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Antilipolytic Action of Insulin on Adipocytes in OLETF Rats : Differences in Sensitivity According to Adipose Tissue Site and in Age-Related Changes

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ABSTRACT

To investigate the mechanisms of visceral fat accumulation at the cellular level, we studied the effects of insulin on lipolysis in adipose cells isolated from Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are a model of spontaneous type 2 diabetes and visceral adiposity. Using 5×10^4 subcutaneous and mesenteric adipose cells isolated from OLETF rats and normal control Long-Evans Tokushima Otsuka (LETO) rats at 8 weeks and 16 weeks of age, we studied the antilipolytic action of insulin by exposing these cells to 10^{-6} M isoproterenol, thereby eliciting lipolysis. Samples were treated with insulin (10^{-11} to 10^{-7} g/mL), and the lipolysis-inhibitory action of insulin was assessed by means of the insulin concentration required to inhibit the maximum lipolytic response by 50% (IC50). In LETO rats, the maximum response to isoproterenol-induced lipolysis did not differ between 8 and 16 weeks of age or between mesenteric and subcutaneous adipose cells. However, in OLETF rats, the maximum response to lipolysis was significantly greater in mesenteric adipose cells than in subcutaneous adipose cells at both 8 and 16 weeks of age. Also, the maximum response to lipolysis was significantly lower in subcutaneous adipose cells and tended to be lower in mesenteric adipose cells at 16 weeks of age than at 8 weeks of age. The IC50 in OLETF rats was significantly lower at 16 weeks of age than at 8 weeks of age in both mesenteric and subcutaneous adipose cells. The IC50 was also slightly but not significantly lower in mesenteric adipose cells than in subcutaneous adipose cells. Our findings show an age-related increase in sensitivity to the antilipolytic action of insulin in OLETF rats which differed with the location of the adipose tissue. (Jikeikai Med J 2004 ; 51 : 13-7)

Key words : visceral fat, subcutaneous fat, body fat distribution, insulin, antilipolytic action, Otsuka Long-Evans Tokushima Fatty rats

INTRODUCTION

Accumulated body fat can be broadly classified as subcutaneous or visceral. Factors that contribute to the location of fat accumulation include 1) gender differences ; 2) qualitative differences between different adipose cells ; 3) dietary habits, such as high-fat or high-sucrose diets ; and 4) genetic factors. We have previously reported that the Otsuka Long-Evans Tokushima Fatty (OLETF) rat, an animal model of spontaneously obese type 2 diabetes[1,2], shows accumulation of visceral fat compared to the genetically-obese Zucker fatty rat, which shows accumulation of subcutaneous fat[3]. We have also noted

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changes over time in the distribution of body fat in the OLETF rat, in which the ratio of intra-abdominal fat to subcutaneous fat increases over that in normal control Long-Evans Tokushima Otsuka (LETO) rats only after 30 weeks of age[3]. Thus, as these rats age their obesity shifts from subcutaneous fat to visceral fat, where age-related differences in insulin secretion might play a role. Also, several hormones control lipolysis in human white adipose cells. In particular, catecholamines promote β receptor-medited lipolysis, as well as $\alpha 2$ receptor-mediated inhibition of lipolysis, whereas insulin primarily inhibits lipolysis.

This study aimed to clarify cellular changes in body fat distribution associated with the progression of type 2 diabetes. We analyzed adipose cells from OLETF rats and LETO rats for differences in the sensitivity to the antilipolytic action of insulin, in the location of adipose tissue, and in age-related changes.

