Molecular Medicine 5: 361-371, 1999

Molecular Medicine

Original Articles

Regulation of Macrophage Migration Inhibitory Factor (MIF) Expression by Glucose and Insulin in Adipocytes In Vitro

Shinji Sakaue,[1] Jun Nishihira,[2] Junichi Hirokawa,[1] Haruhiko Yoshimura,[1] Toshiro Honda,[1] Kenji Aoki,[1] Seiichi Tagami,[1] and Yoshikazu Kawakami[1]

[1]First Department of Medicine and [2]Central Research Institute, Hokkaido University School of Medicine, Sapporo, Japan

Communicated by R. Bucala. Accepted April 14, 1999.

Abstract

Background: It has been reported that macrophage migration inhibitory factor (MIF) stimulated insulin secretion from pancreatic islet β-cells in an autocrine manner, which suggests its pivotal role in the glucose metabolism. According to this finding, we evaluated MIF expression in cultured adipocytes and epididymal fat pads of obese and diabetic rats to investigate its role in adipose tissue.

Materials and Methods: The murine adipocyte cell line 3T3-L1 was used to examine MIF mRNA expression and production of MIF protein in response to various concentrations of glucose and insulin. Epididymal fat pads of Otsuka Long-Evans Tokushima fatty (OLETF) and Wistar fatty rats, animal models of obesity and diabetes, were subjected to Northern blot analysis to determine MIF mRNA levels.

Results: MIF mRNA of 3T3-L1 adipocytes was up-regulated by costimulation with glucose and insulin. Intracellular MIF content was significantly increased by stimulation, whereas its content in the culture medium was decreased. When the cells were treated with cytochalasin B, MIF secretion in the medium was increased. Pioglitazone significantly increased MIF content in the culture medium of 3T3-L1 cells. However, MIF mRNA expression of both epididymal fat pads of OLETF and Wistar fatty rats was down-regulated despite a high plasma glucose level. The plasma MIF level of Wistar fatty rats was significantly increased by treatment with pioglitazone.

Conclusion: We show here that the intracellular glucose level is critical to determining the MIF mRNA level as well as its protein content in adipose tissue. MIF is known to play an important role in glucose metabolism as a positive regulator of insulin secretion. In this context, it is conceivable that MIF may affect the pathophysiology of obesity and diabetes.

Introduction

Macrophage migration inhibitory factor (MIF) was originally identified more than 30 years ago as a lymphokine produced by activated T cells (1,2). MIF was recently found to be secreted from the pituitary gland and monocytes and macrophages, and has the potential to exacerbate endotoxemia (3,4). To date, a much broader role for MIF has been reported. It functions as an essential mediator in T cell activation and delayed-type hypersensitivity (5,6). It should also

Address correspondence and reprint requests to: Dr. Shinji Sakaue, First Department of Medicine, Hokkaido University School of Medicine, Sapporo 060-8638, Japan. Phone: 81-11-716-1161 (ext. 5911); Fax: 81-11-706-7899; E-mail: sakaue-s@tb3.so-net.ne.jp

be noted that MIF counterregulates immunosuppressive effects of glucocorticoid (7). MIF is not limited to immune cells and is ubiquitously expressed in various cells, including pancreatic β-cells and adipocytes (8–10). Recently, it was reported that MIF positively regulated insulin secretion from pancreatic β-cells, depending on the glucose concentration (9). This finding suggests the possibility that MIF plays an important role in the glucose metabolism.

Adipose tissue is not only an energy deposit but also secretes a variety of bioactive molecules such as leptin, plasminogen activator inhibitor-1, and tumor necrosis factor α (TNF-α) (11–13). In particular, TNF-α expression of adipocytes is increased in obesity and exerts an inhibitory effect on the insulin signal transduction system, leading to insulin resistance (14,15). We cloned rat MIF cDNA, reported its physicochemical properties, and succeeded in the crystallization of both human and rat MIF (16–19). Following this, we reported the expression of MIF in adipocytes by Northern blot, Western blot, and immunohistochemical analyses (10). In addition, we revealed that 3T3-L1, a murine adipocyte cell line, secreted MIF in response to TNF-α, and this process was mediated by tyrosine kinase (20). These findings prompted us to investigate the role of MIF in adipose tissue.

In this study, we demonstrated that glucose and insulin could stimulate MIF mRNA expression in adipocytes in vitro. Furthermore, we found that intracellular MIF content increased, whereas the content in the culture medium decreased. On the other hand, we found downregulation of the MIF mRNA level in adipose tissues of two different animal models of non-insulin-dependent diabetes mellitus (NIDDM)—Otsuka Long-Evans Tokushima fatty (OLETF) rats and Wistar fatty rats. On the basis of these results, we discuss here the possible role of MIF in adipose tissue with regard to the pathophysiology of obesity and diabetes.

Materials and Methods

Reagents

Dulbecco's modified Eagle's medium (DMEM), d-biotin, penicillin, streptomycin, insulin, isobutylmethylxanthine, dexamethasone, bovine serum albumin (BSA), and collagenase were obtained from Sigma (St. Louis, MO). Cytochalasin B was from Aldrich (Milwaukee, WI); fetal calf serum (FCS) was from Equitech (Ingram, TX); a random primer labeling kit was from Takara (Kyoto, Japan); and [α-^{32}P]dCTP was from NEN (Boston, MA). Isogen RNA extraction kits were from Nippon Gene (Toyama, Japan), and nylon membranes (Biodyne A) were obtained from Pall BioSupport (East Hills, NY). All other chemicals used were of analytical grade. Pioglitazone was a kind gift from Takeda (Osaka, Japan).

Recombinant rat MIF was purified as previously described (17). A polyclonal MIF antibody specific for rat MIF was generated by immunizing New Zealand white rabbits with purified recombinant rat MIF as described previously (21,22).

