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Nutritional Regulation of Plasminogen Activator Inhibitor-1, Leptin and Resistin Gene Expression in Obese Mouse*

Hyun-Jung Lee, Jeong-Lye Yang[1], Young Hwa Kim[1] and Yangha Kim[2§]

Department. of Food and Nutrition, Changwon University, Changwon 641-773, Korea
[1]Research Institute of Human Ecology, Changwon University, Changwon 641-773, Korea
[2]Department of Food and Nutritional Sciences, Ewha Womens University, Seoul 120-750, Korea

PAI-1 (plasminogen activator inhibitor-1), leptin, and resistin are synthesized and secreted by fat cells of rodents and have recently been postulated to be an important link to obesity. This study was conducted to identify the nutritional regulation of PAI-1, leptin, and resistin gene expression in *ob/ob* mice. The mice were divided into four groups according to nutritional status: control, 48 hour fasting, 48 hour-fasting/12 hour-refeeding, and 48 hour-fasting/24 hour-refeeding. The mRNA levels of each peptide were measured by semi-quantitative RT-PCR. In visceral fat tissue, the level of PAI-1 mRNA increased markedly when 48h-fasted animals were refed with a high carbohydrate-low fat diet. However, fasting/refeeding did not appreciably change PAI-1 mRNA levels in subcutaneous fat tissue. Similar results were obtained for resistin mRNA levels in both types of fat tissues. These findings suggest that visceral adipose tissue might be more sensitively involved in the nutritional regulation of PAI-1 and resistin gene expression compared to subcutaneous fat tissue. The level of leptin mRNA decreased markedly in the 48h-fasted animals, and increased markedly when 48h-fasted animals were refed with a high carbohydrate-low fat diet. The nutritional regulation of leptin mRNA showed similar patterns in both types of fat tissues. In conclusion, the nutritional regulation of gene expression encoding PAI-1, resistin, and leptin from adipocytes may vary according to the type of adipose tissue.

Key words : plasminogen activator inhibitor-1, leptin, resistin, nutrition, gene expression

INTRODUCTION

According to the recent National Health and Nutrition Examination Survey,[1)] it was reported that 23% of Korean people were obese. This obesity can be defined as a positive energy balance, caused by an excessive accumulation of fat tissue where surplus energy is changed into fat through energy intake being higher than energy consumed. Especially, as people get older, the frequency of obesity becomes greater because, as physical movement lessens, energy consumption decreases compared to energy intake. Obesity can be divided into upper-body (central fat deposition) and lower-body obesity, according to the location of fat distribution in the body. Upper-body obesity indicates the deposition of fat in the abdomen; this is referred to as the 'apple-shaped' body and it is frequent in male obesity. Central fat deposition has a closer association than lower-body obesity with the diseases of adults such as diabetes, hypertension and cardiovascular disease. So it can be postulated that abdominal fat is one of the risk factors that threatens a healthy and high-quality life-style.[2)]

The adipose tissue is known as the tissue that has a simple function: that of storing excess energy as fat and making this available as energy when needed by the body. However, recent research has shown that fat cells secrete substances such as PAI-1 (plasminogen activator inhibitor-1), leptin, TNF-α, adipsin and resistin, indicating that adipose tissue has an additional endocrine function by secreting substances controlling energy balance, insulin resistance and the immune system.[3,4)] The amount of fat accumulation in the adipose tissue appears to control the level of gene expression of the above proteins, resulting in the energy balance being controlled by the level of exertion.

In 1991, Sawdey[5)] found that PAI-1, one of the risk factors for cardiovascular disease, was synthesized and secreted by the mammary adipose tissues. Particularly in humans, PAI-1 was found to be more actively synthesized in the visceral adipose tissue than in subcutaneous adipose tissue. Weight loss resulting from energy intake reduction did not change the PAI-1 level in the subcutaneous adipose tissue but decreased the

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§ To whom correspondence should be addressed.

