*Molecular and Cellular Biochemistry* **299:** 93–98, 2007.
DOI: 10.1007/s11010-005-9049-7


# Decreased bodyweight without rebound and regulated lipoprotein metabolism by gymnemate in genetic multifactor syndrome animal

Hong Luo,[1-3] Akiko Kashiwagi,[2] Toshiyuki Shibahara[2] and Kazuo Yamada[1]

[1]*Department of Pathophysiological and Therapeutic Science Division of Medical Biochemistry, Faculty of Medicine, Tottori University, Yonago 683-8503, Japan;* [2]*Division of Laboratory Animal Science, Research Center for Bioscience and Technology, Tottori University;* [3]*International Department, The Japan Society for the Promotion of Science (JSPS), Tokyo, Japan*

Published online: 12 May 2006

## Abstract

*Objective*: The aim of this work was to find obesity control method without rebound. In our previous studies, gymnemate extracted from *Gymnema sylvestre*, inhibited oleic acid absorption. The Otsuka Long-Evans Tokushima Fatty (OLETF) rat, a genetic multifactor syndrome model, exhibits progressive overweight, hyperlipidemia and hyperglycemia. The effect of gymnemate on obesity in OLETF was investigated. *Methods*: Three groups were divided ($n = 4$–8): (1) OLETF-gymnemate, gymnema water extract (containing gymnemate) diet (62.5 g/kg) and water (2.5 g/kg) were supplied 2 weeks from 26–28 weeks, following it general diet and water were fed 3 weeks to observe if it rebound, (2) OLETF-control and (3) the counterpart Long-Evans Tokushima Otsuka rats as normal-control. *Results*: With gymnemate treatment, the food and water intake were decreased about 1/3 and 2/3, along with body weight reduced 57.2±6.4 and 75.5±6.3 g during 1 and 2 weeks respectively. In the end of experiment (3 weeks after gymnemate withdrawal), the body weight was decreased to no significant difference with normal-control. The total cholesterol was decreased about 1/3, moreover LDL+VLDL (low-density and very-low-density lipoprotein) cholesterol decreased about 1/2. The proportion of HDL (high-density lipoprotein) cholesterol to the total cholesterol was increased. The serum triglyceride was decreased to the 1/4 of OLETF control. The level of serum cholesterol and triglyceride was no significant difference in gymnemate group with normal group. *Conclusion*: Supplementation with gymnemate promoted weight loss by its ability to reduce hyperlipidemia, which was no withdrawal rebound: an important discovery. Supplementation with gymnemate is a novel therapeutic tool for weight management, especially in multifactor syndrome. (Mol Cell Biochem **299:** 93–98, 2007)

*Key words*: dyslipidaemia, HDL, herb treatment, LDL, multifactor syndrome, obesity

## Introduction

Obesity has become a global health epidemic, which accompanies with dyslipidaemia, insulin resistance, hypertension and arteriosclerosis generally. This association is referred to as the multifactor syndrome, which also names cardiovascular dysmetabolic syndrome, insulin resistance syndrome or syndrome X. Some of them are multiple congenital

*Address for offprints*: Hong Luo, Faculty of Medicine, Tottori University, Yonago 683-8503, Japan (Email: luo_jsps@hotmail.com)

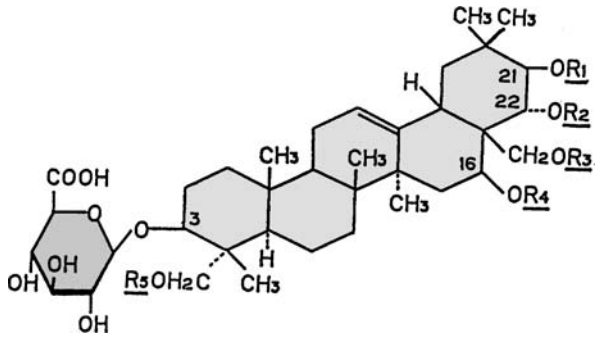


*Fig. 1.* Chemical structure of gymnemate.

anomalies. Diet regimen, bodyweight and lipid control to reduce varies possible complications such as myocardial infarction, fatty liver, microvascular and neurosystem disorder are broadly accepted as basic treatment. The withdrawal rebound could not be ignored in the treatment, because unstable metabolic control is more dangers than persistent obesity. The aim of this study was to find a stable control method without withdrawal rebound in the treatment [1–4].

