

THE ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN OBESITY-ASSOCIATED TYPE 2 DIABETES IN MICE

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Abstract – Macrophage migration inhibitory factor (MIF) is implicated in the pathogenesis of several inflammation-related diseases, including obesity and type 2 diabetes (T2D). However, MIF deficiency itself promotes obesity and glucose intolerance in mice. Here we show that the introduction of a high-fat diet (HFD) further aggravates the parameters of obesity-associated T2D: weight gain and glucose intolerance. Furthermore, in contrast to MIF-KO mice on standard chow, HFD-fed MIF-KO mice develop insulin resistance. Although the clinical signs of obesity-associated T2D are upgraded, inflammation in MIF-deficient mice on HFD is significantly lower. These results imply that MIF possesses a complex role in glucose metabolism and the development of obesity-related T2D. However, the downregulation of inflammation upon MIF inhibition could be a useful tool in short-term T2D therapy for preventing pancreatic islet deterioration.

Keywords: Macrophage migration inhibitory factor, type 2 diabetes, obesity, inflammation

INTRODUCTION

Type 2 diabetes (T2D) is a heterogeneous disease characterized by hyperglycemia resulting from the progressive development of insulin resistance accompanied by defects in insulin secretion (Stumvoll et al., 2005). Apart from a genetic predisposition, the risk of developing T2D in humans increases with age, obesity, cardiovascular disease (hypertension, dyslipidemia) and a lack of physical activity. A sedentary lifestyle combined with energy-rich diets leads to obesity, which often precedes the onset of the disease. Recent findings describe obesity as a state of chronic, low grade inflammation as increased levels of inflammatory cytokines (tumor necrosis factor- α – TNF- α , interleukin-6 – IL-6, interleukin-1 β – IL-1 β) and acute-phase reactants (C-reactive protein – CRP, haptoglobin, fibrinogen) are seen in obese subjects (Donath and Shoelson, 2011; Lumeng and

Saltiel, 2011). Furthermore, elevated levels of IL-1 β , IL-6 and CRP are predictive of T2D (Pascual et al., 2005; Viardot et al., 2010). These detrimental molecules are derived from the adipose tissue of the obese, but also from the immune cells (Donath and Shoelson, 2011).

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine associated with the development of many immune diseases, including T1D (Cvetkovic et al., 2005; Stosic-Grujicic et al., 2008), T2D and obesity (Ghanim et al., 2004; Church et al., 2005). Macrophages have been identified as a primary source of MIF (Calandra et al., 1994). Interestingly, human and murine adipocytes are also a source of MIF (Hirokawa et al., 1997; Skurk et al., 2005). MIF is elevated in the serum of patients with T2D (Yabunaka et al., 2000), and mononuclear cells of the obese demonstrate higher levels of MIF (Dan-

dona et al., 2004). In addition, MIF can cause damage to pancreatic beta cells in cooperation with high levels of nutrients (Saksida et al., 2012).

We have previously shown an increase in systemic and locally produced MIF in C57BL/6 mouse fed a high-fat diet (HFD), which correlated with the weight gain and the developed glucose intolerance (Saksida et al., 2012). The aim of this study was to investigate further the role of MIF in diet-induced obesity by feeding mice lacking the *Mif* gene with a high-fat food. Having in mind the inflammatory *milieu* of obesity, we measured the serum levels of CRP, IL-6 and tumor growth factor- β (TGF- β).

MATERIALS AND METHODS

Material

Unless stated otherwise, all chemicals were purchased from Sigma-Aldrich, St Louis, MO, USA.

Animals

The breeding stock of *Mif* gene deficient (MIF-KO) mice on the C57BL/6 (B6) background was kindly provided by Dr Yousef Al-Abed (The Feinstein Institute for Medical Research, North Shore LIJ Health System, New York, USA) and the mice were further bred using homozygous MIF-KO animals. MIF-KO mice were housed along with their wild type (WT) C57BL/6 counterparts under standard conditions (non-specific pathogen free) in the Animal Facility at the Institute for Biological Research "Siniša Stanković". The experiments were approved by the local Ethical Committee (# 12/07) and conducted in accordance with local and international legislation regarding the well-being of laboratory animals.

