Macrophage migration inhibitory factor deficiency leads to age-dependent impairment of glucose homeostasis in mice

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Abstract

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine produced by many cells and tissues including pancreatic β-cells, liver, skeletal muscle, and adipocytes. This study investigates the potential role of MIF in carbohydrate homeostasis in a physiological setting outside of severe inflammation, utilizing Mif knockout (MIF−/−) mice. Compared with wild-type (WT) mice, MIF−/− mice had a lower body weight, from birth until 4 months of age, but subsequently gained weight faster, resulting in a higher body weight at 12 months of age. The lower weight in young mice was related to a higher energy expenditure, and the higher weight in older mice was related to an increased food intake and a higher fat mass. Fasting blood insulin level was higher in MIF−/− mice compared with WT mice at any age. After i.p. glucose injection, the elevation of blood insulin level was higher in MIF−/− mice compared with WT mice, at 2 months of age, but was lower in 12-month-old MIF−/− mice. As a result, the glucose clearance during intraperitoneal glucose tolerance tests was higher in MIF−/− mice compared with WT mice until 4 months of age, and was lower in 12-month-old MIF−/− mice. Insulin resistance was estimated (euglycemic–hyperinsulinemic clamp tests), and the phosphorylation activity of AKT was similar in MIF−/− mice and WT mice. In conclusion, this mouse model provides evidence for the role of MIF in the control of glucose homeostasis.


Introduction

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine involved in both innate and adaptive immunity. Originally described as a T-cell-derived product, MIF has now been shown to be released by numerous cell types and to be involved in many inflammatory and autoimmune diseases (reviewed in Calandra & Roger (2003)).

Beyond its role in inflammation, many lines of evidence indicate that MIF plays an important role in carbohydrate metabolism. MIF is released from the pancreatic islets in a glucose-dependent fashion (Waeb er et al. 1997, Johansson et al. 2003, Allaman-Pillet et al. 2004), and potentiates the glucose-induced release of insulin from β-cells in an autocrine loop (Waeb er et al. 1997). In addition, MIF is produced by many peripheral tissues, where it induces an alteration of glucose metabolism especially during the inflammatory response (Benigni et al. 2000, Kumagi et al. 2001, Atsumi et al. 2007). While most of these studies were performed in the setting of stress, several reports have also documented an association between MIF, obesity, and insulin resistance (IR) in a physiological environment. MIF secretion and serum concentration are increased in obese individuals or in patients with type 2 diabetes (Church et al. 2005, Skurk et al. 2005, Herder et al. 2008), and a polymorphism in the MIF gene promoter has been linked to obesity (Sakaue et al. 2006). In addition, several reports have described that an elevated level of MIF is associated with decreased β-cell function and IR (Benigni et al. 2000, Church et al. 2005, Sakaue et al. 2006).

Finally, in vitro studies have shown that mouse adipocytes treated with MIF, or anti-MIF monoclonal antibody, had altered insulin-mediated glucose transport and insulin receptor signal transduction (Biddinger & Kahn 2006). Taken together, these data suggest that MIF is associated...
with obesity and IR, and may promote the development of type 2 diabetes, but a clear causal relationship could not be established so far.

Here, we aimed to explore the potential role of MIF on carbohydrate homeostasis under physiological conditions without severe inflammation using a Mif knockout mouse model.

Materials and Methods

Animals

Wild-type (WT) C57BL/6 (B6) mice were obtained from the colony bred at the University of Geneva School of Medicine in core facility and Mif knockout B6 (MIF−/−) mice from a colony bred at the University of Lausanne (Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland). MIF−/− B6 × 129/Sv mice were generated by gene targeting as described previously (Bozza et al. 1999). These mice were backcrossed for seven generations onto a B6 background. MIF−/− mice were maintained by mating between homozygous mice on the B6 background. MIF deficiency was confirmed by reverse transcription (RT)-PCR and western blotting as previously described (Calandra et al. 1994). Of note, the B6 mice used for the backcrossing were the same as the controls, thus ensuring the homogeneity of the studied groups.

