Letter to the Editor

Decreased Plasma MANF Levels are Associated with Type 2 Diabetes*

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Type 2 diabetes mellitus (T2DM) is a multifactorial disease caused by both genetic and environmental factors. Although many genes have been reported to be involved in T2DM, much is still unknown about other genes that are involved in the disease and its progression. Therefore, the exploration of new factors plays a pivotal role in the development of new methods and strategies to prevent this chronic disease.

Mesencephalic astrocyte-derived neurotrophic factor (MANF) is a soluble secretory protein located in the endoplasmic reticulum[1]. MANF has primarily been shown to play neuroprotective and neurorestorative roles in the nervous system[1]. Recent studies indicate that MANF plays a potential role in food intake and energy homeostasis[2] and is involved in the regulation of metabolic diseases. Loss of MANF accelerated lipogenesis and aggravated HepG2 cell steatosis, while MANF overexpression inhibited lipogenesis and rescued HepG2 cell steatosis resulting from free fatty acid treatment[3]. Most importantly, mice with systemic MANF deficiencies had severe diabetes, and MANF protected β cells against glucotoxicity and was indispensable for the proliferation and survival of pancreatic beta cells[4]. Thus, it is reasonable to expect that circulating MANF levels may be significantly decreased during the diabetic state. However, there are contradictory results regarding the levels of circulating MANF in diabetic patients. Plasma MANF concentration was increased compared to controls, in newly diagnosed prediabetes and that T2DM[5] and in children with T1DM[6]. These results led us to investigate whether elevated circulating MANF levels are simply a compensatory response, especially in T2DM patients. Therefore, the aims of the present study were to investigate the relationship between the plasma MANF concentration and T2DM and to determine the clinical factors that are correlated with MANF levels in this population.

One hundred seventy-four individuals were enrolled in this study from 12/2015 to 01/2018 during their admission to the Second Affiliated Hospital of Chongqing Medical University or during an outpatient health check-up. Subjects were divided into 3 groups: 1) healthy controls with normal body mass index (BMI < 25 kg/m^2) and normal fasting glucose, n = 40; 2) overweight and obese subjects with BMI > 25 kg/m^2, n = 78; and 3) T2DM patients with durations of disease of 1–5 years and no obvious complications, n = 56. The present study was approved by the Ethics Committee of The Affiliated Second Hospital of Chongqing Medical University (Chongqing, China), was conducted in accordance with the Declaration of Helsinki, and had the consent of all participants. T2DM was diagnosed according to the 1999 WHO diagnostic criteria. All of the participants enrolled were aged 20–60 years. The exclusion criteria for the study were as follows: 1) patients with types of diabetes other than T2DM, including type 1 diabetes, gestational diabetes or other specific types of diabetes, or who were taking multiple antidiabetic drugs, including insulin; 2) patients with liver and renal dysfunction, thyroid dysfunction, infectious diseases, severe cardiovascular vascular diseases, malignant tumors, or mental diseases. Subjects were further divided into subgroups according to sex.

All peripheral blood samples were collected between 7 am and 9 am after 10–12 h of overnight fasting. Anthropometric parameters, such as height and weight, were measured according to standardized protocols. Systemic systolic blood pressure (SBP) and diastolic blood pressure (DBP)

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were the averages of 3 consecutive measurements acquired by a mercury sphygmomanometer. The levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), free fatty acids (FFA) and uric acid (UA) were determined by standard enzymatic assays. Fasting plasma glucose (FPG) levels were measured by the glucose oxidase method, and glycosylated hemoglobin (HbA1c) levels were measured by ion-exchange high-performance chromatography. Plasma insulin levels were measured using a chemiluminescence assay. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting plasma glucose (FPG, mmol/L) × fasting insulin (FINS, mU/L)/22.5.

The levels of circulating MANF (Abcam, Cambridge, MA, USA), osteopontin (R&D Systems, Minneapolis, MN, USA) and adiponectin (Wuhan USCN Business CO., Ltd, Wuhan, Hubei, China) were measured by commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol.

