Nutritional Sciences 9(3), August, 2006, pp.173-178

Wax Gourd Reduces Adipose Depots and Fat Cell Sizes without Affecting the Levels of Leptin in Rats Fed Medium Fat*

Keum Jee Kang§

Department of Food and Nutrition, DukSung Women's University, Seoul 132-714, Korea

The purpose of this study was to investigate the effects of wax gourd on body weight, body fat, triglyceride, leptin and fat cell size in rats fed medium fat. Male Sprague Dawley rats were fed with experimental diet containing total dietary fat at 20% of calorie with wax gourd (WG) for 4 weeks. Forty rats were divided into 4 groups including control, 5, 10, and 15% (w/w) WG supplement. The results showed that weight gain was significantly higher in 5% WG than that in control ($p<0.05$), but was not different among the WG supplement groups. Total fat weight was significantly decreased in both 10 and 15% WG supplement groups than that of control. The levels of triglycerides and free fatty acids in the plasma were significantly decreased, particularly in 15% WG supplement group ($p<0.05$). The levels of leptin was not significantly different among the experimental groups. Fat cell size was significantly decreased in WG supplement groups compared to that of control group ($p<0.05$). Correlation among the parameters demonstrated that weight gain correlated positively with total fat weight, the levels of leptin and triglycerides. Taken these results together, 15% WG supplement diet substantially reduces total fat weight, triglycerides and free fatty acids levels and fat cell size in the rat fed midium fat.

Key words: Wax gourd, Body weight, Leptin, Triglyceride, Fat depots, Fat cell size

Received June 5, 2006; Revised July 20, 2006; Accepted August 7, 2006

INTRODUCTION

Obesity is considered as one of risk factors for many diseases such as diabetes mellitus, hypertension, hyperlipidemia and cardiovascular diseases.[1-3] According to 2001 National Health and Nutrition Survey,[4] it has been reported that 26.3% of Korean people are considered to be obese. Thus, the prevention and treatment of obesity are becoming important issues, and should deserve more attention than they do now.

The increase and decrease in the body fat depend on the number and size of adipocytes.[5] Thus the increases in the number and size of adipocyte lead to the increase in adipose tissues. Once the number and size of fat cells increase, they increase exponentially with the excess energy taken. Thus, it would be difficult to reduce the number of fat cells through the diets and exercise, although it is possible to reduce the size of the fat cells through the same efforts.[6,7]

leptin is reported as closely associated with controling body weight in human.[8] Leptin is produced and secreted in fat cells. The levels of leptin varies according to individual nutritional status. It has been reported that leptin also increases thermogenesis and physical activities, and decreases food intakes and body fat.[8-11]

In obese people, the triglyceride level of the plasma and the activities of lipogenic enzymes in the liver are expected to be high.[12,13] Due to the high levels of activities of lipogenic enzymes, the biosynthesis of fatty acids increases, leading to higher levels of triglyceride in the plasma.[14] Although the levels of free fatty acids released into the blood stream from the fat pads are controlled by lipases present in the adipose tissues, the efflux of free fatty acids is proportional to total lipid contents present in the adipose tissues.[15,16] Therefore, they could increase fat biosynthesis and storage in the liver.

The prevention and medical treatment of various diseases have been possible by using plant resources traditionally without causing any harmful effects although the scientific basis is not established. It is now high time that we should establish any functional roles of plant

* This study was supported by 2005 Research Fund of Institute of Natural Science Research, DukSung Women's University.
§ To whom correspondence should be addressed.
(E-mail : kjkang@duksung.ac.kr)

resources used traditionally at various levels of chemistry and physiology. Wax gourd (WG) is an annual plant and the taste of oval fruits as a pumpkin is good and has been used for folk medicine.[17,18] Phytochemical screening of wax gourd using bioassay-guided separation has shown the presence of 4 triterpenes and 2 sterols together with a flavonoid C-glycoside, an acylated glucose and a benzyl glycoside.[19] It is said that WG leads to warm body temperature and has effect in stimulating urination, alleviating cough, detoxification and constipation.[20] Lim et al.[21] demonstrated the effect of WG on diabetes mellitus. According to the reports from Kang et al.[22] and Hong et al.,[23] WG supplementation reduced body fatness in rats and human, respectively.