WT and MIF−/− mice were kept and maintained in the same conditions in the animal facility at University of Geneva School of Medicine, and had free access to food and water. Temperature was maintained between 19 and 21 °C with a daily light exposure of 12 h (0700–1900 h). WT and MIF−/− male mice were used for the experiments and classified into four groups: 1–2-month-old mice (referred to as 2-month-old), 3–4-month-old mice (4-month-old), 5–6-month-old mice (6-month-old), and 12-month-old mice. This study was conducted under protocols approved by the institutional animal care and use committee and by Geneva’s veterinarian state office.

Food intake, energy expenditure, locomotor activity, respiratory exchange ratio, and lipid consumption

Mice (7–8 per group) were kept in special calorimetry system cages for 8 days for acclimatization. Then, mice were housed individually in a custom-made 12-cage calorimetry system (LabMaster; TSE Systems, Bad Homburg, Germany) (http://www.medecine.unige.ch/lfaculte/services/phenotypage/), and following an additional 48 h of adaptation period analyzed for food intake, energy expenditure, respiratory exchange ratio (RER), and locomotor activity. Data were collected over 48 h.

Body composition

Body composition (fat and lean masses) was measured with peripheral dual-energy X-ray absorptiometry (PIXImus; GE–Lunar, Madison, WI, USA).

Micro-computed tomography

To assess the volume of adipose tissue, micro-computed tomography (microCT) was performed in mice as previously described (Montet et al. 2007). Delineation of fat was straightforward, since fat has a negative Hounsfield unit compared with positive values for muscle and bone (Seidell et al. 1989). After reconstruction, fat deposition was manually segmented with OsiriX Medical Image software (OsiriX Foundation, Geneva, Switzerland) (Rosset et al. 2004) in the following anatomical compartments: for the thorax: 1) subcutaneous, 2) axilar, and 3) interscapular; and for the abdomen: 1) subcutaneous, 2) visceral, 3) retroperitoneal, and 4) epididymal. Volume of fat was automatically generated from the segmented region (Veyrat-Durebex et al. 2009).

Intraperitoneal glucose, insulin, and pyruvate tolerance tests

For the intraperitoneal glucose tolerance test (IPGTT) and the pyruvate tolerance test (IPPTT), age-matched WT and MIF−/− mice were injected i.p. with glucose (2 g/kg body weight) or pyruvate (2 g/kg body weight) after 12 h of fasting. Blood glucose concentration was assessed at 0, 5, 15, 30, 45, 60, 120, and 180 min with a glucometer (Medisense Precision Xtra, Abbott) on whole blood sampled from the tail vein into heparinized tubes. The area under the curve (AUC) of glucose above baseline value was calculated by the trapezoidal method. For the IPGTT, plasma insulin concentration was assessed prior to and 15 min after glucose injection. Blood was centrifuged at 500 g for 20 min at 20 °C, and plasma was stored at −20 °C. Insulin levels were quantified by Ultra Sensitive Mouse Insulin ELISA (Mercodia, Uppsala, Sweden). IPGTT stimulation index was calculated by dividing the stimulated plasma insulin level measured 15 min after glucose injection by the mean basal insulin value.

Insulin tolerance tests (ITTs) were performed by injecting 0.75 U/kg human regular insulin i.p. (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) in mice fasting for 4 h. Blood glucose concentration was assessed at 0, 15, 30, 45, 60, and 120 min.

IR was also evaluated by the surrogate index homeostasis model assessment (HOMA-IR) calculated as follows:

\[
\text{HOMA-IR} = \frac{\text{fasting serum insulin in mU/l}}{\text{fasting blood glucose in mmol/l}} \times 22.5
\]

Euglycemic–hyperinsulinemic clamps

Mice were fasted for 3 h and anesthetized with Pentobarbital (80 mg/kg i.p.; Abbott Laboratories). Hepatic glucose production (HGP) and glucose infusion rate (GIR) to maintain euglycemia were measured under basal and insulin-stimulated (18 mU/kg per min; Actrapid HM; Novo Nordisk) conditions using d-[3-3H]glucose (16.6 μCi/kg per min; Perkin Elmer, Waltham, MA, USA), as previously described (Burcelin et al. 2002).
Biochemical analyses

