Cellular and Molecular Biology

Li *et al. Cell. Mol. Biol.*2016, 62 (12): 102-108
ISSN: 1165-158X
doi: 10.14715/cmb/2016.62.12.18

Original Research

Body girdle reduced fat deposition and altered other body parameters in rats

T-R. Li[1#], B-C. Chen[2#], Y-F. Zou[2], J. Zhang[2], W. Zhang[3], B. Dong[4], X. Li[1#*], X-F. Chen[3#*]

[1] Department of Intervention and Vascular Surgery, Peking University Third Hospital, Beijing, China
[2] Department of Dermatology, Peking University Shenzhen Hospital, Shenzhen, China
[3] Biomedical Research Institute, Shenzhen Peking University-the Hong Kong University of Science and Technology Medical Center, Shenzhen, China
[4] National Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China

Abstract: Many women favor in wearing foundation garments to shape their body and show satisfactory figures. However, few investigations have been conducted on the physiological impact of wearing tight garments on the body. In this study, we used girdled rats that were fed with a high fat diet to investigate their physiological condition including alterations in food intake, body weight, fat deposition, and hormone concentrations. Over the experiment period, girdled rats maintained normal plasma and liver cholesterol and triglyceride. Leptin level in girdled rats was significantly lower than that in normal control. The fat tissue of girdled rats was more active in secretion of leptin, which might be mediated by mTOR signaling. Girdled rats showed no difference in hematology analysis during the experiment period. This study showed that a body girdle can significantly reduce fat deposition and alter other body parameters in rats.

Key words: Fat deposition, girdle bound, leptin, mTOR, rat.

Introduction

Obesity is increasingly prevalent in the world. People try to lose extra body weight by various means, such as changing diet and exercise. Women, who wished to show a slimmer body shape and build self-confidence, also turned to wear foundation garments (1). Foundation garments, including corsets, girdles and waist nippers, are a type of lingerie for body shaping. However, there is limited information regarding the physiological impact of wearing tight garments on body composition and functional variables such as food intake, body weight, fat deposition, and hormone concentrations.

The diet-induced fat deposition in bodies can be metabolized through energy expenditure, excretion and lipolysis. Food intake and energy expenditure are regulated by a circulating adipocyte-derived protein called leptin (2). Leptin administration is the reason for both decrease in food intake and increase in thermogenesis (3-5). Energy restriction was related to low leptin levels, while overfeeding increased circulating leptin levels (6,7). Thus, it appears that circulating leptin in humans reflects adipose stores and plays a key role in peripheral lipid metabolism (8-10).

Leptin directly activates resident macrophages to form lipid droplets and enhances eicosanoid production via activation of the PI3K/ mTOR pathway (11). Important roles for mTOR in leptin signaling have been established to control food intake in hypothalamic centers and in peripheral cells to regulate lipid metabolism and inflammation (12). Leptin-induced mTOR activation may be associated with obesity-related pathophysiological conditions (13,14).

In this study, we used girdled rats that were fed with a high fat diet to investigate how physiological condition changed over 2-week periods and 4-week periods. This study demonstrates that a girdle resulted in major changes in rat feeding behavior, body composition and lipid metabolism. During the initial 2 weeks period, the girdled rats showed more food consuming, much lower body fat and much lower grade of liver steatosis than the control animals, which was partly due to increased physical activity in the girdled rats. After 4 weeks, however, the girdled rats seemed to be adapted to wearing the girdle and begun to show increases in adiposity and the associated negative effects, although liver steatosis was still less than that in the control animals.

Materials and Methods

Animal and diets

Sprague–Dawley (SD) male rats (200±12g, n=24), purchased from Beijing Experimental Animal Center (Beijing, China), were caged individually (40 L× 25H ×20W cm^3) at 20°C and 45% humidity. Light time was 12h per day from 8:00 to 20:00. Rats were treated according to the Guidelines of China Department of Agriculture (Beijing, China), with the approval of the China Agricultural University Animal Care and Use Committee. All rats were surgically gonadectomized. After a 5-d recovery, rats anesthetized with 4 mg/100g body weight/i.p. sodium pentobarbital (Sigma-Aldrich,

Received May 5, 2016; **Accepted** October 25, 2016; **Published** October 31, 2016

*** Corresponding author:** Dr. Xiao-Fan Chen, Biomedical Research Institute, Shenzhen Peking University-the Hong Kong University of Science and Technology Medical Center. No. 1120, Lianhua Road, Shenzhen, Guangdong, China. 518036. Email: littlecanva@163.com or Dr. Xuan Li, Department of Intervention and Vascular Surgery, Peking University Third Hospital, No. 49, Huayuan North Road, Beijing 100191, China. Email: xuanli@vip.sina.com.