In the previous study, we reported the effects of WG on the anti-obesity in rats fed high fat (40%/Kcal). Also, we questioned how the same levels of WG influence on the body fat in rats fed on a medium fat diet (20%). Therefore, we investigated the effects of WG on the anti-obesity in rats fed medium fat diet.

MATERIALS AND METHODS

1. Experimental Design and Diets

Forty male Sprague Dawley rats weighing about 70 g were used in this study. After adapting the rats to the environments and chow diet for one week, they were divided into 4 groups consisting of 10 rats each by the random method and fed with experimental diet for 4 weeks. The experimental diets were composed of 60% of carbohydrate, 20% protein and 20% fat in terms of their calories (Table 1). The calorie and nutrients of the dried wax gourd powder were calculated on the basis of raw WG presented in the Food Composition Table of Korean Dietary Allowances (2000).[24] WG was obtained in a form of freshly-dried powder from a market in Chungdo-gun, Kyungsangbuk-do in 2001. After purchasing the wax gourd powder, it was stored at 4℃ until use. The WG powder was supplemented at 0 (control), 5, 10 and 15% (w/w) of the AIN 93 maintenance diet, respectively. Five percent WG level was determined according to previous reports.[21,23] The rest of groups included 2-fold (10%) and 3-fold of (15%) the concentration.

Table 1. Diet composition of experimental groups (g/kg diet)

Ingredients \ Dietary groups	Control (I)	5% WG (II)	10% WG (III)	15% WG (IV)
Corn starch	385	358	331	304
Casein	200	196	192	188
Dextrinized corn starch	128	128	128	128
Sucrose	97	97	97	97
Beef tallow	72.5	72.5	72.5	72.5
Corn oil	17.8	16.8	15.8	14.7
Fiber	50	38	26	14
Mineral mix[1)]	35	29	23	17
Vitamin mix[2)]	10	10	10	10
L-Cystine	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5
Tert-buthylhydroquinone	0.014	0.014	0.014	0.014
Wax gourd	0	50	100	150

[1)] AIN 93 mineral mixture
[2)] AIN 93 vitamin mixture

2. Sample Collections

After 4 weeks on their respective experimental diets, the animals were fasted for 12 h. The animals were anesthetized with ethyl ether, and blood was collected from the inferior vena cava into test tubes pre-treated with heparin. The blood was centrifuged at 3000×g for 15 min at 4℃ and the plasma was then collected from the supernatant and was stored at -70℃ for further analysis. Epididymal fat pads, abdominal and perirenal fats were removed and washed in saline solution (0.9% NaCl). After washing and blotting the remaining saline for dryness, the pads were weighed. One gram of epididymal fat pads was kept in 4% formalin in phosphate buffered saline (PBS) in order to determine the sizes of fat cells.

1) Body weight, Food Intake and Food Efficiency

Body weights were measured at the same designated time once a week. Food intakes were measured at a fixed time daily and the food efficiency was obtained by dividing the increase in the body weight by total intake during the experimental period.

2) Triglyceride, Free Fatty Acid and Leptin Levels in Plasma

Triglyceride was analyzed by using a kit provided by Youngdong Pharmaceutical Company (Seoul, Korea). Free fatty acid was also analyzed with a kit (Shin Yang Co, Seoul, Korea) using SICDA-NEFAZYNE method. Leptin was measured by using the RIA kit (Linco Research, INC).

3) Size of Fat Cells in the Epididymal Fat Pads

The epididymal fat pads in paraffin were stained with hematoxylin-eosin (HE) after sectioning in 1 mm thickness section, and the images were recorded and analyzed by an Image Analyzer (Bioquant RND, Nashiville, TN, USA) to determine the fat cell size.[25]

3. Statistical Analysis

All the results were analyzed by using the general linear model (GLM) of the Statistical Analysis System (SAS) program. Duncan's multiple range tests were used to determine the statistical differences at $p<0.05$. All results were expressed by using mean ± standard deviation. The correlations between the parameters were tested by Pearson's correlation coefficient.

RESULTS

1. Food Intake, Body Weight Gain and Food Efficiency

Table 2 showed the average daily food intakes, food efficiency and body weight gains of the rats after 4 weeks on their respective diets. Food intake was significantly lower in 15% WG group than those in control and 5% WG groups ($p<0.05$). Food efficiency was the highest in both 10 and 15% WG groups, while it was the lowest in control group. The weight gain was significantly higher in 5% WG (7.42±0.55g) than those in the control group ($p<0.05$).