Statistical analyses were performed using SPSS statistical software (Version 23.0, SPSS Inc., Chicago, Illinois, USA). Normal distributed variables are shown as the mean ± standard deviation (SD), while skewed distributed as the median (interquartile range). Variables were analyzed by either ANOVA followed by the Bonferroni test or the Kruskal-Wallis 1-way test followed by the Mann-Whitney *U* test to compare variables between groups. Spearman correlation, linear stepwise regression analyses were performed to determine variables that were independently correlated with serum MANF. Trends test across increasing quartiles were estimated by the Cochran-Armitage. *P* values < 0.05 were considered statistically significant.

Table 1 shows the anthropometric and metabolic characteristics of the three groups. Compared to control and T2DM groups, there were more males in the overweight/obese group. Subjects in both the overweight/obese and T2DM groups had higher BMI, SBP, DBP, HOMA-IR, TG, LDL-C, FFA, and serum UA than control group (all $P < 0.05$). As expected, FBG and HbA1c were higher in the T2DM group than in either of the other two groups (both $P < 0.05$). Furthermore, T2DM patients had lower HDL-C levels than control

Table 1. Basic characteristics of all the subjects

Variable	Controls (*n* = 40)	Overweight/Obese (*n* = 78)	T2DM (*n* = 56)
Sex (male/female)	18/22	48/29	22/34
Age (years)	42.5 ± 7.56	44.0 (35.8, 49.0)	44.6 ± 8.4
BMI (kg/m²)	22.02 ± 1.61	27.00 (26.11, 29.02)*	25.80 ± 2.90*
SBP (mmHg)	119.0 ± 10.6	125.0 (119.8, 130.0)*	124.5 ± 9.1*
DBP (mmHg)	71.0 (66.0, 80.0)	76.0 ± 7.1*	75.6 ± 7.5*
HbA1c (%)	4.87 ± 0.52	5.12 ± 0.59	8.03 ± 1.06*,#
FBG (mmol/L)	4.9 (4.5, 5.2)	5.22 ± 0.48	8.23 (6.44, 9.23)*,#
FINS (mU/L)	6.73 (5.55, 8.45)	9.42 (7.36, 13.07)*	6.70 (4.08, 10.30)#
HOMA-IR	1.48 (1.13, 1.93)	2.25 (1.67, 3.06)*	2.21 (1.30, 3.21)*
TG (mmol/L)	0.95 ± 0.32	1.30 (0.91, 1.99)*	1.51 (1.19, 1.94)*
TC (mmol/L)	4.40 ± 0.70	4.98 ± 0.82*	4.73 ± 1.01
HDL (mmol/L)	1.58 ± 0.30	1.47 ± 0.31	1.14 ± 0.33*,#
LDL (mmol/L)	2.42 ± 0.62	2.98 ± 0.74*	2.84 ± 0.75*
FFA (mmol/L)	0.32 ± 0.12	0.43 (0.28, 0.58)*	0.45 ± 0.20*
UA (μmol/L)	195.0 (157.3, 233.8)	370.9 ± 98.2*	319.01 ± 91.35*,#

Note. Normal distributed variables are shown as the mean ± standard deviation (SD), while skewed distributed as the median (interquartile range). *vs.* controls, $^{*}P < 0.05$; *vs*. overweight/obesity, $^{\#}P < 0.05$. BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, HbA1c glycosylated hemoglobin, FBG fasting blood glucose, FINS fasting insulin, HOMA-IR homeostasis model assessment of insulin resistance, TG triglycerides, TC total cholesterol, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, FFA free fatty acids, UA uric acid.

and overweight/obese patients ($P < 0.05$).