Serum lipids were quantified using enzyme-based kits (Randox Laboratories Ltd, Antrim, UK) as previously described (James & Pometta 1990). Interleukin (IL)6, IL10, monocyte chemotactic protein (MCP) 1, γ interferon (γ IFN), tumour necrosis factor α (TNFα), and IL12p70 levels in mouse sera were detected using the mouse inflammation cytokine cytometric bead assay kits from BD Biosciences (San Jose, CA, USA) according to the manufacturer's instructions. Plasma leptin was determined using Multiplex Immunoassay Technology Xmap (MILLIPLEX mouse endocrine panel, 4 Plex; Millipore, St Quentin en Yveline, France).

Total RNA isolation and real-time RT-PCR

The expression of Acc, Fas, Scd-1, Lpl, and ribosomal protein S29 (Rps29) mRNAs was evaluated by quantitative RT-PCR analysis. Total mRNAs from the epididymal, subcutaneous, and perirenal (retroperitoneal) white adipose tissues were extracted by Qiagen RNEasy Midi extraction kit (Qiagen) according to the manufacturer's instructions. RT was performed using Superscript II RT RNA polymerase (Qiagen) as previously described (Serre-Beinier et al. 2009). For quantitative PCR, amplification of genes was performed from 3-125 ng cDNA using the SYBR green PCR Master Mix (Applied Biosystems, Warrington, UK), and an ABI7500 machine (Applied Biosystems, Foster City, CA, USA). Results were normalized to the expression levels of the Rps29 expression gene, used as housekeeping gene. Primer sequences for the targeted mouse genes are described in Table 1.

Western blot for insulin signaling in skeletal muscle and the liver

Frozen tissues (red gastrocnemius and the liver) were mechanically homogenized in sample buffer 1× (62.5 mmol/l Tris–HCl, pH 7.4, 1 mmol/l EDTA, 2% (w/v) SDS, 5% (v/v) glycerol, and 1% (v/v) 2-mercaptoethanol), supplemented with protease inhibitor cocktail (Roche Diagnostics), and 10 mmol/l phosphatase inhibitors sodium orthovanadate (Sigma) and sodium pyrophosphate (Sigma). After a 10 min centrifugation at 13 000 g and 4 °C, the supernatant was collected and stored at −20 °C. Protein concentrations of all samples were determined with the amido black method (Schaffner & Weissmann 1973), and 10 µg of total protein were loaded on an 8% SDS-PAGE gel. Electrophoresed samples were electroblotted onto polyvinylidene fluoride (PVDF) membranes (Immobilon-P, Millipore) in the presence of 0.01% (w/v) SDS and 20% (v/v) methanol using a constant current of 450 mA for 1 h. The membranes were saturated for 1 h at room temperature in a 10 mmol/l Tris–HCl buffer (pH 7.4) containing 150 mmol/l NaCl, 0.1% (v/v) Tween-20, and 5% BSA, and then incubated overnight at 4 °C with antibody. Antibodies against AKT and phospho-AKT (Ser473) were obtained from Cell Signaling Technology (Danvers, MA, USA). Antibody against actin was obtained from Sigma. Detection was performed using HRP-conjugated secondary antibodies and an enhanced chemiluminescence detection system (Amersham Biosciences). Quantifications were then performed using the ChemiDoc XRS from Bio-Rad Laboratories and Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

Results were presented as means ± s.e.m. Differences between two groups were examined for statistical significance using the unpaired Mann–Whitney U test. Statistical differences among three or more groups were examined by one-way ANOVA followed by Scheffè’s post hoc test. A P value <0.05 was considered significant.