[#] These authors contributed equally to this work.

St. Louis, MO, US), were fitted with a girdle bound by elastic textile cloth from the neck to the belly (n=12). To prevent tearing and biting, each rat was equipped with a plastic funnel surrounding the head, allowing the rats to eat and drink. A separate group of funneled but not clothed rats were used as controls (n=12).

All rats received a high fat diet comprised of regular chow supplemented with 15% butter and 1.25% cholesterol (wt/wt) as described previously(15). Food and water were available. Body weight was monitored every 3 days, and feed consumption was recorded everyday. Feces were collected over two periods of three consecutive days as described below.

Food was withdrawn for 12h prior to sampling. Six rats from each group were killed after 2 weeks, and the remaining rats were killed after 4 weeks. Rats were anaesthetized to kill by blood sampling via cardiac puncture. Body length and body weight were determined. Tissues were removed, weighed and stored in liquid nitrogen until further analysis. Additionally, liver samples for histology were cut from the same location (0.5 cm from central vein) of each rat and fixed in buffered formalin.

Digestibility

To evaluate digestibility, feces were collected over two periods of three continuous days. The first period was on days 10-12 and the second period was on days 24-26. Feces were collected from 8:00 to 18:00 on the collection day. All feces for individual rats were pooled and stored at -20°C. Fecal samples were dried in a vacuum-freeze dryer (Tofflon Freezing Drying Systems, Shanghai, China), ground through a 1 mm screen, thoroughly mixed and collected for chemical analysis. All analyses were conducted in duplicate and repeated if the results differed by more than 5%. Feed and fecal energy (GE) were measured with an adiabatic oxygen bomb calorimeter (Parr Instruments, Moline, IL). Digestible energy (DE) was then calculated by subtraction of fecal energy from feed energy. Energy digestibility was the ratio of DE to GE.

Animal activity

To evaluate rat activity in the cage, we adopted the method described by Saibaba. We selected six rats from each treatment at the first day of week 2, and week 4 to record their behavior. Cages to be observed were gently eased part-way out of the rack and the rats then were acclimatized to the new position for 30 min. Cameras (Logitech) were fixed on the rack in order to capture the rat behavior. During each minute of observation, the behavior was scored by 1/0 sampling at 30s and 60s intervals. The behavior patterns were modified as follows:

1. Locomote: walk or run around the cage.
2. Sniff: sniff the cage, air or substrate but with the nose above the sawdust.
3. Stand-upright: stand on hind legs in the center of the cage.
4. Stand-stare: stand still with all 4 feet on the ground and apparently stare ahead.
5. Climb: on the bars of the cage or with the fore paws up on the walls of the cage.
6. Dig: scratch or dig in the sawdust.
7. Autogroom: self-groom the body fur, including lick paws if this was immediately followed by autogroom.
8. Sit: crouched in corner of the cage.
9. Feed/drink: take food from the hopper and/or manipulate it with the mouth or lick the water spout.
10. Defecate.

Measurement of plasma and chemical variables

Plasma glucose was determined with Glucose Colorimetric Assay Kit (Ann Arbor, Cayman, MI). Serum insulin and leptin levels were assayed with ELISA kits (Rapidbio, West Hills, CA). Plasma lipids and hepatic triglyceride concentrations were determined with enzymatic methods (BD, San Jose, CA).

Histological analysis

Formalin-fixed tissues were processed, embedded in paraffin, cut into slides and then stained with hematoxylin and eosin. Liver sections were also stained with Milligan's Trichrome Stain to examine fibrosis (Sigma, St. Louis, MO). Lipid accumulation was determined by Oil Red O (ORO) staining of cryo-sectioned tissue slides as previous description (15).

Western blotting

Tissue samples were pulverized in liquid nitrogen and homogenized in ice-cold buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.1 mM EDTA, 1×Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Scientific, US). After centrifuged at 13000 g for 15 min at 4°C, supernatants were sampled. Protein concentration was determined with the Pierce BCA Protein Assay Kit (Thermo Scientific). Samples were subsequently diluted with 5× Laemmli sample buffer and heated in boiling water for 5 min. Equal amounts of total protein were electrophoresed on 10% (w/v) SDS-polyacrylamide gels and then transferred onto a polyvinylidene fluoride (PVDF) membrane (Amersham, US). Membranes were blocked in 5% fat-free dry milk in Tris-buffered saline (TBS) for 3 h , followed incubation with primary antibodies overnight at 4°C. After washed 5 times with TTBS, the membranes were incubated at room temperature for 40 min with secondary antibodies, and developed with Supersignal West Pico detection kit (Pierce, Rock-ford, IL, USA) according to manufacturer's instructions. Signals were detected on Fujifilm LAS-3000. All primary and secondary antibodies were purchased from Cell Signaling Technology.