Table 2. Effects of wax gourd on food intake, body weight gain and food efficiency in rats fed on a medium-fat diet for 4 weeks

Dietary group[1)]	Food intake	Body weight changes			Food efficiency
		Initial	Final	weight gain	
	(g/day)	(g)	(g)	(g/day)	(g/day)
I	17.36±1.18[ab2)]	71.10±3.30[NS]	264.25±15.80[NS]	6.89±0.53[b]	0.090±0.004[c]
II	17.87±1.32[a]	71.50±3.14	279.22±17.51	7.42±0.55[a]	0.103±0.003[b]
III	16.65±0.52[bc]	71.15±2.83	267.85±12.03	7.03±0.41[ab]	0.105±0.003[ab]
IV	16.07±0.92[c]	71.25±2.60	265.40±17.83	6.94±0.56[ab]	0.107±0.004[a]

[1)] I, fat 20Cal%; II, fat 20Cal% + wax gourd 5%;
III, fat 20Cal% + wax gourd 10%; IV, fat 20Cal% + wax gourd 15%
Number of rats: 9~10 in each group
[2)] Values sharing common superscripts in the same column are not significantly different at $p<0.05$

2. Weights in the Epididymal Fat Pads, Abdominal and Perirenal Fat

To analyze internal fats, the epididymal, abdominal and perirenal fats that consist of large proportion of body fat were isolated and measured for weight/100g body weight (Table 3). There was no significant difference among groups in the epididymal fat pads and perirenal fat. However, abdominal fat was significantly reduced in the groups of both 10 (0.69±0.16g) and 15% WG (0.70±0.14g) groups compared to that of control group

Table 3. Effects of wax gourd on the weight of epididymal fat pad, abdominal fat and perirenal fat in rats fed on a medium-fat diet for 4 weeks (g/100g BW)

Dietary groups[1)]	Epididymal fat pad	Abdominal fat	Perirenal fat	Total fat[2)]
I	1.42±0.29[NS]	1.18±0.59[a3)]	0.27±0.10[NS]	2.87±0.77[a]
II	1.55±0.36	0.87±0.23[ab]	0.23±0.05	2.65±0.58[ab]
III	1.35±0.26	0.69±0.16[b]	0.24±0.05	2.27±0.44[b]
IV	1.27±0.20	0.70±0.14[b]	0.23±0.05	2.20±0.26[b]

[1)] I, fat 20Cal%; II, fat 20Cal% + wax gourd 5%;
III, fat 20Cal% + wax gourd 10%; IV, fat 20Cal% + wax gourd 15%
Number of rats: 9~10 in each group
[2)] Total fat includes epididymal fat pad, abdominal fat and perirenal fat
[3)] Values sharing common superscripts in the same column are not significantly different at $p<0.05$

(1.18±0.59g) ($p<0.05$). When all the epididymal, abdominal and perirenal fats were considered together, they were significantly increased in the control group (2.87±0.77g) compared to 10 (2.27±0.44g) and 15% WG (2.20±0.26g) groups ($p<0.05$).

3. The Level of Triglyceride, Leptin and Free Fatty Acids in Plasma

The level of plasma triglyceride, leptin and free fatty acids of rats are shown in Table 4. The level of triglyceride was significantly decreased in wax gourd 15% (42.38±15.93 mg/dl) as compared to other three groups ($p<0.05$). There was no difference among control, 5 and 10% WG groups in the triglyceride level. Plasma free fatty acid concentration was increased in control group than those in all the WG supplement groups. Among the WG supplement groups, it was significantly lower in 15% WG group (331.54±71.32 μEq/L) than those in 5 (444.89±106.32 μEq/L) and 10% WG groups (463.39±86.44 μEq/L) ($p<0.05$). There was no significant difference in the level of leptin among the experimental groups.