First, we focused on how the MANF levels changed under T2DM conditions compared to those in normal control persons. The circulating concentrations of MANF were significantly lower in the T2DM group [(1.50 ± 2.08) ng/mL] than those in either the overweight/obese group [(4.63 ± 2.62) ng/mL, $P < 0.01$] or the control group [(3.68 ± 2.10) ng/mL, $P < 0.01$]. Additionally, the MANF concentrations in the overweight/obese group were higher than those in the control group ($P = 0.05$) (Figure 1). The osteopontin levels were significantly higher in T2DM patients [(84.60 ± 21.21) ng/mL] than those in both the control [(29.15 ± 9.98) ng/mL, $P < 0.01$] and overweight/obese [(34.65 ± 7.16) ng/mL, $P < 0.01$] groups and were also higher in the overweight/obese group than in the control group. The adiponectin levels were significantly lower in T2DM patients [(4.35 ± 4.17) ng/mL] than those in overweight/obese participants [(4.78 ± 2.77) ng/mL, $P < 0.05$] and the controls [(7.44 ± 2.97) ng/mL, $P < 0.01$], while the adiponectin levels were significantly lower in the overweight/obese group than those in the control group ($P < 0.01$). No significant sex differences were observed in the MANF, osteopontin or adiponectin levels in the entire group, and the MANF levels showed no significant sex distribution in either the control or T2DM groups.

The altered circulating levels of MANF in the overweight/obese and T2DM groups prompted us to explore the correlation between MANF and related indexes. We found that plasma MANF levels were significantly and negatively associated with HbA1c ($r = -0.413$, $P < 0.001$), FBG ($r = -0.452$, $P < 0.001$), and osteopontin ($r = -0.523$, $P < 0.001$) and positively correlated with HDL ($r = 0.447$, $P < 0.001$), as shown in Supplementary Table S1 available in www.besjournal.com. After adjusting for sex and age, MANF was still significantly and negatively correlated with HbA1c ($r = -0.393$, $P < 0.001$), FBG ($r = -0.376$, $P < 0.001$), and osteopontin ($r = -0.537$, $P < 0.001$) and positively correlated with HDL ($r = 0.393$, $P < 0.001$). Next, we performed linear multiple regressions to determine the variables that were independently associated with the circulating MANF levels. The results showed that osteopontin, HDL and BMI were independently related with the circulating MANF. The multiple regression equation was $Y_{MANF} = -1.05 - 0.05X_{osteopontin} + 1.26X_{HDL} + 0.177X_{BMI}$.

Furthermore, to explore whether the prevalence rates of the overweight/obese or T2DM groups increased with increasing plasma MANF, we further divided the MANF levels into quartiles (Supplementary Table S2 available in www.besjournal.com). As predicted, the percentage of individuals with T2DM was higher in the first quartile (76.8%), and only 23.2% of individuals with T2DM were in the other 3 quarters combined. However, in the control group, most individuals were in Q2–Q4 (92.5%), while only a small portion were in the first quartile (7.5%). This was the same distribution as the overweight/obese group. Particularly when the concentrations were analyzed by the Cochran-Armitage trend test, the relative risks for T2DM

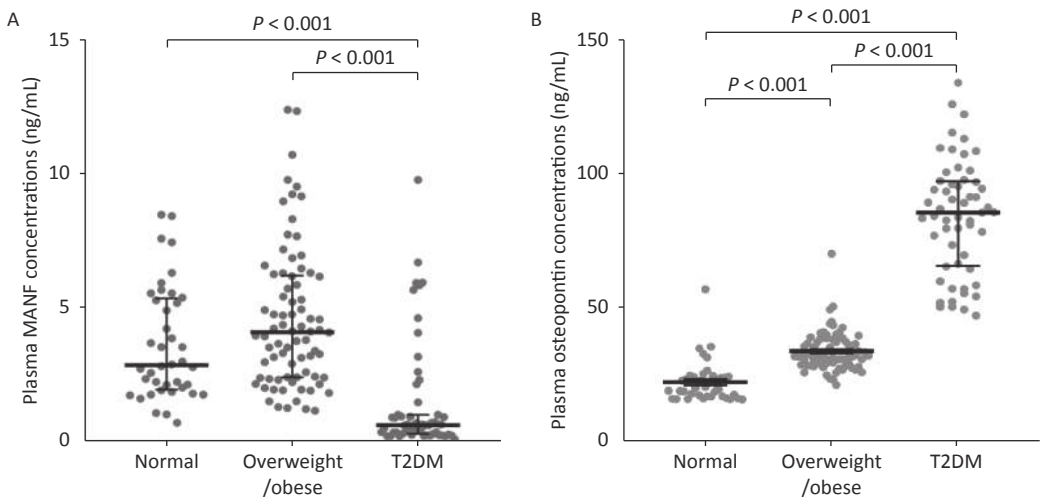

Figure 1. Comparison of the plasma MANF levels among the different groups. (A) Comparison of the plasma MANF levels among the control, overweight/obese, and T2DM groups; (B) Comparison of the plasma osteopontin levels among the control, overweight/obese, and T2DM groups. Compared with normal control or overweight/obese.

increased significantly with decreasing MANF levels ($P < 0.05$).