Results

MIF deficiency affects body weight gain and whole-body energy homeostasis

At birth, MIF−/− mice had 13% lower body weight compared with age-matched WT mice (1.34 ± 0.12 vs 1.54 ± 0.15 g respectively, P<0.01). Body weight gain was lower in MIF−/− mice from the day of birth until 2 months of age leading to a 17% lower body weight in MIF−/− mice at 2 months of age compared with age-matched WT mice (Fig. 1A). Over time, MIF−/− mice gained weight faster than WT mice (Fig. 1A), and by 4 months, body weights of MIF−/− and WT mice were similar. At 12 months, MIF−/− mice had an 18% higher body weight than WT mice (Fig. 1A).

To determine whether the difference in body weight gain between MIF−/− and WT mice could be a consequence of a difference in their total energy balance, we determined their whole-body energy homeostasis at 2, 4, and 12 months of age.

Table 1 PCR primers for quantitative real-time PCR

<table>
<thead>
<tr>
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<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>Acc</td>
<td>5′-TTGCTACCCGAGCAGATTGA-3′</td>
<td>5′-CCAACATTTTGCCAGTTTTC-3′</td>
</tr>
<tr>
<td>Fas</td>
<td>5′-GCAAACGGGCTCTCTTCT-3′</td>
<td>5′-GGGTGTGTCAGGGAAT-3′</td>
</tr>
<tr>
<td>Scd-1</td>
<td>5′-GGCGATGTTCCAGGAGGTACTAC-3′</td>
<td>5′-AGGGTGCCAGATGAAG-3′</td>
</tr>
<tr>
<td>Lpl</td>
<td>5′-CCAGGCGATGCGAACAT-3′</td>
<td>5′-CCAGGCGATGCGAACAT-3′</td>
</tr>
<tr>
<td>Rps29</td>
<td>5′-GCCGAGGTCTCGTCTTG-3′</td>
<td>5′-GCCGAGGTCTCGTCTTG-3′</td>
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by analysis of food intake, RER, and locomotor activities. These attributes were assessed during the dark and light phases when animals were in active and resting states respectively. MIF deficiency did not affect metabolic flexibility as judged by a similar decrease in RER values between dark (active) and light (resting) cycles in MIF−/− and WT mice at 2, 4, and 12 months of age (data not shown). At 4 months, MIF−/− mice consumed 37% more food than WT mice in the active dark cycle (Fig. 1B), whereas food intakes at 2 and 12 months were similar between MIF−/− and WT mice (Fig. 1B).

Ambulatory activity in the dark was lower in MIF−/− mice than WT mice at 2 months of age (Fig. 1C), and was similar at 4 and 12 months of age (Fig. 1C). Fine locomotor activity in the dark was higher in MIF−/− mice than WT mice at 4 months of age, and was similar at 2 and 12 months of age (Fig. 1D). Fine locomotor activity comprises mastication activity and fine limb movements associated with food intake; therefore, the correlation between fine activity (Fig. 1D) and food intake (Fig. 1B) at 4 months of age was expected.

The energy expenditure was also assessed during dark and light phases. While 2-month-old MIF−/− mice presented a higher energy expenditure in the light cycle than age-matched WT mice (Fig. 1E), no difference was measured between MIF−/− and WT mice at 4 and 12 months of age (Fig. 1E).

Overall, these data suggest that the lack of MIF during embryological development induced a lower body weight at birth. Later, MIF deficiency in young 2-month-old mice

![Figure 1](https://example.com/figure1.png)

**Figure 1** MIF deficiency affects body weight gain and whole-body energy homeostasis. (A) Evolution of body weight from 2 to 12 months of age in male WT (black bars; n=10–17) and MIF−/− (white bars; n=16–21) mice fed a normal chow diet according to age. Food intake (B), ambulatory (C), and fine (D) activities, and energy expenditure (E) of 2-, 4-, and 12-month-old WT (black bars) and MIF−/− (white bars) mice were determined in the active (dark) and resting (light) conditions. Results are mean ± S.E.M. n=10–12 per group. (F) Fat and lean content of WT (black and striped bars; n=3–7) and MIF−/− (white and gray bars; n=2–8) mice at 2, 4, and 12 months of age. Fat and lean weights were each determined using dual-energy X-ray absorptiometry (DXA), and fat and lean contents were expressed in % of total body weight. Results are mean ± S.E.M. *P<0.05 versus age-matched WT mice.
decreased the locomotor activity, increased the energy expenditure, and decreased the body weight gain. Starting from 4 months of age, MIF deficiency induced an increased food intake and a higher body weight gain.