PAI-1 level in visceral adipose tissue.[6] The above results showed that the PAI-1 in the visceral adipose tissue might be controlled more closely compared to the subcutaneous adipose tissue; however, no study has been conducted of how PAI-1 synthesis is controlled by diet.[7-9]

Recently, the discovery of the *ob* gene and 'leptin' protein coded by this gene opened up a new possibility for understanding the physiological mechanisms of obesity.[10] In obese mice (*ob/ob* mice), the production of mutant leptin mRNA was found to be increased and this resulted in the repression of secretion of normal leptin into the blood. When *ob/ob* mice were treated with leptin, food intake decreased, and increases in energy consumption resulted in reductions in body weight and body fat. These results suggest that leptin has an endocrine function in controlling body fat through changes in food intake and energy balance.[11,12] Kennedy *et al.*[13] have suggested that increases in the size of adipose tissues results in the sending of satiety signals to the brain which reduces eating, and consequently energy intake is reduced. Earlier studies on humans and animals have shown positive correlations among fat volume, blood leptin and leptin mRNA in adipose tissue. The decreased appetite and energy consumption of the *ob/ob* mouse brought about by the leptin treatment suggests the possibility that leptin might be a satiety signal that travels from adipose tissue to the brain.

In addition, resistin is a 12.5-kDa sized cystein-rich protein which is secreted by adipose tissue.[3] Resistin is also called ADSF (adipocyte-specific secretary factor), and, true to its name, it is only expressed in adipose tissue. Kim *et al.*[14] reported that resistin gene expression was substantially increased by the differentiation of fat cells. Also, the amount of resistin mRNA decreased substantially in fasting or diabetes animals, and increased substantially through refeeding or insulin treatment.[14] These results show that the expression of resistin is related to adipogenesis. Steppan *et al.*[3] reported similar experimental results to Kim[14]; that is, when animals are fasted, serum resistin and the resistin mRNA levels in adipose tissues have markedly decreased, and have dramatically increased by refeeding. Also, levels of serum resistin decreased when animals were treated with the anti-diabetic drug, thiazolidinediones (TZDs).

Given the high rate of synthesis of PAI-1 in adipose tissue, the high PAI-1 concentration in cardiovascular disease, and correlations between hyperinsulinemia and PAI-1, it can be supposed that PAI-1 must be related to fat accumulation and insulin resistance as well as to fibrinolysis inhibition. The diseases of human adults such as diabetes and cardiovascular disease are known to have a close association with abdominal obesity, and therefore can be at least partly controlled through healthy diets. Therefore, it is important to study how dietary patterns regulate PAI-1 synthesis, in order to prevent abdominal fat accumulation, insulin resistance and cardiovascular disease. This study was conducted to investigate the nutritional regulation of the gene expression of PAI-1, leptin, and resistin in *ob/ob* mice.

MATERIALS AND METHODS

Animals and Diets

Obese mice (C57BL/6J, 8 weeks-old, Charles River, USA) were housed individually in a controlled environment: at a temperature of 22 ± 2℃, a relative humidity of 55 ± 5%, and a 12-hour light cycle (the ligh-period was 06:00-18:00h). All mice were fed with standar diet (Purina, USA) for a 10 day stabili- zation period. Then the mice were divided into 4 groups according to nutritional status: a control group, a 48h-fasting group (fasting group), a 48h-fasting/ 12h-refeeding group (12h-refeeding), and a 48h-fasting/ 24h-refeeding group (24h-refeeding). The refeeding groups were fed with a high carbohydrate-low fat diet (TD 8812, Teklad, USA) to induce efficient lipogenesis after fasting. The composition of the high carbohydrate-low fat diet was as follows: casein 200g/kg diet, sucrose 438g/kg diet, corn starch 150g/kg diet and corn oil 100g/kg diet. Mice were given free access to food and water for the experimental period.

Collection of samples

The animals were anesthetized with ether, and the epididymal and subcutaneous fat pads were carefully dissected out. These adipose tissues were frozen in liquid nitrogen and stored at -70℃ until analysis.