Induction of obesity-associated T2D

C57BL/6 and MIF-KO mice (8 weeks old, 35 mice per group) were fed for 16 weeks with either a control diet (CD – 10% fat, 3.7 kcal/g) or a high-fat diet (HFD – 60% fat, 5.1 kcal/g) obtained from Harlan, Madison, USA.

Intraperitoneal insulin (IPITT) and glucose tolerance tests (IPGTT)

IPITT was performed by insulin injection (0.8 μ g/g body weight) to fed mice, while IPGTT was done on fasted mice by injection of D-glucose (2 mg/g body weight). Blood glucose (from the tail vein) was measured at specific time points.

Serum analysis

The animals were bled from the orbital plexus and the sera were stored at -80°C . The sera were examined using ELISA kits for insulin (Mercodia, Uppsala, Sweden), CRP (Anogen, Ontario, Canada), leptin (R&D, Minneapolis, MN, USA), triglycerides (Wako Chemicals, Richmond, VA, USA) and with paired antibodies for IL-6 (eBioscience, San Diego, CA, USA), and TGF- β (R&D) according to the manufacturers' instructions.

Statistical analysis

The results from 2-3 experiments were presented as means \pm SD. To analyze the significance of the differences between various treatments, analysis of variance (ANOVA) followed by the Student-Newman-Keuls test for multiple comparisons, or the Student's *t*-test were used as appropriate. A *p* value less than 0.05 was considered to be significant. The statistical package used was Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA).

RESULTS

MIF deficiency further promotes obesity in HFD-fed mice

By about 15 weeks of age, the weight gain of MIF-KO mice on CD became significantly higher compared to B6 mice on the same diet (Fig. 1A). The introduction of the HFD to 8-week-old MIF-KO mice resulted in a more profound difference in body weight after 9 weeks of feeding compared to the same strain on the CD diet. Interestingly, the development of obesity in B6 and MIF-KO on HFD followed a similar