We have found that gymnemate (GA), a mixture of triterpene of glucuronides (Fig. 1), which was extracted from a traditional herb of *Gymnema sylvestre*, not only inhibited glucose and maltose absorption but also limited oleic acid absorption in small intestine [5–7], which is a good potential candidate.

The Otsuka Long-Evans Tokushima Fatty rat (OLETF), an animal model of multifactor syndrome, exhibits a progressive polyphagia, dislipidaemia, hyperglycemia and rapid body weight gain at last accompaniment of varies system disorder [8–12]. The effects of GA on the body weight, food and water intake, triglyceride, total cholesterol, HDL (high-density lipoprotein) cholesterol, as well as LDL (low-density lipoprotein) and VLDL (very low-density lipoprotein) cholesterol levels in OLETF were investigated during and following it treatment.

## Materials and metods

*Animals*

Male 4 weeks old OLETF and Long-Evans Tokushima Otsuka Rat (LETO) rats that were kindly supplied by Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan), were housed individually in a air condition animal room of Laboratory Animal Science, Research Center for Bioscience and Technology, Tottori University with temperature of 23 °C, humidity of 50% and 12 hours light/dark circles (7 am to7 pm). They had free access to standard food CE-2 (CLEA Japan, INC, Tokyo) and tap water until the experiment start. The care and treatment of the animals conformed to Tottori University guidelines for the ethical treatment of laboratory animals. From 24 to 26 weeks the rats were divided into 3 groups ($n = 4$–8):

(1) GA group in OLETF, gymnema water extract (containing GA) was mixture in diet (62.5 g/kg CE-2) and water (2.5 g/kg) for 2 weeks, following GA withdrawal general diet was fed for 3 weeks to observe if it rebound.
(2) Control of OLETF, standard food and water was fed continuously.
(3) Normal control, the counterpart LETO as normal control. The food and water was same with OLETF control.

To observe if it rebound, the general diet was fed for 3 weeks following the GA feeding period.

*Materials*

Gymnema water extract (containing GA) was kindly supplied by Maruzen Pharmaceuticals Company, Ltd (Osaka, Japan).

*HDL, LDL and VLDL*

The blood samples were collected following anesthetization with intraperitoneal injection of 50 mg/kg body weight sodium pentobarbital (Dainippon Pharmaceutical, Osaka, Japan) at 1:00 to 2:00 pm. The serum was separated by centrifugation at 4 °C. The serum total triglyceride, total cholesterol, LDL + VLDL and HDL cholesterol were measured by commercial kits (Wako, Osaka, Japan).

*Statistical analysis*

Statistical analysis was performed with t test by Microsoft Excel for a Macintosh computer. All data are presented as mean ± SME. $P < 0.05$ was considered to be a significant difference

## Results

*Body weight*

The bodyweight of OLETF was progressive increased. Before GA treatment, the body weight was 630.0 ± 9.5 g. Two weeks later, it increased to 669.0 ± 20 g. In the end of experiment the bodyweight achieved to 680 ± 5.6 g in the OLETF, about 1.5 folds of LETO (480.5 ± 2.2 g). With GA treatment,