Total RNA extraction

Total RNA was extracted using a RNAwizTM kit (Ambion, USA). The RNA concentration of adipose tissue was determined spectrophotometrically.

Semiquantitative reverse transcriptase-polymerase chain reaction

The levels of PAI-1, leptin, and resistin mRNA were determined by the semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). The ß-actin was used as a control housekeeping gene.

Reverse transcription (RT) was performed using total RNA (4 μg) in a final volume of 20$\mu\ell$ solution (pH 8.3) containing 5X buffer (250 mM Tris-HCl; 375 mM KCl; 15 mM $MgCl_2$; and 50 mM DTT; Promega, USA), 1 mM dNTPs, 200 U moliney leukemia virus reverse transcriptase (Promega, USA), and 30 pmole oligo dT_{19} (Promega, USA). The reaction mixture was incubated at

37℃ for 1h and then at 75℃ for 15min. The first strand cDNA for ß-actin was diluted serially. The amount of cDNA for each sample was determined by the ß-actin reference. The cDNA was mixed with 10X Taq polymerase buffer, 0.2mM dNTPs, 0.125U Super Taq polymerase (Super-Bio, Korea), and 0.25㎛M forward and reverse primers. PCR amplification was carried out on an automated DNA thermal cycler (Applied Biosystems, GeneAmp® PCR System 2700, USA).

The sequences of the forward primers were as follows : PAI-1, 5-ATG GAA GAC CCC TTT CTT AG-3 ; resistin, 5-CTA TTT TCA ACC AGA GGC AC-3 ; leptin, 5-TCT ATC AAC AGG TCC TCA CC-3 ; and ß-actin 5-GGA CCT GAC AGA CTA CCT CA-3. The sequence of the reverse primers were as follows : 5-CCT TCC ATT GTC TGA GT-3 ; 5-TCC TTC CAC CAT GTA GTT TC-3 ; 5-ACT GTT GAA GAA TGT CCT GC-3 ; and 5-GTT GCC AAT AGT GAT GAC CT-3.

The sequence for temperature cycling was as follows : 95°℃ for 5 min; denaturation at 95℃ for 15sec; annealing at 50℃ for 30sec employing 25 cycles for PAI-1, 27 cycles for resistin and leptin, and 20 cycles for ß-actin; extension at 72℃ for 40sec; and then a final extension at 72℃ for 10min.

RESULTS AND DISCUSSION

The regulation of PAI-1, leptin and resistin gene expression induced by fasting and fasting/refeeding in the adipose tissues of *ob/ob* mice was found to be as follows. In the epididymal fat tissue, PAI-1 gene expression did not differ between the control and the fasting groups, but was increased in the refeeding groups compared to the control and fasting groups; the highest PAI-1 gene expression was found in the 24h refeeding group (Fig 1A). The fact that the level of PAI-1 mRNA was dramatically increased by refeeding with a high carbohydrate-low fat diet after fasting suggests that the expression of PAI-1 is related to fat synthesis. In a study of non-obese and obese humans,[8] adipocyte PAI-1 mRNA levels were twice as high in the obese group and PAI-1 secretion from adipose tissue was higher in the obese group; also, PAI-1 secretion was related to lipid content and to the volume of fat cells. In an experiment with mice,[15] the results suggested that adipose tissue may be an important contributor to increases in PAI-1 levels observed in the plasma under obese conditions. The results of a study by Samaid et al.[16] support our results as they reported that PAI-1 mRNA was dramatically up-regulated in adipose tissues from *ob/ob* mice compared with lean mice. However, in our study, the level of PAI-1 mRNA in the subcutaneous adipose tissue of the 24h refeeding group was a little higher than in the other groups; the remaining groups showed no differences (Fig 1B). These results suggest that the regulation of PAI-1 gene expression by nutritional status might be more sensitive in epididymal adipose tissue than in subcutaneous adipose tissue, and this corresponds to other experimental results in humans. Bastelica *et al.*[17] reported that levels of PAI-1 mRNA were higher in the visceral fat depot than in the subcutaneous fat depot, and suggested that PAI-1 expression might be more active in the visceral fat depot.

