J. Soc. Cosmet. Scientists Korea
Vol. 37, No. 2, June 2011, 171-176

홍화씨 추출물 유래 세로토닌 유도체의 지방전구세포 분화억제 효능에 대한 연구

정 은 선 · 김 승 범 · 김 무 한 · 신 승 우 · 이 종 성† · 박 덕 훈

바이오스펙트럼(주) 생명과학연구소

(2011년 6월 1일 접수, 2011년 6월 10일 수정, 2011년 6월 13일 채택)

Inhibitory Effects of Serotonin Derivatives on Adipogenesis

Eun sun Jung, Seung beom Kim, Moo han Kim, Seong woo Shin, Jong sung Lee†, and Deok hoon Park

Biospectrum Life Science Institute, Eines Platz 11th FL, 442-13 Sangdaewon-dong, Seongnam-si, Gyunggi-do 435-833, Korea

(Received June 1, 2011; Revised June 10, 2011; Accepted June 13, 2011)

요 약: N-(p-Coumaroyl)serotonin과 N-Feruloylserotonin은 홍화씨에서 유래하는 특이적인 세로토닌유도체로 주로 식물이 병원균을 방어하기 위해서 만들어내는 hydroxycinnamic acid amides계 물질이다. 본 연구에서는 N-(p-Coumaroyl) serotonin과 N-Feruloylserotonin이 지방전구세포의 지방분화에 미치는 영향을 oil-red O염색과 triglyceride 양 측정, GPDH 활성 측정 등을 통해 알아보았고, 그 결과 두 물질 모두 유의적으로 지방세포분화를 억제함을 관찰하였다. 효능 비교물질로 세로토닌을 처리했을 때 세로토닌 자체로는 지방분화에 유의미한 효과는 관찰되지 않았다. N-(p-Coumaroyl) serotonin과 N-Feruloylserotonin은 또한 우수한 항산화능을 보여주었다. 이러한 결과를 통해, N-(p-Coumaroyl)serotonin과 N-Feruloylserotonin이 지방세포분화억제를 통한 항비만 소재로서의 가능성을 확인하였다.

Abstract: N-feruloylserotonin (FS) and N-(pcoumaroyl) serotonin (CS), serotonin derivatives, which have been isolated as major and unique phenolics of safflower seed extract (SSE), are member of hydroxycinnamic acid amides and are implicated in the defense against pathogen infection and insect feeding. In this study, we evaluate inhibitory effects of N-(p-Coumaroyl)serotonin and N-Feruloylserotonin on adipogenesis using oil-red O staining, triglyceride and GPDH activity. we found that while serotonin itself did not suppress differentiation of preadipocytes into adipocytes, N-(p-Coumaroyl)serotonin and N-Feruloylserotonin inhibited the differentiation of preadipocytes into adipocytes in a concentration-dependent manner. In addition, they showed antioxidant effects in DPPH assay. Taken together, these results show that N-feruloylserotonin (FS) and N-(pcoumaroyl) serotonin (CS) suppress differentiation of preadipocytes, suggesting the possibility that these serotonin derivatives can be utilized as an anti-obesity agent.

Keywords: *serotonin derivatives, anti-obesity, adipogenesis, GPDH, triglyceride*

1. 서 론

Obesity is a chronic metabolic disorder that results from the imbalance between energy intake and energy expenditure. It is characterized by enlarged fat mass and elevated lipid concentration in blood[1,2]. The amount of fat mass is increased when the number and/or size of adipocytes are multiplied by proliferation and differentiation. Differentiated adipocyte stores fatty acids (FAs) in the form of triglycerides (TGs) in their cytoplasm. Adipocyte differentiation is the sequential cascade of transcriptional events that control adipogenesis. The central players are peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/en-

† 주 저자 (e-mail: jslee@biospectrum.com)

hancer binding protein α (C/EBPα), both of which are required to coordinate the expression of adipogenic genes (e.g, aP2, SCD, pyruvate carboxylate)[3]. Expression of CEBPβ and CEBPδ precedes both CEBPα and PPARγ[4]. Other transcription factors important to the regulation of adipocyte differentiation include two members of the basic helix-loop-helix family. These members, adipocyte determination- and differentiation-dependent factor 1 (ADD1) identified in rodent, and sterol regulatory element binding protein 1 (SREBP1), recognized as the human homolog to ADD1, were independently identified as a transcription factors regulating both adipocyte differentiation and cholesterol-regulated transcription[5]. A number of studies have demonstrated that natural compounds like capsaicin, EGCG, genistein, berberine, conjugated linoleic acid and baicaline inhibited adipogenesis via suppression of PPAR and CEBP expression[6-11].