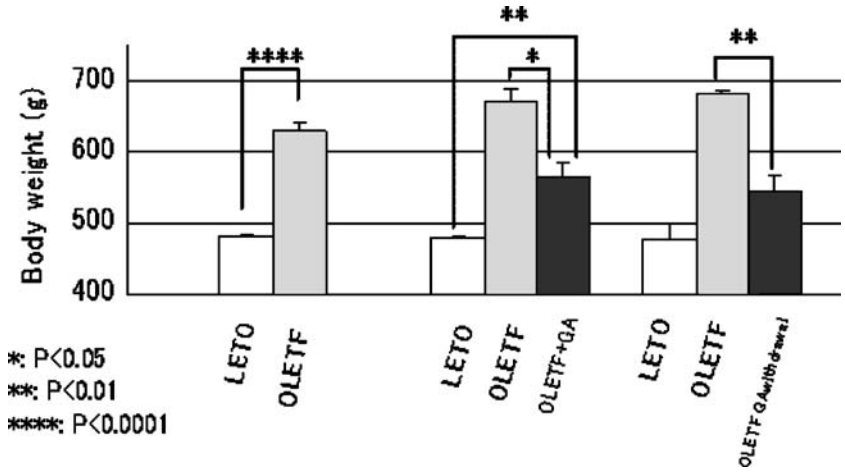


*Fig. 2*. Inhibitory effects of gymnemate on body weight of OLETF. In the OLETF group the bodyweight increased time dependently. In the pre-GA treatment duration (left), the bodyweight was about 150 grams more in OLETF than that in LETO, that was 200 grams after 5 weeks (right). With GA treatment for 2 weeks, the bodyweight was decreased (middle). In the end of experiment (right, 3 weeks after gymnemate withdrawn), the body weight was no significant difference with non-diabetic control.

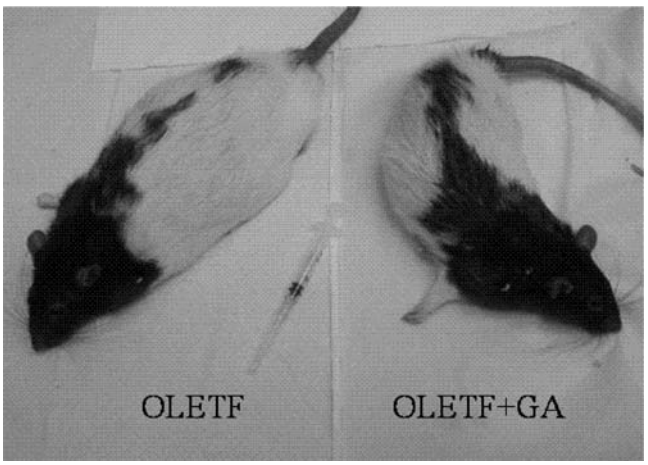


*Fig. 3*. OLETF with or without GA treatment. The photo was taken 3 weeks after GA withdrawal.

the increase was stop not only, but also to the opposite direct: decrease (57.2 ± 6.4 g and 75.5 ± 6.3 g during a week and two weeks respectively). After 3 weeks of GA withdrawal, the body weight was no significant difference with normal control (544 ± 22.8 g *vs*. 475.2 ± 24.3 g) (Figs. 2–3)

*Food and water consumption*

With GA treatment, the food and water consumption in OLETF were decreased about 1/3 and 2/3 that was similar with or even lower than that in LETO (Fig. 4).

*Serum triglyceride*

Serum triglyceride was suppressed by GA. With GA treatment, the serum triglyceride was decreased to about the 2/7 of OLETF control (68.6 ± 11.9 *vs*. 245.6 ± 11.7 mg/dl, $p < 0.0001$), which was no significant difference with normal control (70.0 ± 10.0 mg/dl). After 3 weeks of GA withdrawal, the triglyceride (69.6 ± 5.9 mg/dl) kept no significant difference with normal control (62.9 ± 9.1 mg/dl), however in OLETF control the serum triglyceride achieved 298.3 ± 43.4 mg/dl (more than 4 folds of GA group, $P < 0.0001$, Fig. 5).