The increased body weight observed in 12-month-old MIF-/- mice was associated with a higher proportion of fat tissue and a lower proportion of lean tissue (Fig. 1F). In vivo analysis of adipose tissue distribution in 12-month-old MIF-/- and WT mice using microCT indicated a similar fat distribution between subcutaneous, retroperitoneal, intraperitoneal, and epididymal fat pad areas.

The increased fat mass observed in 12-month-old MIF-/- mice did not correlate either with changes in RNA expression of the lipogenic enzymes acetyl CoA carboxylase.

**Figure 2** MIF deficiency leads to impaired glucose tolerance. (A–C) Blood glucose concentrations during intraperitoneal glucose tolerance tests in overnight fasted WT (black symbols) and MIF-/- (white symbols) mice at 2 (A), 4 (B), and 12 (C) months of age. Results are mean ± S.E.M., n = 6–18 per group. (D) Areas under the curve for WT (black bars) and MIF-/- (white bars) mice. Results are mean ± S.E.M., n = 6–18 per group. (E) Blood insulin concentrations during glucose tolerance tests in WT (black bars) and MIF-/- (white bars) mice before (0) and 15 min (15) after i.p. glucose injection. Results are mean ± S.E.M., n = 5–18 per group. (F) Stimulation indices of WT (black bars) and MIF-/- (white bars) mice. Stimulation indices were calculated by dividing the stimulated plasma insulin levels measured 15 min after glucose injection by the mean basal insulin values. Results are mean ± S.E.M., n = 5–18 per group. *P < 0.05 versus age-matched WT mice.
MIF knockdown impairs glucose homeostasis

V Serre-Beinier, C Tosso and others.

Figure 3 MIF deficiency does not lead to peripheral insulin resistance. (A and B) Blood glucose concentrations during insulin tolerance tests in 4 h fasted WT (black symbols) and MIF−/− (white symbols) mice at 4 (A) and 12 (B) months of age. Results are mean ± S.E.M., n = 6–12 per group. (C) Whole-body glucose utilization rate, glucose infusion rate (GIR), was calculated in steady-state euglycemic–hyperinsulinemic conditions (18 mU/kg per min) in WT (black bar) and MIF−/− (white bar) mice at 12 months of age. Results are mean ± S.E.M., n = 5 per group. (D) The rate of glucose disappearance (RD) was determined before (basal) and at the end (stimulated) of euglycemic–hyperinsulinemic clamps in the same groups of mice, using D-[3-3H]glucose. Results are mean ± S.E.M., n = 5 per group. (E) Insulin signaling in the red gastrocnemius muscle in basal conditions (∼ in western blot and B in densitometry) and after euglycemic–hyperinsulinemic clamps (+ in western blot and I in densitometry) in 12-month-old MIF−/− and WT mice. Western blots revealed the expression of total AKT and the phosphorylated forms (pAKT). Quantification of the ratio of phosphorylated AKT to total AKT was performed using the ChemiDoc XRS and Quantity One software. Results are mean ± S.E.M. of three mice per group.

