Therefore, in order to know the role of IGF-1 in the regulation of TLR2/TNF-α co-expressing adipocytes, we analyzed the effects of IGF-1 on the TLR2 and TNF-α mRNA expressions in 3T3-L1 adipocytes (Fig. 3A). The TNF-α mRNA level was increased by the stimulation of a mixture of myristic and palmitic acids [4]. The incubation of 3T3-L1 cells with hGH did not inhibit the increased expression of TNF-α by FFAs. In contrast, IGF-1 completely inhibited the FFA-induced increase in TNF-α mRNA expression. Furthermore, IGF-1 almost inhibited all of the FFA-induced TLR2 mRNA expression in 3T3-L1 adipocytes. A flow cytometry analysis of single adipocytes prepared from 3T3-L1 adipocytes showed that the FFA-induced increase in the population of TLR2/TNF-α co-expressing adipocytes was largely inhibited by the incubation with IGF-1 (Fig. 3B). These results are in consistent with the observations made using in vivo models (see Figs. 1 and 2), thereby suggesting that IGF-1, not GH, reduces the number of TLR2/TNF-α co-expressing adipocytes in visceral fat.

Low-dose GH supplementation reduces the TLR2 mRNA expression of visceral fat before an obvious change of fat volume in obese mice

We finally examined the effect of low-dose GH supplementation on TLR2 mRNA expression in visceral fat in obese mice in order to know the relationship of TLR2 expression and fat volume in visceral fat. The plasma IGF-1 concentration significantly increased in ob/ob mice supplemented with low-dose GH (GH-ob) in comparison to ob/ob mice in the absence of supplementation (control-ob) (Fig. 4A). Measurements of the fat volume using a CT scan showed no significant difference in either the visceral or the subcutaneous fat volume between the GH-ob and the control-ob mice (Fig. 4B). In contrast, the TLR2 mRNA expression levels of visceral fat tissue were significantly decreased in the GH-ob mice in comparison to those in the control-ob mice (Fig. 4C). These results indicate that low-dose GH supplementation caused the decrease in TNF-α expression in the visceral fat before the obvious change in the visceral fat volume in the obese mice.

Discussion

An abnormal expression of cytokines in adipocytes, particularly in the visceral regions, causes the onset of metabolic syndrome through the development of insulin resistance [2]. We have shown that TNF-α expression is induced in adipocytes accumulated in the visceral, and not in the subcutaneous, regions, using a cell transplantation model [3]. The TNF-α expression in the visceral fat is closely associated with the increased population of TLR2/TNF-α co-expressing adipocytes in response to a high-fat intake [4]. The identification of the TLR2/TNF-

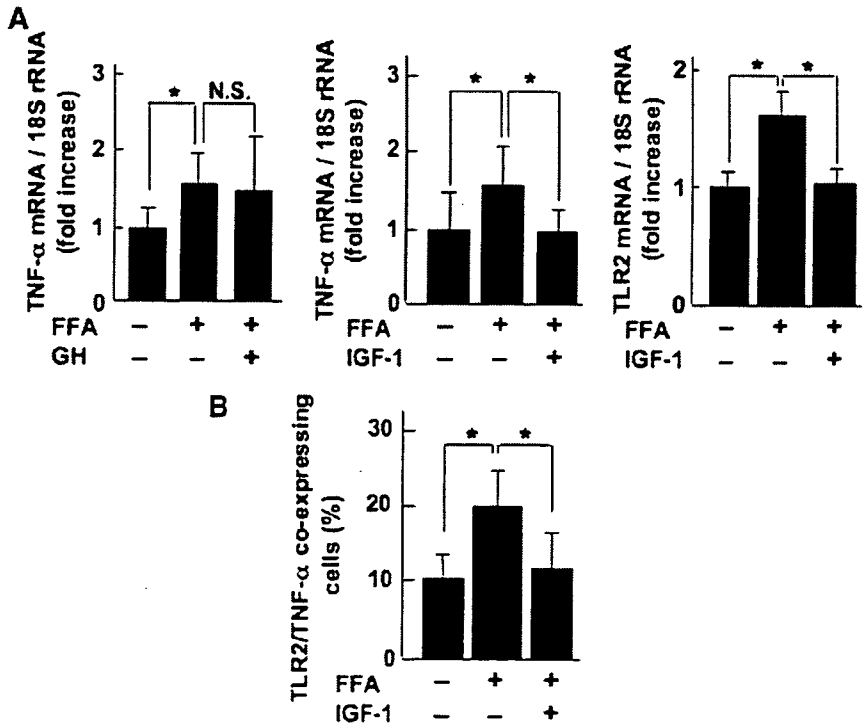


Fig. 3. Effects of GH or IGF-1 on FFA-induced TLR2 and TNF-expressions in 3T3-L1 adipocytes. (A) Serum-starved 3T3-L1 adipocytes treated with 1 mM FFA in the presence or absence of hGH or IGF-1 for 8 h. Quantitative RT-PCR was used to measure the expression level of TNF-α gene or TLR2 gene $n = 6$. $^*P < 0.05$. (B) Flow cytometric analyses of TLR2/TNF-α co-expressing adipocytes in 3T3-L1 adipocytes. Serum-starved 3T3-L1 adipocytes treated with 1 mM FFA in the presence or absence of IGF-1 for 8 h, and analyzed by FACS Calibur. The averaged populations of TLR2/TNF-α co-expressing adipocytes in the total cells (20,000 cells) were expressed ($n = 3$). $^*P < 0.05$.

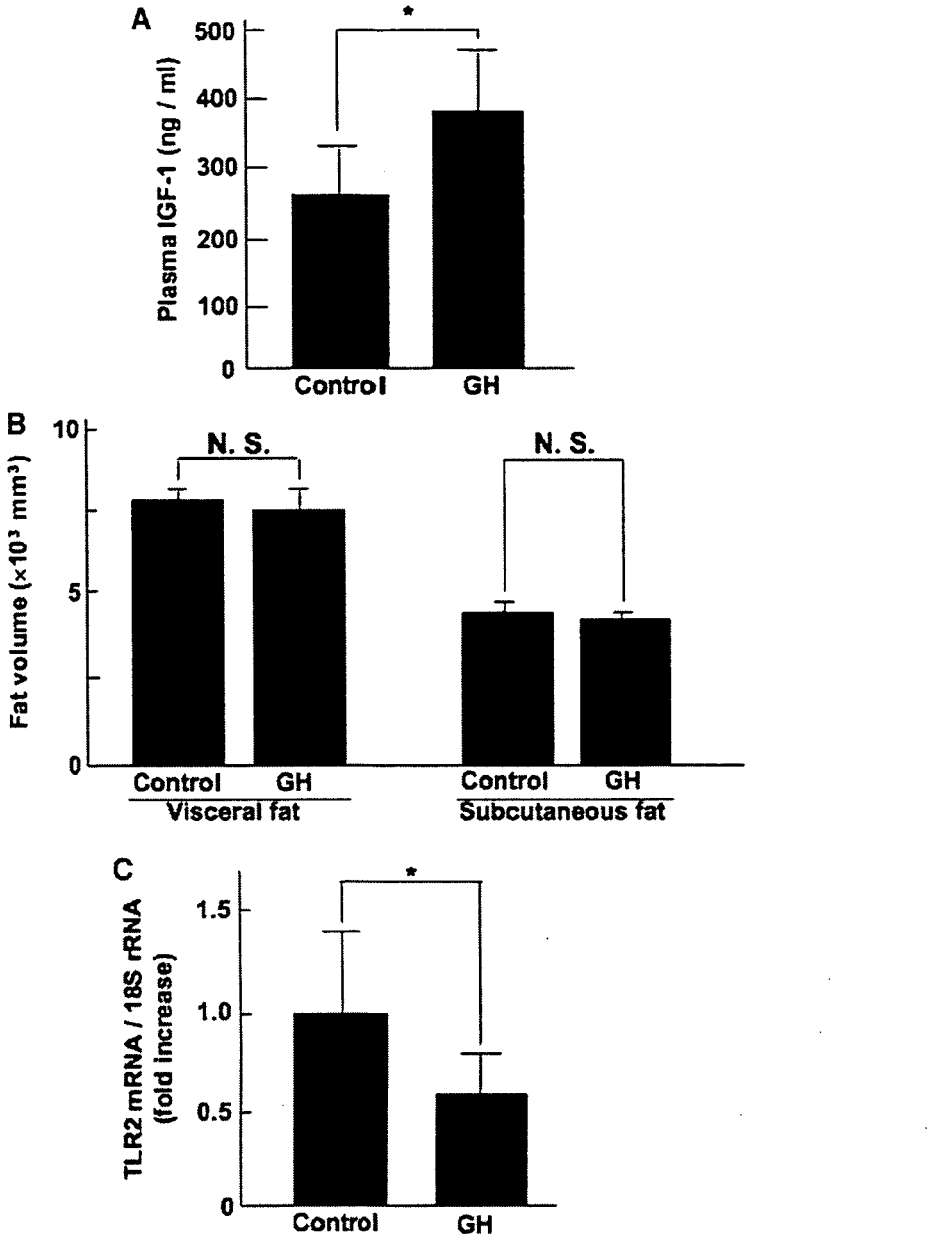


Fig. 4. Effects of low-dose hGH supplementation on the fat volume and TLR2 mRNA expression of visceral fat. Male ob/ob mice were supplemented with hGH (0.5 mg/kg/day) (GH) or PBS (Control) for 4 weeks. (A) The plasma mouse IGF-1 concentration was measured. $n = 7$. $^{*}P < 0.05$. (B) Visceral or subcutaneous fat volumes at 4 weeks after administration of hGH or PBS alone was measured by using CT. $n = 7$. $^{*}P < 0.05$. (C) The TLR2 mRNA expression levels in mesenteric fat tissue were measured by RT-PCR. $n = 7$. $^{*}P < 0.05$.

α co-expressing adipocytes as a regulator of TNF-α expression in the visceral fat suggested that the regulation of the occurrence of pathogenic adipocytes in the visceral fat is important for the improvement of TNF-α-mediated insulin resistance.

Low-dose GH supplementation research has recently focused on the regulation of insulin resistance accompanied by visceral obesity. Yuen et al. found that low-dose GH therapy (0.1 mg/day) improved insulin sensitivity in GH-deficient adults and also notably in subjects with the metabolic syndrome [7]. Johansson showed that GH treatment of obese men reduces the abdominal fat mass, and improved the accompanied metabolic abnormalities [6]. These clinical studies indicate that low-dose GH supplementation is potentially beneficial for metabolic abnormalities accompanied by visceral obesity, in contrast to the glucose intolerance due to the GH overproduction in acromegaly. In this context, there are relevant studies regarding the heterogenous effect of GH on metabolic abnormalities using animal models [13,14]. Based on this background, we performed this study in order to clarify the mechanism for the effect of low-dose GH supplementation on insulin resis-

tance, particularly through the regulation of the population of TLR2/TNF-α co-expressing adipocytes, which has been shown to be related to high-fat-induced insulin resistance [4]. A flow cytometry analysis clearly showed that continuous low-dose GH supplementation reduced the population of TLR2/TNF-α co-expressing adipocytes in visceral regions, and improved insulin resistance. These results using high-fat fed mice are inconsistent with the above clinical observations in obese subjects [6]. We then studied the mechanism of low-dose GH supplementation-mediated inhibition of high-fat induced TLR2 and TNF-α expressions in visceral fat using another model. The GH continuously supplemented from the subcutaneously implanted cells reduced the high-fat induced insulin resistance, and the effect was abolished by the neutralization of IGF-1, a mediator of GH action [15]. The cancellation of GH-mediated action was also observed in the inhibition of TLR2 expression in the visceral fat. Thus, our study showed that IGF-1 was a key molecule in the low-dose GH supplementation for the regulation of TLR2 and TNF-α expressions in visceral fat. The results obtained from cultured adipocytes supported the role of IGF-1 in the effect of low-dose GH supplementation.

The effect of IGF-1 on apoptosis and adipogenesis have been shown in primary cultured adipocytes [16,17]. Our results suggested that TLR2 is one of the genes regulated by IGF-1 in 3T3-L1 cells. The induction of TLR2 expression in high-fat intake could be protected by low-dose GH supplementation through the effect of IGF-1 on visceral adipocytes. The study using ob/ob mice suggested that the effect of IGF-1 on the suppression of TLR2 expression is not necessarily linked to the changes in visceral fat volume. The identification of IGF-1 as a regulator of TLR2 mRNA expression in adipocytes may contribute to the elucidation of the heterogenous functions of GH in various metabolic states. Recent clinical trials suggested that the effects of low-dose GH supplementation are mediated by its ability to increase IGF-1 without the induction of lipolysis [18]. The studies of IGF-1-mediated function on visceral adipocytes may be important for the further therapeutic application of low-dose GH (or IGF-1) supplementation in patients with metabolic syndrome and insulin resistance.

Acknowledgments

We thank for Drs. K. Aragane and J. Kusunoki (Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd) for their assistance of fat volume measurement. These studies were supported by grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology to N.R., Y.S., and H.B.

References

[1] S.M. Grundy, Metabolic syndrome: a multiplex cardiovascular risk factor, J. Clin. Endocrinol. Metab. 92 (2007) 399–404.

[2] L. Hutley, J.B. Prins, Fat as an endocrine organ: relationship to the metabolic syndrome, Am. J. Med. Sci. 330 (2005) 280–289.

[3] M. Shibasaki, K. Takahashi, T. Itou, S. Miyazawa, M. Ito, J. Kobayashi, H. Bujo, Y. Saito, Alterations of insulin sensitivity by the implantation of 3T3-L1 cells in nude mice. A role for TNF-alpha? Diabetologia 45 (2002) 518–526.