*Serum cholesterol*

In the GA group, the serum cholesterol was decreased to about 2/3 of OLETF control (85.0±6.5 *vs*. 128.6±3.0 mg/dl, $P < 0.05$), which was no significant difference with normal control (101.6 ± 1.2 mg/dl). In the end of experiment, the cholesterol in GA group was kept no significant difference with normal control although GA withdrawal (113.0 ± 6.5 mg/dl *vs*. 102.5 ± 8.5 mg/dl, $P > 0.05$), while that in OLETF control achieved 157.5 ± 12.5 mg/ml ($p < 0.05$ *vs*. GA group), moreover LDL+VLDL cholesterol decreased about 1/2 (from 84.7 ± 7.1 to 48.9 ± 10.1 mg/dl, $P < 0.05$).

The percentage of HDL-cholesterol in serum was more than LDL+VLDL-cholesterol 3 weeks after GA withdrawal in GA treatment OLETF, contrasted to OLETF-control. The Containing of HDL and LDL+VLDL-cholesterol in LETO-control was half-and-half simultaneously (Fig. 6).

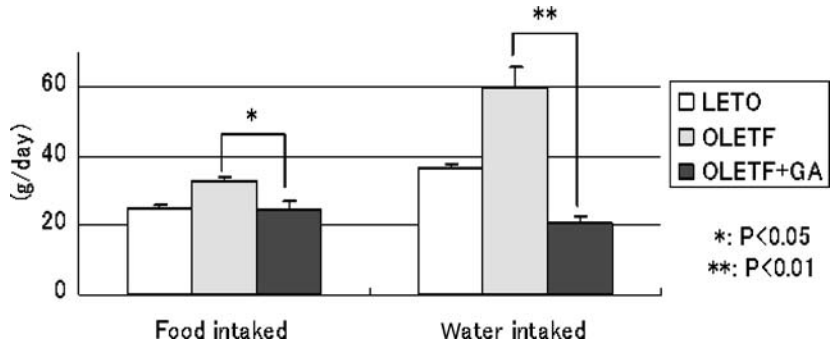


*Fig. 4*. Inhibitory effects of GA on food (left) and water (right) intake.

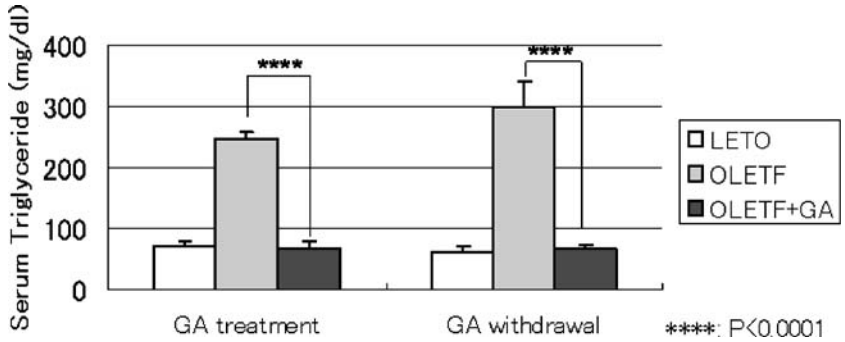


*Fig. 5*. Inhibitory effects of GA on serum triglyceride. The triglyceride increased time dependently in OLETF that was 4 to 5 folds of LETO. With GA treatment, the triglyceride decreased to normal lever (left) and was kept even GA withdrawal (right).

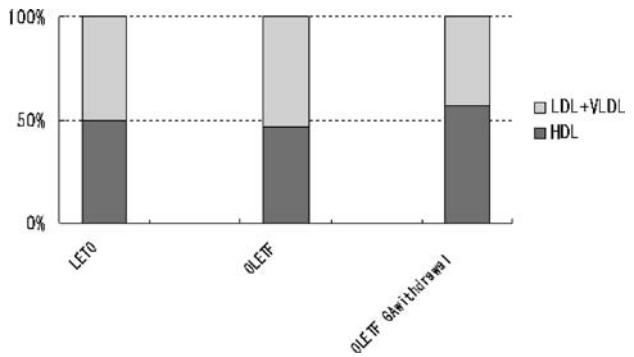


*Fig. 6*. Effects of GA on the containing of serum cholesterol. The proportion of HDL to total cholesterol was increased by GA. The sample was taken 3 weeks after GA withdrawal.