Safflower (*Carthamus tinctorius* L.) seeds have been used as a traditional herbal medicine in Korea and other Asian countries. Serotonin derivatives, N-(p-Coumaroyl) serotonin (CS) and N-Feruloylserotonin (FS), have been identified as major and unique phenolics in defatted safflower seed extract (SSE)[12]. These serotonin derivatives has been reported to have antioxidative properties, cholesterol-lowering activity and protective effects on postischemic myocardial dysfunction[13-15]. Rho *et al.* showed that N-feruloylserotonin (FS) and N-(pcoumaroyl) serotonin (CS) strongly inhibited the melanin production of *Streptomyces bikiniensis* and B16 melanoma cells in comparison with a known melanogenesis inhibitor, arbutin[16].

Until now, no study has investigated the effect of serotonin derivatives on differentiation of preadipocytes. In this study, we investigated the inhibitory effects of serotonin derivatives on adipogenesis by measuring oil-red O staining, triglyceride and GPDH activity in 3T3-L1.

2. Materials and Methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen, Inc. (Invitrogen, CA, USA). Serotonin, isobutylmethylxanthine, dexamethasone, and insulin were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-feruloylserotonin (FS) and N-(pcoumaroyl) serotonin (CS) were provided by Dr. KH Back (Department of Biotechnology, Chonnam National University, KOR)[17].

2.2. Cell Culture Treatment

Cell culture and stimulation. 3T3-L1 preadipocytes (ATCC, Manassas, VA) were seeded in 6 well plate at a density of 15 × 104 cells/well. Cells were grown in phenol red-free DMEM supplemented with 10 % charcoal-stripped FBS at 37 ℃ in a 5 % CO_2 humidified atmosphere. To induce differentiation, 2-day post-confluent 3T3-L1 preadipocytes (day 0) were incubated for 3 days with differentiation medium 0.5 mM isobutylmethylxanthine, 0.25 μM dexamethasone, and 1 μg/mL insulin in phenol red-free DMEM supplemented with 10 % charcoal-stripped FBS. Then preadipocytes were maintained in and refed every 2 days with maintenance medium [phenol red-free DMEM supplemented with 10 % charcoal-stripped FBS and 1 μg/mL insulin]. To examine the effect of serotonin derivatives on adipocyte differentiation, 2-day post-confluent 3T3-L1 preadipocytes were stimulated for 3 days with the differentiation medium in the presence or absence of the indicated concentrations of serotonin derivatives. The medium was then replaced with the maintenance media in the presence or absence of the indicated concentrations of serotonin derivatives every 3 days until the end of the experiment at day 9. Serotonin derivatives was reconstituted in DMSO and stored at -20 ℃.

2.3. Oil Red-O Staining Assay

Culture dishes were washed twice in phosphate-buffered saline and fixed for 30 min in phosphate-buffered saline containing 4 % formaldehyde. After a single wash in water, cells were stained with oil red-O for 30 min. After the staining, dishes were washed twice in water and photographed. Oil red-O was prepared by

diluting a stock solution (0.5 g of oil red-O (Sigma, MO, USA) in 100 mL of isopropanol) with water (3 : 2), followed by filtration.

2.4. Cytotoxicity Assay

This assay was performed after 3T3-L1 preadipocytes were induced to differentiate into adipocytes in the presence of herbal extracts. The general viability of cultured cells was determined by the reduction of WST-8(2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4- disulfophenyl)-2H- tetrazolium, monosodium salt) (Dojindo Laboratories, Japan) to a highly water-soluble formazan dye. To each well, 10 μL of WST-8 solution was added. Cells were then incubated at 37 ℃ for 3 h and the absorbance was measured at 450 nm using a spectrophotometer (Power Wave, Bio-tek Inc, VT, USA).