Animals

Male OLETF and Long-Evans Tokushima Otsuka (LETO) rats were kindly provided by Otsuka (Tokushima, Japan), and male Wistar fatty rats and their lean littermates were provided by Takeda. Animal studies conformed to the Regulations for Animal Experiments of the Institute for Animal Experimentation, Hokkaido University School of Medicine. The animals were housed under a constant light–dark cycle (lights on from 6:00 A.M. to 6:00 P.M.) and had free access to food and water. Pioglitazone was given to five Wistar fatty rats as a food admixture at 30 mg/kg for 1 week. As a result, the rats were administered a dose of 3–4 mg/kg body weight/day. Blood samples for feeding blood glucose were collected at 9:00 P.M. and those for fasting blood glucose and plasma insulin were obtained in the morning after an overnight fast. Blood samples were carefully obtained from tail veins of the awake rats with an 18-gauge needle. After anesthesia by diethyl ether and halothane, blood was collected from the abdominal aorta in a heparinized tube, and rats were sacrificed to obtain epididymal fat pads. Plasma was obtained by centrifugation and was kept at −20°C until use.

Cell Culture

Murine adipose cell line 3T3-L1 was purchased from the American Type Culture Collection (Rockville, MD). Cells were grown in DMEM containing 25 mM HEPES, 100,000 U/liter penicillin, 100 mg/liter streptomycin, and 8 mg/liter d-biotin (medium A) with 10% FCS in a humid atmosphere of 5% CO_2 at 37°C. Post-confluent cells were exposed to medium A containing 10% FCS, 0.5 mM isobutylmethylxanthine, 1 μM dexamethasone, and 10 μg/ml insulin to induce

differentiation. After 72 hr, the medium was changed to medium A supplemented with 10% FCS and 5 μg/ml insulin, and then cells were fed every 3 days. At 9 days after the induction, the cells were exposed to medium A containing 1% FCS for 24 hr, and subjected for use.

Assays of Glucose and Insulin

Blood glucose was measured with a glucose analyzer (ANTSENSE, Daikin, Osaka, Japan) according to the manufacturer's protocol. Plasma insulin levels were determined by means of a rat insulin radioimmunoassay (RIA) kit (Incstar, Stillwater, MN).

Adipocyte Isolation from Rat Adipose Tissue

Mature adipocytes were isolated from epididymal fat pads of 30-week-old and 46-week-old OLETF and LETO rats by treatment of collagenase as described by Rodbell, with slight modifications (23). In brief, fat pads were washed in sterile phosphate-buffered saline (PBS), minced, and incubated for 1 hr at 37°C with gentle shaking in DMEM containing 25 mM HEPES (pH 7.4) and 1 mg/ml collagenase. The tissue sample was filtered through a nylon mesh (250-μm pore size), and adipocytes were separated by low-speed centrifugation (×1000 rpm, 5 min).

Northern Blot Analysis

Northern blot analysis was carried out as previously described (16). In brief, total RNAs of the rat epididymal fat and murine 3T3-L1 adipocytes were extracted and separated by electrophoresis on agarose gels containing 0.6 M formaldehyde and blotted onto nylon membrane filters. The hybridization was carried out with the rat MIF cDNA probe radiolabeled by [α-^{32}P]dCTP using a random primer DNA labeling kit. The hybridization was performed in a solution containing the radiolabeled cDNA probe, 50% formamide, 0.75 M NaCl, 1% sodium dodecylsulfate (SDS), 20 mM Tris-HCl (pH 7.5), 2.5 mM EDTA, 0.5× Denhardt's solution (1× Denhardt's solution; 0.2% BSA, 0.2% polyvinylpyrolidone, 0.2% Ficoll), and 10% dextran sulfate overnight at 43°C. After hybridization the filters were washed with 2× standard saline citrate (SSC) (1× SSC; 0.15 M NaCl, 0.015 M sodium citrate) at room temperature, followed by 0.2× SSC containing 0.1% SDS at 60°C, and then subjected to autoradiographic analysis. As a control, the same filters were probed with the coding region of rat β-actin cDNA radiolabeled as described above. The quantitative analysis was carried out with an MCID Image Analyzer (Fuji Film, Tokyo, Japan).

Enzyme-Linked Immunosorbent Assay (ELISA) of MIF

ELISA for MIF was performed at room temperature throughout the procedure. The anti-rat MIF antibody dissolved in PBS (50 μl) (4 μg/ml) was added to each well of a 96-well microtiter plate, which was then left to sit for 30 min. The plate was washed three times with distilled water. All wells were filled with PBS containing 0.5% BSA for blocking and left for 20 min. After removal of the blocking solution, the samples were added in duplicate to individual wells and incubated for 1 hr. Aliquots of culture medium were collected to examine secreted MIF content. To determine the intracellular content of MIF, 3T3-L1 adipocytes were homogenized with sonication in PBS containing a cocktail of protease inhibitors (1 mM benzamidine, 1 mM phenylmethylsulfonylfluoride, 1 mM ethylenediaminetetraacetic acid, and 1 μg/ml leupeptin) and centrifuged at 12,000 × *g*, and supernatants were subjected to the assay. After the plate was washed three times with PBS containing 0.05% Tween 20 (washing buffer), 50 μl of biotin-conjugated anti-MIF antibody was added to each well. Following incubation for 1 hr, the plate was again washed three times with the washing buffer. Avidin-conjugated horseradish peroxidase was added to each well, and the microtiter plate was incubated for 15 min. After washing three times, the substrate solution (50 μl) was added to each well. The substrate solution (10 ml) contained 8 mg of o-phenylenediamine and 4 μl of 30% H_2O_2 in citrate phosphatase buffer (pH 5.0). After incubation for 20 min, the reaction was terminated with 25 μl of 4 N sulfuric acid. The absorbance was measured at 492 nm using an ELISA plate reader (Bio-Rad, Model 3550). Intracellular MIF contents were normalized by total protein of cell lysates measured with a Micro BCA protein assay kit (Pierce, Rockford, IL).