## Discussion

Multifactor syndrome characterized by the clustering of insulin resistance and hyperinsulinemia. It is often associated with dyslipidemia (hyperlipidemia); essential hypertension; abdominal obesity; glucose intolerance or non-insulin-dependent diabetes mellitus (diabetes mellitus, type II); and an increased risk of cardiovascular events [1–4, 8].

OLETF is an inbred strain of Long-Evans rats that was developed from outbred Long-Evans stock in 1983, and the strain was established in 1992 through selective breeding by Tokushima Research Institute (Japan) [12]. The OLETF rats that naturally develop insulin resistance at the age of 16 weeks and type II diabetes at the age of 30 weeks were used as a type II diabetes animal model generally. In fact, besides diabetes OLETF exhibits a progressive polyphagia, dislipidemia, hypertension and rapid body weight gain earlier than the hyperglycemia, which resembles human multifactor syndrome [13–14].

GA is a group of saponin with triterpenoid structure that improves glucose metabolism and diabetes. Although the mechanism of the action of GA has not been fully understood, some of them have been reported such as: relaxation of intestinal smooth muscle and inhibition of intestinal peristalsis, inhibition of potassium channel, regulation of the unstirred layer function, limitation of the paracellular absorption, stimulation of the nitric oxide production [5–7, 15] in intestine, decrease of the gastric inhibitory peptide secretion [16], and increase of the permeability in pancreas [17]. About 6 years ago, we have discovered that Gymnema inhibited the absorption of oleic acid, which suggest the possibility of GA inhibiting lipid absorption [7]. Next year, increase of fecal steroid excretion was reported also by Nakamura *et al*. [18].

Before that time, although blood triglyceride tendency to decease during long time application of GA has been recorded in our group, the effect was believed as a result of improving glucose metabolism [19]. After that few effects on serum lipid have been reported, but all of them are in experiment diabetic or dislipidemia induced by diets or drugs. Recently Preuses *et al*, reported that Gymnema sylvestre extract combination of (−)-hydroxycitric acid (HCA-SX), a natural extract of from *Garcinia cambogia* can improve lipid metabolism and reduce body weight in obese patients, but have not provided the data of GA without HCA-SX [20]. In high fat diet Wistar rat, GA deceases the serum cholesterol as well as triglyceride and increases fecal lipid excretion, but hardly affects bodyweight [21]. Inhibiting the increase of bodyweight by long time GA treatment has been reported by our group [19]. In ob/ob and db/db mice, *Gymnema yunnanense* extract reduces blood glucose and bodyweight [22]. Until now, decrease the bodyweight and lipid simultaneously even after GA withdrawal in progress obesity has not been seen.

In our knowledge, it was 1st study that the improvement of lipid metabolism and decrease bodyweight without rebound in genetic multiply metabolic syndrome animal model. Moreover the proportion of HDL to total cholesterol was increased. The reason for our result may be due to the animal model and method for GA treatment. The doses of GA in our experiment were decided by food and water consumption (the dose of GA in proportion to taking food and water). There was a positive proportion of body weight to taking food and water; as a result the dose was regulated by the degree of polyphagia, an important factor for obesity and dislipidemia. The GA treatment and food intake were simultaneous, that made it possible to more effectively down regulation of lipid especially cholesterol absorption by decrease the oleic acid reabsorption from intestine.

