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

Hyun-Jung Lee, Jeong-Lye Yang, Young Hwa Kim and Yangha Kim

Department of Food and Nutrition, Changwon University, Changwon 641-773, Korea

1Research Institute of Human Ecology, Changwon University, Changwon 641-773, Korea

2Department 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, 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.

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, adipin and resistin, indicating that adipose tissue has an additional endocrine function by secreting substances controlling energy balance, insulin resistance and the immune system. 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 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

*This research was supported by grants from Ministry of Health & Welfare (01-PJ1-PG3-22000-0015).

Accepted: May 9, 2003

1To whom correspondence should be addressed.
PAI-1 level in visceral adipose tissue. 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. 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. 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. Kennedy et al. have suggested that increases in the size of adipose tissues result in the sating of saticity 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 saticity signal that travels from adipose tissue to the brain.

In addition, resistin is a 12.5-kDa sized cysteine-rich protein which is secreted by adipose tissue. Resistin is also called ADSF (adipocyte-specific secretory factor), and, true to its name, it is only expressed in adipose tissue. Kim et al. 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. These results show that the expression of resistin is related to adipogenesis. Steppan et al. reported similar experimental results to Kim; 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°C, a relative humidity of 55 ± 5%, and a 12-hour light cycle (the light-period was 06:00-18:00h). All mice were fed with standard diet (Purina, USA) for a 10 day stabi lization 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°C until analysis.

Total RNA extraction
Total RNA was extracted using a RINawizTM 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 β-actin was used as a control housekeeping gene.

Reverse transcription (RT) was performed using total RNA (4 μg) in a final volume of 20 μl solution (pH 8.3) containing 5X buffer (250 mM Tris-HCl; 375 mM KCl; 15 mM MgCl2; and 50 mM DTT; Promega, USA), 1 mM dNTPs, 200 U moliney leukemia virus reverse transcriptase (Promega, USA), and 30 pmole oligo dT19 (Promega, USA). The reaction mixture was incubated at