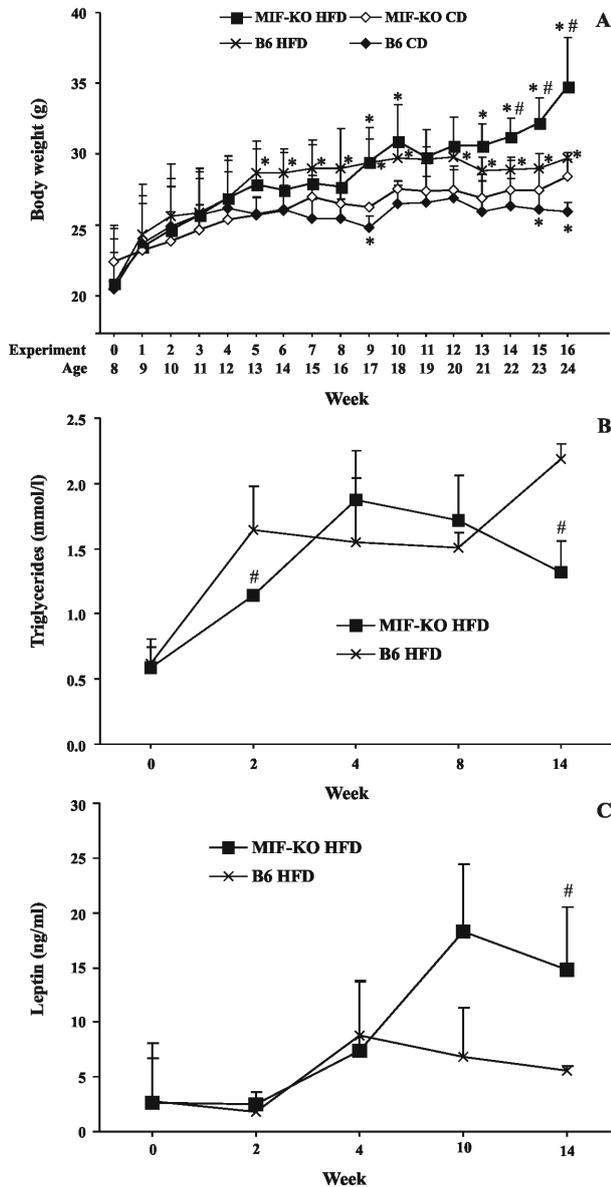


Fig. 1. The effect of MIF deletion on obesity, triglyceride and leptin levels. Body weight of B6 and MIF-KO mice on CD and HFD was measured weekly (A). Triglycerides from serum of non-fasting mice (B). Serum leptin concentration (C). Data are presented as mean \pm SD, $n=15-50$, * indicates $p<0.05$ vs. MIF-KO CD, # indicates $p<0.05$ vs. B6 HFD.

pattern until week 14 of the experiment when the MIF-KO mice started to gain more weight than the B6 mice (Fig. 1A). Both strains on HFD increased their triglycerides over time, but triglyceride levels

in the MIF-KO mice were lower at week 14 (Fig. 1B). At the same time point, serum leptin was significantly higher in the MIF-KO mice compared to the B6 mice (Fig. 1C). Seemingly, both strains on CD had normal leptin levels throughout the experiment (not shown).

MIF deletion influences insulin resistance

MIF-KO mice on CD showed slightly higher levels of fasting glucose and impaired glucose tolerance compared to B6 on the same diet (Fig. 2A, B). HFD elevated fasting glycemia and further promoted glucose intolerance in the MIF-KO mice (Fig. 2A, B). IPITT resulted in normal glucose disposal in both strains on CD (Fig. 2C). However, in the same test the MIF-KO mice on HFD were unable to lower the serum glucose properly, indicating a marked insulin resistance that was greater than the one seen in B6 on HFD (Fig. 2C). The serum insulin concentration remained normal in the mice on CD, but increased over time in both strains on HFD (Fig. 2D).

MIF deletion prevents HFD-induced changes in serum inflammatory markers

Obesity is usually associated with the chronic presence of slightly elevated proinflammatory mediators. Notably, serum CRP and IL-6 levels were downregulated in the HFD-fed MIF-KO mice compared to the B6 mice on the same diet (Fig. 3A, B), while the TGF- β concentration declined over time in the sera of both mouse strains on HFD (Fig. 3C). The levels of all mentioned parameters in the CD-fed mice did not change throughout the experiment (not shown).

DISCUSSION

This study shows that the absence of MIF simultaneously promotes diet-induced obesity and insulin resistance in mice, and abrogates chronic inflammation.

MIF is implicated in the development of obesity and T2D in humans (Ghanim et al., 2004; Church et al., 2005) and in B6 mice (Saksida et al., 2012).

