Different Effect of Growth Hormone on the Triglyceride Metabolism in Visceral and Subcutaneous Adipose Tissues of Rats

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Abstract To clarify the mechanism by which visceral adipose tissue developed in growth hormone (GH) deficient patients, and was reduced by GH supplementation, the effects of GH on the triglyceride metabolism in the visceral and subcutaneous adipose tissues of the rats were compared.

Visceral and subcutaneous adipose tissues were obtained from omentum and abdominal wall. [^{14}C]deoxyglucose uptake and the triglyceride synthetic activity from [^{14}C]oleic acid, and heparin-releasable lipoprotein lipase activity were not changed by the addition of GH (10 ng/mL) in both visceral and subcutaneous adipose tissues, but the lipolysis by GH (10 ng/mL) was much more remarkable in the visceral adipose tissue than in subcutaneous adipose tissue, whereas adrenaline-induced lipolysis occurred almost equally in both tissues.

These results suggest that the preponderance of visceral adipose tissue in GH deficiency might partly be due to the lack of GH-preferential lipolysis in visceral adipose tissue.

Key words: growth hormone, lipolysis, visceral adipose tissue

Introduction

In growth hormone (GH) deficiency, an increase in body fat is known to occur (1). Recently, it has been reported that the visceral fat is dominant, and is reduced by GH supplementation (2). Generally, visceral fat dominance is associated with many coronary risk factors such as hyperlipidemia, glucose intol-

erance, and hypertension (3, 4). Thus it is thought to be a risk factor for ischemic coronary heart disease. Rosén *et al.* (5) reported the high incidence of cardiovascular disease in panhypopituitarism patients taking routine supplement therapy, and suggested that no supplement of GH might contribute to it. In Japan, the study group on panhypopituitarism, supported by the Japan Ministry of Health and Welfare, reported that GH-deficient patients with panhypopituitarism supplemented with other hormones showed a high incidence of cardiovascular risk factors such as

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hyperlipidemia, diabetes mellitus, and hypertension compared with GH-normal patients (6). So, it is important to clarify the mechanism by which visceral fat accumulates in the GH deficient state.

As for the regulation of triglyceride metabolism in the adipose tissue, synthetic and catabolic pathways can be divided. We investigated the effects of GH on the [^{14}C] deoxyglucose uptake, triglyceride synthesizing activities from [^{14}C] oleic acids and heparin-releasable lipoprotein lipase activities of the rat visceral and subcutaneous adipose tissues, as a synthetic pathway. As a catabolic pathway, lipolytic activity with GH and adrenaline were compared in both adipose tissues.

Materials and Methods

Animals

The animals used were 8 weeks old Wistar strain male rats. Subcutaneous adipose tissues were obtained from the inguinal area of the abdominal wall. Visceral adipose tissues were from the omentum. The adipose tissues were kept in Dulbecco modified Eagle medium containing 10 % fetal calf serum at room temperature.

Materials

Human recombinant GH was kindly provided by Sumitomo Pharmaceutical Co., Ltd. (Osaka, Japan) and Pharmacia AB (Stockholm, Sweden). Insulin was obtained from Sigma. Adrenaline was purchased from the Daiichi Pharmaceutical Co., Ltd. Fatty acids were measured by enzymatic methods using NEFA kit-U purchased from Nipponshoji Co. Ltd. (Osaka, Japan) with an autoanalyzer.

Glucose Uptake

After decapitation, the adipose tissues were removed quickly. The tissues were cut into pieces (2×2 mm) by scissors. One hundred mg was placed in a test tube in 0.5 mL of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 3 % bovine serum albumin. 2-deoxy-D- [^{14}C] glucose (1 μCi) was added to the medium in the presence or absence of insulin (100 nM) or GH (10 ng/mL) and the tissues were incubated for 1 hour at 37 °C. Then, the minced tissues were washed by phosphate buffer saline 3 times. The radioactivities incorporated into the tissues were counted by scintillation counter (Beckman).

Triglyceride Synthesizing Activity

Minced fat tissues (100 mg) were put into the reaction mixture containing [^{14}C]oleic acids (1 μCi), 5 % bovine serum albumin in 1 mL of Dulbecco modified Eagle medium-10 mM Tris HCl pH 7.4, with 100 nM insulin or 10 ng/mL GH. The reaction mixture was incubated for 60 min at 37 °C. The reaction was stopped by the addition of 5 mL of Dole's extraction mixture (isopropanol:heptane:1N H_2SO_4 =40:10:1) (7). Then, 3 mL of heptane and 2 mL of water were added and were shaken. Three mL of heptane layer was removed and poured into another test tube. Three mL of 0.05N NaOH-50 % ethanol mixture was added and after shaking for 5 min, 0.5 mL of the upper layer was removed and the radioactivity was counted.