2.5. Triglyceride Assay

3T3-L1 Adipocytes were washed with PBS and harvested into Tris buffer (pH 7.5) containing EDTA, sonicated to homogenize the cell suspension, and assayed for total triglyceride with the Triglyceride assay kit (Cayman Chemical, MI, USA). An aliquot of the homogenate was used to determine protein content with a protein assay kit (Pierce, IL, USA).

2.6. Glycerol-3-Phosphate Dehydrogenase (GPDH) Activity

3T3-L1 Adipocytes were washed twice with PBS and harvested into 25 mM Tris buffer (pH 7.5) containing EDTA and DTT. Levels of GPDH activity was measured using GPDH activity assay kits (Takara Bio inc, Japan) following the same methodology outlined in Kozak *et al.* [18].

2.7. DPPH Assay

DPPH (diphenyl-p-picrylhydrazyl) (Sigma, MO, USA), a stable nitrogen-centered free radical, was dissolved in methanol for 5 min to give a 200 μM solution. The tested compounds were added to DPPH of equal volume in a 96-well microplate as quadruplicates, along with sample blanks and controls. The concentration (absorption) of DPPH during the 30 min observation time was measured at 540 nm. The decrease in absorption at 540 nm was correlated with the scavenging action of the tested compound. Data were presented as the mean ± standard deviation. Experiment was performed in duplicate and repeated three times.

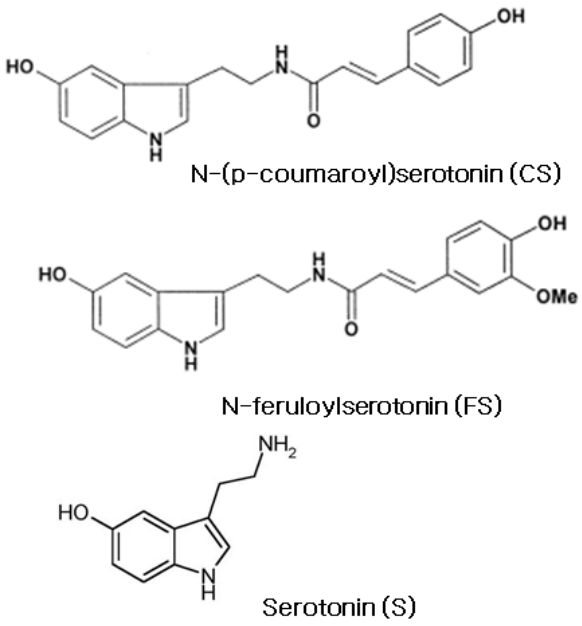


Figure 1. Structure of serotonin derivatives.

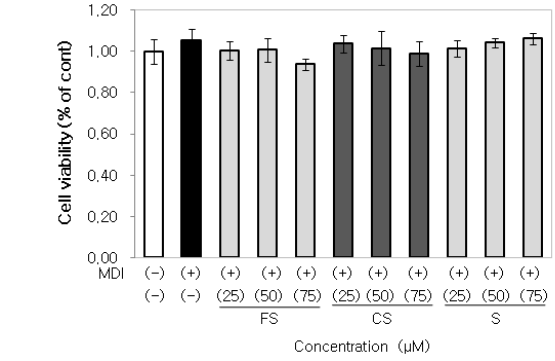
2.8. Statistics

The statistical significance of the data was determined by Student's *t*-test. $p < 0.05$ was considered significant.

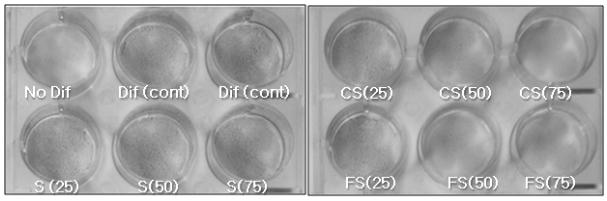
3. Results and Discussion

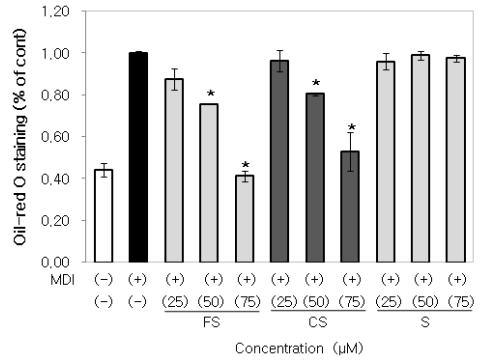
3.1. Serotonin Derivatives Inhibits Lipid Accumulation in 3T3-L1 Adipocyte Serotonin