Statistical Analysis

Results are expressed as the mean ± SD. All data were compared with the Student's *t*-test for unpaired data. *P*-values <0.05 were considered to be statistically significant.

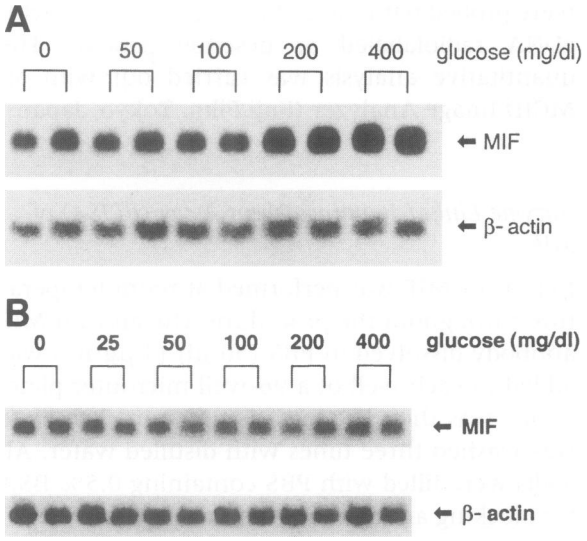

Fig. 1. Glucose-dependent induction of MIF mRNA expression in 3T3-L1 adipocytes. Total RNA (20 μg each) was extracted from cells and MIF mRNA expression was analyzed by Northern blot as described in Materials and Methods. Cells were incubated (A) in medium A containing 1% FCS and 5 μg/ml of insulin for 24 hr with 0, 50, 100, 200, and 400 mg/dl of glucose, and (B) in medium A containing 1% FCS without insulin for 24 hr with 0, 25, 50, 100, 200, and 400 mg/dl of glucose.

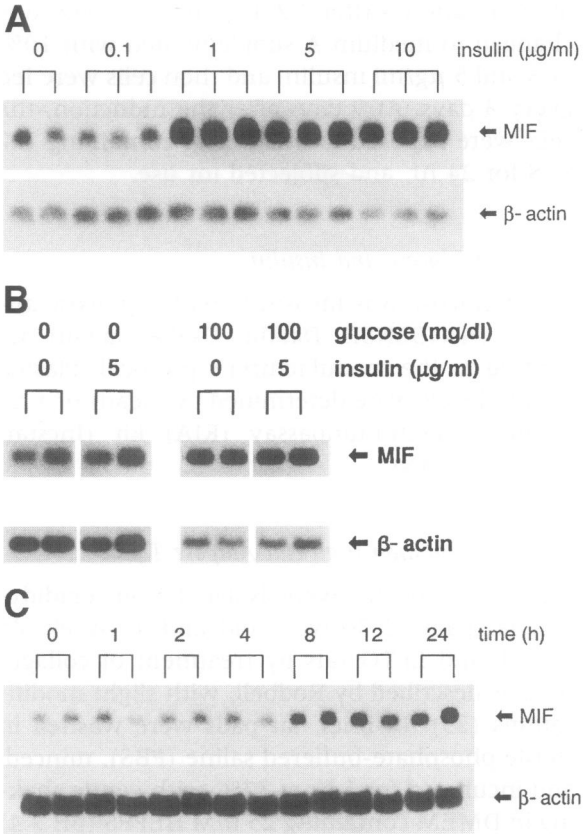

Fig. 2. Insulin-dependent induction of MIF mRNA expression in 3T3-L1 adipocytes. Total RNA (20 μg each) was extracted from cells and MIF mRNA expression was analyzed by Northern blot as described in Materials and Methods. Cells were incubated (A) in medium A containing 1% FCS for 24 hr with 0, 0.1, 1, 5, and 10 μg/ml insulin, (B) in medium A in the absence or presence of 100 mg/dl glucose containing 1% FCS with 0 and 5 μg/ml insulin for 24 hr, and (C) in medium A with 1% FCS and 5 μg/ml insulin for 0, 1, 2, 4, 8, 12, and 24 hr.

Results

Glucose- and Insulin-Dependent Induction of MIF mRNA Expression in 3T3-L1 Adipocytes

To investigate the role of MIF in adipose tissues with regard to obesity and diabetes, we first examined the MIF mRNA level of 3T3-L1 adipocytes in the presence of various concentrations of glucose and insulin. 3T3-L1 adipocytes were exposed to medium A containing 1% FCS without insulin for 24 hr at 9 days after induction, and then incubated in the presence of glucose or insulin for 24 hr. MIF mRNA of 3T3-L1 adipocytes was up-regulated by glucose in a dose-dependent manner, ranging from 50 to 400 mg/dl with a 2.2-fold increase at the dose of 400 mg/dl (Fig. 1A); however, the up-regulation of MIF mRNA was not seen in the absence of insulin (Fig. 1B). A time-course study showed that the maximum level was reached at 12 hr after glucose stimulation, and remained at a plateau up to at least 24 hr (data not shown). In response to insulin, MIF mRNA was markedly induced, reached the maximum level at the dose of 1 μg/ml with a 2.8-fold increase, and was slightly decreased at the doses of 5 and 10 μg/ml (Fig. 2A). Up-regulation by insulin was only observed when 3T3-L1 adipocytes were incubated with 400 mg/dl glucose, but no significant increase was observed in the presence of 100 mg/dl glucose or in the absence of glucose (Fig. 2B). In a time-course study, up-regulation of the MIF mRNA level was seen at 8 hr after insulin stimulation and remained high up to 24 hr (Fig. 2C). These in vitro results indicated that both glucose and insulin were required for up-regulation of MIF mRNA.