Obesity has become a world health problem. The obese reasons can be divided in to environmental and inheritable factors. The environmental factors include overeat, high fat and high calorie food, lacking of physical activity, sedentary lifestyle, high stress, depression, alcohol and lower socioeconomic status [1–3, 23–29]. Following the clone of *ob* gene in mouse [30], various obesity relative genes have been reported. The obesity in OLETF are due to multiple genetic mutations [31] such as absence of cholecystokinin A receptors (CCK-AR) and dorsomedial hypothalamic over expression of neuropeptide Y (NPY) gene. Accordingly, it overeats and has a decreased responsiveness to ingested fats [9–11, 32]. About 33% of obesity are hereditary obesity [23], which are more difficult to control generally. In our study, the obesity and hyperlipidemia were inhibited by GA in genetic multifactor syndrome animal. It can be suggested that GA not only prevented the sedentary lifestyle obesity but also inhibited the genetic obesity by improved the cholesterol metabolism and inhibited polyphagia.

# Conclusion

(1) GA inhibited the hyperlipidemia and overweight in genetic multifactor syndrome animal.
(2) The inhibitory effects were due to not only directly suppression the intestinal absorption but also suppression innate polyphagia, a key reason of diabetes and obesity.
(3) GA improved lipid metabolism: increasing the proportion of HDL cholesterol to the total cholesterol alone with decreasing total cholesterol and triglyceride.
(4) The inhibitory effects were without rebound after GA withdrawal, an important discovery.
(5) GA may be expected to reduce the complication morbidity and mortality of obesity especially in genetic multifactor syndrome.

# Acknowledgments

This work is supported by The Japan Society for the Promotion of Science (JSPS), Tokyo, Japan with a grant No of 15.03129.

# References

1. Ozsahin AK, Gokcel A, Sezgin N, Akbaba M, Guvener N, Ozisik L, Karademir BM.: Prevalence of the metabolic syndrome in a Turkish adult population. Diabetes Nutr Metab 17: 230–234, 2004
2. Daskalopoulou SS, Mikhailidis DP, Elisaf M: Prevention and treatment of the metabolic syndrome. Angiology 55: 589–612, 2004
3. Hexeberg S, Retterstol K: Hypertriglyceridemia – diagnostics, risk and treatment. Tidsskr Nor Laegeforen 124: 2746–9, 2004
4. Moon YS, Kashyap ML: Pharmacologic treatment of type 2 diabetic dyslipidemia. Pharmacotherapy 24: 1692–713, 2004
5. Luo H, Imoto T, Hiji Y: Inhibitory effect of voglibose and gymnemic acid on maltose absorption in vivo. World J Gastroenterol 7: 270–274, 2001
6. Luo H, Wang LF, Imoto T, Hiji Y: Inhibitory effect and mechanism of acarbose combined with gymnemic acid on maltose absorption in rat intestine. World J Gastroenterol 7: 9–15, 2001
7. Wang LF, Luo H, Miyoshi M, Imoto T, Hiji Y, Sasaki T: Inhibitory effect of gymnemic acid on intestinal absorption of oleic acid in rats. Can J Physiol Pharmacol. 76: 1017–1023, 1998
8. Timar O, Sestier F, Levy E: Metabolic syndrome X: A review. Can J Cardiol. 16: 779–789, 2000
9. De Jonghe BC, Hajnal A, Covasa M.: Increased oral and decreased intestinal sensitivity to sucrose in obese, prediabetic CCK-A receptor-deficient OLETF rats. Am J Physiol Regul Integr Comp Physiol 288: R292–R300, 2005
10. Moran TH: Cholecystokinin and satiety: Current perspectives. Nutrition. 16: 858–865, 2000
11. Bi S, Ladenheim EE, Schwartz GJ, Moran TH: A role for NPY overexpression in the dorsomedial hypothalamus in hyperphagia and obesity of OLETF rats. Am J Physiol Regul Integr Comp Physiol 281: R254–R260, 2001
12. K Kawano, T Hirashima, S Mori, Y Saitoh, M Kurosumi and T Natori: Spontaneous long-term hyperglycemic rat with diabetic complications.