Serotonin (5-HT) has been implicated as a critical factor in the short-term (meal-by-meal) regulation of food intake[19]. Novel selective agonist of the 5-HT (2C) receptor has been developed for weight loss therapy, but the direct effect of serotonin on differentiation of adipocyte is remained unknown. So we also investigated the effect of serotonin (S) on adipogenesis to compare with the efficacy of N-feruloylserotonin (FS)



(a)





(b)

Figure 2. Effect of serotonin derivatives on adipocyte differentiation. To determine the effect of serotonin derivatives on adipocyte differentiation, 3T3-L1 preadipocytes were differentiated into adipocytes in the presence or absence of the indicated concentrations of serotonin derivatives analyzed 14 days after the induction of differentiation. (a) cell viability, (b) oil red O staining. Data are expressed as mean ± S.D., * $p < 0.05$ compared with untreated control. Results were confirmed by three independent experiments.

and N-(pcoumaroyl) serotonin (CS).

Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with the indicated concentrations of serotonin derivatives every 2 days for 9 days. No significant effect on cell viability has been observed with serotonin derivatives concentrations under 75 μM

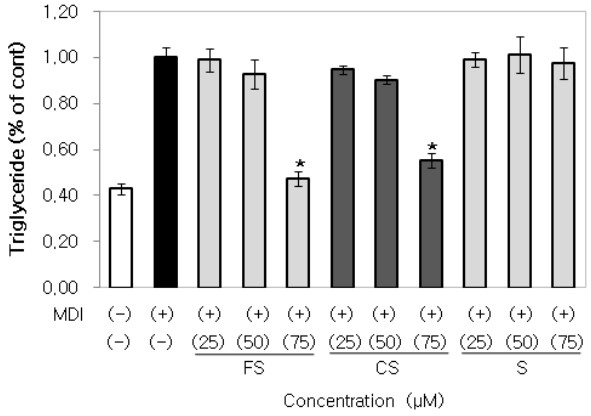


Figure 3. Effects of serotonin derivatives on triglyceride contents. This triglyceride contents assay was performed after the incubation of 3T3-L1 preadipocytes in the presence or absence of indicated concentrations of serotonin derivatives for 14 days at 37 °C in a 5 % CO_2 atmosphere. Cellular triglyceride contents was determined according to the protocol described in materials and methods and was expressed as the mean ± S.D. All values were significant (* $p < 0.05$) compared with values for untreated control.

(Figure 2A). When preadipocytes differentiated into adipocytes, morphological alterations were observed due to the accumulation of lipid droplets in the cytoplasm. As evidenced by Oil Red O staining, N-feruloylserotonin (FS) and N-(pcoumaroyl) serotonin (CS) significantly decreased lipid accumulation compared with control cells (Figure 2B). Serotonin showed no effect on lipid accumulation.

3.2. Serotonin Derivatives Inhibits Cellular Triglyceride Content and GPDH Enzyme Activity in 3T3-L1 Adipocyte

To further characterize the effects of N-feruloylserotonin (FS) and N-(pcoumaroyl) serotonin (CS) on differentiation, cellular triglyceride content and GPDH enzyme activity were measured. By the time of full differentiation at day 9, cells treated with N-feruloylserotonin (FS) and N-(pcoumaroyl) serotonin (CS) inhibited triglyceride accumulation compared with the controls (Figure 3). Consistent with the observed reduction in triglyceride accumulation, GPDH activity was reduced by N-feruloylserotonin (FS) and N-(pcoumaroyl) serotonin (CS) (Figure 4).