Table 4. Effects of wax gourd on the levels of plasma leptin, triglyceride and free fatty acids in rats fed a medium-fat diet for 4 weeks

Dietary groups[1)]	Triglyceride (mg/dl)	Free fatty acid (Eq/L)	Leptin (pg/ml)
I	65.93±22.36[a2)]	591.16±123.47[a]	226.6±96.82[NS3)]
II	71.88±28.18[a]	444.89±106.32[b]	201.89±46.36
III	80.28±23.16[a]	463.39±86.44[b]	180.6±51.50
IV	42.38±15.93[b]	331.54±71.32[c]	185.4±41.59

[1)] I, fat 20Cal%; II, fat 20Cal% + wax gourd 5%;
III, fat 20Cal% + wax gourd 10%; IV, fat 20Cal% + wax gourd 15%
Number of rats: 9~10 in each group
[2)] Values sharing common superscripts in the same column are not significantly different at $p<0.05$
[3)] NS: not significant

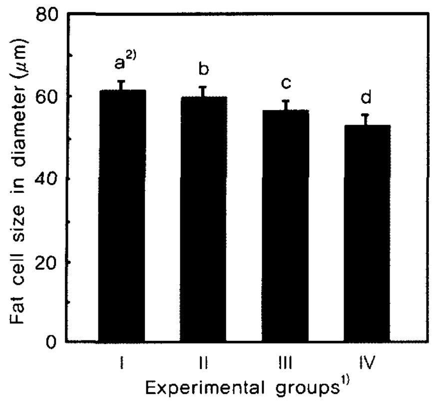

Fig. 1. Effects of wax ground on fat cell size in rats fed a medium-fat diet for 4 weeks

[1)] I, fat 20Cal%; II, fat 20Cal% + wax gourd 5%; III, fat 20Cal% + wax gourd 10%; IV, fat 20Cal% + wax gourd 15% Number of rats: 9~10 in each group

[2)] Different superscripts are significantly different at $p<0.05$

4. Size of Fat Cells

Figure 1 shows the summarized results obtained by histological analysis of epididymal fat pad to determine fat cell size with the image analyzer. It was the largest in control group, and the size of fat cells was significantly decreased according to the increased concentration of WG supplement ($p<0.05$) showing the effects of WG on the fat cell sizing.

DISCUSSION

1. The Effect of Wax Gourd on Anti-Obesity

Recently, researches on the physiological activities of plant materials are being actively carried out. Among the materials, WG is, in particular, known to be effective in diabetes and obesity.[21-23]

We used a medium fat (20%) diet that is the amount generally recommended daily in this study. As shown in Table 2, the food intake was the lowest and food efficiency was the highest in 15% WG supplement group, respectively. However, there was no significant difference found among the groups in the total weight gain. According to Hong's report,[23] the rate of increase in body weight was decreased in the rat fed with 1% WG powder, and there was 3.6 kg of decrease found in body weight in 30 women who have been administered for 28 days with 30% WG powder and 24 g granules consisting of traditional herbal mixture every day. It appears that our results are not consistent with the Hong's results that showed decreasing effect in body weight although we used comparatively higher proportion of WG powder in the diet. This may reflect different fat types used in his (lard) and our (beef tallow) study. In our study, the analysis of adipose depots suggests that there was no significant difference among groups between the epididymal fat pads and perirenal fat in the adipose tissue mass (Table 3). However, abdominal fat was significantly reduced in the groups of both 10 (0.69±0.16 g) and 15% WG (0.70±0.14 g) groups compared to that of control group (1.18±0.59g) ($p<0.05$). When all the epididymal, abdominal and peri-renal fat were added, they were significantly increased in the control group (2.87±0.77g) compared to 10 (2.27±0.44 g) and 15% WG (2.20±0.26 g) groups ($p<0.05$). Hong[23] demonstrated a decreased body weight in the rats fed with WG powder compared to that of control animals, showing a consistent effect of WG on the body fat. In our previous study, it was found that body weight increase was the lowest without any difference in body fat of rats fed 40% fat diet in 15% WG group. In this study, 15% WG supplement showed beneficial effect in decreasing body fat but without affecting body weight (Table 3). This suggests that the inhibitory effects of WG supplement in de novo lipogenesis are more clear when the fat content in the diet is relatively low as in the medium fat diet.[26)]

It has been also reported that higher body weight shows higher level of plasma triglyceride.[27)] The abdominal adipocyte is relatively large and different from the subcutaneous adipocyte. Therefore, abdominal adipocyte maintains high lipoprotein lipase activity, leading to the increase in the blood level of the free fatty acids by active degradation of triglycerides. In turn, the increased free fatty acids are taken by adipocytes, re-accumulating as forms of triglyceride in adipose tissues.[28)] In our study, the level of plasma triglyceride and free fatty acid in 15% WG group was significantly lower than those in both control and other WG groups (Table 4). It was suggested that less lipogenesis occur in 15% WG group due to the lower triglyceride level in the plasma. This is also another evidence for the effectiveness of WG for anti-obesity.