Hematology

Blood samples were stored with K2-EDTA for less than 1 h before analysis. Blood was assayed with a Mindray Auto Hematology Analyzer (Shenzhen, China), delivering a standard package of blood variables (Invitros 2000) including the red (RBC) and white (WBC) blood cells, platelets (PLT) and the percentages of three WBC subpopulations – lymphocytes (LY), monocytes (MON) and granulocytes (GRA), values of mean platelet volume (MPV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), content of hemoglobin (HGB), red cell distribution width (RDW), plateletcrit (PCT), platelet distribution width (PDW) and hematocrit (HCT) (16).

Subpopulations of white blood cells were recognized according to their size: LY between 30 and 100 fL, MON between 100 and 150 fL and GRA between 150 and 450 fL (Invitros 2000). MPV, PDW, PCT were parameters related to platelets whereas HGB, HCT, MCV, MCH, MCHC, RDW to erythrocytes.

Statistical Analysis

Results are expressed as means ± SEM. In all statistical tests, differences were considered significant at $P<0.05$. One way ANOVA followed by a Bonferroni t-test was performed to compare the difference between groups. All analyses and calculation were conducted with PASW statistics 18.0 (SPSS).

Results

Girdled rats showed increased food intake and increased activity

To evaluate the body overall health condition, we analyzed rat blood by examination of regular hematological parameters, such as the numbers of red blood cells (RBC), white blood cells (WBC), lymphocytes, granulocytes, and platelets (Table 1). The results showed that all hematological parameters were in the normal physiological range regardless of treatment. In week 2, platelets of the girdled rats displayed a slightly increase, while other lymphocyte indices were normal.

The initial body weights of rats were closely similar. However, the body weight of girdled rats decreased significantly by 7% over the first four days while the control rats displayed a slightly increase in body weight (Figure 1A). After day 4 both groups of rats gradually increased body weight at a similar rate (Figure 1B). It indicated that, after an initial period of adaption to the girdle, body weight was no longer influenced by the girdle.

To evaluate the feed digestibility, we collected rat feces in three independent days on Days 10, 11, 12 (period 1) and Days 22, 23, 24 (period 2), analyzed the feed gross energy and fecal energy, and calculated the corresponding digestible energy (DE) and feed energy digestibility. The results showed that during the initial sampling period, girdled rats consumed 16% more feed (Figure 2A) with 18% higher DE (Figure 2B) compared with the controls, but both groups lost similar amounts of energy via the feces and showed similar energy digestibility. As the treatment processed to the second period, none of these parameters showed any differences between the two groups (data not shown). It indicated an adaption period for rats wearing the girdle. These results demonstrate that girdled rats had higher energy intake with a similar metabolic feed efficiency, implying a behavioral difference between girdled rats and controls.

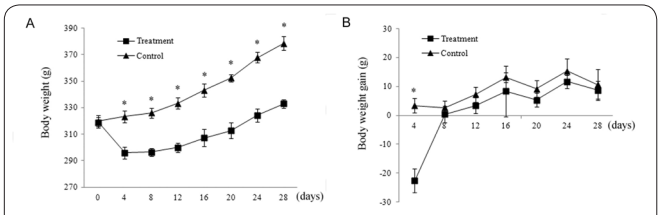

Figure 1. Body weight and body weight gain of girdled rats. (A) The change of body weight of girdled rats. (B) The change of body weight gain of girdled rats. During the experiment period, rat body weight was measured every four days. The results were expressed as mean±SEM (n=12 before week 2, n=6 after week 2). P value of less than 0.05 was considered to be statistically significant.

The behavior of girdled and control rats were markedly different from the control rats (Figure 3). In both week 2 and week 4, girdled rats showed more active behaviors (including locomote, sniff, stand-upright, climb, dig and autogroom) but less sitting in the cages than their control counterparts. Such increased active behaviors could imply increased energy expenditure in the girdled rats.