[4] K. Murakami, H. Bujo, H. Unoki, Y. Saito, High fat intake induces a population of adipocytes to co-express TLR2 and TNFalpha in mice with insulin resistance, Biochem. Biophys. Res. Commun. 354 (2007) 727–734.

[5] C.W. Ahn, C.S. Kim, J.H. Nam, H.J. Kim, J.S. Nam, J.S. Park, E.S. Kang, B.S. Cha, S.K. Lim, K.R. Kim, H.C. Lee, K.B. Huh, Effects of growth hormone on insulin resistance and atherosclerotic risk factors in obese type 2 diabetic patients with poor glycaemic control, Clin. Endocrinol. (Oxf.) 64 (2006) 444–449.

[6] G. Johannsson, P. Marin, L. Lonn, M. Ottosson, K. Stenlof, P. Bjorntorp, L. Sjostrom, B.A. Bengtsson, Growth hormone treatment of abdominally obese men reduces abdominal fat mass, improves glucose and lipoprotein metabolism, and reduces diastolic blood pressure, J. Clin. Endocrinol. Metab. 82 (1997) 727–734.

[7] K.C. Yuen, D.B. Dunger, Persisting effects on fasting glucose levels and insulin sensitivity after 6 months of discontinuation of a very low-dose GH therapy in adults with severe GH deficiency, Clin. Endocrinol. (Oxf.) 64 (2006) 549–555.

[8] T. Hirata, H. Unoki, H. Bujo, K. Ueno, Y. Saito, Activation of diacylglycerol O-acyltransferase 1 gene results in increased tumor necrosis factor-alpha gene expression in 3T3-L1 adipocytes, FEBS Lett. 580 (2006) 5117–5121.

[9] M. Shibasaki, K. Takahashi, T. Itou, H. Bujo, Y. Saito, A PPAR agonist improves TNF-alpha-induced insulin resistance of adipose tissue in mice, Biochem. Biophys. Res. Commun. 309 (2003) 419–424.

[10] M. Ito, H. Bujo, K. Takahashi, T. Arai, I. Tanaka, Y. Saito, Implantation of primary cultured adipocytes that secrete insulin modifies blood glucose levels in diabetic mice, Diabetologia 48 (2005) 1614–1620.

[11] H. Unoki, H. Bujo, S. Yamagishi, M. Takeuchi, T. Imaizumi, Y. Saito, Advanced glycation end products attenuate cellular insulin sensitivity by increasing the generation of intracellular reactive oxygen species in adipocytes, Diabetes Res. Clin. Pract. 76 (2007) 236–244.

[12] H.S. Kooistra, G. Voorhout, P.J. Selman, A. Rijnberk, Progestin-induced growth hormone (GH) production in the treatment of dogs with congenital GH deficiency, Domest. Anim. Endocrinol. 15 (1998) 93–102.

[13] M.A. Salem, Effects of the amino-terminal portion of human growth hormone on glucose clearance and metabolism in normal, diabetic, hypophysectomized, and diabetic-hypophysectomized rats, Endocrinology 123 (1988) 1565–1576.

[14] M.A. Heffernan, A.W. Thorburn, B. Fam, R. Summers, B. Conway-Campbell, M.J. Waters, F.M. Ng, Increase of fat oxidation and weight loss in obese mice caused by chronic treatment with human growth hormone or a modified C-terminal fragment, Int. J. Obes. Relat. Metab. Disord. 25 (2001) 1442–1449.

[15] E. Corpas, S.M. Harman, M.R. Blackman, Human growth hormone and human aging, Endocr. Rev. 14 (1993) 20–39.

[16] M.N. Dieudonne, R. Pecquery, M.C. Leneveu, Y. Giudicelli, Opposite effects of androgens and estrogens on adipogenesis in rat preadipocytes: evidence for sex and site-related specificities and possible involvement of insulin-like growth factor 1 receptor and peroxisome proliferator-activated receptor gamma2, Endocrinology 141 (2000) 649–656.

[17] P. Fischer-Posovszky, H. Tornqvist, K.M. Debatin, M. Wabitsch, Inhibition of death-receptor mediated apoptosis in human adipocytes by the insulin-like growth factor I (IGF-I)/IGF-I receptor autocrine circuit, Endocrinology 145 (2004) 1849–1859.

[18] K.C. Yuen, D.B. Dunger, Therapeutic aspects of growth hormone and insulin-like growth factor-I treatment on visceral fat and insulin sensitivity in adults, Diabetes Obes. Metab. 9 (2007) 11–22.

Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 561 (2007) 206–213
www.elsevier.com/locate/ejphar

Effect of PPARα activation of macrophages on the secretion of inflammatory cytokines in cultured adipocytes

Kentaro Murakami [a], Hideaki Bujo [b,*], Hiroyuki Unoki [c], Yasushi Saito [a]

[a] Department of Clinical Cell Biology, Chiba University Graduate School of Medicine, Chiba 260-8670, Japan
[b] Department of Genome Research and Clinical Application, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan
[c] Division of Applied Translational Research, Chiba University Graduate School of Medicine, Chiba 260-8670, Japan

Received 8 June 2006; received in revised form 20 December 2006; accepted 21 December 2006
Available online 20 January 2007

Abstract

The relationship between adipocytes and infiltrated macrophages in fat tissue is important for the pathogenesis of insulin resistance through the activation of cytokines. Peroxisome proliferator-activated receptors (PPARs) play a role in the regulation of cytokine secretion in these cells. We studied the effect of the PPARα activation of macrophages on the modulation of the tumor necrosis factor α (TNFα) expression in adipocytes using a cell culture system. A conditioned medium of lipopolysaccharide (LPS)-stimulated RAW264.7 cells, a macrophage cell line, induced the level of TNFα mRNA in 3T3-L1 adipocytes. This effect was inhibited by the addition of neutralizing antibody against interleukin 6 (IL-6) in the conditioned medium or the preincubation of RAW264.7 cells with a specific PPARα agonist, K-111 (2,2-dichloro-12-(4-chlorophenyl)dodecanoic acid). K-111 reduced both the IL-6 production and mRNA expression in RAW264.7 cells, and its effect was stronger than that of rosiglitazone, a PPARγ agonist. The activation of the stress-activated protein kinase/c-Jun NH2-terminal kinase (SAPK/JNK) pathway and nuclear factor kappa B (NF-κB) subunits of p65 was significantly inhibited by K-111. The blocking of IL-6 production through the SAPK/JNK pathway or by transfection with siRNA specific for IL-6 abolished the inhibitory effect of K-111 on the TNFα expression in the 3T3-L1 adipocytes. As a result, the IL-6 produced by RAW264.7 cells is an inducer of TNFα expression in 3T3-L1 adipocytes, and the IL-6 secretion is inhibited by the activation of PPARα. The PPARα activators may suppress the pathogenetical secretion of TNFα in the adipocytes through the functional modulation of the infiltrated macrophages.

Keywords: Adipocyte; PPARα; Macrophage; Fat tissue; IL-6; TNFα

1. Introduction

Insulin resistance is linked to a wide array of metabolic disorders leading to atherosclerosis, such as hypertension, dyslipidemia, or disturbed glucose tolerance (Ginsberg, 2000; Hayden and Reaven, 2000; Reaven, 1995). A cluster of these abnormalities is now recognized as metabolic syndrome (Report of a WHO Consultation, 1999; Expert Panel on Detection, 2001). We have previously shown that visceral accumulation of adipose tissue, and not subcutaneous accumulation, causes systemic insulin resistance through the increased tumor necrosis factor α (TNFα) secretion from adipocytes using a cell-transplanted model (Shibasaki et al., 2002). Resistin is also another possible molecule, which regulates the systemic insulin sensitivity, possibly through the TNFα activation in the model mice (Kitagawa et al., 2004). In this context, the accumulated visceral fat secretes other cytokines, such as Vascular Endothelial Growth Factor (VEGF) (Miyazawa-Hoshimoto et al., 2005). As a result, the cytokine secretion of adipocytes accumulated in the visceral area seems to play an important role in the pathogenesis of insulin resistance and the related vascular diseases in humans. Recent transcriptional profiling experiments using animal models have pointed to a striking regulation of the inflammatory cytokines in adipose tissue, thus suggesting that macrophage infiltration into adipose tissue could be integral to these pathogenic changes (Weisberg et al., 2003; Xu et al., 2003). Interleukin 6 (IL-6) is one of the inflammatory cytokines which link both adipocytes and macrophages. The expression of IL-6 in fat tissue is elevated in individuals demonstrating

* Corresponding author. Tel.: +81 43 226 2091; fax: +81 43 226 2095.
E-mail address: hbujo@faculty.chiba-u.jp (H. Bujo).

0014-2999/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.12.037

obesity with insulin resistance (Mohamed-Ali et al., 1998, 1997; Vozarova et al., 2001; Straub et al., 2000; Fernandez-Real et al., 2001).

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, which belong to the nuclear receptor family. PPARγ plays a pivotal function in the differentiation of adipocytes (Adams et al., 1997; Rosen and Spiegelman, 2001). In fact, chronic treatment with PPARγ activators improves the degree of glucose homeostasis by increasing the insulin sensitivity in various animal models of obesity and diabetes as well as in humans (Miyazaki et al., 2001; Hirose et al., 2002). However, a previous study showed that PPARγ agonists do not obviously suppress the IL-6 production in macrophage (Thieringer et al., 2000). PPARα was first identified for its role in the regulation of both the lipid and carbohydrate metabolisms, and subsequent data have also demonstrated that it exhibits a potent anti-inflammatory activity (Sheu et al., 2002; Delerive et al., 1999). Therefore, in this study we analyzed the effect of a PPARα agonist on the inflammatory cytokine expressions in adipocytes through the macrophage-derived IL-6 pathway using cultured cells, in order to elucidate the possible involvement of PPARα activation in the pathogenetical link between adipocytes and infiltrated macrophages in fat tissue.

2. Materials and methods

2.1. Materials

K-111 (2,2-dichloro-12-(4-chlorophenyl)dodecanoic acid, purity 99%), was synthesized at the Research Laboratories of Kowa company (Tokyo, Japan). Rosiglitazone was given from Takeda Pharmaceutical company (Osaka, Japan). SP600125 (Anthra[1,9-cd]pyrazol-6(2*H*)-one, purity 98%) was purchased from BIOMOL international L.P. (Plymouth Meeting, PA, USA) and these samples were used as solution of various concentrations in dimethyl sulfoxide (DMSO) purchased from SIGMA-Aldrich Company Ltd. (St. Louis, MO, USA). The final concentrations of DMSO were less than 0.1% where the cell viability was not affected. RAW264.7 cells and 3T3-L1 cells were obtained from ATCC (Dainippon Pharmaceutical, Osaka, Japan). DMEM containing 10 mM glucose (DMEM-L) medium and DMEM containing 25 mM glucose (DMEM-H) medium were obtained from SIGMA-Chemicals (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Gemini-Bio Products (Woodland, CA, USA). Lipopolysaccharide (LPS: *Escherichia coli* 0127 B8) was purchased from SIGMA-Chemicals (St. Louis, MO, USA). IL-6 Enzyme-Linked Immunosorbent Assay (ELISA) kits and Anti-mouse IL-6 neutralizing antibody were obtained from R&D systems (Minneapolis, MN, USA).

2.2. Cell culture

RAW264.7 cells were maintained in DMEM-L medium supplemented with 10% FBS, and gentamicin sulfate (20 mg/ml) at 37 °C under humidified 5% CO_2/95% air. 3T3-L1 adipocytes were differentiated while referring to the method described previously (Rubin et al., 1977). Briefly, preadipocytes were grown to confluence after which they were cultured for 3 days in DMEM-H, 10% FBS, and antibiotics (culture medium) further supplemented with 10 μg/ml insulin, 0.5 mM isobutylmethylxanthine and 0.25 μM dexametazone.

The cells had accumulated fat droplets after an additional 3 days in the culture medium with 5 μg/ml insulin followed by 3–6 days in culture medium. All stimulations were carried out in DMEM-H without any additions.

2.3. Preparation of RAW264.7-conditioned medium (CM)

RAW264.7 cells were cultured in DMEM-L with 10% FBS in 5% CO_2/95% humidified air at 37 °C. The cells were treated with or without K-111 (30 μM) and/or SP600125 (10 μM) and then were incubated for 18 h, followed by the addition of LPS (1 μg/ml). After 8 h, the medium was changed with or without K-111 (30 μM) and/or SP600125 (10 μM). After 18 or 36 h, the medium was collected and centrifuged at 300 ×*g* for 5 min. The supernatant was concentrated by Centoricon (MILLIPORE Corporation, Billerica, MA, USA), and then was sterilized by filtrating through a 0.22 μm filter, and used as RAW264.7-CM.

2.4. Cytokine production assay using ELISA

RAW264.7 cells suspended in DMEM-L with 10% FBS were placed in multi-well culture plates, and treated with K-111 (30 μM), rosiglitazone (30 μM) or SP600125 (10 μM) followed by the addition of LPS (1 μg/ml). The mixture was incubated at 37 °C for 8 h. The culture medium was then subjected to centrifugation at 300 ×*g* for 5 min, and the concentrations of the cytokines in the supernatant were measured using commercial ELISA kits according to the manufacturer's instructions.

2.5. Measurement of mRNA levels using quantitative real-time reverse transcriptase-polymerase chain reaction

IL-6 and TNFα mRNA expression was determined by quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was isolated from RAW264.7 cells or 3T3-L1 adipocytes with ISOGEN (Nippon gene, Tokyo, Japan) and 1 μg RNA was reverse transcribed. The amplification of each target cDNA was performed with TaqMan PCR reagent kits in the ABI PRISM 7700 sequence detection system according to the protocols provided by the manufacturer (PE Applied Biosystems, Foster City, CA). The primer/probe sets of IL-6 and TNFα were purchased from the manufacturer (PE Applied Biosystems, Foster City, CA), and then were used for the amplification step. IL-6 and TNFα mRNA expressions were calculated relative to 18S ribosomal RNA (rRNA).