carboxylase (57.5 ± 15.0 vs 100.0 ± 28.4 in MIF−/− and WT mice respectively), fatty acid synthase (70.1 ± 19.5 vs 100.0 ± 27.7 in MIF−/− and WT mice respectively), stearoyl-CoA desaturase 1 (168.7 ± 38.5 vs 161.6 ± 43.4 in MIF−/− and WT mice respectively), and lipoprotein lipase (87.2 ± 14.6 vs 100.0 ± 29.2 in MIF−/− and WT mice respectively), or changes in serum levels of cholesterol (70.6 ± 7.7 vs 71.4 ± 5.3 mg/dl in MIF−/− and WT mice respectively).
respectively), triglycerides (55.3 ± 9.5 vs 56.0 ± 8.4 mg/dl in MIF−/− and WT mice respectively), and nonesterified fatty acid (1.3 ± 0.3 vs 1.1 ± 0.3 mmol/l in MIF−/− and WT mice respectively). Despite their increased fat content, IL6, γIFN, and TNFα levels were not changed in older 12-month-old MIF−/− mice, whereas IL10 (72.8 ± 2.2 vs 28.5 ± 7.8 pg/ml in MIF−/− and WT mice respectively, P < 0.05), MCP1 (100.6 ± 8.9 vs 65.8 ± 6.4 pg/ml in MIF−/− and WT mice respectively, P < 0.05), and leptin (200.8 ± 33.2 vs 101.1 ± 15.1 pmol/l in MIF−/− and WT mice respectively, P < 0.05) were increased.

MIF deficiency leads to impaired glucose tolerance

To determine whether differences in whole body energy homeostasis and body composition between MIF−/− and WT mice were associated with changes in glucose homeostasis, IPGTTs were performed on 1-, 2-, 4-, and 12-month-old MIF−/− and WT mice. Results observed for 1- and 2-month-old MIF−/− and WT mice were similar, and only results for 2-month-old mice are presented. Fasting (time 0) glycemia was similar between MIF−/− and WT mice at all ages (Fig. 2A–C). After glucose injection, MIF−/− mice showed higher blood glucose clearance compared with WT mice (Fig. 2A). At 2 and 4 months of age, blood insulin level 15 min after glucose injection in MIF−/− mice was similar to that of WT mice and not elevated over fasting blood insulin level (0 min in IPGTT; Fig. 2E). Accordingly, 2-month-old MIF−/− mice secreted more insulin in response to glucose than WT mice with a stimulation index significantly higher (Fig. 2F). Since 4 months of age, the amount of insulin released after glucose stimulation in MIF−/− mice did not change, while the basal insulin release increased leading to a reduction in stimulation index (Fig. 2F).

The HOMA-IR was 4.7- and 5.5-fold higher for MIF−/− mice than in WT mice at 4 and 12 months of age respectively.

All IPGTT results taken together indicate that MIF deficiency is associated with an age-dependent increase in fasting insulin level and a decrease in insulin stimulation index, leading to impaired glucose tolerance in older mice.

MIF deficiency does not lead to peripheral insulin resistance

The level of IR was assessed by ITTs (Fig. 3A and B). Glycemic profiles analyzed for 120 min after insulin injection were similar in MIF−/− and WT mice at 4 (Fig. 3A) and 12 months of age (Fig. 3B).
In addition, euglycemic–hyperinsulinemic clamp tests at high insulin level showed similar levels of peripheral IR in 12-month-old MIF−/− and WT mice. Indeed, during the clamps, we observed that a similar GIR was required to maintain normal glucose levels in MIF−/− and WT mice (Fig. 3C). These similar GIRs were associated with similar rates of glucose disappearance (Fig. 3D) as well as similar suppression of the HGP (data not shown) in both groups. At steady state, similar serum glucose (5.7 ± 0.3 vs 6.2 ± 0.2 mmol/l for MIF−/− and WT mice respectively) and insulin levels (12.9 ± 5.3 vs 15.6 ± 3.1 ng/ml for MIF−/− and WT mice respectively) were observed for the MIF−/− and WT mice. At the end of the clamp tests, muscle and liver samples were collected, total proteins extracted, and western blots performed. Again, similar levels of AKT phosphorylation were taking place in MIF−/− and WT mice, in muscle without or after insulin perfusion (Fig. 3E).

In order to assess neoglucogenesis in the liver, IPPTTs were performed in 12-month-old mice and demonstrated lower levels of glucose in MIF−/− mice compared with WT mice (Fig. 4A; AUC was 282.6 ± 49.7 and 693.1 ± 215.0 for MIF−/− and WT mice respectively, P=0.053). Again, higher levels of AKT phosphorylation were observed in MIF−/− mice compared with WT mice in basal conditions (without insulin perfusion; Fig. 4B). This result suggests a decreased neoglucogenesis in these mice, likely related to their higher basal insulin level. After insulin stimulation, similar levels of AKT phosphorylation were taking place in MIF−/− and WT mice (Fig. 4B).