Effect of girdle on tissue weights

From week 2 to week 4, the control rats were keeping with increased body weight (C2 vs. C1, P=0.003), while

Table 1. Body indices and hormone levels of girdled bound treated rats at week 2 and week 4.

		2 Weeks		4 Weeks	
		Control (C1)	Treatment (T1)	Control (C2)	Treatment (T2)
Ratio of Tissue Weight to BW (%)	Heart	0.27±0.01	0.29±0.01	0.25±0.01	0.29±0.01‡§
	Liver	3.14±0.06	3.21±0.13	2.99±0.09	3.00±0.33
	Stomach	0.46±0.01	0.49±0.01*	0.40±0.01††	0.45±0.01‡
	Thymus	0.040±0.00	0.05±0.00*	0.04±0.01	0.05±0.01
	Kidney	0.60±0.01	0.66±0.03	0.51±0.02††	0.61±0.01‡‡
	Spleen	0.18±0.01	0.18±0.01	0.18±0.02	0.17±0.01
	Pericadial fat	0.23±0.01	0.16±0.02**	0.20±0.02	0.18±0.01
	Mesenteric fat	1.31±0.05	0.73±0.06**	1.10±0.07†	0.83±0.11
	Perirenal fat	1.78±0.21	0.42±0.07**	1.78±0.22	0.83±0.16‡‡§
	Body fat	3.32±0.25	1.31±0.11**	3.07±0.27	1.84±0.26‡
	Bust girth (cm)	15.54±0.17	15.02±0.18	16.03±0.23	15.46±0.18
	Abdomen girth (cm)	17.66±0.25	16.56±0.17*	18.40±0.36	17.26±0.41
	Body length (cm)	22.92±0.30	22.2±0.37	23.69±0.11†	23.03±0.17‡
	Body weight (g)	333.2±4.28	299.9±3.51**	378.4±5.25†	333.0±3.33‡‡
	Blood glucose (mmol/L)	5.82±0.26	5.22±0.29	5.86±0.24	5.52±0.56
	Insulin (mU/L)	0.267±0.037	0.257±0.009	0.560±0.079	0.475±0.105
	Leptin (ng/mL)	1.034±0.203	0.163±0.016**	0.509±0.074	0.308±0.070

Ratio of tissue weight is equal to tissue weight divided by body weight. Data are mean±SEM, n=12 at week 2 and n=6 at week 4. Statistics were performed with ANOVA (SPSS). *: T1 vs C1; †: C2 vs C1; ‡: T2 vs C2; §: T2 vs T1.
$P<0.05$ (*,†,‡,§) and $P<0.01$(**,††,‡‡,§§) were considered significantly different. BW: body weight.

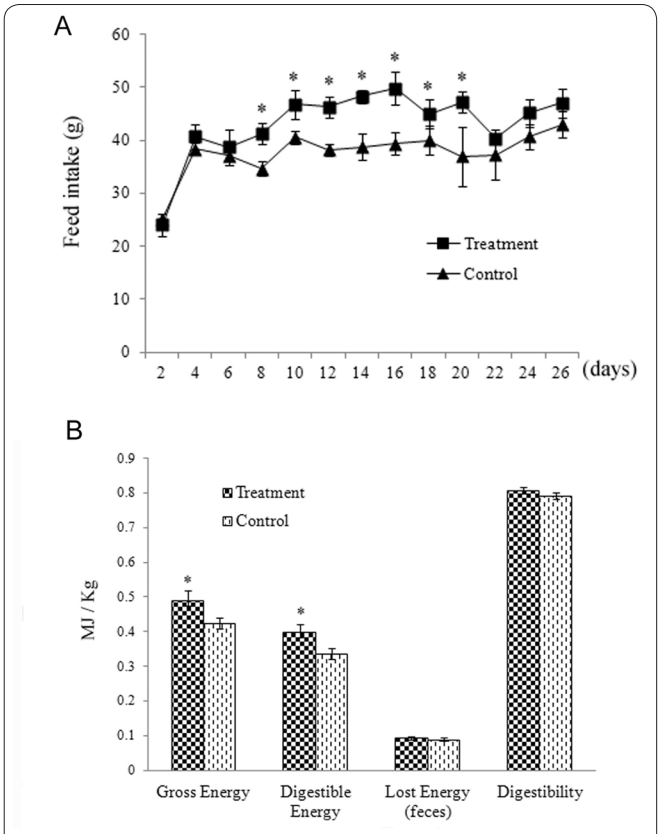

Figure 2. Feed intake and digestible energy of girdled rats. (A) Feed intake of girdled rats. (B) Digestible energy of girdled rats. Feed consumption was recorded every day. To evaluate the feed digestibility, rat feces were collected in three independent days on Days 10, 11, 12 (period 1) and Days 22, 23, 24 (period 2). All analyses were conducted in triplicate. Feed gross energy (GE) and fecal energy were measured with an adiabatic oxygen bomb calorimeter. Digestible energy (DE) was calculated by subtraction of fecal energy from feed gross energy. Energy digestibility was the ratio of DE to GE. The results were expressed as mean±SEM (n=12 before week 2, n=6 after week 2). P value of less than 0.05 was considered to be statistically significant.