2.6. Preparation of cytosol extracts

RAW264.7 cells suspended in DMEM-L with 10% FBS were placed in 6-well plates (2 ml/well), and incubated at 37 °C in the absence or presence of K-111 (30 μM). After 30 min, each well

was treated with LPS (1 μg/ml) for the times indicated in each figure. The cells were washed with ice-cold phosphate-buffered saline (PBS) and collected on ice. Cytosol extracts from these cells were isolated by NE-PER Nuclear and Cytoplasmic extraction reagents (PIERCE Biotechnology, Rockford, IL, USA), according to the manufacturer's instructions.

2.7. Western blot analysis

RAW264.7 cells suspended in DMEM-L with 10% FBS were placed in 6-well plates (2 ml/well), and incubated at 37 °C in the absence or presence of K-111 (30 μM). After 18 h, each well was treated with LPS (1 μg/ml) for the times indicated in each figure. The cells were washed with ice-cold PBS and lysed in radio-immunoprecipitation assay (RIPA) buffer (whole cell lysates).

Western blot analysis was conducted essentially as previously described using ECL reagent (Amersham Pharmacia Biotech, Piscataway, NJ). Phosphorylated extracellular signal-regulated kinase (ERK) 1/2 (p44/42 mitogen-activated protein kinase (MAPK)), p38MAPK, stress-activated protein kinase/c-Jun NH2-terminal kinase (SAPK/JNK) and nuclear factor kappa B (NF-κB) p65, or total ERK1/2, p38MAPK, SAPK/JNK and IκBα were detected using rabbit polyclonal antibodies from Cell Signaling Technology (Beverly, MA). Whole cell lysates were used for the assay of phosphorylated ERK1/2, p38MAPK and SAPK/JNK and cytosol extracts were used for the assay of IκBα and phosphorylated NF-κB p65.

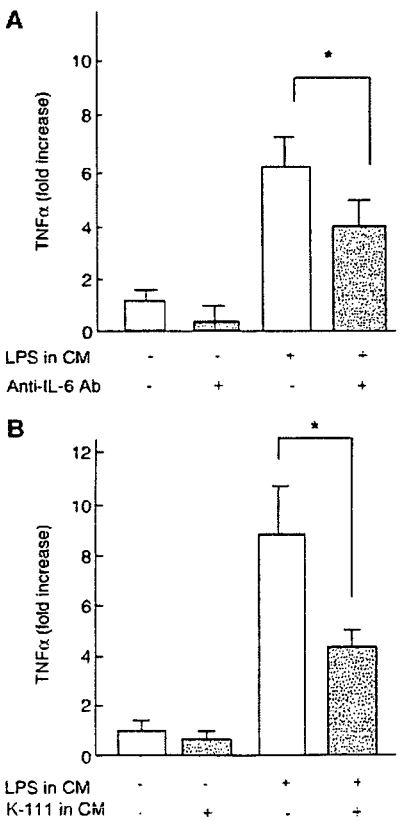


Fig. 1. Effect of K-111 on the potency of RAW264.7 cells of the conditioned medium for the TNFα mRNA expression in 3T3-L1 cells. A. 3T3-L1 adipocytes were treated with a conditioned medium of RAW264.7 cells, which were added until they reached 10%, in the absence or presence of IL-6-neutralizing antibodies for 48 h. B. 3T3-L1 adipocytes were treated with conditioned medium of RAW264.7 cells with or without K-111, which were added until they reached 10% for 48 h. Isolated total RNA was transcribed into cDNA, and then was analyzed by real-time RT-PCR using specific primers for TNFα. Levels of TNFα mRNA expression in each treatment are presented as a fold increase of that in untreated cells. *$P<0.05$ ($n=4$–5).

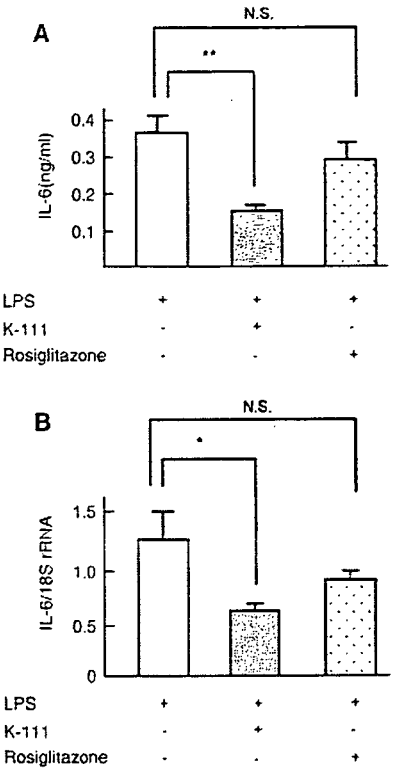


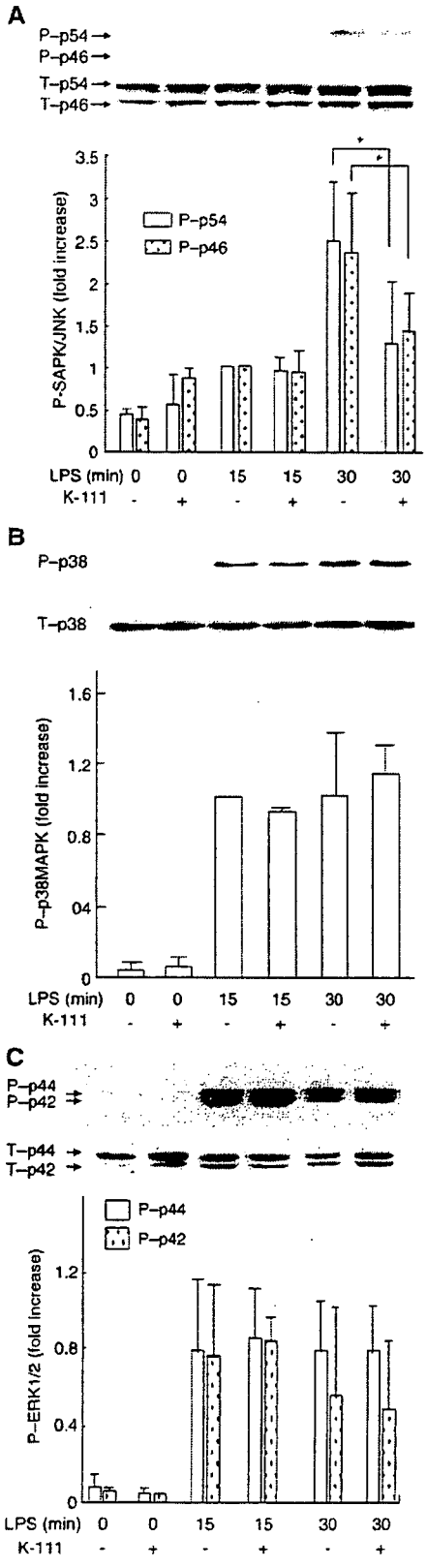
Fig. 2. Effect of K-111 on the secretion and gene expression of IL-6 in RAW264.7 cells. A. RAW264.7 cells were pretreated with or without 30 μM of K-111 or rosiglitazone for 18 h before the addition of LPS (1 μg/ml). The concentrations of IL-6 in the culture supernatants 8 h after stimulation by LPS were measured using ELISA. B. Total RNA as isolated 8 h after stimulation by LPS, and transcribed into cDNA. IL-6 mRNA level was analyzed using real-time RT-PCR. *$P<0.05$, **$P<0.01$ ($n=3$–5). N.S., not significant.

2.8. siRNA transfection

RAW264.7cells were transfected with 5 nM siRNA to IL-6 or All Star Negative Control siRNA (Qiagen, Germany) using Hiperfect transfection reagent (Qiagen) as described by the manufacturer. The treated cells were then subjected to the following experiments.

2.9. Statistical analysis

The results are shown as the means±S.D. Differences between various treatments were analyzed by unpaired Student's t tests with P values <0.05 considered to be significant.



3. Results

3.1. Effect of a specific PPARα agonist, K-111, on the TNFα expression in 3T3-L1 adipocytes through the action for RAW264.7 cells

In order to determine the effect of the PPARα activation of macrophages for the regulation of cytokine secretion from adipocytes through IL-6 secretion from macrophages, we studied the effect of a conditioned medium with RAW264.7, a macrophage cell line, on the TNFα expression in cultured adipocytes, 3T3-L1 cells. For this aim, 3T3-L1 adipocytes were incubated with or without 10% (v/v) LPS-stimulated RAW264.7-CM for 48 h. Real-time RT-PCR showed the expression of TNFα mRNA in 3T3-L1 adipocytes to be significantly induced with the conditioned medium of RAW264.7 cells (Fig. 1A). The mRNA level of TNFα was increased by 6-fold of the control. The increased level of TNFα mRNA was significantly inhibited in the presence of neutralizing antibody against IL-6 (1 μg/ml). These results indicated that IL-6, which is secreted from RAW264.7 cells, is a major inducer of the TNFα expression in 3T3-L1 adipocytes.

We next analyzed the effect of CM of RAW264.7 cells pretreated with a specific PPARα agonist, K-111 on the TNFα expression in 3T3-L1 adipocytes (Fig. 1B). Pretreatment of CM with K-111 reduced its induction activity for TNFα mRNA expression in 3T3-L1 cells. As a result, the PPARα activation was found to cause a decrease in the TNFα expression in 3T3-L1 adipocytes, which was induced by the LPS-stimulated RAW264.7-CM.

3.2. K-111 inhibits IL-6 transcription and secretion in LPS-stimulated RAW264.7 cells

Based on the obtained results, we next studied the effect of K-111 on the IL-6 transcription and secretion in the cultured macrophages, and compared our findings with those for rosiglitazone, a potent PPARγ agonist. The results of ELISA showed that K-111 (30 μM) significantly reduced the IL-6 production after stimulation with LPS (1 μg/ml) in RAW264.7 cells (Fig. 2A). On the other hand, rosiglitazone (30 μM) did not show a significant decrease in the secreted IL-6 level, and this finding was consistent with that of a previous report (Thieringer et al., 2000). Therefore, in order to determine whether the effect is caused at the transcription level of the IL-6 gene, the mRNA level was analyzed after the exposure of LPS (1 μg/ml) in the presence of K-111 using real-time RT-PCR. K-111 (30 μM) significantly reduced the IL-6 mRNA level (Fig. 2B). These

Fig. 3. Effect of K-111 on the MAPK activation in RAW264.7 cells. RAW264.7 cells were pretreated with or without 30 μM of K-111 for 18 h before the addition of LPS (1 μg/ml). Samples (each containing 7.5 μg of protein) were analyzed by Western blotting using antibodies against phospho(p)-SAPK/JNK or total(T)-SAPK/JNK (A), phospho(p)-p38 or total (T)-p38 (B), and phospho(p)-ERK1/2 (p44/42 MAPK) or total (T)-ERK1/2(p44/42 MAPK) (C), respectively. Each signal was scanned and calculated as a fold increase of the signal in the stimulation of LPS for 15 min. $^{*}P<0.05$ ($n=3$–4).

results show that PPARα activation inhibits IL-6 at the transcriptional and secretional levels in cultured macrophages.

3.3. K-111 suppresses the activation of SAPK/JNK in RAW264.7 cells

To elucidate the reason why the PPARα activation of macrophage suppresses the IL-6 secretion in cultured macrophage, we analyzed the effect of K-111 on the phosphorylation of ERK1/2, SAPK/JNK, and p38MAPK in RAW264.7 cells.

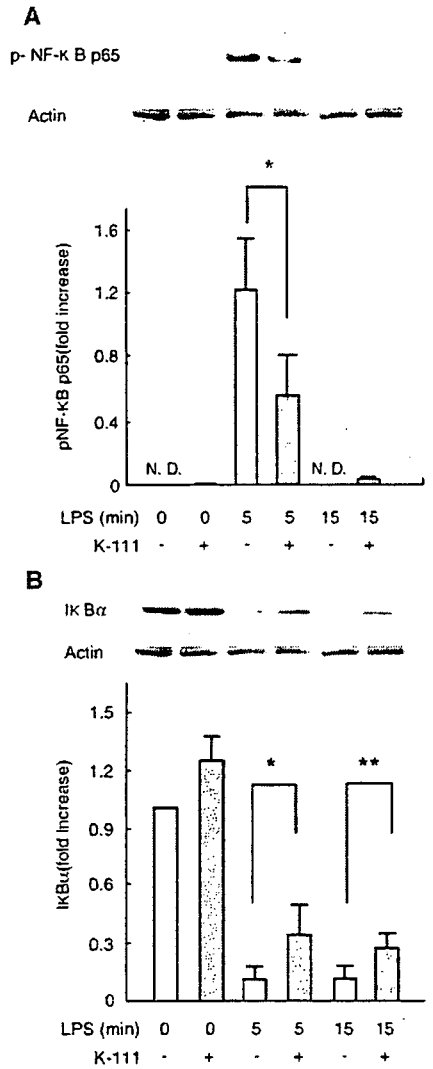


Fig. 4. The effect of K-111 on the LPS-induced NF-κB activation in RAW264.7 cells. RAW264.7 cells were preincubated at 37 °C for 18 h with or without K-111 (30 μM), and then were treated with 1 μg/ml of LPS for 0–15 min. Next, cytosol extracts (each containing 10 μg of protein) were prepared and analyzed by Western blotting using an antibody which specifically recognizes phospho(p)-NF-κB p65 (A). The cytosol extracts (each containing 10 μg of protein) were also analyzed by Western blotting using an antibody which specifically recognizes IκBα (B). Western blotting using an antibody against Actin was used as control. Each signal was scanned and calculated as a fold increase of the signal in the stimulation of LPS for 5 min (A) or for 0 min (B). $^{*}P<0.05$, $^{**}P<0.01$ ($n=3$). N.D., not detected.