In the present study, we uncovered important differences from control MANF levels in both high-risk populations and T2DM. This is crucial supplementary data to those described in previous studies. The main findings of this study were as follows: 1) MANF concentrations are significantly decreased in diabetic individuals; 2) the circulating concentrations of MANF are significantly correlated with indexes of glucolipid metabolism; 3) compared to the lower quartile, the relative risk for T2DM is dramatically increased in the upper quartile of MANF levels; and 4) BMI, HDL and osteopontin are optimal independent predictors of circulating MANF in this study cohort.

Our finding was in contrast with previous studies that showed that the plasma MANF concentration was increased in newly diagnosed T2DM as well as T1DM children compared with that in healthy controls[4]. Considering that our overweight/obese and prediabetic patients as well as newly diagnosed T1DM or T2DM patients in other studies may all be at the early stage of diabetes, we believe that the elevated circulation MANF in these patients is only a temporary response of the human body to the insult of metabolic stress. Emerging evidence shows that ER stress plays an important role in the pathogenesis of obesity, insulin resistance and T2DM[1]. MANF could regulate the UPR and act as an anti-ER stress factor[1]. Recombinant MANF or overexpression of MANF could partly protect mouse and human beta cells from endoplasmic reticulum (ER) stress-induced beta cell death and potentiate mouse and human beta cell proliferation[1]. Therefore, in the early stage of T2DM, a compensatory increase in MANF may work as an anti-ER stress protector against disease progression, similar to the increased insulin secretion in the early stage of T2DM, but as the disease progresses with a long duration of glucotoxicity and/or lipotoxicity, MANF expression is decreased, which in turn, exacerbates the disease. This was further verified by the negative correlation of MANF with FBG and HbA1c. Most importantly, the relative risk for T2DM was dramatically increased in the upper quartile of MANF levels compared with the lower quartile of MANF levels. Thus, our present study showed the actual state of MANF in T2DM and suggested that MANF could be a new therapeutic candidate for protecting the body from ER stress caused by lipotoxicity and glucotoxicity.

Osteopontin is a multifunctional glycoprotein closely correlated with obesity and insulin resistance[7]. In the present study, our finding is in accordance with what Yamaguchi H et al. reported in their research[8]. Furthermore, preoperative serum osteopontin levels might be useful for predicting 3-year T2DM remission independent of weight loss in patients undergoing bariatric surgery[9]. Genetic osteopontin deficiency protected mice from obesity-induced insulin resistance and excess gluconeogenesis[10]. We found a significant negative correlation between plasma osteopontin and MANF levels, and osteopontin levels significantly and independently predicted plasma MANF levels. Both *in vivo* and *in vitro* studies showed that osteopontin could induce ER stress in liver cells and exacerbate insulin resistance in obesity, and MANF is a well-established ER stress-response protein that combines with GRP78 for localization in the endoplasmic reticulum, separates from GRP78 to the cytoplasm or circulation once ER stress is initiated, and plays a protective role in numerous ER stress-associated diseases, such as diabetes[1]. Moreover, MANF deficiency leads to ER stress and induces the UPR signaling pathway, especially the PERK/eIF2α pathway[1]. Therefore, we considered that osteopontin and MANF may be correlated with each other by ER stress; however, this is the first report on the correlation of the two factors in T2DM, and much remains to be discovered about how they affect each other under diabetic conditions.

MANF expression is wide expressed in mammalian tissues[6], and the exact source of plasma MANF remains to be studied. We agreed with Emilia et al. that the basal level of MANF detected in human serum is not likely to originate from β-cells[6]. However, whether the elevated plasma MANF was correlated with ER-stressed tissues such as liver and pancreas under obese or prediabetes and the decreased plasma MANF was related to the failure of these organs are worth future research.