Production of MIF in 3T3-L1 Adipocytes by Glucose and Insulin

We examined the MIF content in the culture medium of 3T3-L1 adipocytes and its intracellular content. After incubation in the presence of

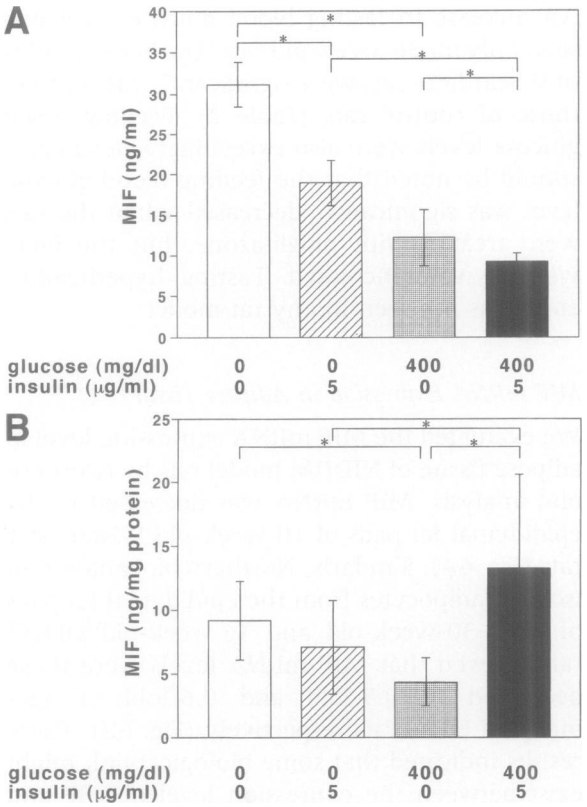

Fig. 3. Effect of glucose and insulin on MIF production by 3T3-L1 adipocytes. (A) Aliquots of culture medium of 3T3-L1 adipocytes incubated in the indicated concentrations of glucose and insulin for 24 hr were collected (n = 10). (B) After 24-hr incubation, the cells were homogenized and the supernatants were prepared as described in Materials and Methods (n = 10). MIF concentrations were measured with ELISA. Data are shown as the mean ± SD. *$p < 0.05$.

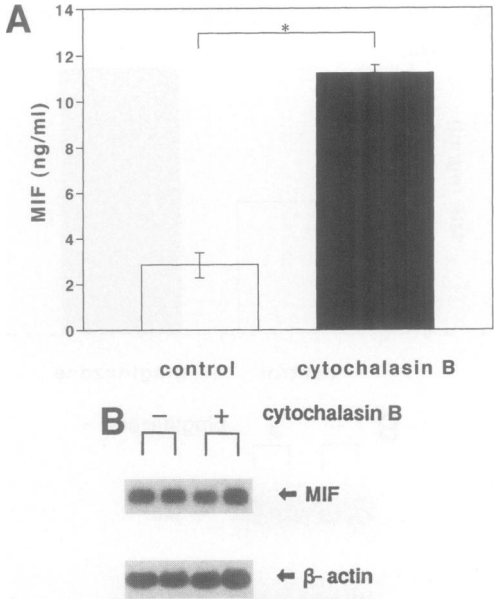

Fig. 4. Effect of cytochalasin B on MIF secretion from adipocytes. 3T3-L1 adipocytes were treated with 5 μg/ml of insulin for 12 hr, and then the medium was changed to one containing 20 μM of cytochalasin B for 12 hr. (A) MIF protein levels in the media were determined with ELISA (n = 4). Data are shown as the mean ± SD. *$p < 0.05$. (B) Total RNA was obtained from cells and then MIF mRNA expression was analyzed by Northern blot.

the various concentrations of glucose and insulin for 24 hr, aliquots of the culture medium and supernatants of cell lysates were collected, and MIF concentrations were measured with ELISA. Incubation of adipocytes with either glucose or insulin caused a decrease in secreted MIF content in the medium (Fig. 3A). The decrease in MIF content in the medium was more obvious when the adipocytes were costimulated with both glucose and insulin. On the other hand, the intracellular MIF protein level was increased by incubation with glucose and insulin in accordance with the up-regulation of MIF mRNA (Fig. 3B).

To further examine the effects of glucose and insulin with respect to intracellular and extracellular MIF contents, 3T3-L1 adipocytes were treated with cytochalasin B (20 μM) for 12 hr after incubation with insulin for 12 hr. The treatment with cytochalasin B induced a significant increase in MIF content in the culture medium (Fig. 4A). However, the MIF mRNA level was minimally changed (Fig. 4B). These results suggested that glucose transport into the cell was critical for determination of intracellular and extracellular MIF content. Glucose and insulin might stimulate uptake of MIF by adipocytes and promote its turnover rate.

Effect of Pioglitazone on MIF Protein Production by 3T3-L1 Adipocytes

Pioglitazone is known as an anti-diabetic agent and has a pharmacological action to improve insulin resistance. We examined the effect of pioglitazone on MIF mRNA expression and MIF protein production by 3T3-L1 adipocytes. When the adipocytes were incubated with 5 μM pioglitazone for 24 hr, MIF protein content in the culture medium was increased to about 2-fold that of the untreated cells, but the MIF mRNA expression level was minimally changed (Fig. 5A,B). These results showed that pioglitazone had the potential to enhance MIF secretion in the culture medium of the adipocytes.