Lipoprotein Lipase Activity

The adipose tissue (100 mg/mL) was incubated with heparin (10 $\mu\text{g/mL}$) at 37 °C for 15 min in 2 mL of Dulbecco modified Eagle

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medium. Then the lipoprotein lipase activity of the medium was assayed. The reaction mixture for measurement of lipase consisted of 20 μ g of triolein emulsified with 0.02 % Triton X-100, 5 % bovine serum albumin and high density lipoprotein (5 μ g protein) in 100 mM Tris HCl pH 8.0 in 250 μ L. Released free fatty acids were measured by the enzymatic method stated above.

Lipolysis

The adipose tissues were cut into pieces. One hundred mg of tissue was put into a medium containing 4 % bovine serum albumin in 2 mL of Dullbecco's modified Eagle medium. GH was added from 0.1-100 ng/mL. Adrenaline was added from 0.01 to 100 ng/mL. Incubation was performed for 3 hours at 37 °C. Released free fatty acids in the medium were measured by the enzymatic method as stated above.

Results

The Effect of Growth Hormone on the Triglyceride Synthesizing Pathway

The effect of GH on the triglyceride synthesizing pathways in visceral and subcutaneous adipose tissues was investigated, comparing to insulin. The [14 C] deoxyglucose uptake is shown in the upper panel of Fig. 1. Insulin enhanced the glucose uptake in visceral fat, but scarcely at all in the subcutaneous adipose tissue. GH did not show an enhancing effect in either of the tissues.

Triglyceride synthesizing activities from [14 C] oleic acids are shown in the middle panels. Insulin also enhanced the triglyceride synthesis from oleic acids only in visceral adipose

Fig. 1 Effect of insulin and growth hormone on triglyceride metabolism in mesenteric and subcutaneous adipose tissues.

The materials are shown in Materials and Methods. For [14 C] deoxyglucose uptake, the incubation was performed for 60 min. For triglyceride synthetic activities from [14 C] oleic acid, the incubation was performed for 2 hours. Heparin releasable lipoprotein lipase activity was measured using the medium incubated with fat tissues and heparin (10 μ g/mL) for 15 min.

tissue. GH did not show any effect on triglyceride synthesis from free fatty acids. Lipoprotein lipase activities released from adipose tissue with heparin are shown in the lower panel. Insulin and GH did not affect the lipoprotein lipase activity in both adipose tissues.

The Effect of GH on Lipolytic Activity

For the effect of GH on the catabolism of triglycerides, lipolytic activities were studied. The time course of the lipolysis is shown in Fig. 2, right. Adrenaline (10 ng/mL) enhanced the lipolytic activity both in the subcutaneous

Fig. 2 Hormone-induced FFA mobilization from subcutaneous and mesenteric adipose tissue

Fig. 3 Hormone-induced FFA mobilization from subcutaneous and mesenteric adipose tissue

and the visceral adipose tissues. The rate was a little higher in visceral adipose tissue than in subcutaneous adipose tissue. Whereas, GH (1 ng/mL) enhanced the lipolytic activity in the visceral adipose tissues predominantly and scarcely at all in subcutaneous adipose tissue as shown in Fig. 2, right.

The dose effect of hormones is shown in Fig. 3. Adrenaline showed maximum activity with 1 ng/mL, and a little higher in visceral adipose tissue than subcutaneous adipose tissue.

GH showed maximum activity at 0.1 ng/mL, and became a plateau above 1 ng/mL. This value was nearly the concentration of the human plasma levels. The lipolytic rates by GH were far higher in the visceral adipose tissue than in the subcutaneous adipose tissue.

Discussion

As for triglyceride synthetic activity of GH, there have been several reports (8, 9). Among them, the lipogenic action was reported (9). We could not observe any effect of GH on visceral and subcutaneous adipose tissue. GH might be lipogenic only in the GH-deprived condition. Further studies are required. In any case GH appeared to have little effect on the triglyceride synthesizing pathway.