the girdled rats showed no increased body weights (T2 vs. T1) (Table 2). Similarly, most tissues of girdled rats showed slightly higher relative weights (T1 vs. C1; T2 vs. C2), which might be due to the stable body weights from week 2 to week 4. The exception was body fat (comprised of pericardial fat (p=0.004), mesenteric fat (p=0.000) and peri-renal fat (p=0.000)) which were lower in the girdled rats at week 2 compared to the control (T1 vs. C1, P=0.000).

Additionally, girdle bound altered the relative weights of specific tissues in time course. For example, in week 2, the relative stomach weight was 7% less than the control (P=0.013), while in week 4, the girdle effect was extended to more than 11% (P=0.026) on this relative stomach weight. The relative weight of kidney was not significantly different from the control in the first 2 weeks but it grew into 16% more than the control (P=0.001) in week 4. It indicated that girdle bound might impact on feed intake and body fluid exchanging via kidney. During the first two weeks of treatment, girdle bound profoundly lowered the pericardial fat by 30% (P=0.000), mesenteric fat by 40% (P=0.000) and peri-renal fat by 76% (P=0.000), thus the total body fat was decreased by more than 50% (T1 vs. C1, P=0.000).

However, as the girdle bound processed to week 4, the relative fat weights were increased slightly (T2 vs. C2, P=0.011), as the pericardial and mesenteric fats showed no statistically different from the control, and the peri-renal fat content grew into 50% of the control (p=0.008). It demonstrated that body girdle was able to effectively control the fat deposition in the early period upon the girdle bound. As the bound restriction processed, girdled bodies turned to grow fat which might be due to the body adaption. Blood glucose and plasma insulin levels were not changed.

Effect of the girdle on on lipid metabolism

After feeding with a high fat diet, control rats exhibit higher plasma and liver cholesterol and triglyceride than girdled rats (Table 3). Liver histology also demons-

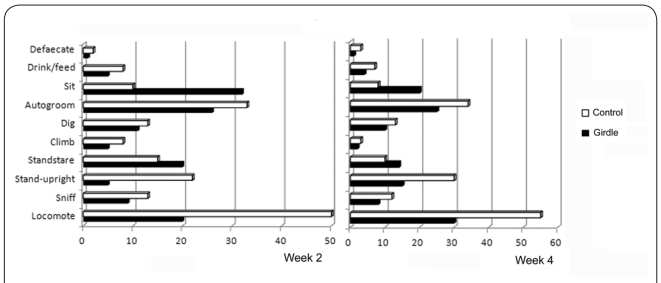

Figure 3. Activity comparison between girdled and control rats. Six rats were selected from each treatment at the first days of week 2 (A) and week 4 (B) respectively to record behavior every 30s for 30 min. The behavior was allocated to one of the ten behavior (locomote, sniff, stand-upright, stand-stare, climb, dig, autogroom, sit, feed/drink, defecate). The behavior was scored 1 or 0 according to the on-time appearance.

Table 2. Serum and liver lipids and serum chemical parameters of girdled bound treated rats at week 2 and week 4.

		2 weeks		4 weeks	
		Control (C1)	**Treatment (T1)**	**Control (C2)**	**Treatment (T2)**
Serum	Total-cholesterol (mmol/L)	2.76±0.21	2.49±0.44*	2.84±0.43	2.08±0.28†
	HDL-cholesterol (mmol/L)	0.94±0.01	1.35±0.04	1.56±0.03	1.82±0.02
	Triglyceride (mmol/L)	2.8±0.2	1.8±0.2*	2.6±0.2	1.6±0.3†
	AST (U/L)	76.4±5.7	82.7±7.4	101.7±9.8	119±9.7
	ALT (U/L)	33.4±2.6	35.1±4.4	57.8±4.9	43.6±5.7
	BUN (mmol/L)	5.69±0.63	7.52±1.09	8.62±0.42	7.49±0.88
Liver	Triglyceride (μmol/g)	12.5±0.4	7.5±0.6*	12.4±0.7	8.1±0.7†
	Cholesterol (μmol/g)	7.1±0.3	7.5±0.4	7.7±0.4	7.1±0.6

Data are mean±SEM, n=12 at week 2 and n=6 at week 4. Statistics were performed with ANOVA (SPSS). *: T1 vs C1; †: T2 vs C2. P<0.05 (*,†) was considered significantly different.