Materials and Methods

Animals

Male OLETF rats and LETO rats were obtained at 4 weeks of age from the Tokushima Research Institute (Otsuka Pharmaceutical Co., Tokushima). The animals were housed in plastic cages ($320 \times 270 \times 175$ mm) in an animal room with controlled temperature (23 ± 2°C) and relative humidity ($55 \pm 15\%$) and a 12-hour light/dark cycle (lights on at 0700 hours). Rats were supplied with standard chow (CE-2; CLEA Japan, Inc., Tokyo) and tap water ad libitum until 8 or 16 weeks of age. At both 8 and 16 weeks of age, the body weights of OLETF rats (348.0 ± 1.7 g and 470.3 ± 9.8 g) were significantly greater than those of LETO rats (268.0 ± 1.2 g and 412.7 ± 14.9 g). The guidelines for Laboratory Animal Facilities of the Jikei University School of Medicine were followed for the care and use of the animals in this study.

Methods

Isolated adipocytes were prepared from fat specimens with collagenase[4-7]. Using collagenase (collagenase type I [222 U/mg], GIBCO-BRL, Grand Island, NY, USA), adipose cells (5×10^4 cells/well) were collected from subcutaneous and mesenteric adipose tissue in male OLETF rats at 8 and 16 weeks of age ($n=4$) and age-matched male LETO rats ($n=4$), and cultured in the presence of 0 mol/l or 10^{-6} mol/l isoproterenol ((−) isoproterenol-(+)-bitartrate salt, Sigma Chemical Co., St. Louis, MO, USA), thereby eliciting lipolysis. The antilipolytic action of insulin (Wako Pure Chemical Industries Ltd. Osaka, Japan) was investigated at concentrations of 10^{-11} to 10^{-7} g/ml (staged in 3-fold-concentration increments) and 0 g/ml. When the reaction was completed after 3 hours at 37°C, the amount of free fatty acids released into the supernatant was determined (NEFA C-test, Wako) and used as a measure of lipolytic activity. Insulin-induced inhibition of lipolysis was evaluated in terms of the insulin concentration required to inhibit the maximum lipolytic response by 50% (IC50).

Statistical analysis

All numerical values are expressed as means ± SEM. The statistical significance of differences between groups was assessed with Student's t-test. A p value of <0.05 was regarded as indicating statistical significance.

Results

In the absence of isoproterenol, no lipolysis was observed in subcutaneous or mesenteric adipose cells obtained from either OLETF rats or LETO rats (Fig. 1).

LETO rats showed no major differences in the maximum lipolytic response to isoproterenol at 8 and 16 weeks of age in either mesenteric or subcutaneous adipose cells. In contrast, OLETF rats showed significantly greater maximum lipolytic responses in mesenteric than in subcutaneous adipose cells at both 8 and 16 weeks of age. Also, the maximum response to lipolysis was significantly lower for subcutaneous adipose cells, and tended to be lower for mesenteric adipose cells, at 16 weeks of age than at 8 weeks of age (Table 1).

Insulin-induced inhibition of lipolysis (IC50) in OLETF rats was significantly lower at 16 weeks than