Insulin promoted glucose uptake mainly in visceral adipose tissue, and also triglyceride synthesis. These results suggest that visceral adipose tissue development is promoted by insulin. This fact was also supported by the report by Inadera (10). The lipolytic action of GH had been controversial (11, 12) but now,

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direct action has been demonstrated (12). We could also demonstrate the direct action, because lipolysis did not require a lag phase.

The lipolytic activities were clearly different between visceral and subcutaneous adipose tissues, and that from the visceral fat was much more sensitive for GH than the subcutaneous one.

The mechanism by which triglyceride is hydrolyzed in adipocytes is still controversial. In the case of adrenaline, activation of hormone sensitive lipase by protein kinase C was proposed (13). Okuda *et al.* (14) proposed that phosphatidylcholine on the surface of lipid droplets might be a regulatory factor for lipolysis in fat cells, and adrenaline ruined the barrier role of the phospholipid between the substrate and hormone sensitive lipase. But the lipolytic mechanism for GH is not entirely clear so it is difficult to discuss the mechanism by which GH had different effects on visceral and subcutaneous adipose tissues. Moller *et al.* (15) reported that complementary deoxyribonucleic acid (cDNA) of GH was transfected into Chinese hamster ovary cells and expressed GH receptor into the cell surface, and observed enhanced lipolysis so GH-induced lipolytic activity might be mediated by GH receptor. The difference in the GH-induced lipolysis between visceral and subcutaneous adipose tissues might be due to the difference of the GH receptor expression in both tissues. Further study is required.

In summary, it is suggested that GH reduced the visceral adipose tissue mainly by enhancing the lipolytic activity in the visceral adipose tissue.

Further studies are required to determine the effect of GH on visceral fat cell prolifera-

tion and differentiation.

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References

1. Salomon F, Cuneo RC, Hesp R, Sonksen PH. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *N Engl J Med* 1989; 321: 1797-1804.
2. Bengtsson BA, Eden S, Lonn L, Kvist H, Stokland A, Lindstedt G, *et al.* Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. *J Clin Endocrinol Metab* 1993; 76: 309-17.
3. Lapidus L, Bengtsson C, Larsson B, Pennert K, Rybo E, Sjostrom L. Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. *BMJ* 1984; 289: 1257-61.
4. Ster MP, Haffner SM. Body fat distribution and hyperinsulinemia as risk factors for diabetes and cardiovascular disease. *Arteriosclerosis* 1986; 6: 123-30.
5. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet* 1990; 336: 285-8.
6. Irie M, Miyashita Y, Itoh Y, Shirai K. The complications in panhypopituitarism in Japan, in Report of study group of Min-

- istry of Health and Welfare on Hypopituitarism. 1994: 37-9.
7. Dole VP. A relation between non-esterified fatty acid in plasma, and the metabolism of them, *J Clin Invest* 1956; 35: 150-4.
 8. Davidson MB. Effect of growth hormone on carbohydrate and lipid metabolism. *Endocr Rev* 1987; 8: 115-31.
 9. Smal J, De Meyts P. Role of kinase C in the insulin-like effects of human growth hormone in rat adipocytes. *Biochem Biophys Res Commun* 1987; 147: 1232-40.
 10. Inadera H, Ishikawa Y, Shirai K, Saito Y, Yoshida S. Proliferation and triglyceride synthesizing activity of fibroblast-like cells derived from epididymal and subcutaneous adipose tissue of rats. *Scand J Clin Lab Med* 1993; 53: 225.
 11. Frigeri LG, Khoo JC, Robel G. Absence of lipolytic activity from purified human growth hormone in cultured 3T3-L1 adipocytes. *Horm Res* 1983; 17: 197-201.
 12. Dietz J, Schwartz J. Growth hormone alters lipolysis and hormone-sensitive lipase activity in 3T3-F442A adipocytes. *Metabolism* 1991; 40:8, 800-6.
 13. Belfrage P, Fredrikson G, Olsson H, Strfors P. Hormonal regulation of adipose tissue lipolysis by reversible phosphorylation of hormone-sensitive lipase. *Prog Clin Biol Res* 1982; 102: 213-23.
 14. Okuda H, Saito Y, Matsuoka N, Takeda E, Kumagai A. Role of phospholipid in adrenaline-induced lipolysis and cyclic AMP production. *J Biochem* 1978; 83: 887-92.
 15. Möller C, Emtner M, Arner P, Norstedt G. Growth hormone regulation of lipid metabolism in cell transfected with growth hormone receptor cDNA. *Mol Cell Endocrinol* 1994;99: 111-7.