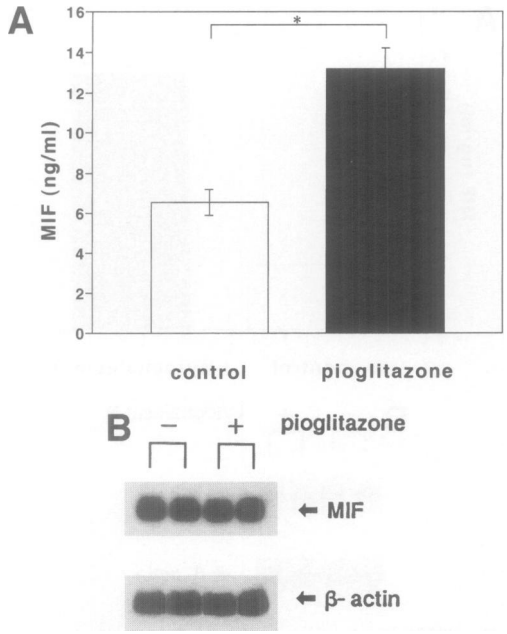

Fig. 5. Effect of pioglitazone on MIF secretion from adipocytes. 3T3-L1 adipocytes were incubated in medium A containing 1% FCS with or without 5 μM of pioglitazone for 24 hr. (A) MIF protein levels in media were determined with ELISA (n = 7). Data are shown as the mean ± SD. *p < 0.05. (B) Total RNA was obtained from cells and then MIF mRNA expression was analyzed by Northern blot.

Characteristics of Animals

The body weight of 30-week-old OLETF rats was significantly higher than that of LETO rats; however, no significant difference between the two groups of rats was seen at the age of 46 weeks (Table 1). Feeding blood glucose levels in both 30-week-old and 46-week-old rats were significantly elevated, especially in 46-week-old rats. An increase in fasting blood glucose level was seen only for 30-week-old rats. The body weights of Wistar fatty rats were significantly higher than those of control rats (Table 2). Feeding blood glucose levels were also exceedingly elevated. It should be noted that the feeding blood glucose level was significantly decreased when the rats were treated with pioglitazone, but the body weights were increased. Fasting hyperinsulinemia was not seen in any rat model.

MIF mRNA Expression in Adipose Tissue

We evaluated the MIF mRNA expression level in adipose tissue of NIDDM model rats by Northern blot analysis. MIF mRNA was decreased in the epididymal fat pads of 10-week-old Wistar fatty rats (Fig. 6A). Similarly, Northern blot analysis of isolated adipocytes from the epididymal fat pads of both 30-week-old and 46-week-old OLETF rats showed that MIF mRNA levels were those decreased to 0.5-fold and 0.6-fold of age-matched LETO rats respectively (Fig. 6B). These results indicated that some biological link might exist between the expression level of MIF and the pathophysiological states of obesity and diabetes.

Plasma MIF Concentration

The plasma MIF levels of OLETF rats of the 30-week-old and 46-week-old age-groups and of Wistar fatty rats were minimally changed (Fig. 7A,B), despite the fact that MIF production in the adipose tissue of obesity/diabetes model rats was markedly decreased, as shown in Figure 6. As for the experiment using pioglitazone, we found that plasma MIF levels of Wistar fatty rats were significantly increased by treat-

Table 1. Characteristics of LETO and OLETF rats

	30 weeks		46 weeks	
	LETO	**OLETF**	**LETO**	**OLETF**
Body weight (g)	485 ± 21	613 ± 33*	570 ± 26	593 ± 30
Fasting blood glucose (mg/dl)	117 ± 9	167 ± 31*	108 ± 23	180 ± 70
Feeding blood glucose (mg/dl)	132 ± 12	172 ± 11*	119 ± 11	422 ± 117*
Fasting plasma insulin (μU/ml)	4.5 ± 4.3	3.1 ± 2.2	2.9 ± 2.3	2.6 ± 0.2

Mean ± S.D. *p < 0.05 versus age-matched LETO rats (n = 4).

Table 2. Characteristics of Wistar fatty and its control rats

	Control	Fatty	Fatty + pio.[a]
Body weight (g)	294 ± 15	340 ± 16*	381 ± 21**
Fasting blood glucose (mg/dl)	114 ± 12	125 ± 19	101 ± 3**
Feeding blood glucose (mg/dl)	142 ± 9	481 ± 54*	197 ± 19**
Fasting plasma insulin (μU/ml)	4.2 ± 3.9	6.3 ± 1.6	8.9 ± 2.8

Mean ± S.D. *$p < 0.05$ versus control rats, **$p < 0.05$ versus Wistar fatty rats ($n = 5$).
[a]Fatty + pio., Wistar fatty rats treated with pioglitazone.

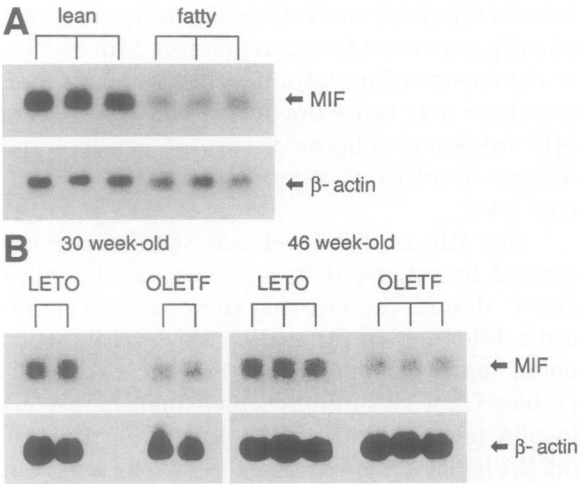

Fig. 6. Differential expression of MIF mRNA in adipose tissues of diabetic and nondiabetic rats. (A) Total RNA was obtained from epididymal fat pads of 10-week-old Wistar fatty ($n = 3$) and lean littermate rats ($n = 3$). (B) Epididymal fat pads from 30-week-old ($n = 2$) and 46-week-old ($n = 3$) OLETF and LETO rats were carefully separated into adipocytes and vascular-stromal fractions as described in Materials and Methods, and then total RNA (20 μg each) was obtained from adipocyte fractions. MIF mRNA expression was analyzed by Northern blot. Representative results of each group are shown.