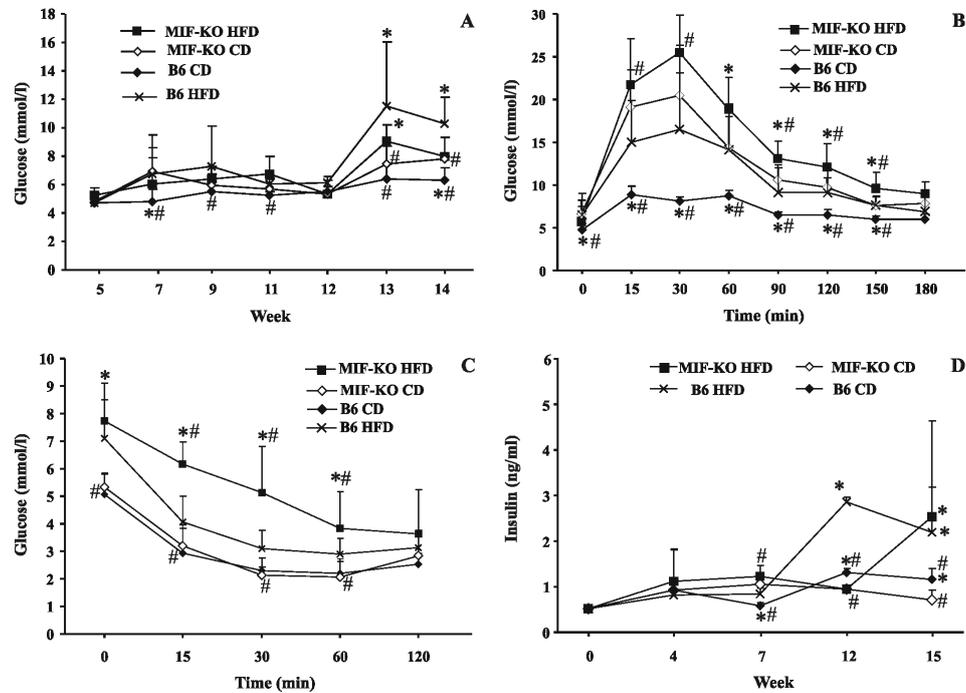


Fig. 2. MIF absence provoked glucose intolerance and insulin resistance. Blood was taken from the tail vein of fasting animals on a weekly basis and glucose was measured (A). IPGTT was performed on fasting mice at week 7 of the experiment (B). IPITT was performed at week 7 of the experiment (C). Serum insulin concentration (D). Data are presented as mean \pm SD, $n=10-15$, * indicates $p < 0.05$ vs. MIF-KO CD, # indicates $p < 0.05$ vs. B6 HFD.

Additionally, MIF deficiency protects mice from the development of glucose intolerance in atherosclerotic-prone mice (Verschuren et al., 2009) or during endotoxemia (Atsumi et al., 2007). However, results obtained by Serre-Beinier et al. (2010) suggest that MIF-KO mice spontaneously develop obesity and glucose intolerance in adult age (12 months old). Interestingly, these mice did not develop insulin resistance. Our experiments confirm their findings, although our colony of MIF-KO mice developed glucose intolerance by the age of 8 weeks, while the introduction of HFD exacerbated the parameters of obesity and T2D, including the appearance of insulin resistance.

The normal serum insulin and insulin tolerance seen in CD-fed MIF-KO mice suggests that insulin receptor and signaling are fully operative. In addition, endotoxemic MIF-KO mice display normal glucose uptake (Atsumi et al., 2007). Therefore, the defect may reside in MIF-KO islets' capacity for in-

sulin secretion. Indeed, impaired IPGTT confirmed that MIF-KO islets might possess an innate defect in insulin secretion what is in accordance with *in vivo* studies by Serre-Beinier et al. (2010). The observed impairment might stem from MIF's positive influence on the insulin secretion pathway (Waeber et al., 1997).

Recent data suggest that MIF blood or serum levels correlate with leptin levels in obese subjects (Sheu et al., 2008; Sumarac-Dumanovic et al., 2009). Indeed, the leptin serum was higher in the HFD-fed MIF-KO compared to the B6 mice approximately at the time when a significant difference in weight gain between the two strains was observed.