Otsuka Long-Evans Tokushima Fatty (OLETF) strain. Diabetes 41: 1422–1428, 1992
13. Yoshida Y, Ohyanagi M, Iwasaki T: Chronological changes of alpha-adrenoceptor-mediated vascular constriction in Otsuka-Long-Evans-Tokushima fatty rats. Hypertens Res: 26: 559–567, 2003
14. Yagi K, Kim S, Wanibuchi H, Yamashita T, Yamamura Y, Iwao H: Characteristics of diabetes, blood pressure, and cardiac and renal complications in Otsuka Long-Evans Tokushima Fatty rats. Hypertension 29: 728–735, 1997
15. Luo H: Possible participation of NO and EDHF in the relaxation of rat intestinal circular muscle induced by Gymnema water extracts containing gymnemic acids. Yonago Igaku Zasshi 50: 22–31, 1999
16. Fushiki T, Kojima A, Imoto T, Inoue K, Sugimoto E: An extract of Gymnema sylvestre leaves and purified gymnemic acid inhibits glucose-stimulated gastric inhibitory peptide secretion in rats. J Nutr 122: 2367–2373, 1992
17. Persaud SJ, Al-Majed H, Raman A, Jones PM: Gymnema sylvestre stimulates insulin release in vitro by increased membrane permeability. J Endocrinol 163: 207–212, 1999
18. Yumiko Nakamura, Yukari Tsumura, Yasuhide Tonogai and Tadashi Shibata: Fecal steroid excretion is increased in rats by oral administration of gymnemic acids contained in *Gymnema sylvestre* leaves. Journal of Nutrition 129: 1214–1222, 1999
19. Terasawa H, Miyoshi M and Toshiaki Imoto T: Effects of long-term administration of gymnema sylvestre watery-extract on variations of body weight, plasma glucose, serum triglyceride, total cholesterol and insulin in wistar fatty rats. Yonago Acta Medica 37: 117–127, 1994
20. Preuss HG, Bagchi D, Bagchi M, Rao CV, Dey DK, Satyanarayana S: Effects of a natural extract of (-)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and Gymnema sylvestre extract on weight loss. Diabetes Obes Metab 6: 171–180, 2004
21. Shigematsu N, Asano R, Shimosaka M, Okazaki M: Effect of administration with the extract of Gymnema sylvestre R. Br leaves on lipid metabolism in rats. Biol Pharm Bull 24: 713–717, 2001
22. Xie JT, Wang A, Mehendale S, Wu J, Aung HH, Dey L, Qiu S, Yuan CS: Anti-diabetic effects of Gymnema yunnanense extract. Pharmacol Res 47(4): 323–329, 2003
23. Stunkard AJ: Current views on obesity. Am J Med 100: 230–236, 1996
24. Jequier E. Pathways to obesity: Int J Obes Relat Metab Disord 260: S12–S17, 2002
25. Roberts SB, McCrory MA, Saltzman E: The influence of dietary composition on energy intake and body weight. J Am Coll Nutr 2002; 21: 140S–145S, 2002
26. Popkin BM, Paeratakul S, Zhai F, Keyou G: A review of dietary and environmental correlates of obesity with emphasis on developing countries. Obes Res 3: 145S–153S, 1995
27. Campbell I. The obesity epidemic: can we turn the tide?. Heart 89: 35–37, 2003
28. Morley JE: Neuropeptide regulation of appetite and weight. Endocr Rev 8: 256–287, 1987
29. Sobal J, Stunkard AJ: Socioeconomic status and obesity: A review of the literature. Psychol Bull 105: 260–275, 1989
30. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman J M: Positional cloning of the mouse obese gene and its human homologue. Nature 372: 425–432, 1994
31. Nara Y, Gao M, Ikeda K, Sato T, Sawamura M, Kawano K, Yamori Y: Genetic analysis of non-insulin-dependent diabetes mellitus in the Otsuka Long-Evans Tokushima Fatty rat. Biochem Biophys Res Commun 241: 200–204, 1997
32. Schwartz, G J; Whitney, A; Skoglund, C; Castonguay, T W; Moran, T H: Decreased responsiveness to dietary fat in Otsuka Long-Evans Tokushima fatty rats lacking CCK-A receptors. Am J Physiol 277: R1144–R1151, 1999