It has been reported that the level of leptin increases when the body fat increases.[11,29)] In our study, the plasma leptin concentration was not significantly affected by WG supplement (Table 4). However, leptin level was slightly lower in 15% WG group than those in the other groups. This may be another close association of 15% WG with less accumulation of fat.

Fat cell size can be an indicator for energy accumulation in adipose tissues.[30)] Although the number of fat cells, once formed, would not decrease by weight reduction, it is known that the size of fat cell could be controlled by the diet.[31] The fact that the fat cell sizes shown by an image analyzer were smaller in 15% WG group than those in other groups (Fig.1) further indicates that wax gourd may decrease triglyceride synthesis, leading to accumulation of less fat.[32] Our results demonstrate that 15% WG powder supplement in the diet are the most effective in inhibiting hypertrophy of fat cells in rats.

2. Correlation of the Parameter

When we considered the correlations between the body fat and the related factors, the weight gain was positively correlated with total fat (epididymal fat, abdominal fat and perirenal fat), leptin and TG ($p<0.01$, Table 5). This suggested that the rate of increase in the body weight becomes high if body fat increases. Also total fat was positively correlated with the leptin level in plasma ($p<0.001$). Although fat cell volume and leptin expression showed a positive correlation,[32] we found no correlation between them in our study (Table 5).

The inhibitory effect of wax gourd on body fat may be caused by either the decreased activities of lipogenic enzymes or the decreased activity of lipoprotein lipase involved in the lipid efflux into tissues or the increased activity of hormone-sensitive lipase. These possible explanations should be addressed in separate experiments to elucidate the biochemical mechanisms involved in the inhibitory roles of wax gourd.

In conclusion, the findings in this study indicate that medium fat diet containing 15% of wax gourd decreased adipose depots and fat cell size. But, wax gourd supplementation did not significantly affect the leptin level. It might be emphasized that anti-obesity effect of WG supplement was more apparent in rats fed medium fat (20%) diet rather than 40% fat diet, and 15% of WG supplement maximized the anti-obesity effect.

Table 5. Correlation coefficients between parameters of rats fed experimental diets for 4 weeks

	Weight gain	Total[1)] fat	Leptin[2)]	Free fatty acid	Tri-glyceride	Fat cell size
Weight gain	.	0.3681*	0.3554**	0.0115	0.4361**	-0.0615
Total fat	.	.	0.6511***	0.2034	0.1650	0.1955
Leptin[1)]	.	.	.	0.1403	0.2468	0.1376
Free fatty acid[1)]	.	.	.	.	0.0192	0.0349
Triglyceridep	.	.	.	.	.	0.1140
Fat cell size	.	.	.	.	.	.

[1)] Total fat include the epididymal, abdominal and perirenal fat
[2)] The levels of leptin, free fatty acid and triglyceride (TG) are obtained from the plasma
*** $p<0.001$
** $p<0.01$
* $p<0.05$