Many reports indicate that MIF is actually positioned at the top of the inflammatory cascade and that MIF deficiency benefits almost every condition with underlying inflammation processes; but it also reduces the ability of cells to produce TNF- α , IL-1,

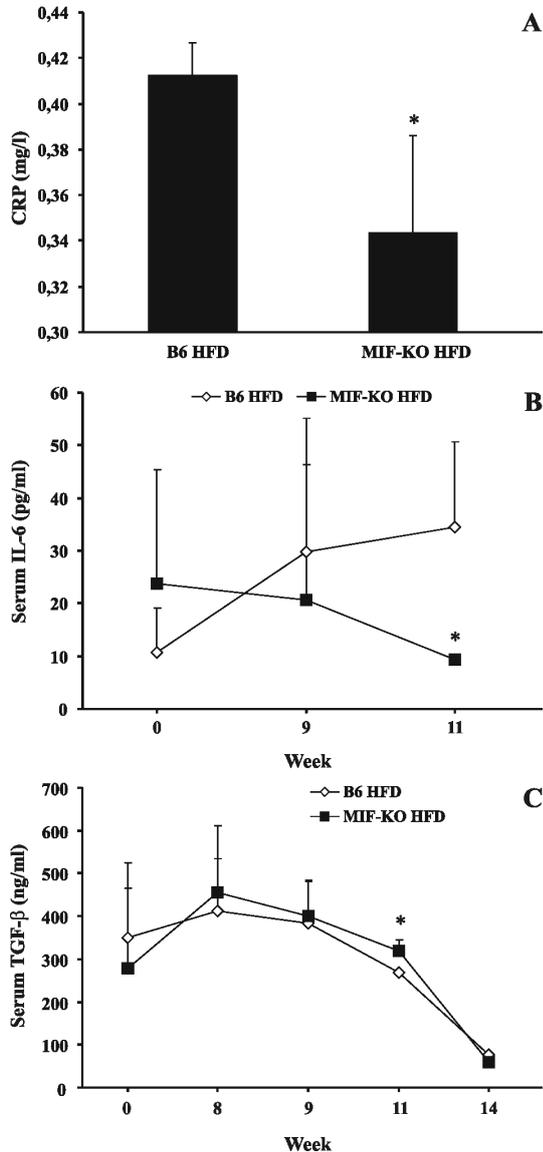


Fig. 3. The influence of HFD on inflammatory markers in MIF-KO mice. Serum CRP (week 12 of the experiment) (A). Serum IL-6 concentration (B). Σερυμ ΤΓΦ-β concentration (C). Data are presented as mean±SD, n=5-15, * indicates $p < 0.05$ vs. B6 HFD.

IL-6, IL-17, IL-23 (Cvetkovic and Stosic-Grujicic, 2006; Stosic-Grujicic et al., 2009). More specifically, it has been shown that MIF absence reduces the chronic inflammation associated with obesity and atherosclerotic disease (Verschuren et al., 2009). Similarly, our results suggest that the reason for the

downregulation of inflammation in HFD-fed MIF-KO mice could be MIF deficiency itself. However, HFD resulted in a significant drop of serum TGF-β over time in both strains, indicating that MIF deficiency affected only the pro-inflammatory arm of the immune response.

One of MIF's functions is the counter-regulation of glucocorticoid immunosuppressive action. Apart from interfering with immune response, the excess of glucocorticoids affects insulin signaling and inhibits glucose-stimulated insulin release *in vitro* (Lambillotte et al., 1997; Staab and Maser, 2010). Therefore, in the absence of MIF, glucocorticoids lack their natural opponent and the observed defects in islet insulin secretion and the suppression of inflammation could be consequences of elevated glucocorticoids.

CONCLUSION

This study suggests that the role of MIF in obesity and T2D is complex. On one side, MIF is clearly important for retaining the normal function of beta cells, while on the other, MIF acts as a booster of the inflammation that underlines T2D development. Also, having in mind that MIF in a nutrient-rich environment can induce beta cell apoptosis, therapy with MIF blockers at specific time points during T2D pathogenesis may prolong the time without exogenous insulin treatment. Therefore, research focused on the determination of the exact time for anti-MIF therapy during HFD-induced obesity-associated T2D is warranted.