Taken together, these results demonstrate the absence of IR in MIF−/− mice.

Discussion

MIF is a proinflammatory cytokine known to be involved in various acute and chronic inflammatory diseases. Increasing evidences suggest a relationship between MIF, inflammation, and impaired glucose metabolism as suggested, among others, by the assessment of Mif promoter polymorphism (reviewed in Herder et al. (2008), Stosic-Grujicic et al. (2008) and Toso et al. (2008)).

In the present study, we characterized the effects of Mif gene disruption on whole-body energy homeostasis and glucose metabolism. Of note, MIF−/− mice were bred as homozygous, and were not littermates of the WT controls. In order to minimize the risk of potential bias, a backcrossing has been performed using the same B6 mice as the controls, thus ensuring an appropriate homogeneity between groups.

First, we observed that MIF deficiency alters embryological development, and leads to a lower body weight at birth. After birth, we observed that MIF-deficient mice had a higher body weight gain and a higher fat content than WT mice that correlated with higher food intake and fine activity. We demonstrated that the absence of MIF leads to impaired glucose tolerance.

In vivo glucose-stimulated insulin secretion increases very early in MIF−/− mice (from 1 month of age), while basal insulin secretion increases only after 4 months of age at the time of higher food intake. This suggests that fasting insulin levels remain low during the first month of development in mice (up to 4 months of age). In addition, the observed higher glucose-stimulated insulin levels can explain the increased glucose tolerance in young 1-, 2-, and 4-month-old MIF−/− mice.

In contrast, in older MIF−/− mice (12 months of age), basal insulin secretion was increased compared with age-matched WT mice, while glucose-stimulated insulin secretion did not change. As a result, insulin stimulation indices decreased with age, and were very low in older 12-month-old MIF−/− mice compared with WT mice. These observations could account for the impaired glucose tolerance observed in old MIF−/− mice.

The effect of MIF on insulin secretion has been evaluated in earlier in vivo and in vitro studies. It has been shown that once released, MIF has a positive autocrine action on the secretion of insulin, which ultimately leads to lower levels of glucose and MIF (Waeb er et al. 1997). These observations are based on the increased in vitro glucose-induced insulin release from rodent islets with the addition of recombinant MIF. In addition, the use of neutralizing anti-MIF antibodies or the downregulation of Mif gene expression (by constitutive expression of Mif antisense RNA) decreases glucose-induced insulin secretion in perfusion studies performed with rat islets or an insulin-producing cell line (INS). In contrast, in our experiments, MIF deficiency increased in vivo glucose-induced insulin release in mice. These discordant findings may reflect the different experimental models used. Waeb er et al. (1997) worked with isolated rat islets and the INS rat ß-cell line in acute MIF inhibition conditions, whereas our study was performed in mice with MIF genetic deficiency.

In an effort to better understand the cause of the altered IPGTT profiles in MIF−/− mice, various tests were conducted to assess peripheral IR, including euglycemic–hyperinsulinemic clamp tests, ITTs, and the assessment of the levels of AKT phosphorylation. Taken together, these results demonstrate the absence of IR in MIF−/− mice. The causes of the decreased glucose tolerance observed in old MIF−/− mice during the IPGTT experiments remain to be further explored, but may be linked to a lower level of peripheral insulin sensitivity, not detected by the present tests, to a proportionally low increase in insulin secretion after glucose stimulation and/or to the secretion of a less potent form of insulin (MIF and insulin are secreted in the same granules; Waeb er et al. 1997).