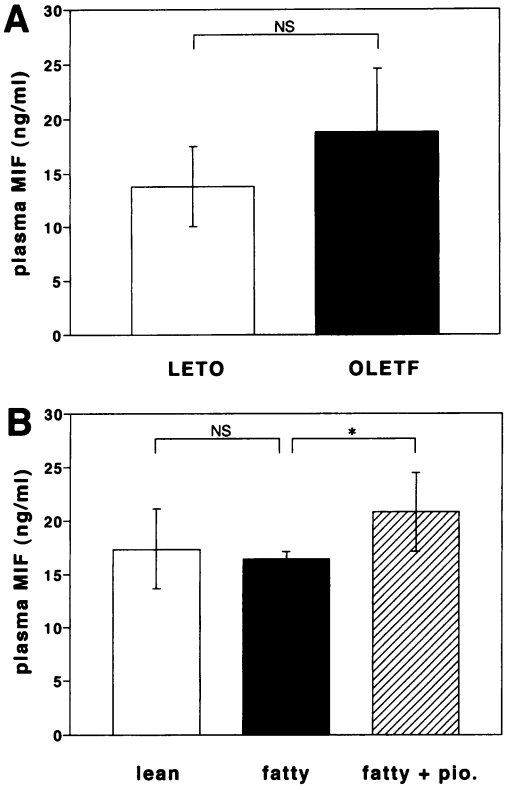

Fig. 7. Plasma MIF levels of diabetic and nondiabetic rats. (A) 30-week-old ($n = 4$) and 46-week-old ($n = 4$) OLETF and LETO rats, and (B) 10-week-old Wistar fatty rats ($n = 5$), those treated with pioglitazone ($n = 5$), and their lean littermates ($n = 5$) were evaluated. Plasma MIF levels were measured by ELISA. Data are shown as the mean ± SD. *$p < 0.05$; NS, not significant; fatty + pio., Wistar fatty rats treated with pioglitazone.

ment with this agent (21 ± 3.7 ng/ml) compared with rats of the same age-group (16 ± 0.7 ng/ml) (Fig. 7B).

Discussion

In this study we demonstrated that MIF mRNA expression of cultured 3T3-L1 adipocytes was markedly up-regulated by costimulation with glucose and insulin in a dose-dependent manner. In association with glucose metabolism, it was reported that MIF mRNA expression was induced by glucose in pancreatic islet β-cells and isolated rat pancreatic islets (9). The present results on adipocytes are consistent with this finding and suggest that MIF mRNA could be induced by glucose beyond cell types. However, it should be noted that not only glucose but also insulin is essential for the up-regulation of MIF

mRNA in adipocytes. This observation suggests that glucose uptake by the cells in response to insulin may be critical for the regulation of MIF mRNA expression.

For regulation of the intracellular glucose level, glucose transporter (GLUT) plays a pivotal role, by promoting uptake of glucose by cells such as adipocytes. There are several types of GLUT with distinct characteristics. For example, GLUT2 present in islet β-cells transports glucose into the cells independent of insulin (24). Accordingly, a high blood glucose level readily increases the intracellular glucose level of pancreatic islet β-cells via GLUT2. Therefore, it is expected that MIF mRNA will be expressed in the islet β-cells as a result of a high glucose level as previously reported (9). On the other hand, GLUT4 expressed in adipocytes stimulates glucose transport in an insulin-dependent manner (25). Thus, the glucose level in adipose tissue could be up-regulated by insulin, which results in the up-regulation of MIF mRNA only when these cells are sensitive to insulin. This is consistent with the finding that no significant increase in MIF mRNA level by stimulation with glucose and insulin was observed in undifferentiated 3T3-L1 cells, which do not express GLUT4 (data not shown).

Costimulation of adipocytes with glucose and insulin induced an increase in intracellular MIF content, in accordance with the up-regulation of mRNA expression. On the other hand, the stimulation caused a significant decrease in secreted MIF content. The reason for this decrease is not fully understood, but it is likely that the costimulation with glucose and insulin not only stimulated MIF mRNA expression but also enhanced the uptake of MIF protein by the adipocytes. To investigate the possible mechanism of MIF uptake by the cells, we examined the effect of cytochalasin B, an inhibitor of glucose transport. Cytochalasin B appeared to suppress MIF uptake by the cells, resulting in an increase in MIF content in the culture medium. Although cytochalasin B has other pharmacological effects, such as disorganization of actin bundle patterns, the current result strongly suggests that glucose transport played a pivotal role in determining intracellular and secreted MIF levels.

For further investigation, we employed two animal models, OLETF and Wistar fatty rats, in this study. These are known as animal models of NIDDM with obesity (26,27). Although MIF mRNA expression of adipocytes was up-regulated by glucose in vitro, its expression in adipose tissue obtained from OLETF and Wistar fatty rats was by contrast reduced despite high blood glucose levels. The discrepancy between these results from in vitro and in vivo experiments could be explained by increased or decreased glucose uptake by adipocytes and adipose tissue, respectively. In vivo, sensitivity of peripheral adipocytes in obese and diabetic rats to insulin is usually decreased, which results in a decrease in the intracellular glucose level, concomitant with the down-regulation of MIF mRNA. Thus, decreased sensitivity of peripheral tissues to insulin might be the major cause of the decreased MIF mRNA level in vivo because both insulin and glucose are equally important for up-regulating MIF mRNA, as demonstrated in vitro. In other words, insulin resistance may cause the decreased expression of MIF mRNA in adipose tissues of obesity- and diabetes-model rats, despite a high plasma glucose level.