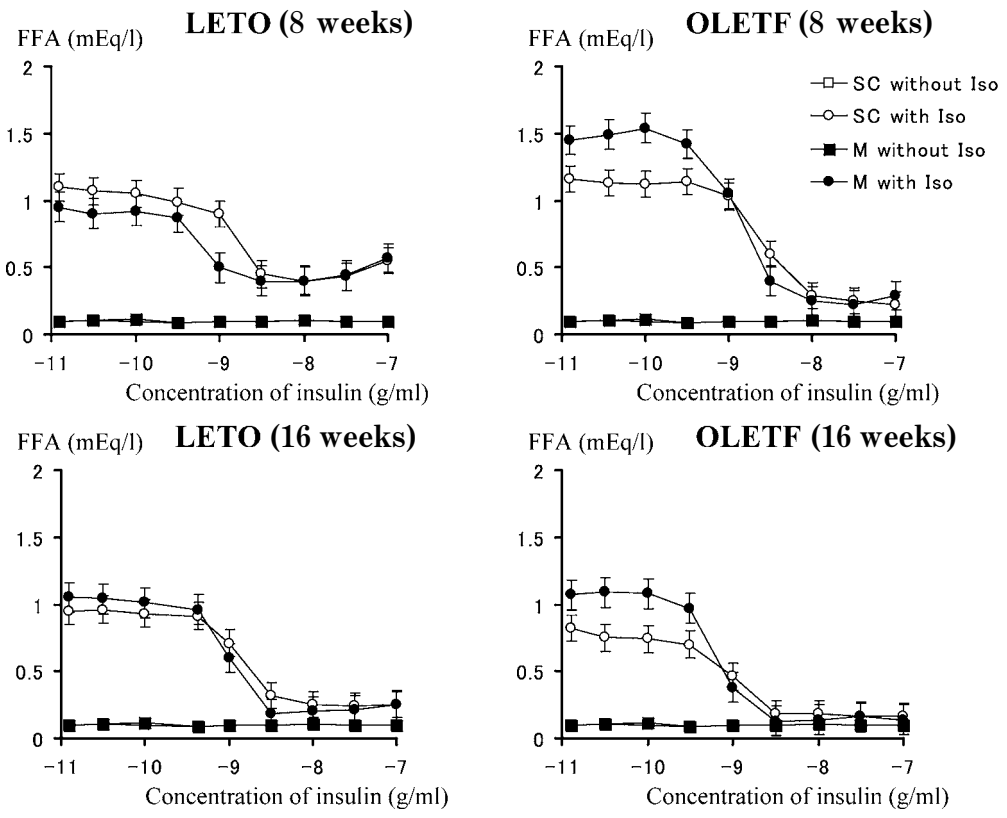

Fig. 1. Effect of insulin on lipolytic activity of mesenteric adipose cells and subcutaneous adipose cells induced by isoproterenol in LETO and OLETF rats. SC: subcutaneous adipose cells, M: mesenteric adipose cells, Iso: isoproterenol, FFA: free fatty acid

Table 1. The maximum lipolytic response to isoproterenol in adipose cells from LETO and OLETF rats

	LETO rats		OLETF rats	
	Subcutaneous adipose cell	Mesenteric adipose cell	Subcutaneous adipose cell	Mesenteric adipose cell
8-week-old	1.18±0.12	1.00±0.21	1.11±0.04	1.53±0.11*
16-week-old	1.05±0.18	1.17±0.01	0.79±0.06†	1.27±0.09**

The amounts of free fatty acids released into the supernatant (mEq/l) are shown.
*$p<0.05$, **$p<0.01$ vs subcutaneous adipose cell, †$p<0.01$ vs 8-week-old

Table 2. The insulin concentration required to inhibit the isoprpterenol-induced maximum lipolytic response by 50% (IC50) in LETO and OLETF rats

	LETO rats		OLETF rats	
	Subcutaneous adipose cell	Mesenteric adipose cell	Subcutaneous adipose cell	Mesenteric adipose cell
8-week-old	1.96±0.38	1.95±0.93	3.80±0.90	1.62±0.25
16-week-old	0.81±0.26	0.95±0.00	1.06±0.17*	0.61±0.11*

The amounts of free fatty acids released into the supernatant (mEq/l) are shown.
*$p<0.05$ vs 8-week-old

at 8 weeks of age, both in mesenteric and subcutaneous adipose cells. The IC50 values for mesenteric adipose cells were slightly but not significantly lower than those for subcutaneous adipose cells (Fig. 1, Table 2).

DISCUSSION

Even at 8 weeks of age body weight was significantly higher in OLETF rats than in age-matched LETO rats. Although we did not measure plasma levels of glucose, insulin, or lipids in the present study, we have previously reported that oral glucose tolerance in 5-week-old OLETF rats was significantly impaired compared with that in age-matched LETO rats[3]. Glucose intolerance in OLETF rats increased markedly with age, and plasma glucose levels at 60 minutes after glucose loading reached 300 mg/dl at 10 weeks of age and 350 mg/dl at 18 weeks of age. Plasma insulin levels during oral glucose tolerance testing in 10-week-old and 18-week-old OLETF rats were significantly higher than those in age-matched LETO rats. Plasma levels of triglycerides and total cholesterol in nonfasting OLETF rats were significantly higher than those in LETO rats even at 9 weeks of age, and hyperlipidemia became more marked with advancing age[2].