These intracellular pathways have been shown to be involved in the transcription of inflammatory cytokines. The kinetics of MAPK activation stimulated with 1 μg/ml LPS in subconfluent RAW264.7 cells was examined using a Western blot analysis (Fig. 3). SAPK/JNK and p38 were activated by LPS, thus reaching their maximal activities within 30 min, and ERK1/2 was also potently activated at 30 min (maximum at 15 min). Pretreatment of the cell with 30 μM of K-111 for 18 h significantly inhibited the LPS-induced phosphorylation of SAPK/JNK (Fig. 3A). The phosphorylation of p38MAPK and ERK1/2 was not significantly inhibited (Fig. 3B and C). These results suggest that the inhibitory effect of K-111 on the activation of IL-6 secretion in RAW264.7 cells is possibly caused by the suppression of the SAPK/JNK pathway.

3.4. K-111 inhibits NF-κB activation in RAW264.7 cells

The IL-6 expression is known to be mediated by NF-κB activation followed by signals through the SAPK/JNK pathway in human monocytes (Tuyt et al., 1999). Therefore, the suppression of the SAPK/JNK pathway, and IL-6 mRNA and protein expression by K-111 suggests that the decrease in IL-6 mRNA is mediated by the deactivation of NF-κB in RAW264.7 cells. The effect of K-111 on LPS-induced activation of NF-κB was evaluated by Western blot analysis using the cytosol extracts from RAW264.7 cells. RAW264.7 cells were preincubated with 30 μM of K-111, and then with LPS (1 μg/ml) for 0–30 min. LPS significantly induced the phospho(p)-NF-κB subunits of p65 in cytosol within 5 min, and then the induction was inhibited in the presence of K-111 (Fig. 4A). We next analyzed the effect of K-111 on the LPS-induced IκBα degradation, since the NF-κB activation is regulated by the steady-state levels of its inhibitor IκBα protein. RAW264.7 cells were pretreated with K-111 (30 μM) for 18 h prior to stimulation with LPS (1 μg/ml). Thereafter, the cytoplasmic IκBα levels were analyzed by Western blot analysis. Stimulation with LPS decreased the IκBα signal intensity within 15 min (Fig. 4B), and re-appeared at 30 min (data not shown). The IκBα degradation level decreased in the presence of K-111 for 15 min. The effect of K-111 on IL-6 transcription in macrophage is thus possibly mediated by the inhibition of NF-κB activation.

3.5. Blocking of SAPK/JNK pathway abolishes the effect of K-111 on the IL-6 secretion of RAW264.7 and TNFα expression of 3T3-L1 adipocytes, respectively

In order to know the role of IL-6 secretion mediated by SAPK/JNK pathway in the action of K-111 in activated macrophages on the following inhibition of TNFα expression in adipocytes, we analyzed the effect of blocking of SAPK/JNK pathway on the inhibition of K-111 for the IL-6 production and TNFα expression in RAW264.7 cells and 3T3-L1 adipocytes, respectively (Fig. 5). For this purpose, RAW264.7 cells were pretreated with 10 μM SP600125, a specific SAPK/JNK inhibitor (Bennett et al., 2001; Han et al., 2001), before the K-111 treatment. SP600125 significantly decreased the IL-6 production after stimulation with LPS (1 μg/ml). The treatment

of K-111 in the pretreated RAW264.7 cells did not show any significant change in the level of IL-6 production in SP600125-treated RAW264.7 cells (Fig. 5A). We then analyzed the effects of CM of LPS-stimulated RAW264.7 cells pretreated by SP600125 on the TNFα expression in 3T3-L1 adipocytes. The pretreatment of CM with SP600125 significantly reduced the LPS-induced level of the TNFα mRNA expression in 3T3-L1 adipocytes. The treatment of K-111 on the SP600125 pretreatment of RAW264.7 cells did not show a significant change in the inhibitory effect on the TNFα expression in 3T3-L1 adipocytes (Fig. 5B). Furthermore, the

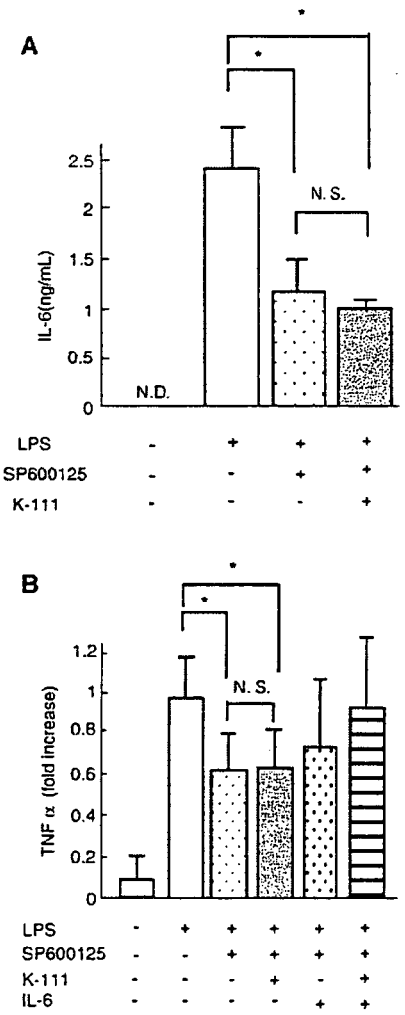


Fig. 5. Effect of blocking of SAPK/JNK pathway on the K-111 induced inhibition for IL-6 secretion in RAW264.7 cells and TNFα expression in 3T3-L1 adipocytes. A. RAW264.7 cells were pretreated with or without 10 μM SP600125 in the presence or absence of 30 μM K-111 for 18 h before the addition of LPS (1 μg/ml). The concentrations of IL-6 in the culture supernatants 24 h after stimulation by LPS were measured using ELISA. *$P<0.05$. N.S., not significant ($n=3$). N.D., not detected. B. 3T3-L1 adipocytes were treated with CM of RAW264.7 cells at 10% of medium for 48 h. Isolated total RNA was transcribed into cDNA, and analyzed by real-time RT-PCR using specific primers for TNFα. Levels of TNFα mRNA expression in each treatment are presented as a fold increase of the signal of the LPS-stimulated RAW264.7-CM-treated cells. *$P<0.05$. N.S., not significant ($n=5$).

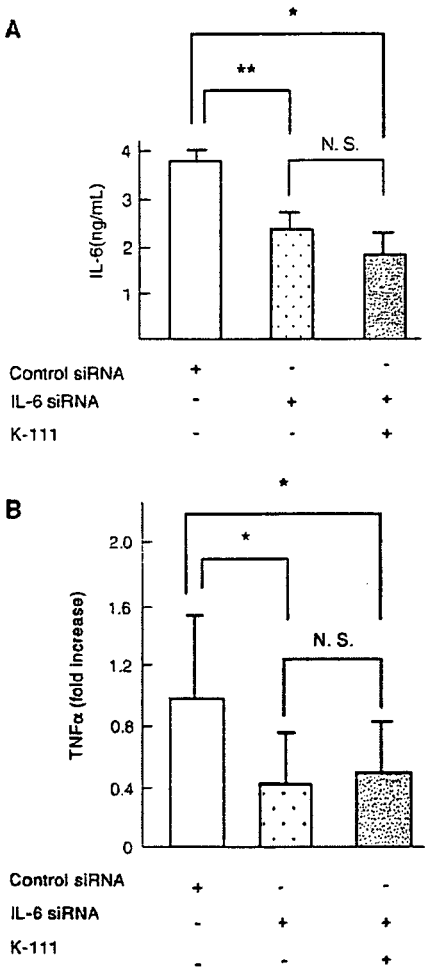


Fig. 6. The effect of the IL-6 gene knockdown using the specific siRNA in RAW264.7 cells on the inhibitory action of K-111 for the TNFα expression in 3T3-L1 adipocytes. RAW264.7 cells were transfected with siRNA to IL-6 or control siRNA. Transfected cells were incubated with 30 μM K-111 for 18 h before the addition of LPS (1 μg/ml). A. The IL-6 concentration in the culture supernatants incubated for 36 h after stimulation with LPS for 8 h. *$P<0.05$, **$P<0.01$. N.S., not significant ($n=3$). B. TNFα mRNA levels in 3T3-L1 adipocytes incubated with CM of RAW264.7 cells at 10% of medium for 48 h. Total RNA was transcribed into cDNA, and analyzed by real-time RT-PCR using specific primers for TNFα. Levels of TNFα mRNA expression are presented as fold increases of that in 3T3-L1 cells incubated with the CM of RAW264.7 cells transfected with control siRNA. *$P<0.05$. N.S., not significant ($n=6$–10).

decreased mRNA level of TNFα recovered after the addition of recombinant IL-6 in the medium of 3T3-L1 adipocytes.

3.6. The knockdown of IL-6 gene in RAW264.7 cells abolishes the effect of K-111 on the TNFα expression in 3T3-L1 adipocytes

We finally knockdowned IL-6 gene using its specific siRNA in order to clarify the role of IL-6 gene expression in RAW264.7 cells in the induction of TNFα expression in 3T3-L1 adipocytes. The transfection with IL-6 siRNA significantly reduced the levels of LPS-induced IL-6 secretion compared to that with control siRNA. Treatment of K-111 had no significant effect on LPS-induced IL-6 production in the IL-6 knockdown cells

(Fig. 6A). We therefore analyzed the effect of CM of LPS-stimulated RAW264.7 cells transfected with IL-6 siRNA on the TNFα expression in 3T3-L1 adipocytes. The TNFα expression induced by CM of IL-6 knockdown cells was significantly decreased compared to the expression level by the CM of control cells (Fig. 6B). K-111 did not change the TNFα expression level induced by the CM of IL-6 knockdown RAW264.7 cells. These results indicate that the effect of K-111 on the inhibition of TNFα expression in 3T3-L1 adipocytes is mediated by the decreased in IL-6 gene expression in RAW264.7 cells.

4. Discussion

Our study using the activated (LPS-stimulated) macrophage cell line, RAW264.7, showed the conditioned medium of activated macrophages to increase the level of TNFα mRNA in 3T3-L1 adipocytes, and this effect was largely dependent on the IL-6 secretion from the activated macrophages. The preincubation of LPS-stimulated RAW264.7-CM with K-111 suppressed the TNFα expression in 3T3-L1 adipocytes induced by the CM of activated macrophage. K-111, a potent PPARα agonist, inhibited the transcription and subsequent production of IL-6. This effect was caused by the suppression of the activation of SAPK/JNK and the NF-κB translocation in the cells. These effects were evident in comparison to those of rosiglitazone, a selective PPARγ agonist. The blocking of IL-6 production by the inhibition of the SAPK/JNK pathway or siRNA specific for the IL-6 gene abolished the inhibitory effect of K-111 on the TNFα expression in 3T3-L1 adipocytes. These results using cultured macrophages and adipocytes indicate that K-111 suppresses the IL-6 secretion in macrophages through both the SAPK/JNK and NF-κB pathways, thereby reducing the TNFα secretion in 3T3-L1 adipocytes. The PPARα agonists are thus suggested to be able to modulate the interaction of macrophages and adipocytes in visceral fat, which have been proposed to cause systemic insulin resistance through the activation of cytokines derived from visceral fat.

In macrophages, the biosynthesis of cytokines is regulated at multiple levels, thus involving a multitude of signal transduction pathways. LPS binds to LPS-binding protein (LBP), which activates the MAPKs (ERK1/2, p38 and SAPK/JNK) signals, and then the transcription factors, NF-κB (Sancéau et al., 1995). To characterize the mechanism of the inhibitory effect of PPARα activation on IL-6 production, we analyzed the effect of K-111 on the activation of MAPKs by LPS. K-111 inhibited the LPS-induced JNK activation, but not the activation of ERK1/2 and p38. The activation of PPARα also suppressed the phosphorylation of NF-κB subunits of p65, and the degradation of IκBα was stimulated by LPS. These results indicate that PPARα activation probably suppresses the activation of NF-κB by the inhibition of the degradation of IκBα protein. The PPARα ligands were demonstrated to inhibit the IL-1β-induced IL-6 secretion, thereby inducing the IκBα mRNA and protein expression in smooth muscle cells and hepatocytes (Delerive et al., 1999, 2000). IκBα protein induction mainly occurs in the nucleus, which may reduce the NF-κB-binding activity (Delerive et al., 2000). In this study, the PPARα activation was thought to suppress IL-6 in macrophages through a similar mechanism. In agreement with our observations, fibrates are thus not considered to affect the LPS-induced IL-6 transcription in PPARα-deficient mice (Delerive et al., 1999). PPARα-deficient splenocytes produced, in response to LPS stimulation, two to three times more IL-6 than the splenocytes from wild-type mice (Poynter and Daynes, 1998). Furthermore, our results showed the effects of PPARα activation to be obvious in comparison to those of PPARγ agonist, rosiglitazone. K-111 was recently characterized by Meyer et al. (1999) as a potent PPARα activator, without the activation of PPARγ. An anti-diabetic potency with insulin sensitizing and lipid-lowering activities was demonstrated in rodent models of type 2 diabetes (Pill and Kuhnle, 1999). Furthermore, K-111 reduced hyperinsulinaemia without changing the blood glucose levels in obese rhesus monkeys (Schafer et al., 2004). A previous study also showed that PPARγ agonists do not obviously suppress IL-6 in macrophage (Thieringer et al., 2000). As a result, PPARα may play a role in the inflammatory response in fat tissues. K-111, therefore, seems to also show a significant suppression of the inflammatory effect induced by LPS in macrophages. Many cytokines and other factors are produced and released by the fat tissue, and this observation has recently been recognized as the chronic inflammatory state of visceral obesity (Wellen and Hotamisligil, 2003). The cells implanted into visceral, and not subcutaneous, areas express TNFα mRNA, and the bioactive peptide secreted causes decreased insulin sensitivity in muscle (Shibasaki et al., 2002).