The MIF mRNA level was significantly decreased in adipose tissues in vivo, as described above, despite the fact that there was no significant difference in plasma insulin level between obese and diabetic rats and their controls (Tables 1, 2). This finding shows that the plasma insulin level did not solely determine the MIF mRNA level of adipose tissue in vivo as it did not in vitro. Sensitivity of adipose tissues to insulin is more critical for induction of MIF mRNA via up-regulation of the intracellular glucose level. An increase in glucose uptake by adipocytes in response to insulin might be directly linked to up-regulation of MIF mRNA.

TNF-α mRNA is overexpressed in adipose tissues of some obese animals and humans when insulin resistance occurs (13,28). This is because TNF-α has the potential to suppress the expression of GLUT4 in response to insulin by regulating its intracellular signaling pathway, which leads to insulin resistance (29). Therefore, it is hypothesized that obesity causes insulin resistance mainly from overexpression of TNF-α in adipose tissue (15). We previously reported that TNF-α increased the MIF mRNA level and MIF secretion into culture medium of 3T3-L1 adipocytes (10,20), but in contrast, we found a marked decrease in the MIF mRNA level in adipose tissue of obese and diabetic rats. The precise biological reason for the decrease in MIF mRNA level in vivo remains to be elucidated. MIF up-regulates TNF-α, and vice versa (4). Accordingly, it is speculated that MIF mRNA might be down-regulated so as not to stimulate TNF-α expres-

sion. Moreover, plasma MIF might be elevated in the state of obesity/diabetes to overcome insulin resistance, stimulating secretion of insulin from pancreatic β-cells. However, the mechanism of insulin resistance in adipose tissue with respect to TNF-α levels is still controversial (30,31). In this context, further study on TNF-α and MIF mRNA levels in adipose tissues should be carried out to elucidate the pathophysiological role of MIF in obesity and diabetes.

In this study, plasma MIF levels of OLETF rats were slightly elevated, although this elevation was not statistically significant. For humans, we found that serum MIF levels in diabetic patients are significantly elevated compared to those of nondiabetic controls (unpublished data, M. Yabunaka). The elevated plasma MIF level may be due to a decreased turnover rate of MIF—namely, impaired uptake and degradation of MIF by adipose tissue. Increased plasma MIF would stimulate insulin secretion from pancreatic β-cells to compensate for the pathological state of diabetes mellitus.

Furthermore, we administered pioglitazone to Wistar fatty rats to improve insulin resistance, then measured the plasma level of MIF. Pioglitazone, a thiazolidinedione derivative, is known as a ligand for the peroxisome proliferator-activated receptor γ (PPARγ) (32). This agent induces differentiation of adipocytes, which results in improved insulin sensitivity in peripheral tissue in vivo (33–35). The plasma MIF level was significantly increased by treatment with pioglitazone (Fig. 3B), along with a decreased blood glucose level (Table 2). Consistent with this, 3T3-L1 adipocytes treated with pioglitazone secreted more MIF protein than nontreated cells (Fig. 5A). As for the pharmacological action, pioglitazone blocked the inhibitory effect of TNF-α on glucose uptake, but did not stimulate insulin-stimulated glucose uptake in 3T3-L1 adipocytes (36). Thus, the increase in MIF content in culture medium in response to pioglitazone may not be related to intracellular glucose levels. Maturation of adipocytes might be intimately related with MIF production, because mature adipocytes produce more MIF protein than premature cells (20). Indeed, pioglitazone is known to have the potential to stimulate cell differentiation (33). Thus, the increase in plasma MIF level caused by pioglitazone might be due to differentiation of adipocytes in vivo.

In summary, we have demonstrated that MIF mRNA expression was induced by glucose and insulin, and intracellular MIF protein was increased in cultured adipocytes. Secreted MIF protein in the medium was decreased, which might be on account of enhanced uptake of MIF protein by the adipocytes. In vivo, we found decreased expression levels of MIF mRNA in adipose tissues of obese and diabetic rat models, which may be due to decreased sensitivity of peripheral tissues to insulin. Since MIF functions as a positive regulator of insulin secretion (9), administration of recombinant MIF may stimulate insulin secretion from pancreatic β-cells, resulting in prevention of hyperglycemia. Taken together, these results indicate that MIF is involved in various biological responses of adipocytes and that its expression should be delicately balanced by glucose and insulin. Further studies in this area are currently under way.

Acknowledgments

This research was supported in part by grants-in-aid from the Ministry of Education, Science and Culture of Japan (no. 0970144) and the Takeda Science Foundation. We are grateful to Miss Yuka Mizue of Sapporo Immunodiagnostic Laboratory for her technical assistance.

References

1. Bloom BR, Bennett B. (1966) Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science* **153:** 80–82.
2. David JR. (1966) Delayed hypersensitivity in vitro: its mediation by cell free substances forms by lymphoid cell-antigen interaction. *Proc. Natl. Acad. Sci. U.S.A.* **56:** 72–77.
3. Bernhagen J, Calandra T, Mitchell RA, et al. (1993) MIF is a pituitary-derived cytokine that potentiated endotoxaemia. *Nature* **365:** 756–759.
4. Calandra T, Bernhagen J, Mitchell RA, Bucala R. (1994) The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *J. Exp. Med.* **179:** 1895–1902.
5. Bacher M, Metz CN, Calandra T, et al. (1996) An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. *Proc. Natl. Acad. Sci. U.S.A.* **93:** 7849–7854.
6. Bernhagen J, Bacher M, Calandra T, et al. (1996) An essential role for macrophage migration inhibitory factor (MIF) in the delayed-type hypersensitivity reaction. *J. Exp. Med.* **183:** 277–282.