Visceral and subcutaneous adipose cells from LETO rats did not differ in the extent of catecholamine-induced lipolysis. In contrast, in OLETF rats mesenteric adipose cells showed significantly more pronounced catecholamine-induced lipolysis than did subcutaneous adipose cells. Accelerated lipolysis is generally associated with greater adipose cell diameter ; i.e., lipolysis increases as cells become larger[8]. In OLETF rats both mesenteric and subcutaneous adipose cells show age-related increases in diameter, although the diameter of mesenteric adipose cells is clearly greater than that of subcutaneous adipose cells[9]. We suspect that this difference in cell diameter contributed to the difference in catecholamine-induced lipolysis between mesenteric and subcutaneous adipose cells from OLETF rats in the present study.

Adipose cells from LETO rats showed no major age-related changes in sensitivity to the antilipolytic action of insulin. In contrast, findings in both mesenteric and subcutaneous adipose cells were significantly higher for the 16-week-old OLETF rats than for the 8-week-old rats. These findings are consistent with the progression of obesity with aging in OLETF rats.

In human subjects isolated visceral fat adipose cells are less sensitive to the antilipolytic action of insulin than are subcutaneous adipose cells[10]. Differences in insulin receptor affinity and insulin action at the postreceptor level may contribute to this difference in sensitivity, but studies of the possible mechanisms have not been published. Our present study disagrees with studies of human isolated adipose cells[10] in finding that visceral adipose cells were more sensitive to antilipolytic actions of insulin than were subcutaneous adipose cells. We cannot explain the reason for this difference. However, given that no lipolysis was observed in the absence of catecholamine, our study focused on the antilipolytic actions of insulin in catecholamine-induced lipolysis, whereas the study in human isolated adipose cells[10] focused on the antilipolytic actions of insulin on lipolysis in the absence of catecholamines. Thus, this difference in focus might be responsible for the apparently contradictory results. Another difference is that the experiment with isolated human adipose cells[10] used nonobese subjects, whereas our study used OLETF rats with visceral adiposity. The mesenteric adipose cells from OLETF rats have larger diameters than do subcutaneous adipose cells[9]. This difference in cell diameter is assumed to be more marked in OLETF rats than in nonobese subjects and may have contributed to the differing results between the two studies. Future studies should compare adipose cells of the same diameter or use a conversion factor to correct for differences in cell diameter.

In addition to studies showing aging-associated changes in body fat distribution in OLETF rats[3], a 5-year prospective epidemiologic study in second-generation Japanese-Americans has demonstrated that reduction in early insulin secretion preceded the accumulation of visceral fat, with a significant negative correlation shown between baseline early insulin

secretion and the amount of change (increase) in visceral fat during the subsequent 5-year period of the study[11].

In OLETF rats, because insulin secretion gradually decreases with aging, adipose cells in different parts of the body differ in their sensitivity to the resulting weaker inhibition of lipolysis. This difference in sensitivity might contribute to changes in body fat distribution. In addition to inhibiting lipolysis, insulin reportedly promotes lipogenesis, and visceral fat appears to be more strongly affected than is subcutaneous fat[12,13]. We therefore suggest that reductions in insulin secretion may attenuate lipogenesis, particularly in subcutaneous fat, which is less sensitive to the lipogenesis-promoting action of insulin, and that this lesser sensitivity might contribute to changes in body fat distribution.

We conclude that the gradual reductions in insulin secretion as impaired glucose tolerance progresses to type 2 diabetes in both humans and OLETF rats bring about shifts in obesity from subcutaneous fat to visceral fat, given their differences in sensitivity to the lipolysis-inhibitory and lipogenesis-promoting effects of insulin.

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