Table 3. Hematology of girdled bound treated rats at week 2 and week 4.

	2 Weeks		4 Weeks	
	Control (C1)	Treatment (T1)	Control (C2)	Treatment (T2)
WBC (10^9/L)	4.16±0.64	4.48±0.59	6.84±0.65	4.74±1.36
Lymph# (10^9/L)	3.1±0.59	3.25±0.57	5.02±0.65	3.44±1.02
Gran# (10^9/L)	0.96±0.14	1.1±0.14	1.64±0.28	1.24±0.29
Lymph (%)	73.16±3.47	70.83±3.51	72.64±4.44	69.56±1.81
Mon (%)	2.7±0.23	2.65±0.23	2.82±0.27	2.52±0.27
Gran (%)	24.14±3.33	26.53±3.35	24.54±2.37	27.8±1.87
RBC (10^{12}/L)	8.71±0.50	7.73±0.20	7.67±0.18	7.68±0.07
HGB (g/L)	141.2±10.5	121.5±3.4	126±2.3	125.6±3.0
HCT (%)	48.02±3.32	41.75±0.71	41.88±0.62	41.62±0.83
MCV (fL)	55.08±0.76	54.175±0.75	54.7±0.67	54.26±0.70
MCH (pg)	16.12±0.30	15.68±0.22	16.4±0.24	16.32±0.35
MCHC (g/L)	293.0±2.1	290.5±3.3	300.2±1.6	301.2±4.9
RDW (%)	15.06±0.21	14.5±0.43	14.68±0.54	14.24±0.24
PLT (10^9/L)	467.6±116.2	858.5±21.4*	982.2±56.5	721±52.0†
MPV (fL)	6.52±0.19	6.3±0.13	6.4±0.10	6.34±0.19
PDW	15.06±0.12	14.9±0.07	14.92±0.06	14.88±0.12
PCT (%)	0.299±0.076	0.541±0.023*	0.602±0.042	0.460±0.046

Data are mean±SEM, n=12 at week 2 and n=6 at week 4. Statistics were performed with ANOVA (SPSS). *: T1 vs C1; #: T2 vs C2. P<0.05 (*,†) was considered significantly different.

Red blood cells (RBC); white blood cells (WBC); platelets (PLT); lymphocytes (LY); monocytes (MON); granulocytes (GRA); mean platelet volume (MPV); mean cell volume (MCV); mean cell hemoglobin (MCH); mean cell hemoglobin concentration (MCHC); hemoglobin (HGB); red cell distribution width (RDW); plateletcrit (PCT); platelet distribution width (PDW); hematocrit (HCT).

trates significantly lower fat deposition in girdled rats (Figure 4). At week 2, control livers displayed mainly macro steatosis which progressed to macro and micro steatosis at week 4. The girdled rats had relative normal livers throughout the treatment period.

Lower circulating leptin concentrations in girdled rats were hugely decreased in at week 2. But in the following two weeks, leptin levels were not different from the control rats. In the first two weeks, the relative adipose tissue weight of girdled rats was 1.310±0.112% (T1) and that of the control rats was 3.323±0.249% (C1). The adipose tissue ratio was 3.23 vs 1.31 (C1 vs. T1, 2.47:1). The ratio of leptin between control and treatment was 1.034 vs 0.163 (C1 vs. T1, 6.34:1). In the week 4, the adipose tissue ratio became 3.071 vs 1.844 (C2 vs. T2, 1.67:1) and the leptin level ratio turned into 0.509 vs 0.308 (C2 vs. T2, 1.65:1). These results showed that the adipose tissue of girdled rats secreted low leptin in the first two weeks, but the treatment adipose tissue became more active in leptin secretion as the girdle bound processed into the week 4,

Leptin secretion is regulated by a number of factors, including activation of mTOR(12). mTOR levels were significantly higher in the peri-renal adipose tissue of girdled rats in week 2 compared with the levels in control rats. However, by week 4, the mTOR levels of girdled rats were the same as those of control rats (Figure 5). It implied that in the early period of girdle bound, the adipose tissues of girdled rats were regulated to secrete leptin, which in turn regulated body weight, food consumption, lipolysis, energy consumption and body temperature. This regulatory effect was subsequently attenuated as girdled bound processed to week 4.