In summary, we showed that the activation of PPARα inhibited the expression of TNFα in 3T3-L1 adipocytes by suppressing IL-6 production in macrophages using a culture system. Our findings therefore suggest that PPARα activation may improve insulin resistance through the inhibitory effect on the interaction between macrophages and adipocytes in visceral fat tissue in humans. These results obtained using cultured cells need to be further analyzed in other systems to elucidate the significance of PPARα regulation in fat tissue.

References

Adams, M., Montague, C.T., Prins, J.B., Holder, J.C., Smith, S.A., Sanders, L., Digby, J.E., Sewter, C.P., Lazar, M.A., Chatterjee, V.K., O'Rahilly, S., 1997. Activators of peroxisome proliferators activated receptor gamma have depot specific effects on human preadipocyte differentiation. J. Clin. Invest. 100, 3149–3153.

Bennett, B.L., Sasaki, D.T., Murray, B.W., O'Leary, E.C., Sakata, S.T., Xu, W., Leisten, J.C., Motiwala, A., Pierce, S., Satoh, Y., Bhagwat, S.S., Manning, A.M., Anderson, D.W., 2001. SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. Proc. Natl. Acad. Sci. U. S. A. 98, 13681–13686.

Delerive, P., De Bosscher, K., Besnard, S., Vanden Berghe, W., Peters, J.M., Gonzalez, F.J., Fruchart, J.C., Tedgui, A., Haegeman, G., Staels, B., 1999. PPARα negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-κB and AP-1. J. Biol. Chem. 274, 32048–32054.

Delerive, P., Gervois, P., Fruchart, J.C., Staels, B., 2000. Induction of IkBα expression as a mechanism contributing to the anti-inflammatory activities of PPARα activators. J. Biol. Chem. 275, 36703–36707.

Expert panel on detection, evaluation and treatment of high blood cholesterol in adults, 2001. Executive Summary of the Third Report of the National

Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 285, 2486–2497.

Fernandez-Real, J.M., Vayreda, M., Richart, C., Gutierrez, C., Broch, M., Vendrell, J., Ricart, W., 2001. Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women. J. Clin. Endocrinol. Metab. 86, 1154–1159.

Ginsberg, H.N., 2000. Insulin resistance and cardiovascular disease. J. Clin. Invest. 106, 453–458.

Han, Z., Boyle, D.L., Chang, L., Bennett, B., Karin, M., Yang, L., Manning, A.M., Firestein, G.S., 2001. c-Jun N-terminal kinase is required for metalloproteinase expression and joint destruction in inflammatory arthritis. J. Clin. Invest. 108, 73–81.

Hayden, J.M., Reaven, P.D., 2000. Cardiovascular disease in diabetes mellitus type 2: a potential role for novel cardiovascular risk factors. Curr. Opin. Lipidol. 11, 519–528.

Hirose, H., Kawai, T., Yamamoto, Y., Taniyama, M., Tomita, M., Matsubara, K., Okazaki, Y., Ishii, T., Oguma, Y., Takei, I., Saruta, T., 2002. Effects of pioglitazone on metabolic parameters, body fat distribution, and serum adiponectin levels in Japanese male patients with type 2 diabetes. Metabolism 51, 314–317.

Kitagawa, Y., Bujo, H., Takahashi, K., Shibasaki, M., Ishikawa, K., Yagui, K., Hashimoto, N., Noda, K., Nakamura, T., Yano, S., Saito, Y., 2004. Impaired glucose tolerance is accompanied by decreased insulin sensitivity in tissues of mice implanted with cells that overexpress resistin. Diabetologia 47, 1847–1853.

Meyer, K., Volkl, A., Endele, R., Kuhnle, H.F., Pill, J., 1999. Species differences in induction of hepatic enzymes by BM 17.0744, an activator of peroxisome proliferator-activated receptor alpha (PPARα). Arch. Toxicol. 73, 440–450.

Miyazawa-Hoshimoto, S., Takahashi, K., Bujo, H., Hashimoto, N., Yagui, K., Saito, Y., 2005. Roles of degree of fat deposition and its localization on VEGF expression in adipocyte. Am. J. Physiol., Endocrinol Metabol. 288, E1128–E1136.

Miyazaki, Y., Glass, L., Triplitt, C., Matsuda, M., Cusi, K., Mahankali, A., Mahankali, S., Mandarino, L.J., DeFronzo, R.A., 2001. Effect of rosiglitazone on glucose and non-esterified fatty acid metabolism in Type II diabetic patients. Diabetologia 44, 2210–2219.

Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D.R., Miles, J.M., Yudkin, J.S., Klein, S., Coppack, S.W., 1997. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J. Clin. Endocrinol. Metab. 82, 4196–4200.

Mohamed-Ali, V., Pinkney, J.H., Coppack, S.W., 1998. Adipose tissue as an endocrine and paracrine organ. Int. J. Obes. 22, 1145–1158.

Pill, J., Kuhnle, H.F., 1999. BM 17.0744: a structurally new antidiabetic compound with insulin-sensitizing and lipid-lowering activity. Metabolism 48, 34–40.

Poynter, M.E., Daynes, R.A., 1998. Peroxisome proliferator-activated receptor α activation modulates cellular redox status, represses nuclear factor-κB signalling, and reduces inflammatory cytokine production in aging. J. Biol. Chem. 273, 32833–32841.

Report of a WHO Consultation. Definition, diagnosis and classification of diabetes mellitus and its complications, 1999. Part 1 Diagnosis and classification of diabetes mellitus. World Health Organization, Department of Noncommunicable Disease Surveillance, Geneva.

Reaven, G.M., 1995. Pathophysiology of insulin resistance in human disease. Physiol. Rev. 75, 473–486.

Rosen, E.D., Spiegelman, B.M., 2001. PPAR: a nuclear regulator of metabolism, differentiation, and cell growth. J. Biol. Chem. 276, 37731–37734.

Rubin, C.S., Lai, E., Rosen, O.M., 1977. Acquisition of increased hormone sensitivity during in vitro adipocyte development. J. Biol. Chem. 252, 3554–3557.

Sancéau, J., Kaisho, T., Hirano, T., Wietzerbin, J.J., Kaisho, T., 1995. Triggering of the human interleukin-6 gene by interferon-γ and tumor necrosis factor-α in monocytic cells involves cooperation between interferon regulatory factor-1, NFκB, and Sp1 transcription factors. J. Biol. Chem. 270, 27920–27931.

Schafer, S.A., Hansen, B.C., Volkl, A., Fahimi, H.D., Pill, J., 2004. Biochemical and morphological effects of K-111, a peroxisome proliferator-activated receptor (PPAR)α activator, in non-human primates. Biochem. Pharmacol. 68, 239–251.

Sheu, M.Y., Fowler, A.J., Kao, J., Schmuth, M., Schoonjans, K., Auwerx, J., Fluhr, J.W., Man, M.Q., Elias, P.M., Feingold, K.R., 2002. Topical peroxisome proliferators activated receptor-alpha activators reduce inflammation in irritant and allergic contact dermatitis models. J. Invest. Dermatol. 118, 94–101.

Shibasaki, M., Takahashi, K., Itou, T., Miyazawa, S., Ito, M., Kobayashi, J., Bujo, H., Saito, Y., 2002. Alterations of insulin sensitivity by the implantation of 3T3-L1 cells in nude mice. A role for TNF-alpha? Diabetologia 45, 518–526.

Straub, R.H., Hense, H.W., Andus, T., Scholmerich, J., Riegger, G.A., Schunkert, H., 2000. Hormone replacement therapy and interrelation between serum interleukin-6 and body mass index in postmenopausal women: a population-based study. J. Clin. Endocrinol. Metab. 85, 1340–1344.

Thieringer, R., Fenyk-Melody, J.E., Le Grand, C.B., Shelton, B.A., Detmers, P.A., Somers, E.P., Carbin, L., Moller, D.E., Wright, S.D., Berger, J., 2000. Activation of peroxisome proliferator-activated receptor gamma does not inhibit IL-6 or TNF-alpha responses of macrophages to lipopolysaccharide in vitro or in vivo. J. Immunol. 164, 1046–1054.

Tuyt, L.M., Dokter, W.H., Birkenkamp, K., Koopmans, S.B., Lummen, C., Kruijer, W., Vellenga, E., 1999. Extracellular-regulated kinase 1/2, Jun N-terminal kinase, and c-Jun are involved in NF-kappa B-dependent IL-6 expression in human monocytes. J. Immunol. 162, 4893–4902.

Vozarova, B., Weyer, C., Hanson, K., Tataranni, P.A., Bogardus, C., Pratley, R.E., 2001. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. Obes. Res. 9, 414–417.

Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., Ferrante Jr., A.W., 2003. Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. 112, 1796–1808.

Wellen, K.E., Hotamisligil, G.S., 2003. Obesity-induced inflammatory changes in adipose tissue. J. Clin. Invest. 112, 1785–1788.

Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., Tartaglia, L.A., Chen, H., 2003. Chronic inflammation in fat plays a crucial role in the development of obesity related insulin resistance. J. Clin. Invest. 112, 1821–1830.

Received: Sep 17, 2007
Accepted: Nov 5, 2007
Published online: Dec 25, 2007

Original Article

Association of Personality (NEO-Five Factor Inventory) with Eating Behaviors and Physical Activity Levels in Obese Subjects in the Saku Control Obesity Program (SCOP)

Yumi Ohmori [1], Nozomu Suzuki [1], Akemi Morita [1], Naomi Aiba [1], Motohiko Miyachi [1], Shaw Watanabe [1], Yuriko Kikuchi [2], Mitsuru Kimira [3] for SCOP Group

1) National Institute of Health and Nutrition
2) School of Medicine, Keio University
3) Tokyo University of Agriculture

Abstract

BACKGROUND: Obesity is one of the most common risks for lifestyle-related diseases, but the personality of individuals in relation to obesity has not been well studied. We investigated the association of personality traits with physical activity levels and eating behaviors in obese subjects.

METHOD: The subjects were 116 males and 119 females in the Saku Control Obesity Program SCOP study. The influence of personality on obesity was analyzed using a questionnaire from the NEO-FFI. We analyzed the association of physical activity level (measured with an accelerometer) and eating behavior (assessed by a questionnaire) among the three classes (low, average, high) of scores within five personality domains.

RESULTS: Scores in the Neuroticism and Agreeableness domains of females were significantly higher than those of males. There were significant differences among the three classes of Neuroticism and Agreeableness with regard to physical activity levels. Eating behavior was associated with the Neuroticism and Openness domains. The scales of bad eating behavior related to obesity were positively correlated with scores in the Neuroticism domain in both males and females. In males the scale of all categories of eating behavior increased as scores in the Openness domain rose; in females the scale of "perception of constitution and weight" decreased as Openness scores rose.

CONCLUSION: Personality determined by NEO-FFI was related to physical activity level and eating behavior. In particular, the Neuroticism domain had great effects on these parameters.

KEY WORDS: obesity, personality, NEO-FFI, eating behavior, physical activities

Introduction

Obesity appears to be closely correlated with personality, and recent studies have shown that obese people are at increased risk of depression and Neuroticism.[1,2] A study using the NEO-Five Factor Inventory (NEO-FFI) reported that obese females had more neurotic tendencies compared with females who were not obese.[3] In addition, Yoshida et al reported that the effectiveness of treatment for obesity was influenced by differences in personality.[4]

To further investigate the effects of personality traits on obesity, we performed a psychological behavior analysis (NEO-FFI) of obese participants in a weight-loss intervention program. The present NEO-FFI [5] is a shortened version of the Revised NEO Personality Inventory (NEO PI-R). Yoshimura et al translated this version into Japanese, and its reliability and validity have been confirmed.[6] NEO-FFI is a questionnaire that measures personality traits within five domains, and it has been most widely used in the United States. The NEO-FFI has proven useful for the study of health consciousness and behavior in both young and elderly subjects.[7,8]

Although a wide array of research has shown a positive effect of weight loss in the prevention of lifestyle-related diseases, methods to achieve changes in physical activity and eating behaviors in obese individuals have not been well developed. In addition, no studies have examined how personality traits are related to the process of losing weight in obesity education programs. Therefore, we conducted a multifactorial study of the physical activity levels and eating behaviors among obese subjects using the NEO-FFI questionnaire. We also tried to clarify whether individual personality assessment can serve as a valuable tool in such individualized education programs.

Shaw Watanabe, M.D. / National Institute of Health and Nutrition
1-23-1 Toyama, Shinjuku, Tokyo 162-8636, Japan
Tel: +81-3-3203-5721 / Fax: +81-3-3203-8662 / E-mail: watashaw@nih.go.jp

Method

The subjects and methods of this study were described in detail elsewhere in this supplement.[9] Subjects were 235 obese people (116 males and 119 females; 40–64 years) with BMI > 28.3 kg/m² at their last medical check-up at the Saku Central Hospital. The participants gave written informed consent prior to being enrolled in the SCOP study.

The NEO-FFI consists of 60 questions, 12 for each of five personality domains. For each question, subjects express agreement or disagreement on a five-point Likert scale: (1) strongly disagree, (2) disagree, (3) neutral, (4) agree, (5) strongly agree. The NEO-FFI is used to measure the five major domains of individual personalities (Neuroticism, Extroversion, Openness, Agreeableness, and Conscientiousness), which allow for a comprehensive assessment of normal adult personality. Raw scores of Neuroticism, Extroversion, Openness, Agreeableness, and Conscientiousness were converted into T-scores, and each domain was grouped into three classes: high (T = 56 and higher), average (T = 45-55), and low (T = 44 and lower).[10] T-scores have a mean of 50 and a standard deviation of 10 and allow for comparison of individuals across the population.

