Sui, A., Su, T., Shen, X., & Xie, B. (2017). Identification of different macrophage subpopulations with distinct activities in a mouse model of oxygen-induced retinopathy. *International Journal of Molecular Medicine*, *40*(2), 281–292. https://doi.org/10.3892/ijmm.2017.3022