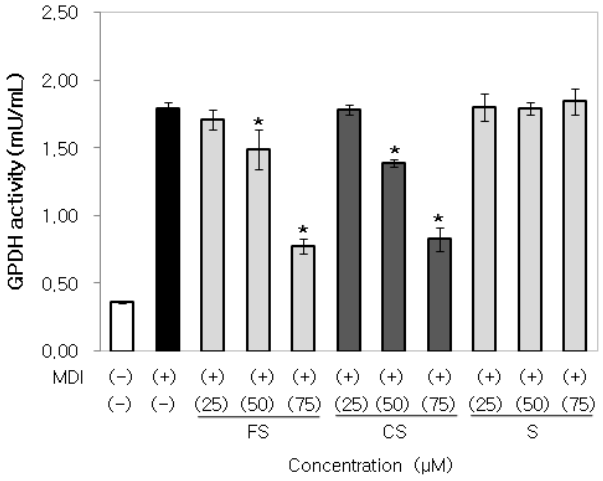


Figure 4. Effects of serotonin derivatives on GPDH activity. This GPDH activity assay was performed after the incubation of 3T3-L1 preadipocytes in the presence or absence of indicated concentrations of serotonin derivatives for 14 days at 37 °C in a 5 % CO_2 atmosphere. GPDH activity was determined according to the protocol described in materials and methods and was expressed as the mean ± S.D. All values were significant (* $p < 0.05$) compared with values for untreated control.

3.3. Antioxidant Effect of Serotonin Serivatives

Adipocytes in obese patients exhibit increased oxidative stress via the activation of reactive oxygen species (ROS) producing systems and inactivation of antioxidant enzymes. Increased oxidative stress in accumulated fat is an important target for the development of new therapies[20].

In order to investigate antioxidant effects of N-feruloylserotonin (FS) and N-(pcoumaroyl) serotonin (CS), we carried out *in vitro* test for diphenyl-p-picrylhydrazyl (DPPH) radical scavenging assay. The DPPH test showed that serotonin derivatives have significant anti-oxidant activities (Figure 5).

4. Conclusions

In conclusion, N-feruloylserotonin (FS) and N-(pcoumaroyl) serotonin (CS) have anti-adipogenic and antioxidant activities, which are beneficial for preventing obesity.

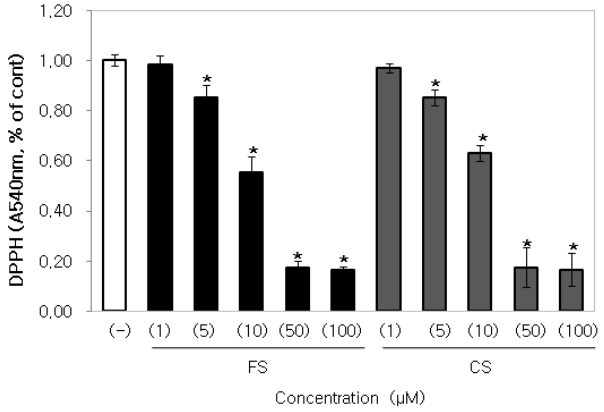


Figure 5. *In vitro* antioxidant activities of serotonin derivatives using DPPH assay. Data are expressed as mean ± S.D., * $p < 0.05$ compared with a control. Results were confirmed by the experiment which was repeated three times in triplicate.

Acknowledgements

This work was supported by a grant from the Korean Ministry of Knowledge and Economy (70011029).

References

1. M. J. Devlin, S. Z. Yanovski, and G. T. Wilson, Obesity: what mental health professionals need to know, *Am. J. Psychiatry*, **157**, 854 (2000).
2. K. Fujioka, Management of obesity as a chronic disease: nonpharmacologic, pharmacologic, and surgical options, *Obes. Res.*, **10**, 116S (2002).
3. S. MJitrapakdee, G. Slawik, M. Medina-Gomez, J. C. Campbell, J. K. Wallace, S. Sethi, O'Rahilly, and A. J. Vidal-Puig, The peroxisome proliferator- activated receptor-gamma regulates murine pyruvate carboxylase gene expression *in vivo*, and *in vitro*, *J. Biol. Chem.*, **280**, 27466 (2005).
4. Z. Cao, R. M. Umek, and S. L. McKnight, Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells, *Genes Dev.*, **5**, 1538 (1991).
5. P. Tontonoz, J. B. Kim, R. A. Graves, and B. M. Spiegelman, ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determi-