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REFERENCES

Atsumi, T., Cho, Y.R., Leng, L., McDonald, C., Yu, T., Danton, C., Hong, E.G., Mitchell, R.A., Metz, C., Niwa, H., Takeuchi, J., Onodera, S., Umino, T., Yoshioka, N., Koike, T., Kim, J.K., and R. Bucala (2007). The proinflammatory cytokine

- macrophage migration inhibitory factor regulates glucose metabolism during systemic inflammation. *J. Immunol.* **179**, 5399-5406.
- Calandra, T., Bernhagen, J., Mitchell, R.A., and R. Bucala (1994). The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *J. Exp. Med.* **179**, 1895-1902.
- Church, T.S., Willis, M.S., Priest, E.L., Lamonte, M.J., Earnest, C.P., Wilkinson, W.J., Wilson, D.A., and B.P. Giroir (2005). Obesity, macrophage migration inhibitory factor, and weight loss. *Int. J. Obes. (Lond)*. **29**, 675-681.
- Cvetkovic, I., Al-Abed, Y., Miljkovic, D., Maksimovic-Ivanic, D., Roth, J., Bacher, M., Lan, H.Y., Nicoletti, F., and S. Stosic-Grujicic (2005). Critical role of macrophage migration inhibitory factor activity in experimental autoimmune diabetes. *Endocrinology*, **146**, 2942-2951.
- Cvetkovic, I., and S. Stosic-Grujicic (2006). Neutralization of macrophage migration inhibitory factor-novel approach for the treatment of immunoinflammatory disorders. *Int. Immunopharmacol.* **6**, 1527-1534.
- Dandona, P., Aljada, A., Ghanim, H., Mohanty, P., Tripathy, C., Hofmeyer, D., and A. Chaudhuri (2004). Increased plasma concentration of macrophage migration inhibitory factor (MIF) and MIF mRNA in mononuclear cells in the obese and the suppressive action of metformin. *J. Clin. Endocrinol. Metab.* **89**, 5043-5047.
- Donath, M.Y., and S.E. Shoelson (2011). Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.* **11**, 98-107.
- Ghanim, H., Aljada, A., Hofmeyer, D., Syed, T., Mohanty, P., and P. Dandona (2004). Circulating mononuclear cells in the obese are in a proinflammatory state. *Circulation*, **110**, 1564-1571.
- Hirokawa, J., Sakaue, S., Tagami, S., Kawakami, Y., Sakai, M., Nishi, S., and J. Nishihira (1997). Identification of macrophage migration inhibitory factor in adipose tissue and its induction by tumor necrosis factor-alpha. *Biochem. Biophys. Res. Commun.* **235**, 94-98.
- Lambillotte, C., Gilon, P., and J.C. Henquin (1997). Direct glucocorticoid inhibition of insulin secretion - An *in vitro* study of dexamethasone effects in mouse islets. *J. Clin. Invest.* **99**, 414-423.
- Lumeng, C.N., and A.R. Saltiel (2011). Inflammatory links between obesity and metabolic disease. *J. Clin. Invest.* **121**, 2111-2117.
- Pascual, G., Fong, A.L., Ogawa, S., Gamliel, A., Li, A.C., Perissi, V., Rose, D.W., Willson, T.M., Rosenfeld, M.G., and C.K. Glass (2005). A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature*, **437**, 759-763.
- Saksida, T., Stosic-Grujicic, S., Timotijevic, G., Sandler, S., and I. Stojanovic (2012). Macrophage migration inhibitory factor deficiency protects pancreatic islets from palmitic acid-induced apoptosis. *Immunol. Cell Biol.* **90**, 688-698.