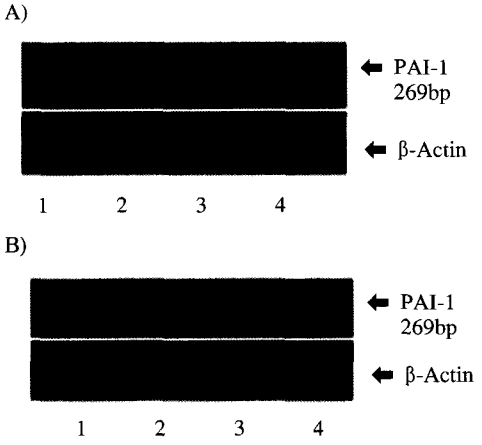

Fig 1. Semi-quantitative RT-PCR of PAI-1 from eididymal A) and subcutaneous B) fat tissues. The ß-actin amplified from the same samples was also shown. Lane 1, normal diet; Lane 2, 48h-fasting; Lane 3, 48h-fasting/12h-refeeding ; Lane 4, 48h-fasting/24h-refeeding.

Obesity can be divided into lower-body type obesity which is related to adipocyte proliferation and upper-body type obesity which is related to adipocyte hypertrophy. Our results suggest that PAI-1 expression is highly related to abdominal obesity which is considered as a adipocytes hypertrophy type obesity. Abdominal obesity is one of the risk factors for cardiovascular disease. Thus, it can be postulated that the higher risk of cardiovascular disease in abdominally-obese persons is due to increases in PAI-1 gene expression in visceral adipose tissue.

Our study found that the level of resistin mRNA increased in epididymal fat tissue by refeeding with a high carbohydrate-low fat diet after fasting (Fig 2A). Not only PAI-1 gene expression, but also resistin gene expression was increased dramatically by refeeding, and these results could explain the relationship observed between resistin gene expression and lipogenesis. It was reported that, when rats were fasted, the serum resistin concentration and the resistin mRNA in the adipose tissue were significantly decreased, and were dramatically increased by refeeding.[14] It can be suggested that blood glucose concentration might be a

more important factor for regulation of resistin gene expression and the rate of lipogenesis, because the level of resistin mRNA in the 24h refeeding group was not higher than that in the control group. McTernaa *et al.*[18] reported that the resistin protein expression in abdominal fat depots was increased compared with the thigh and breast fat depots.

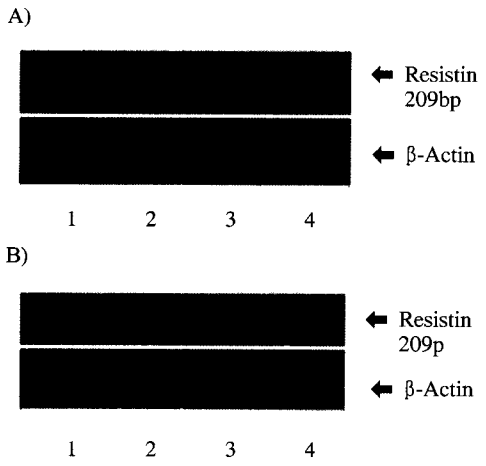

Fig 2. Semi-quantitative RT-PCR of resistin from eididymal A) and subcutaneous B) fat tissues. The β-actin amplified from the same samples was also shown. Lane 1, normal diet ; lane 2, 48h-fasting ; lane 3, 48h-fasting/12h-refeeding ; lane 4, 48h-fasting/24h-refeeding.