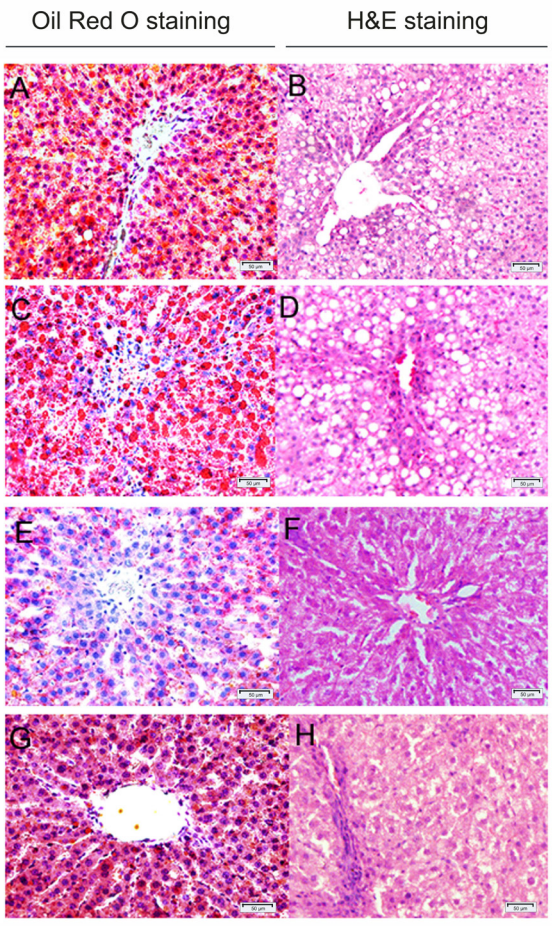

Figure 4. Liver histology of girdle bound treatment on rats. (A~D) Representative liver histology of control rats at week 2 (A,B) and week 4 (C,D). (A,C) specimen stained with oil red; (B,D) specimen stained with H&E. (E~H) Representative liver histology of girdle bound rats at week 2 (E,F) and week 4 (G,H). (E,G) specimen stained with oil red; (F,H) specimen stained with H&E. Girdled rats had maintained relative normal liver histology but the control rats had built steatosis from week 2 and maintained to week 4.

Discussion

Obesity has grown to be a major problem, especially in China where about 20% of the world's obese people

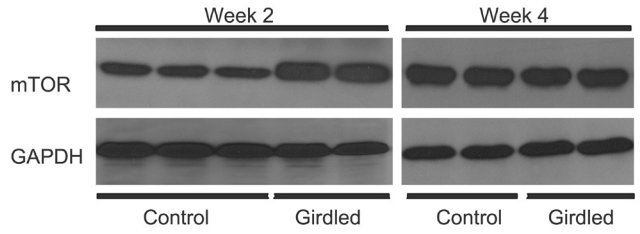

Figure 4. Analysis of mTOR expression in peri-renal adipose tissues of girdle bound treated rats. Western blotting was performed in triplicates and a representative blot is shown.

live (17). Overweight people particularly women have begun to wear body foundation garments to gloss over figure disadvantages and show less appetite. It has been proposed that wearing tight body foundation garments is associated with reduced fat deposition. In this study, we established a rat model wearing a relative flexible cloth girdle while receiving a high fat diet. The head funnel and physical bound did not cause physiological abnormal according to the hematology examination. It suggested that this girdle bound rat model is a valid animal model in the study of girdle effect in physiology and endocrinology, which can facilitate in other studies such as girdle texture and flexibility.

Although girdled rats showed no differences in hematological variables, there were major effects on body composition, liver fatty deposition and circulating lipids. It indicated that the body weight gain of girdled rats similar to that of controls was due to their increased activity and increased food intake. Girdled rats showed much less total body fat than control animals, while circulating leptin concentrations in girdled rats was also lower. It indicated that the girdled rats were becoming accustomized to wearing the girdle.