- Serre-Beinier, V., Toso, C., Morel, P., Gonelle-Gispert, C., Veyrat-Durebex, C., Rohner-Jeanrenaud, F., Calandra, T., Roger, T., James, R.W., Montet, X., Bühler, L., Bosco, D., and T. Berney (2010). Macrophage migration inhibitory factor deficiency leads to age-dependent impairment of glucose homeostasis in mice. *J. Endocrinol.* **206**, 297-306.
- Sheu, W.H., Chang, T.M., Lee, W.J., Ou, H.C., Wu, C.M., Tseng, L.N., Lang, H.F., Wu, C.S., Wan, C.J., and I.T. Lee (2008). Effect of weight loss on proinflammatory state of mononuclear cells in obese women. *Obesity (Silver Spring)*, **16**, 1033-1038.
- Skurk, T., Herder, C., Kräft, I., Müller-Scholze, S., Hauner, H., and H. Kolb (2005). Production and release of macrophage migration inhibitory factor from human adipocytes. *Endocrinology*, **146**, 1006-1011.
- Staab, C.A., and E. Maser (2010). 11beta-Hydroxysteroid dehydrogenase type 1 is an important regulator at the interface of obesity and inflammation. *J. Steroid Biochem. Mol. Biol.* **119**, 56-72.
- Stosic-Grujicic, S., Stojanovic, I., and F. Nicoletti (2009). MIF in autoimmunity and novel therapeutic approaches. *Autoimmun. Rev.* **8**, 244-249.
- Stosic-Grujicic, S., Stojanovic, I., Maksimovic-Ivanic, D., Momcilovic, M., Popadic, D., Harhaji, L., Miljkovic, D., Metz, C., Mangano, K., Papaccio, G., Al-Abed, Y., and F. Nicoletti (2008). Macrophage migration inhibitory factor (MIF) is necessary for progression of autoimmune diabetes mellitus. *J. Cell Physiol.* **215**, 665-675.
- Stumvoll, M., Goldstein, B.J., and T.W. Van Haeften (2005). Type 2 diabetes: principles of pathogenesis and therapy. *Lancet*, **365**, 1333-1346.
- Stumvoll, M., Goldstein, B.J., and T.W. Van Haeften (2005). Type 2 diabetes: principles of pathogenesis and therapy. *Lancet*, **365**, 1333-1346.
- Sumarac-Dumanovic, M., Stevanovic, D., Ljubic, A., Jorga, J., Simic, M., Stamenkovic-Pejkovic, D., Starcevic, V., Trajkovic, V., and D. Micic (2009). Increased activity of interleukin-23/interleukin-17 proinflammatory axis in obese women. *Int. J. Obes. (Lond)*. **33**, 151-156.
- Verschuren, L., Kooistra, T., Bernhagen, J., Voshol, P.J., Ouwens, D.M., van Erk, M., de Vries-van der Weij, J., Leng, L., van Bockel, J.H., van Dijk, K.W., Fingerle-Rowson, G., Bucala, R., and R. Kleemann (2009). MIF deficiency reduces chronic inflammation in white adipose tissue and impairs the development of insulin resistance, glucose intolerance,

and associated atherosclerotic disease. *Circ. Res.* **105**, 99-107.

Viardot, A., Lord, R.V., and K. Samaras (2010). The effects of weight loss and gastric banding on the innate and adaptive immune system in type 2 diabetes and prediabetes. *J. Clin. Endocrinol. Metab.* **95**, 2845-2850.

Waeber, G., Calandra, T., Roduit, R., Haefliger, J.A., Bonny, C., Thompson, N., Thorens, B., Temler, E., Meinhardt, A.,

Bacher, M., Metz, C.N., Nicod, P., and R. Bucala (1997). Insulin secretion is regulated by the glucose-dependent production of islet beta cell macrophage migration inhibitory factor. *Proc. Natl. Acad. Sci. USA*, **94**, 4782-4787.

Yabunaka, N., Nishihira, J., Mizue, Y., Tsuji, M., Kumagai, M., Ohtsuka, Y., Imamura, M., and M. Asaka (2000). Elevated serum content of macrophage migration inhibitory factor in patients with type 2 diabetes. *Diabetes Care*, **23**, 256-258.