7. Calandra T, Bernhagen J, Metz CN, et al. (1995) MIF as a glucocorticoid-induced modulator of cytokine production. *Nature* **377:** 68–71.
8. Bacher M, Meinhardt A, Lan HY, et al. (1997) Macrophage migration inhibitory factor expression in experimentally induced endotoxemia. *Am. J. Pathol.* **150:** 235–246.
9. Weaber G, Calandra T, Roduit R, et al. (1997) Insulin secretion is regulated by the glucose-dependent production of islet β cell macrophage migration inhibitory factor. *Proc. Natl. Acad. Sci. U.S.A.* **94:** 4782–4787.
10. Hirokawa J, Sakaue S, Tagami S, et al. (1997) Identification of macrophage migration inhibitory factor in adipose tissue and its induction by tumor necrosis factor-α. *Biochem. Biophys. Res. Commun.* **235:** 94–98.
11. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature* **372:** 425–432.
12. Samad F, Yamamoto K, Loskutoff DJ. (1996) Distribution and regulation of plasminogen activator inhibitor-1 in murine adipose tissue in vivo. Induction by tumor necrosis factor-α and lipopolysaccharide. *J. Clin. Invest.* **97:** 37–46.
13. Hotamisligil GS, Shargill NS, Spiegelman BM. (1993) Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science* **259:** 87–91.
14. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. (1996) IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance. *Science* **271:** 665–668.
15. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. (1994) Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-α. *J. Clin. Invest.* **94:** 1543–1549.
16. Sakai M, Nishihira J, Hibiya Y, Koyama Y, Nishi S. (1994) Glutathione binding rat liver 13k protein is the homologue of the macrophage migration inhibitory factor. *Biochem. Mol. Biol. Int.* **33:** 439–446.
17. Nishihira J, Kuriyama T, Sakai M, Nishi S, Ohki S, Hikichi K. (1995) The structure and physicochemical properties of rat liver macrophage migration inhibitory factor. *Biochim. Biophys. Acta* **1247:** 159–162.
18. Suzuki M, Murata E, Tanaka I, Nishihira J, Sakai M. (1994) Crystallization and a preliminary X-ray diffraction study of macrophage migration inhibitory factor from human lymphocytes. *J. Mol. Biol.* **265:** 1141–1143.
19. Sugimoto E, Suzuki M, Nakagawa A, Tanaka I, Fujinaga M, Nishihira J. (1995) Crystallization of rat liver macrophage migration inhibitory factor for MAD analysis. *J. Struct. Biol.* **115:** 331–334.
20. Hirokawa J, Sakaue S, Furuya Y, et al. (1998) Tumor necrosis factor-α regulates the gene expression of macrophage migration inhibitory factor through tyrosine kinase-dependent pathway in 3T3-L1 adipocytes. *J. Biochem.* **123:** 733–739.
21. Suzuki H, Kanagawa H, Nishihira J. (1996) Evidence for the presence of macrophage migration inhibitory factor in murine reproductive organs and early embryos. *Immunol. Lett.* **51:** 141–147.
22. Makita H, Nishimura M, Miyamoto K, et al. (1998) Effect of anti-macrophage migration inhibitory factor antibody on lipopolysaccharide-induced pulmonary neutrophil accumulation. *Am. J. Respir. Crit. Care Med.* **158:** 573–579.
23. Rodbell M. (1964) Metabolism of isolated fat cells. I. Effect of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239:** 375–380.
24. Chen L, Alam T, Johnson J, Hughes S, Newgard CB, Unger RH. (1990) Regulation of β-cell glucose transporter gene expression. *Proc. Natl. Acad. Sci. U.S.A.* **87:** 4088–4092.
25. Suzuki K, Kono T. (1980) Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. *Proc. Natl. Acad. Sci. U.S.A.* **77:** 2542–2545.
26. Ikeda H, Shino A, Matsuo T, Iwatsuka H, Suzuoki Z. (1981) A new genetically obese-hyperglycemic rat (Wistar fatty). *Diabetes* **30:** 1045–1050.
27. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T. (1992) Spontaneous long-term hyperglycemic rat with diabetic complications: Otsuka Long-Evans Tokushima fatty (OLETF) strain. *Diabetes* **41:** 1422–1428.
28. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. (1995) Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. *J. Clin. Invest.* **95:** 2409–2415.
29. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. (1994) Tumor necrosis factor a inhibits signaling from the insulin receptor. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 4854–4858.
30. Ishida K, Mizuno A, Murakami T, Shima K. (1996) Obesity is necessary but not sufficient for the development of diabetes mellitus. *Metabolism* **45:** 1288–1295.
31. Murase K, Odaka H, Suzuki M, Tayuki N, Ikeda H. (1998) Pioglitazone time-dependently reduces tumor necrosis factor-α level in muscle and improves metabolic abnormalities in Wistar fatty rats. *Diabetologia* **41:** 257–264.
32. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ. *J. Biol. Chem.* **270:** 12953–12956.
33. Tontonoz P, Hu E, Spiegelman BM. (1994) Stimulation of adipogenesis in fibroblasts by PPARγ2,

a lipid-activated transcription factor. *Cell* **79:** 1147–1156.

34. Hallakou S, Doare L, Foufelle F, et al. (1997) Pioglitazone induces in vivo adipocyte differentiation in the obese Zucker fa/fa rat. *Diabetes* **46:** 1393–1399.
35. Sugiyama Y, Shimura Y, Ikeda I. (1990) Effect of pioglitazone on hepatic and peripheral insulin resistance in Wistar fatty rats. *Arzneimittelforschung* **40:** 436–440.
36. Szalkowski D, White-Carrington S, Berger J, Zhang B. (1995) Antidiabetic thiazolidinediones block the inhibitory effect of tumor necrosis factor-α on differentiation, insulin-stimulated glucose uptake, and gene expression in 3T3-L1 cells. *Endocrinology* **136:** 1474–1481.