Physical activity levels were measured using an accelerometer (Suzuken, Nagoya, Japan); the details of how these levels were assessed is described elsewhere.[11] The device can monitor the number of steps as well as exercise intensity with the acceleration sensor, allowing calculation of the caloric expenditure through physical activity. Each subject received the device 2 weeks before the baseline health check-up. Participants were unable to view the data so that they would not consequently alter their normal routines of physical activity.

Participants'eating behavior was analyzed by a questionnaire in the Manual of Obesity 2006 written by the Japan Society for the Study of Obesity (see Appendix 1).[12,13] The questionnaire's 55 statements are based on the statements given by obese people in a clinical survey, and subjects were asked to agree or disagree on a four-point Likert scale: (1) disagree, (2) sometimes, (3) having a trend, (4) agree. The questionnaire is assessed by categorizing 51 items into the following eight categories: (1) perception of constitution and weight, (2) motivation for eating, (3) unhealthful eating, (4) feeling of fullness and hunger, (5) bad eating habits, (6) contents of diet, (7) unsteady eating pattern, and (8) total of all of them. One is a dummy question. The higher score in this questionnaire indicated worse in eating behavior. Based on each participant's answers; his or her eating behaviors were plotted along these eight axes and used for further analysis. Because we slightly modified the eating behavior questionnaire, the validity of the eight categories was analyzed using principal component analysis.

The associations between personality, physical activity levels, and eating behaviors were analyzed using SPSS® version 14.0 (SPSS Inc., Tokyo). Associations among the mean physical activity levels, eating behavior categories, and NEO-FFI classes were analyzed by ANOVA, Bonferroni test, and Games-Howell multiple comparison. Analysis was administrated according to each subject's sex because selecting the different question from 55 question to assess each category by sex. The database was processed using Excel® 2003 (Microsoft, Redmond, WA, USA) and converted to SPSS.

Results

The mean and standard deviation of NEO-FFI raw scores of the subjects and the distribution of *T*-scores among the low, average, and high classes for each personality domain are shown in ***Table 1*** and ***Figure 1***. The raw scores for both males and females coincided well, except for significantly higher scores in females in the domains of Neuroticism and Agreeableness. Within each sex, there was no significant difference in the distribution of *T*-scores across all domains. Correlation coefficients among the raw scores of the NEO-FFI are shown in ***Table 2***. Significant negative associations were found between Neuroticism and Extroversion and between Neuroticism and Agreeableness in males. In females, however, significantly negative associations existed between Neuroticism and Extroversion, Neuroticism and Agreeableness, and Neuroticism and Conscientiousness, but significantly positive associations were found between Extroversion and Agreeableness, Extroversion and Conscientiousness, and Agreeableness and Conscientiousness.

Table 1 ***The raw score of NEO-Five Factor Inventory***

	male (n = 116)	female (n = 119)	total (n = 235)
Neuroticism	22.8 ± 5.6	25.5 ± 6.8 *	24.2 ± 6.4
Extraversion	25.3 ± 5.6	26.7 ± 6.1	26.0 ± 5.9
Openness	28.2 ± 5.1	28.6 ± 4.5	28.4 ± 4.8
Agreeableness	29.7 ± 4.4	32.2 ± 4.3 *	31.0 ± 4.5
Conscientiousness	28.1 ± 5.2	28.5 ± 5.9	28.3 ± 5.5

Value present the mean ±SD
* Significantly diffelent between sex (P<0.05)

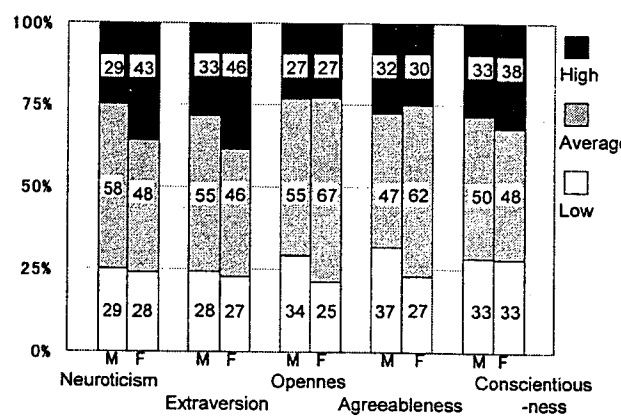


Fig. 1. The distribution of *T*-scores among the low class (*T* = 44 and lower), average class (*T* = 45–55), and high class (*T* = 56 and higher) in each personality domain of the NEO-FFI by sex. The values indicate the number of subjects (males, M, *n* = 116; females, F, *n* = 119)

· Relationship between NEO-FFI Scores and Physical Activity

Although the absolute number of daily step counts was greater in females (8015 ± 3126) than in males (7601 ± 3300), there was no significant difference between these values. METs·h (exercise intensity × time) was similar in males and females: 3.0 ± 1.4 and 3.1 ± 1.3, respectively.

Tables 3 and 4 lists the number of steps and physical activity levels according to *T*-score class among the NEO-FFI domains. In females, daily step counts and METs·h were low in those with low Neuroticism scores, and physical activity was significantly higher in the average class than in the low class of Neuroticism.

Table 2 Correlation coefficients in the scales of the five domain of NEO-Five Factor Inventory

	male : n = 116				female : n = 119			
	Extraversion	Openness	Agreeableness	Conscientiousness	Extraversion	Openness	Agreeableness	Conscientiousness
Neuroticism	-0.30 *	0.08	-0.23 *	-0.12	-0.43 *	0.01	-0.24 *	-0.32 *
Extraversion		0.06	0.11	0.18		-0.02	0.32 *	0.47 *
Openness			0.18	0.16			0.15	0.01
Agreeableness				-0.15				0.25 *

* $p<0.05$

Table 3 The association between the daily step counts and NEO-Five Factor Inventory.class in each domain

		Low class			Average class			High class	
		mean ± SD	(n)		mean ± SD	(n)		mean ± SD	(n)
Neuroticism	male	7178 ± 3284	(26)	male	7479 ± 3424	(57)	male	8242 ± 3069	(28)
	female	6741 ± 2422	(28)	female	8692 ± 3467	(48)	female	8090 ± 2937	(43) * a
Extraversion	male	7945 ± 2118	(27)	male	7512 ± 3848	(52)	male	7455 ± 3222	(32)
	female	7435 ± 3036	(27)	female	7918 ± 2980	(46)	female	8454 ± 3319	(46)
Openness	male	7926 ± 3301	(34)	male	7254 ± 2967	(51)	male	7856 ± 3932	(26)
	female	8303 ± 3371	(25)	female	7789 ± 2632	(67)	female	8310 ± 3999	(27)
Agreeableness	male	8659 ± 3950	(34)	male	6703 ± 2542	(46)	male	7773 ± 3244	(31) * a
	female	8825 ± 3366	(27)	female	7898 ± 2830	(62)	female	7529 ± 3448	(30)
Conscientiousness	male	7428 ± 3180	(32)	male	7364 ± 2624	(50)	male	8200 ± 4361	(29)
	female	7102 ± 2696	(33)	female	8055 ± 3080	(48)	female	8696 ± 3425	(38)

* Significant between groups by ANOVA ($P<0.05$)
a : Significantly different between Low class and Average class ($P<0.05$)
Low class : *T*-scores = 44 and lower
Average class : *T*-scores = 45 - 53
High class : *T*-scores = 56 and higher

Table 4 The association between METs·h and NEO-Five Factor Inventory class in each domain

		Low class			Average class			High class	
		mean ± SD	(n)		mean ± SD	(n)		mean ± SD	(n)
Neuroticism	male	2.8 ± 1.3	(26)	male	3.0 ± 1.5	(57)	male	3.3 ± 1.3	(28)
	female	2.6 ± 1.0	(28)	female	3.4 ± 1.5	(48)	female	3.2 ± 1.2	(43) * a
Extraversion	male	3.2 ± 0.9	(27)	male	3.0 ± 1.7	(52)	male	3.0 ± 1.3	(32)
	female	2.9 ± 1.3	(27)	female	3.1 ± 1.3	(46)	female	3.3 ± 1.4	(46)
Openness	male	3.1 ± 1.4	(34)	male	2.9 ± 1.3	(51)	male	3.1 ± 1.7	(26)
	female	3.2 ± 1.5	(25)	female	3.0 ± 1.1	(67)	female	3.3 ± 1.7	(27)
Agreeableness	male	3.5 ± 1.8	(34)	male	2.6 ± 1.1	(46)	male	3.1 ± 1.3	(31) * a
	female	3.5 ± 1.5	(27)	female	3.1 ± 1.2	(62)	female	2.9 ± 1.5	(30)
Conscientiousness	male	3.0 ± 1.4	(32)	male	2.9 ± 1.2	(50)	male	3.3 ± 1.9	(29)
	female	2.8 ± 1.1	(34)	female	3.2 ± 1.4	(47)	female	3.4 ± 1.5	(38)

* Significant between groups by ANOVA ($P<0.05$)
a : Significantly different between Low class and Average class ($P<0.05$)
Low class : *T*-scores = 44 and lower
Average class : *T*-scores = 45 - 53
High class : *T*-scores = 56 and higher

In males, there were no significant differences in daily step counts and METs·h among the Neuroticism classes. With regard to Agreeableness scores, in males both physical activity measures were lower in the average class than in the low and high classes. In females there was an increase in daily step counts and METs·h as Conscientiousness scores rose, but no significant difference existed among the classes according to ANOVA.

· Association between Eating Behavior and NEO-FFI Scores

The results of the principal component analysis with a varimax rotation for eating behavior are shown by sex in *Table 5*. According to the principal component analysis, 16 factors had eigenvalues > 1.0. For each sex, we chose the five highest factors, where the cumulative percentage for attribution was no less than 40%. In males, these factors were: (1) eat between meals; (2) fast eating·gluttony; (3) uncertainty of hunger; (4) promiscuous eating habits·dining out; and (5) supper conscious. In females the top factors were: (1) comfort eating·Western food; (2) uncertainty of hunger; (3) fast eating; (4) dining out; and (5) promiscuous eating habits. We examined the relationship between principal component scores of these factors and the raw scores in the five personality domains; although some associations were significant, the coefficients were all less than 0.265 (data not shown).

Stronger correlations were found between the eight eating behavior categories within the Manual of Obesity 2006

Table 5-1 Rotated factor loading based on rank correlations of eating behaiver

Male	Factor 1 Eat between meals	2 Fast eating· Gluttony	3 Uncertainty of hunger	4 Promiscuous eating habits· Dining out	5 Supper-conscious
I often eat between meals.	0.817	0.073	0.019	0.142	0.025
I often eat snacks.	0.778	0.061	-0.013	-0.016	0.073
I tend to eat anything when I have nothing to do.	0.671	0.115	0.181	0.089	-0.044
I often eat sweet rolls.	0.585	-0.082	0.048	0.064	0.089
I always keep food around.	0.550	0.124	0.158	0.311	0.030
I often have a midnight snack.	0.543	0.043	-0.021	0.247	0.064
I don't have a sense of hunger and fullness.	0.403	0.214	0.323	0.195	0.106
I eat a meal fast.	0.094	0.825	-0.069	0.107	-0.059
I don't chew well.	0.051	0.811	0.119	0.105	0.074
I eat as putting food into my mouse one after another.	0.043	0.759	0.236	0.010	0.042
I stuff food into my month.	-0.019	0.632	0.140	0.125	0.058
I'm told I eat a lot.	0.390	0.515	0.241	0.024	0.129
I don't satisfied unless I eat my fill.	0.340	0.440	0.033	0.156	0.159
I tend to order more than I can eat at eating out.	0.085	0.414	0.112	0.063	0.191
Just a meal, I can eat my favorite foods a meal	0.336	0.390	0.206	0.001	0.134
I cannot help cooking more than enough.	-0.008	0.198	0.757	0.074	-0.022
I eat a lot at dinner compared with other meals.	0.062	0.283	0.724	0.076	0.212
I'm uncomfortable unless I keep enough food let in a refrigerator.	0.191	0.017	0.625	0.165	0.071
When I find something good at the grocery store, I buy it even if it is not planed.	0.192	0.053	0.483	0.006	0.424
I eat well even if I have a cold.	0.029	0.267	0.399	0.046	-0.093
I have dinner late.	0.012	0.103	0.039	0.768	0.158
I don't have a regular meal rhythm.	0.233	0.183	0.103	0.649	-0.048
I don't have enough time to eat meal.	0.324	0.324	0.176	0.643	-0.012
I am a night person	0.098	-0.039	-0.199	0.554	0.157
I often eat out and have food delivered.	0.007	-0.061	0.274	0.545	0.308
I often buy at the convenience stores.	0.288	0.070	0.071	0.379	0.262
I have much occasions to attend dinner at drinking parties.	0.068	0.016	-0.060	0.145	0.781
I have many social occasions to eat.	0.101	0.219	0.066	0.203	0.681
I drink beer often.	-0.152	-0.009	0.155	0.010	0.572
I am not satisfied when a very few-food items are served at dinner.	0.174	-0.025	0.398	-0.057	0.535
I can't sleep when I feel hungry.	0.045	0.137	0.183	0.107	0.417

Principal components analysis with varimax rotation
Figure in front of each question is the number of each items of questionnaire (see Appendix)