We found that girdled rats had lower fat deposition compared to the control. And the liver histology confirmed the difference of fat deposition in livers. Girdled rats showed higher feed intake but similar feces-loss-energy. Leptin regulates body weight by regulating hunger, food consumption, lipolysis, energy consumption and body temperature (18). It was not surprisingly that girdled rats showed lower leptin levels compared to the control since leptin is predominantly secreted by adipose tissues. However, the fat tissue of girdled rats showed less active in leptin secretion. The secretion of leptin is regulated by multiple factors, and the intrinsic regulatory mechanism is not fully understood. The well established intracellular mediators are mTOR, ATP, Ca^{2+} etc (19). In week 2, mTOR expression was profoundly elevated in adipose tissues of girdled rat, while this augment disappeared in week 4. We do not understand the reasons that girdle bound increased mTOR expression in adipose tissues which subsequently contributed to the leptin secretion, but at least it demonstrated that physical body bound indeed triggered a systemic hormonal alteration which induced the downstream intracellular signal pathway to cascade this signaling.

In a word, girdle bound rat model is a valid animal model in study of girdle effect in physiology and endocrinology, which can significantly reduce fat deposition and altered other body parameters in rats. The study indicates that the wearing of tight foundation garments may have important physiological consequences for women but also that any such consequences may be limited in duration as the women adapts to wearing the garment.

Acknowledgments

This work was supported by the Fundamental Research Funds for the Central Universities (No.212QJ097), National Natural Science Foundation of China (81402600), Shenzhen Research Grant (JCYJ20150403110829622), and the Scientific and Technical Supporting Programs (2012BAD51G00). We thank Dr. Phil A. Thacker for the language revision of this manuscript.

References

1. Phelan ST. Fads and fashions: the price women pay. Primary Care Update for OB/GYNS 2002; 9: 138- 143.
2. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425-432.
3. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 1995; 269: 546-549.
4. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995; 269: 540-543.
5. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 1995; 269: 543-546.
6. Kolaczynski JW, Considine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, et al. Responses of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. Diabetes 1996; 45: 1511-1515.
7. Wisse BE, Campfield LA, Marliss EB, Morais JA, Tenenbaum R, Gougeon R. Effect of prolonged moderate and severe energy restriction and refeeding on plasma leptin concentrations in obese women. Am J Clin Nutr 1999; 70: 321-330.
8. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, et al. Identification and expression cloning of a leptin receptor, OB-R. Cell 1995; 83: 1263-1271.
9. Sobhani I, Bado A, Vissuzaine C, Buyse M, Kermorgant S, Laigneau JP, et al. Leptin secretion and leptin receptor in the human stomach. Gut 2000; 47: 178-183.
10. Fruhbeck G, Gomez-Ambrosi J, Martinez JA. Pre- and postprandial expression of the leptin receptor splice variants OB-Ra and OB-Rb in murine peripheral tissues. Physiol Res 1999; 48: 189-195.
11. Maya-Monteiro CM, Almeida PE, D'Avila H, Martins AS, Rezende AP, Castro-Faria-Neto H, et al. Leptin induces macrophage lipid body formation by a phosphatidylinositol 3-kinase- and mammalian target of rapamycin-dependent mechanism. J Biol Chem 2008; 283: 2203-2210.
12. Cota D, Proulx K, Smith KA, Kozma SC, Thomas G, Woods SC, et al. Hypothalamic mTOR signaling regulates food intake. Science 2006; 312: 927-930.
13. Ren J. Leptin and hyperleptinemia - from friend to foe for cardiovascular function. J Endocrinol 2004; 181: 1-10.
14. Wang Z, Jiang T, Li J, Proctor G, McManaman JL, Lucia S, et al. Regulation of renal lipid metabolism, lipid accumulation, and glomerulosclerosis in FVBdb/db mice with type 2 diabetes. Diabetes 2005; 54: 2328-2335.
15. Huang C, Qiao X, Dong B. Neonatal exposure to genistein ameliorates high-fat diet-induced non-alcoholic steatohepatitis in rats. Br J Nutr 2011; 106: 105-113.
16. Britzina ALYDN. Method to test influence of bio-implants on

blood cells by consecutive measurements. Environ Exp Biol 2010; 2010: 107-110.
17. Saibaba P, Sales GD, Stodulski G, Hau J. Behaviour of rats in their home cages: daytime variations and effects of routine husbandry procedures analysed by time sampling techniques. Lab Anim 1996; 30: 13-21.
18. Li MD. Leptin and beyond: an odyssey to the central control of body weight. Yale J Biol Med 2011; 84: 1-7.
19. Szkudelski T. Intracellular mediators in regulation of leptin secretion from adipocytes. Physiol Res 2007; 56: 503-512.