The high fasting insulin levels measured in older MIF−/− mice coupled to a normal insulin sensitivity of adipose tissue may increase glucose uptake in this tissue and lipogenesis. This could explain the higher fat content observed in these mice. Of note, previous studies have
reported that MIF deficiency can promote adipogenesis in an in vitro adipocyte differentiation model (Atsumi et al. 2007). In the present study, expression levels of the lipogenic enzymes Acc, Fas, Scd-1, and Lpl in 4- and 12-month-old MIF−/− mice were similar to WT ones, suggesting that other genes may be upregulated in MIF−/− mice such as C/EBPα, C/EBPβ, and C/EBPδ, which are transcriptionally activated during adipogenesis and have been shown to be upregulated in MIF-deficient fibroblasts compared with WT fibroblasts during adipocyte differentiation (Atsumi et al. 2007). Obesity has been associated with a low grade inflammatory reaction with macrophage infiltration in white adipose tissue and secretion of proinflammatory cytokines such as IL6 and TNFα.

Despite their increased fat content, the histological assessment of adipose tissue did not show an increased leukocyte infiltration in MIF−/− mice compared with WT mice. IL6 and TNFα levels were not changed in older MIF−/− mice, whereas IL10 and MCP1 as well as leptin were increased. This observation is consistent with previous reports indicating that secretion of the proinflammatory cytokines IL6 and TNFα was unchanged or reduced in MIF−/− mice studied in a setting of inflammation (Amaral et al. 2007, Stosic-Grujicic et al. 2008, Stojanovic et al. 2009). Moreover, Verschuren et al. (2009) showed that genetic deletion of MIF in a mouse model of IR, (the low-density lipoprotein receptor-deficient mouse) reduced macrophage infiltration in white adipose tissue, and lowered both tissue-specific and systemic chronic inflammation. Conversely, genetic deletion of MIF in this mouse model did not affect obesity, and blocked the development of glucose intolerance, suggesting that MIF function in glucose homeostasis is complex.

Several reports have suggested that a low birth weight and a subsequent perinatal increased rate of growth are associated with a later development of obesity and IR. This has been observed in rat and mouse models with bilateral uterine artery ligation or isocalorie low protein nutrition during pregnancy (Simmons et al. 2001, Song et al. 2008, Cripps et al. 2009, Hermann et al. 2009). In addition, these data are also supported by human observations (Baker et al. 1989, Levy–Marchal & Czernichow 2006). In our mouse model, we cannot fully rule out the impact of the low birth weight of the MIF−/− mice in their observed phenotype. However, they show a higher glucose-induced insulin secretion until 4 months of age, and only develop an impaired glucose control late in life (> 6 months). These characteristics are different from those observed in the models of low birth weight, and we speculate that the observed alteration of the glucose metabolism is primarily linked to the absence of MIF. This point will require further assessment. Similar to other peptides, including leptin, ghrelin, or glucagon-like peptide (GLP1), MIF may control glucose and lipid metabolisms in the brain in addition to a possible autocrine regulatory role in insulin-secreting β-cells and adipocytes. Knauf et al. (2005, 2008) have shown that activation of brain GLP1 signaling leads to hyperinsulinemia, IR, and energy storage by a mechanism that did not involve the muscle insulin receptor. GLP1 action could be related to changes in MIF concentration and subsequently to AMPK activity, an enzyme previously shown to be regulated by MIF (Miller et al. 2008).

In summary, we have provided direct evidence that the proinflammatory cytokine MIF plays a key role in insulin secretion and glucose homeostasis. MIF deficiency is linked to hyperinsulinemia, impaired glucose tolerance, and high body fat content in mature mice. These data provide the first evidence that MIF is not only an associated bystander linked to glucose metabolism, but can be a key causative cytokine in the development of metabolic abnormalities. Its role and mechanism of action remain to be further characterized. Thus, MIF appears as a potential target for intervention in various glucose metabolism abnormalities, including type 2 diabetes.

Declaration of interest
VS-B, CT, PM, CG-G, CV-D, FR-J, TR, RWJ, XM, LB, DB, and TB have nothing to declare. TC consults and has received grant support from Baxter AG, and is an inventor on US patent number 7,517,523; 6,998,238; 6,645,493; 6,080,407; and 6,030,615.

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