Table 5-2 Rotated factor loading based on rank correlations of eating behaiver

Female	Factor 1 Comfort eating, Western food	2 Uncertainty of hunger	3 Fast eating	4 Dining out	5 Promiscuous eating habits
I tend to eat fruits and sweets when I see them.	0.772	0.275	0.080	0.134	0.064
Just a meal, I can eat my favorite foods a meal	0.711	-0.014	0.253	-0.116	0.383
I tend to eat anything when I have nothing to do.	0.637	0.180	-0.020	0.257	-0.079
I tend to eat when I see others eating.	0.633	0.336	0.030	0.156	-0.150
I love sweets.	0.611	0.132	0.123	0.073	0.227
I always gain weight whenever I take long holidays.	0.603	-0.016	0.006	0.210	-0.133
I regret after I eat a lot.	0.575	0.282	0.218	0.087	-0.003
I often eat between meals.	0.574	0.124	0.068	0.003	0.237
I don't satisfied unless I eat my fill.	0.565	0.200	0.324	-0.076	0.153
I tend to eat when I am irritated or stressed.	0.554	0.138	-0.044	0.077	0.276
I believe that I gain weight because I like sweets	0.504	0.108	-0.054	0.081	0.048
I'm told I eat a lot.	0.502	0.203	0.250	0.094	-0.086
I eat more western food than Japanese food.	0.468	-0.275	0.118	0.445	0.050
I like greasy food.	0.420	-0.009	0.208	0.233	-0.022
I eat a lot at dinner compared with other meals.	0.136	0.785	0.022	-0.108	0.113
I cannot help cooking more than enough.	0.154	0.750	0.157	-0.215	0.052
I'm uncomfortable unless I keep enough food let in a refrigerator.	0.220	0.687	0.062	0.025	-0.165
I tend to order more than I can eat at eating out.	0.104	0.568	0.063	0.289	0.222
When I find something good at the grocery store, I buy it even if it is not planed.	0.340	0.471	0.166	0.120	0.172
I always keep food around.	0.326	0.403	0.153	0.274	0.152
I eat a meal fast.	0.047	0.053	0.818	0.162	-0.045
I don't chew well.	0.133	0.123	0.776	0.081	0.109
I eat as putting food into my mouse one after another.	0.238	0.159	0.632	0.121	0.126
I often buy at the convenience stores.	0.171	-0.078	0.148	0.755	0.194
I often eat fast food like hamburgers.	0.187	-0.010	0.025	0.718	-0.134
I eat meal a lot.	0.263	-0.145	0.207	0.525	0.090
I often eat out and have food delivered.	-0.059	0.194	0.196	0.507	0.314
I don't have a regular meal rhythm.	0.069	0.124	-0.039	0.040	0.765
I have dinner late.	0.154	-0.004	0.147	0.124	0.717
I often have a midnight snack.	0.250	0.230	0.197	0.010	0.485

Principal components analysis with varimax rotation
Figure in front of each question is the number of each items of questionnaire (see Appendix)

questionnaire and the raw scores in each of the personality domains *(Table 6)*. In females, scores in the eight categories had negative associations with the domains of Extroversion, Openness, Agreeableness, and conscientiousness. In both males and females, however, the associations with Neuroticism were positive. Extroversion scores had significant positive associations with eating behavior scores only in males, whereas Agreeableness and Extroversion scores had significant negative associations with these scores only in females.

We compared the mean score of the eight eating behavior categories by sex with those from a previous study on normal-weight subjects *(Fig. 2)*.[12] In both males and females, the scales of "perception of physical constitution and weight" and "bad habits for eating" in our subjects were much higher than those of the normal-weight subjects.

Diagrams of the eight eating behavior categories by three classes (low, average, high) of Neuroticism and Openness are shown in ***Figure 3*** (no other domains showed significant associations). Significant differences among the three classes of Neuroticism scores were seen in the categories "unhealthful eating" and "feeling of fullness and hunger" in males. In all categories, the higher the Neuroticism class, the higher the eating behavior score. Females showed a similar trend, except "total points," "unsteady eating pattern," and "contents of meals" also showed significant difference between Neuroticism classes. Among the three classes of Openness scores, there were significant differences in the eating behavior categories of "total points," "motivation for eating," "unhealthful eating," and "unsteady eating pattern" in males. In females, "contents of meals," and "perception of constitution and weight" showed a significant difference among Openness classes.

Table 6 ***Correlation coefficients between NEO-Five Factor Inventory scale and principal component score by eating behavior questionnaire***

		Neuroticism	Extraversion	Openness	Agreeableness	Conscientiousness
Male	Total point	0.221 *	0.204 *	0.272 *	0.007	-0.015
	Conception of body constitution and weight	0.090	0.120	0.084 *	-0.142	0.123
	Motivation for eating	0.112	0.204 *	0.273	0.079	0.030
	Unhealthful eating	0.338 *	0.022	0.278 *	-0.082	0.099
	Feeling of fullnes and hunger	0.313 *	0.141	0.155	-0.023	0.070
	Bad habits for eating	0.212 *	0.197 *	0.174	0.058	-0.056
	Contents of meals	0.015	0.192 *	0.111	0.009	-0.136
	Unsteady of eating pattern	0.224 *	0.114	0.316 *	0.051	-0.111
Female	Total point	0.277 *	-0.055	-0.085	-0.133	-0.211 *
	Conception of body constitution and weight	0.143	-0.046	-0.271 *	-0.176	-0.225 *
	Motivation for eating	0.161	0.087	0.012	0.052	-0.106
	Unhealthful eating	0.353 *	-0.136	-0.024	-0.112	-0.090
	Feeling of fullnes and hunger	0.238 *	-0.114	-0.042	-0.168	-0.140
	Bad habits for eating	0.148	-0.003	-0.082	-0.103	-0.114
	Contents of meals	0.240 *	-0.098	-0.208 *	-0.207 *	-0.240 *
	Unsteady of eating pattern	0.203 *	-0.070	0.095	-0.081	-0.192 *

* $p<0.05$

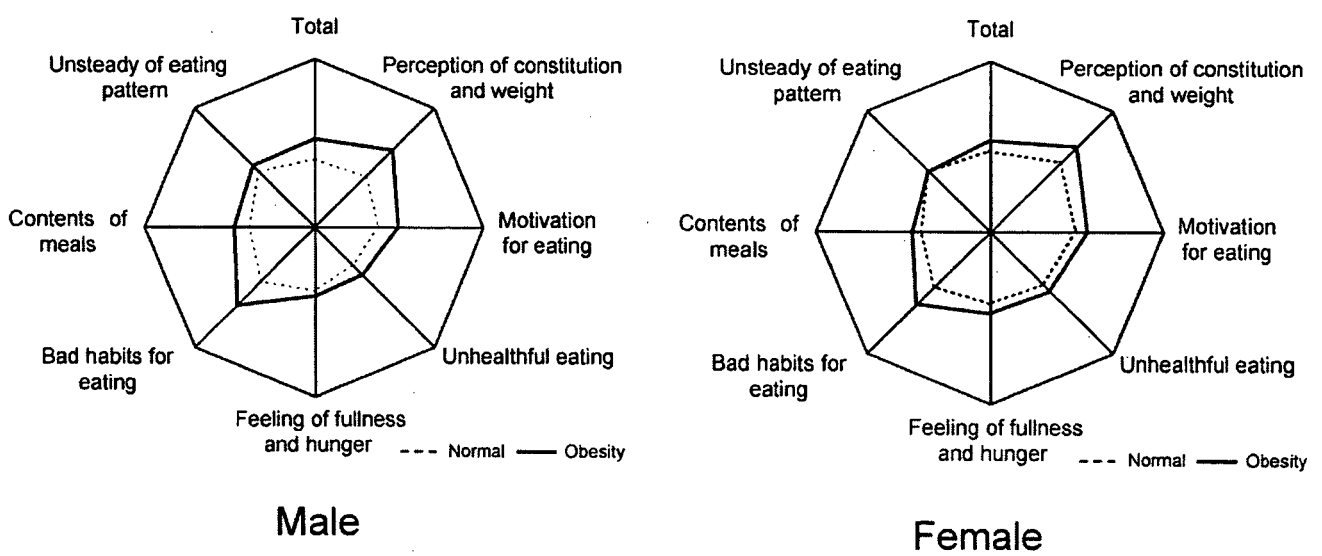


Fig. 2. Comparison between the eating behaviors of obese subjects in this study and normal-weight subjects in a previous study.[12] Displayed are the scores of obese subjects (solid line) and normal-weight subjects (dash line) by sex.

*Fig. 3*a

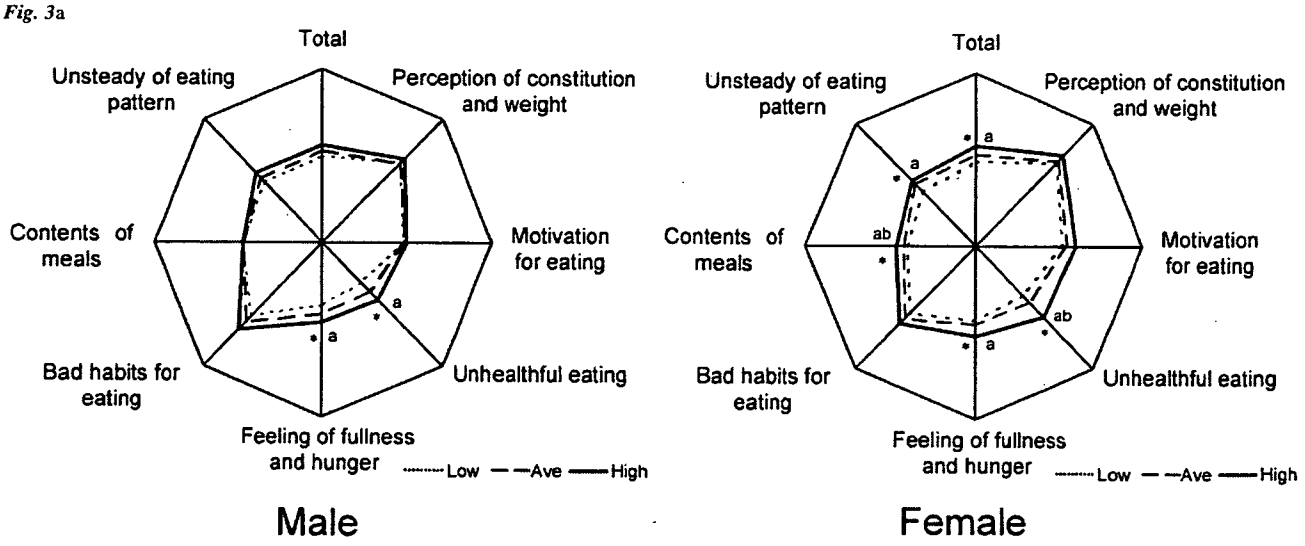


*Fig. 3*b

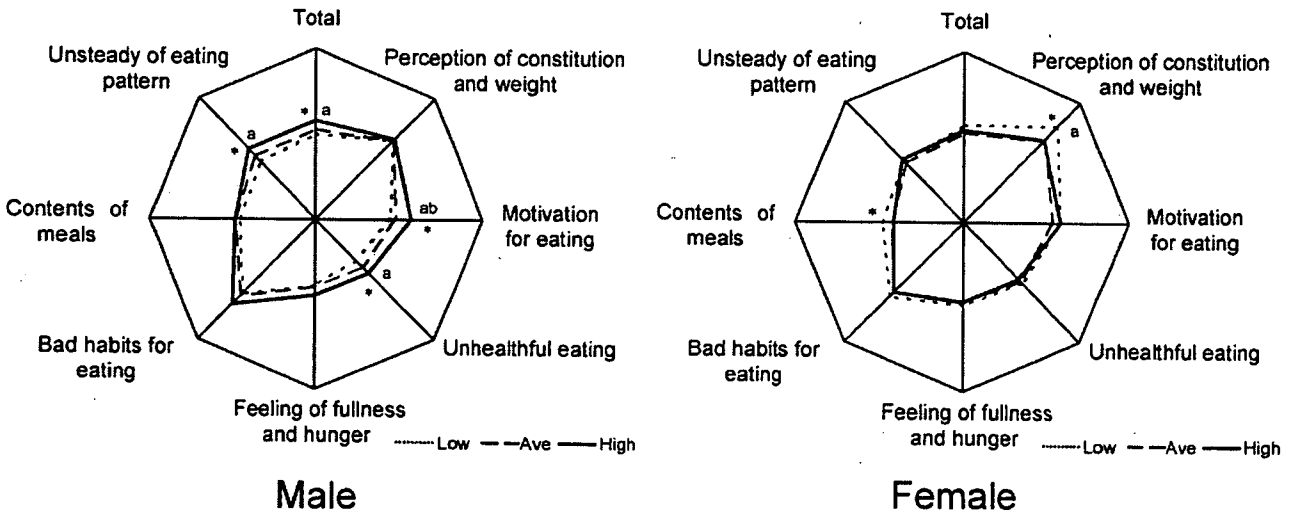


Fig. 3. Comparison of the eating behaviors among subjects in the low, average, and high classes of Neuroticism (a) and Openness (b), separated by sex (males, left; females, right). Asterisks indicate significant difference among the three classes (ANOVA, *p < 0.05). Each superscript indicates significant difference between High vs Low (a: P<0.05), significant between High vs Ave in (b:P<0.05), significant between Ave vs Low (c: P<0.05) by multiple comparison.

Discussion

Basic personality is considered to be static throughout a person's life unless a significant incident occurs.[14] Therefore, personality is an important element that affects an individual's lifestyle, including eating behavior and physical activities. However, the relationship between lifestyle and the personality traits of obese people has not been studied in detail.

According to Costa and McCrae, the five-factor model of personality should not be used to judge the value (i.e., good vs. bad) of particular personality traits;[14] rather it allows for a comprehensive assessment of normal adult personality. Consistency was reported between the five personality domains of the NEO-FFI as rated by the subjects themselves and by close family and friends. This consistency suggests that the questionnaire in the NEO-FFI can be successfully translated into different languages without losing efficacy.

Considering the distribution of NEO-FFI personality domains among the subjects in this study, in females there was a tendency to T-score higher in Neuroticism and Extroversion, although there were no significant differences among the low, average, and high classes in these domains. The mean T-score and the standard deviation of the subjects in this study were 56 and 28, whereas those in the normative population were 50 and 10 for each domain. Thus, compared with the subjects who are representative samples of other area examined in previous studies [6,15] our subjects tended to be somewhat more neurotic and extroverted. It is expected that these differences were influenced from obesity. However, they might are influenced from regional difference. An American national survey[16] showed that the distribution of NEO-FFI was not effected by the differences of age, race and sex. Thus, it is necessary to compare the subjects in same region for more reliable results.