Literature Cited

1) Grundy SM. Multi-factorial of obesity: implications for prevention. *Am J Clin Nutr* 67(suppl):563s-572s, 1998
2) Bray GA. Complications of obesity. *Annals of Internal Medicine* 103:1052-1062,1985
3) Huh KB. Cause of obesity. *Korean J Nutr* 23(5):333-336, 1990
4) National Health and nutrition survey report. Ministry of Health and Welfare, 2001
5) Albu J, Allison D, Boozer CN, Heymsfield S, Kissileff H, Kretser A, Krumhar K, Leibel R, Nonas C, Pi-Sunyer X, Vanltalline T, Wedral E. Obesity Solutions: report of a Meeting. *Nutr Rev* 55(5):150-156, 1977
6) Sisk M, Azin MJ, Hausman DB, Jewell DE. Effect of conjugated linoleic acid on fat pad weights and celluarity in Sprague Dawley and Zucker rats. *FASEB* 3116:A536, 1999
7) Flier JS. The adipocyte:Storage depot or node on the energy information superhighway. *Cell* 80:15-18, 1995
8) Considine RV, Sinha MK, Heiman ML. Serum immunoreactive-leptin concentrations in normal weight and obese humans. *N Engl J Med* 334:292-295, 1996
9) Behme MT. Leptin: Products of the obese gene. *Nutrition Today* 31(4):138-141, 1996
10) Mistry AM, Swick AG, Romos DR. Leptin rapidly lowers food intake and elevates metabolic rates in lean ob/ob mice. *J Nutr* 127:2065-2072, 1997
11) Havel PJ. Role of adipose tissue in body-weight regulation: mechanism regulating leptin production and energy balance. *Proc Nutr Soc* 59(3):359-371, 2000
12) Chan DC, Watts GF, Barrett PH, Mamo JC, Redgrave TG. Markers of triglyceride rich lipoprotein remnant metabolism in visceral obesity. *Clinical Chemistry Feb* 48(2):278-283, 2002
13) Elam MB, Wilcox Hg, Cagen LM, Deng X, Raghow R, Kumar P, Heimberg M, Russell JC. Increased hepatic VLDL secretion, lipoproteins and SREBP-1 expression in the corpulent JCRLA-CP rat. *J Lipid Res* 42(12):2039-2048, 2001
14) Coppack SW, Jenson MD, Miles JM. In vivo regulation of lipolysis in humans. *J Lipid Res* 35:177-193, 1994
15) Jenson MD, Haymond MW, Rizza RA, Cryer Pe, Miles JM. Influence of body fat distribution on free fatty acid metabolism in obesity. *J Clin Invest* 83(4):1168-1173, 1989
16) Bruce R, Godsland I, Walton C, Crock D, Wynn V. Associations between insulin sensitivity and free fatty acid and triglyceride and triglyceride metabolism independent of uncomplicated obesity. *Metabolism* 43(10):1275-1281, 1994
17) Song HS. The Encyclopedia of Crops in Korea. Pulkotnamoo, 1998
18) Ahn DG. The Herb Illustrated-book of Korea. Kyohaksa, 1998
19) Yoshizumi S, Murakami T, Kadoya M, Matsuda H, Yamahara J, Yoshikawa M. Medicinal food stiffs. XI. Histamine release inhibitors from wax gourd, the fruits of Benincasa hispida

cogn. *Cogn Yakugaku Zasshi* 118:188-192, 2001

20) Huh J. The Handbook of Oriental Medicine NamSanDang, 1994

21) Lim SJ, Jeong JG, Kim MW, Choi SS, Han HK, Park JE. Effects of Benincasa hispida intake on blood glucose and lipid level on streptozotocin induced diabetic rats. *Korean J Nutr* 36(4):335-343, 2003

22) Kang KJ, Lim SJ, Jeong JG, Han HK, Choi SS, Kim MH, Kwon SY. Effects of wax gourd on weight, triglyceride, leptin and fat cell size in rats fed on a high fat diet. *Korean J Nutr* 36(5):446-451, 2003

23) Hong SS, Choi SY, Cha SK, Kim HJ, Park HJ, Lee YS. A study on the weight loss effect of wax gourd and the development of processed food from wax gourd. Korea Food Development Institute Report 10, 1999

24) Korean Nutrition Society, Recommended Dietary Allowances for Koreans: 7th ed., 2000

25) Kang KJ, Kim KH, park HS. Dietary conjugated linoleic acid did not affect on body fatness, fat cell sizes and leptin levels in male Sprague Dawley rats. *Nutritional Sciences* 5(3):117-122, 2002

26) Park YH, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. *Lipids* 32(8):853-858, 1997

27) Despres JP. Abdominal obesity as important component of insulin resistant syndrom. *Nutrition* 19:452-459, 1993

28) Jang JY, Choi HJ. Effects of Artenisia Iwayomogi oligosaccharide on the blood lipids, abdominal adipose tissues and leptin levels in the obese rats. *J Korean Nutr* 36(5):437-445, 2003

29) Van Harmelen V, Reynisdottir S, Eriksson P, Thorne A, Hoffstedt J, Lonnqvist F, Arner P. Leptin secretion from subcutaneous and visceral tissue in women. *Diabetes* 47 (6):913-917, 1998

30) Sjostrom L, William-olsson T. Prospective studies on adipose tissue development in man. *Int J Obes* 5:597-604, 1981

31) Williams-roberts S, Worthtngton BS. Nutrition throught the life cycle. Mosby-year book: 7th ed., 2000

32) Guo KY, Halo P, Leibel RL, Zhang Y. Effects of obesity on the relationship of leptin mRNA expression and adipocyte size in anatomically distinct fat depots in mice. *Am J Physiol Regul Integr Comp Physiol* 287:R112-R119, 2004