nation and differentiation, *Mol. Cell. Biol.*, **13**, 4753 (1993).
6. C. L. Hsu and G. C. Yen, Effects of capsaicin on induction of apoptosis and inhibition of adipogenesis in 3T3-L1 cells, *J. Agric. Food. Chem.*, **55**, 1730 (2007).
7. H. K. Kim, C. Nelson-Dooley, M. A. Della-Fera, J. Y. Yang, W. Zhang, and D. L. Hartzell, Genistein decreases food intake, body weight and fat pad weight and causes adipose tissue apoptosis in ovariectomized female mice, *J Nutr.*, **136**, 409 (2006).
8. C. Huang, Y. Zhang, Z. Gong, X. Sheng, Z. Li, and W. Zhang, Berberine inhibits 3T3-L1 adipocyte differentiation through the PPARgamma pathway, *Biochem. Biophys. Res. Commun.*, **348**, 571 (2006).
9. J. M. Brown, M. S. Boysen, S. S. Jensen, R. F. Morrison, J. Storkson, and R. L. Currie, Isomer-specific regulation of metabolism and PPAR γ signaling by CLA in human preadipocytes, *J. Lipid. Res.*, **44**, 1287 (2003).
10. M. H. Cha, I. C. Kim, B. H. Lee, and Y. Yoon, Baicalein inhibits adipocyte differentiation by enhancing COX-2 expression, *J. Med. Food*, **9**, 145 (2006).
11. H. S. Moon, H. G. Lee, Y. J. Choi, T. G. Kim, and C. S. Cho. Proposed mechanisms of (-)-epigallocatechin-3-gallate for anti-obesity, *Chem. Biol. Interact.*, **167**(2), 85 (2007).
12. H. L. Zhang, A. Nagatsu, T. Watanabe, J. Sakakibara, and H. Okuyama, Antioxidative compound-sisolated from safflower (*Carthamus tinctorius* L.) oil cake, *Chem. Pharm. Bull.*, **45**, 1910 (1997).
13. S. H. Cho, H. R. Lee, T. H. Kim, S. W. Choi, W. J. Lee, and Y. Choi, Effects of defatted safflower seed extract and phenolic compounds in diet on plasma and liver lipid in ovariectomized rats fed high-cholesterol diets, *J. Nutr. Sci. Vitaminol.*, **50**, 32 (2004).
14. N. Koyama, K. Kuribayashi, T. Seki, K. Kobayashi, Y. Furuhata, K. Suzuki, H. Arisaka, and T. Nakano, Serotonin derivatives, major safflower (*Carthamus tinctorius* L.) seed antioxidants, inhibit low-density lipoprotein (LDL) oxidation and atherosclerosis in apolipoprotein E-deficient mice, *J. Agric. Food. Chem.*, **54**, 4970 (2006).
15. Y. Hotta, A. Nagatsu, W. Liu, T. Muto, C. Narumiya, X. Lu, M. Yajima, N. Ishikawa, K. Miyazeki, N. Kawai, H. Mizukami, and J. Sakakibara, Protective effects of antioxidative serotonin derivatives isolated from safflower against ostischemic myocardial dysfunction, *Mol. Cell. Biochem.*, **238**, 151 (2002).
16. J. S. Roh, J. Y. Han, J. H. Kim, and J. K. Hwang, Inhibitory effects of active compounds isolated from safflower (*Carthamus tinctorius* L.) seeds for melanogenesis, *Biol. Pharm. Bull.*, **27**(12), 1976 (2004).
17. S. M. Jang, A. Ishihara, and K. Back, Production of coumaroylserotonin and feruloylserotonin in transgenic rice expressing pepper hydroxycinnamoyl-coenzyme A: serotonin N-(hydroxycinnamoyl) transferase, *Plant. Physiol.*, **135**(1), 346 (2004).
18. L. P. Kozak and J. T. Jensen, Genetic and developmental control of multiple forms of L-glycerol 3-phosphate dehydrogenase, *J. Biol. Chem.*, **249**(24), 7775 (1974).
19. J. C. Halford, J. A. Harrold, E. J. Boyland, C. Lawton, and J. E. Blundell, Serotonergic drugs : effects on appetite expression and use for the treatment of obesity, *Drugs*, **67**(1), 27 (2007).
20. S. Furukawa, T. Fujita, M. Shimabukuro, M. Iwaki, Y. Yamada, Y. Nakajima, O. Nakayama, M. Makishima, M. Matsuda, and I. Shimomura, Increased oxidative stress in obesity and its impact on metabolic syndrome, *J. Clin. Invest.*, **114**(12), 1752 (2004).