Costa and Mcrae said that an individual with high scores of Neuroticism tends to be nervous, uneasy, and very sensitive to stress, whereas a person with low scores of Neuroticism tends to be relaxed and stable.[14] A study by Gidi showed a positive

correlation between BMI and Neuroticism scores in both obese and the non-obese females.[3] In addition, previous studies showed that Neuroticism is positively correlated with levels of Eating Disorder and bad eating behavior.[17,18] In this study, females in the high class for Neuroticism showed high scores for eating behaviors. Thus, it is suggested that a person with high scores of Neuroticism may have some problems related to eating behavior. Also, females in the high class for Neuroticism had high daily step counts and high METs·h. Considering the result, people with a low Neuroticism score, who tend to be relaxed, secure, and confident, may not overeat but may also engage in little physical activity.

In males, the scores of all categories of eating behavior increased as Openness scores rose. People with a high degree of Openness tend to be very curious and quick to take positive actions,[10] and their curiosity and activities might cause somewhat of a rise in appetite. In contrast, we found no positive association between Openness scores and eating behaviors among females. Thus, it appears that the effects of Openness on eating behavior differ between males and females.

With regard to Agreeableness, the average class of males showed low values for daily step counts and METs·h, whereas those in the low and high classes had higher physical activity. Meta analysis studying the correlates of personality and physical activities did not show the association between Agreeableness and physical activities until 2006.[19] Subjects of the studies which use NEO-FFI including this meta-analysis were selected from students, cancer survivors, elderly people; were not middle-aged people such as our study. It isn't still clear that there was the association between Agreeableness and physical activities. It is necessary to clearly the association of NEO-FFI and physical activities in greater number of samples with and without obesity in general population including middle-aged people.

According to our analysis based on the NEO-FFI, different personality trait distributions were found between obese people and the general population, so further study regarding personality traits is necessary for the obese population. For instance, among the five personality domains, there was a significant difference in eating behaviors among the three classes of Neuroticism in both males and females, with the scores of nearly all categories of eating behavior increasing as Neuroticism scores rose. The associations between eating behaviors, physical activity levels, and personality traits defined by the NEO-FFI showed that personality analysis can serve as a useful tool in health education. As seen in ***Figure 2***, obese people showed a broader range of scores for the eight categories of eating behavior compared to the general adult population.[12] Using this eating behavior questionnaire, we were able to identify which categories caused more problems for each subject, which can then be used to improve an individual's eating behavior through nutritional education.

Although personality has long been considered to be unchangeable throughout an individual's life, Adil et al. recently reported that the personality scales have changed in a short period of time.[20] Another study reported that the NEO-FFI scales differ between elderly people and college students.[21] Thus, if personality can change over the course of a person's life, these baseline data should help us to elucidate which pre-intervention traits allow for more successful behavior modification with regard to eating behavior and physical activity.

Questionaire of eating behavior

	Question	Question number from Mannual of Obesity
1	I often have a midnight snack.	4
2	I am a night person	18
3	I don't have a regular meal rhythm.	27
4	I often eat between meals.	21
5	I don't have enough time to eat meal.	47
6	I have dinner late.	37
7	I don't eat breakfast.	48
8	I'm often told I eat a lot.	8
9	Just a meal, I can eat my favorite foods a meal	13
10	I don't satisfied unless I eat my fill.	15
11	I regret after I eat a lot.	32
12	I can't sleep when I feel hungry.	39
13	I think about next meal just after a meal.	45
14	I often eat snacks.	11
15	I like strong seasoning.	14
16	I often eat fast food like hamburgers.	30
17	I like greasy food.	43
18	I like noodles.	19
19	I often eat sweet rolls.	40
20	I love sweets.	52
21	I tend to eat left-over food because I don't want to waste.	12
22	I tend to eat when I am irritated or stressed.	16
23	I always keep food around.	23
24	I tend to eat when I see others eating.	24
25	I tend to eat fruits and sweets when I see them.	34
26	I always gain weight whenever I take long holidays.	20
27	I tend to eat anything when I have nothing to do.	31
28	I believe myself to gain weight more easily than others.	42
29	I believe myself to gain weight even by drinking water.	22
30	I eat a meal fast.	1
31	I eat as putting food into my mouse one after another.	55
32	I don't chew well.	25
33	I stuff food into my month.	41
34	I tend to order more than I can eat at eating out.	28
35	I cannot help buying more food than necessary.	33
36	I cannot help cooking more than enough.	38
37	I believe that I gain weight because I like sweets	2
38	I often buy at the convenience stores.	3
39	I eat a lot at dinner compared with other meals.	35
40	I gain weight because I have not sufficient physical activity.	36
41	I'm uncomfortable unless I keep enough food let in a refrigerator.	5
42	When I find something good at the grocery store, I buy it even if it is not planed.	44
43	I drink beer often.	46
44	I am not satisfied when a very few-food items are served at dinner.	17
45	I don't have a sense of hunger and fullness.	49
46	I have many social occasions to eat.	50
47	I don't loose weight although I don't so much.	51
48	I tend not to be hungry before meals.	53
49	I eat meal a lot.	54
50	I gain weight because I lie down soon after I finish meal.	6
51	I have much occasions to attend dinner at drinking parties.	7
52	I get irritated when I'm hungry.	9
53	I eat well even if I have a cold.	10
54	I eat more western food than Japanese food.	29
55	I often eat out and have food delivered.	26

References

1) Faith M, Flint J, Fairburn C, et al. Sex differences in the relationship between personality dimensions and relative body weight. Obes Res 9:647-650, 2001.
2) Wadden T, Butryn M, Sarwer D, et al. Comparison of psychosocial status in treatment-seeking females with class III vs. class I-II obesity. Obesity 14 (Suppl.):90-98, 2006.
3) Gidi R. The big five and self-esteem among overweight dieting and non-dieting females. Eat Behav 7:355-361, 2006.
4) Yoshida S, Murano S, Saito Y, et al. Treatment of obesity by a personality classification oriented program. Obes Res 3 (Suppl. 2):205-209, 1995.
5) Costa PT Jr, McCrae RR. NEO-PI-R professional manual: Reserved NEO Personality and NEO Five-Factor Inventory (NEO-FFI). Psychological Assessment Resources Inc., Odessa, FL, 1992.
6) Yoshimura K, Nakamura K, Ono Y, et al. Reliability and validity of Japanese version of the NEO Five-Factor Inventory (NEO-FFI): a population-based survey in Aomori prefecture. Jpn J Stress Sci 13:45-53, 1998.
7) Kikuchi Y, Inoue T, Ito M, et al. Health consciousness of young people in relation to their personality. J Epidemiol 9:121-131, 1999.
8) Kikuchi Y, Watanabe S. Personality and dietary habits. J Epidemiol 10:191-198, 2000.
9) Watanabe S, Morioka M, Morita A, et al. Strategy and design of the Saku Control Obesity Program. J Epidemiol. (in press)
10) Shimonaka Y, Nakazato K, Gondoh K, et al. NEO-PI-R, NEO-FFI: the Japanese version. Tokyo Shinri Co. Ltd., Tokyo, 2002. (in Japanese)
11) Miyachi M, Ohmori Y, Yamamoto K, et al. Physical activity of obese people. J Epidemiol. (in press)
12) Ohkuma K, Ohkuma M. Modification therapy. Nihon Rinsho 61 (Suppl.):631-639, 2003. (in Japanese)
13) Japan Obesity Association Edit Committee Guidance. Manual of obesity. Ishiyaku Co. Ltd., Tokyo, 114-118, 2001.
14) Costa PT Jr, McCrae RR. The NEO-PI/NEO-FFI manual supplement. Psychological Assessment Resources Inc., Odessa, FL, 1989.
15) Yoshimura K, Ono Y, Nakamura K, et al. Validation of the Japanese version of the Neo-Five-Factor Inventory in a large community sample. Psychol Rep 88:443-449, 2001.
16) Costa PT Jr, McCrae RR, Zonderman AB, et al. Cross-sectional studies of personality in a national sample: 2. Stability in neuroticism, extraversion, and openness. Psychol Aging 1:144-149, 1986.
17) Podar I, Hannus A, Allik J. Personality and affectivity characteristics associated with eating disorders: A comparison of eating disordered, weight-preoccupied, and nomal samples. J Pers Assess 73:133-147, 1999.
18) Brookings JB, Wilson JF. Personality and family-environment predictors of self-reported eating attitudes and behaviors. J Pers Assess 63:313-326, 1994.
19) Rhodes RE, Smith NE. Personality correlates of physical activity: a review and meta-analysis. Br J Sports Med 40:958-965, 2006.
20) Adil H, David H, Ellen J, et al. The use of the NEO-Five Factor Inventory to assess personality in trauma patients: a two-year prospective study. J Orthopaed Trauma 16:660-667, 2002.
21) Kikuchi Y, Watanabe S. Personality of elderly people and dietary habits: science of macrobiotic food. Science Forum Co. Ltd., Tokyo, 147-157, 2002. (in Japanese)

JAAM

ANTI-AGING MEDICINE

Received: Sep 17, 2007
Accepted: Dec 4, 2007
Published online: Jan 29, 2008

Original Article

The Use of a Uniaxial Accelerometer to Assess Physical-activity-related Energy Expenditure in Obese Men and Women: Saku Control Obesity Program (SCOP)

Motohiko Miyachi [1], Yumi Ohmori [1], Kenta Yamamoto [2], Hiroshi Kawano [2], Haruka Murakami [1], Akemi Morita [1], Shaw Watanabe [1]

1) National Institute of Health and Nutrition
2) Waseda University

Abstract

INTRODUCTION: Energy expenditure (EE) associated with physical activity is negatively correlated with prevalence of obesity and related diseases, and exercise plays a major role in prevention and treatment of these diseases. We determined baseline daily step-count and physical activity-related energy expenditure (PAEE) in 230 obese subjects (40–64 years old) participating in the Saku Control Obesity Program. The secondary purpose of this study was to determine the association between abdominal fat and amount of physical activity.

METHODS: Daily step-count and PAEE were measured using a uniaxial accelerometer. The subjects wore the uniaxial accelerometer on their belt from the time they woke up until going to bed for 2 weeks. Adjusted PAEE (METs·h/day) was calculated based on daily PAEE and body weight.

RESULTS AND CONCLUSIONS: Daily step-count, PAEE, and adjusted PAEE were 7,815±3,211 (mean±SD) steps/day, 258±115 kcal/day, and 3.09±1.38 METs·h/day, respectively. There were no significant differences in daily step-count or adjusted PAEE between men and women. Daily step-count and adjusted PAEE were somewhat lower than the reference values for the quantity of physical activity for health promotion (8,000-10,000 steps/day and 3.3 METs·h/day) established by the Ministry of Health, Labour, and Welfare of Japan. BMI, visceral fat area, and abdominal circumference were negatively and weakly correlated with daily step-count and adjusted PAEE ($r=-0.13$ to -0.19, $P<0.05$ to 0.01). These results suggest that the amount of physical activity assessed by uniaxial accelerometry is partially associated with not only systemic obesity but also abdominal obesity.

KEY WORDS: accelerometer, energy expenditure, daily step-count, obesity, physical activity

Introduction

The energy expenditure (EE) associated with physical activity is negatively correlated with the prevalence of obesity and related diseases, such as diabetes, hypertension, and cardiovascular disease, and exercise has been shown to play a major role in the prevention and treatment of these diseases.[1-3] When developing treatment strategies for these diseases, including nutritional education, quantitative information related to physical activity is required to provide more effective goals. Thus, to prevent and treat these diseases more effectively, information regarding physical activity is useful, not only for researchers and healthcare workers but also for the general public.

Activity monitoring based on an accelerometry sensor is a useful method for obtaining objective information on physical activity patterns and for estimating the related EE,[4,5] because this type of sensor (Lifecorder; Suzuken Co. Ltd., Nagoya, Japan) can continuously measure the intensity, duration, and frequency of activity. The device has a unique algorithm for assessment of PAEE, especially unstructured activities. In addition, several studies indicated that the EE during running and walking estimated using this device correspond to the EE measured by indirect calorimetry, and the device was also more effective for measuring EE in free-living conditions as compared with a metabolic chamber.[6,7]

Increasing physical activity and decreasing caloric intake are indispensable for the improvement of excess weight and obesity. The Saku Control Obesity Program (SCOP) is a randomized control crossover study that aims to reduce visceral fat in overweight and obese subjects by interventions of physical activity and diet. Our systematic review suggested that an increase in adjusted PAEE at 10 METs·h/week (1.38 METs·h/day) is necessary to reduce visceral fat area in overweight and obese subjects.[8] The increase in adjusted PAEE corresponds to an increase of nearly 3,000 steps/day. Thus, all SCOP subjects receive physical activity modification education so that their daily step-count increase gradually by 3,000 steps/day. As each subject's target for modification of physical activity depends on the baseline level, accurate baseline measurements of physical activity are needed. The first purpose of the present study was to accurately determine the baseline status of physical activity using a uniaxial accelerometer. Furthermore, there have been few studies of the relationship between abdominal obesity and physical activity. Therefore, the second purpose of this study was to determine the association between visceral fat area measured by CT scan and amount of physical activity estimated by accelerometry.

Motohiko Miyachi, Ph.D. / Program for Health Promotion and Exercise,
National Institute of Health and Nutrition
1-23-1 Toyama, Shinjuku, Tokyo 162-8636 Japan
Tel: +81-3-3203-8061 / Fax: +81-3-3203-1731 / E-mail: miyachi@nih.go.jp