In conclusion, we report novel findings of a significant decrease in circulating MANF levels in patients with T2DM. These findings add new information to previous studies about the changes of MANF in T2DM. More studies are needed to understand the mechanism of MANF in the development of T2DM.

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REFERENCES

1. He M, Wang C, Zhang L. Potential therapeutic role and mechanism of mesencephalic astrocyte-derived neurotrophic factor in metabolic disease. Diabetes Weight Manag J, 2020; 1, 1−8.
2. Yang S, Li SH, Li XJ. MANF: a new player in the control of energy homeostasis, and beyond. Front Physio, 2018; 9, 1725.
3. He M, Wang C, Long XH, et al. Mesencephalic astrocyte-derived neurotrophic factor ameliorates steatosis in HepG2 cells by regulating hepatic lipid metabolism. World J Gastroenterol, 2020; 26, 1029−41.
4. Lindahl M, Danilova T, Palm E, et al. MANF is indispensable for the proliferation and survival of pancreatic β cells. Cell Rep, 2014; 7, 366−75.
5. Wu T, Zhang F, Yang Q, et al. Circulating mesencephalic astrocyte-derived neurotrophic factor is increased in newly diagnosed prediabetic and diabetic patients, and is associated with insulin resistance. Endocr J, 2017; 64, 403−10.
6. Galli E, Härkönen T, Sainio MT, et al. Increased circulating concentrations of mesencephalic astrocyte-derived neurotrophic factor in children with type 1 diabetes. Sci Rep, 2016; 6, 29058.
7. Gómez-Ambrosi J, Catalán V, Ramírez B, et al. Plasma osteopontin levels and expression in adipose tissue are increased in obesity. J Clin Endocrinol Metab, 2007; 92, 3719−27.
8. Yamaguchi H, Igarashi M, Hirata A, et al. Progression of diabetic nephropathy enhances the plasma osteopontin level in type 2 diabetic patients. Endocr J, 2004; 51, 499−504.
9. Carbone F, Adami G, Liberale L, et al. Serum levels of osteopontin predict diabetes remission after bariatric surgery. Diabetes Metab, 2019; 45, 356−62.
10. Kiefer FW, Neschen S, Pfau B, et al. Osteopontin deficiency protects against obesity-induced hepatic steatosis and attenuates glucose production in mice. Diabetologia, 2011; 54, 2132−42.

Supplementary Table S1. Spearman correlation r and linear regression analysis of the variables associated with the circulating MANF levels in the studied subjects

Variable	Spearman		Multiple		
	r	*P*	*b*	*P*	95% *CI*
BMI	0.049	0.519	0.218	0.003	(0.06, 0.29)
SBP	−0.049	0.521	–	–	–
DBP	−0.035	0.648	–	–	–
HbA1c	−0.413	< 0.001	–	–	–
FBG	−0.452	< 0.001	–	–	–
FINS	0.169	0.025	–	–	–
HOMA-IR	−0.025	0.747	–	–	–
TG	−0.156	0.04	–	–	–
TC	0.158	0.038	–	–	–
HDL	0.447	< 0.001	0.168	0.033	(0.11, 2.40)
LDL	0.108	0.158	–	–	–
FFA	0.004	0.955	–	–	–
UA	−0.014	0.852	–	–	–
Osteopontin	−0.523	< 0.001	−0.498	< 0.001	(–0.07, –0.03)
Adiponectin	0.106	0.163	–	–	–

Note. In linear stepwise regression analysis, the values included for analysis were BMI, HbA1c, TG, HDL-C, HOMA-IR, and osteopontin. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FFA, free fatty acids; UA, uric acid.

Supplementary Table S2. Distribution of patients according to MANF quartile

MANF quartile	Control (%)	Overweight/obesity (%)	T2DM (%)
Q1	7.5	3.8	76.8
Q2	35.0	25.6	7.1
Q3	27.5	34.6	5.4
Q4	30.0	35.9	10.7
P trend test	< 0.05	< 0.05	< 0.05

Note. Data are shown as numbers and percentages of the total number in every group according to MANF quartile. T2DM, Type 2 diabetes mellitus; MANF, Mesencephalic astrocyte-derived neurotrophic factor.