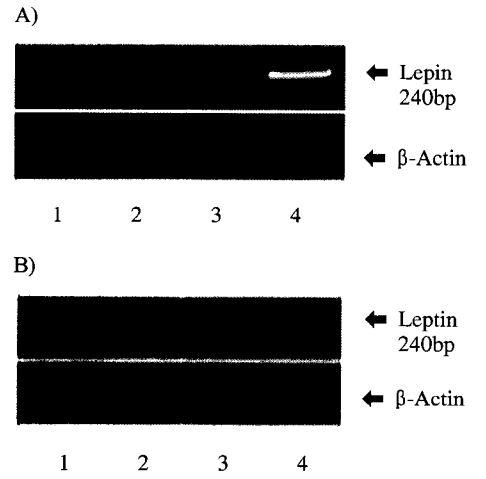

Fig 3. Semi-quantitative RT-PCR of leptin from eididymal A) and subcutaneous B) fat tissues. The β-actin amplified from the same samples was also shown. Lane 1, normal diet ; lane 2, 48h-fasting ; lane 3, 48h-fasting/12h-refeeding ; lane 4, 48h-fasting/24h-refeeding.

It has also been reported that resistin gene expression was regulated by hormones and nutrition in 3T3-L1 adipocytes[19]; the levels of resistin mRNA were significantly up-regulated by high glucose concentrations, and dexamethasone increased both resistin mRNA and protein by 2.5- to 3.5-fold in 3T3-L1 adipocytes. Furthermore, the same study suggested that glucose and dexamethasone affect insulin sensitivity and fat tissue mass, in part by altering the expression and eventual secretion of resistin from adipocytes. Steppan *et al.*[3] reported that circulating resistin levels were decreased by the anti-diabetic drug rosiglitazone, but increased in diet-induced obesity in *ob/ob* mice. These results showed that insulin-stimulated glucose uptake was neutralized by the action of resistin, impling a potential link of resistin between obesity and diabetes.

In subcutaneous adipose tissue, the levels of resistin increased by refeeding with a high carbohydrate-low fat diet after fasting (Fig. 2B). However, in subcutaneous adipose tissue, the level of resistin mRNA in the 24h refeeding group was only a little higher compared to the control group. These results suggest that the regulation of resistin gene expression by nutritional status might be more sensitive in epididymal adipose tissue than in subcutaneous adipose tissue, similar to the case of PAI-1.

The levels of leptin mRNA were highest in the 24h refeeding group and lowest in the fasting group in both epididymal and subcutaneous adipose tissues (3A, 3B). Frederich *et al.*[20] reported that high-fat diets evoke sustained increases in circulating leptin concentrations and in leptin mRNA levels in adipose tissue, and this is attributed to increased amounts of body fat. In human studies[21-23], the levels of leptin mRNA were higher in obese women than in non-obese women, and starvation reduced the leptin mRNA level in abdominal adipose tissues in obese women. These results showed a similar regulatory pattern for leptin gene expression to what we found in our experiments. The restriction of carbohydrates might cause decreases in leptin production, and especially it can be suggested that the fasting condition might work as a key signal for decreases in plasma insulin and leptin. David *et al.*[24] suggested that total adipose mass rather than meal consumption or dietary energy source might be related to leptin level. Also, Ahren *et al.*[25] reported that plasma leptin levels increased when *ob/ob* mice were treated with a high fat diet, implying that leptin gene expression might control body energy balance according to body fat content status.

CONCLUSION

The levels of PAI-1 and resistin mRNA were increased in the epididymal adipose tissues of *ob/ob* mice by refeeding them with a high carbohydrate-low fat diet after 48h fasting; however, the stimulatory effects of this nutritional regime had little effect on resistin gene expression, and had no apparent effect on PAI-1 gene expression, in subcutaneous adipose tissue. These find-

ings suggest that visceral adipose tissue might be more involved in the nutritional regulation of PAI-1 and resistin gene expression compared to subcutaneous adipose tissue. On the other hand, the level of leptin mRNA decreased more markedly in 48h fasting animals than in control animals in both epididymal and subcutaneous adipose tissues. Refeeding markedly in- creased the level of leptin mRNA in both types of adipose tissue. The nutritional regulation of gene expression encoding PAI-1, resistin, and leptin which are secreted from adipocytes may therefore vary according to the type of adipose tissue.

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