The Correlation of Insulin-Like Growth Factor Binding Protein-3 Proteolysis with Insulin Resistance in Obese Adolescents

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The Correlation of Insulin-Like Growth Factor Binding Protein-3 Proteolysis with Insulin Resistance in Obese Adolescents

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ABSTRACT

The correlation of insulin-like growth factor binding protein-3 proteolysis with insulin resistance in obese adolescents

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Purpose: The prevalence of adolescent obesity is increasing, thereby causing an increase of metabolic syndrome in adolescents. Recently, the metabolic roles of insulin-like growth factor binding protein-3 (IGFBP-3) in human adipocytes and animals have been revealed. This study was performed to investigate the role of IGFBP-3 proteolysis in insulin resistance in obese adolescents.

Methods: A total of 197 adolescents aged 12 to 13years were included in this study. They were classified into the obesity group (*n*=56, M:F=25:31), the overweight group (n=41, M:F=19:22), and the control group (*n*=100, M:F=49:51) according to body mass index (BMI) for age and gender. Measurements for anthropometric profiles

(height, weight, and waist circumference) and blood pressure (BP) were taken. Lipid profiles, and levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), high sensitivity C-reactive protein, fasting glucose and fasting insulin were measured. Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated. IGFBP-3 proteolytic fragments were obtained by Western immunoblot assay.

Results: Weight, height, waist circumference, BMI, systolic BP, and diastolic BP were higher in the overweight and obesity groups than in the control group ($\boldsymbol{P} < 0.01$). The levels of triglycerides, low-density lipoprotein cholesterol, ALT, fasting glucose, and fasting insulin were increased in the overweight and obesity groups compared to the control group ($\boldsymbol{P} < 0.01$), while the level of high-density lipoprotein cholesterol was decreased in the obesity group compared to the overweight and control groups ($\boldsymbol{P} < 0.01$). HOMA-IR was significantly increased in the overweight and obesity groups compared to the control group (control *vs*. overweight, 1.5±0.8 *vs*. 2.9±3.2, $\boldsymbol{P} < 0.001$; control *vs*. obesity, 1.5±0.8 *vs*. 3.6±2.2, $\boldsymbol{P} < 0.001$). The optical densities of the IGFBP-3 proteolytic fragments were significantly increased in the overweight and obesity groups compared to controls (control *vs*. overweight, 2.09 ± 1.32 (100%) *vs*. 3.83 ± 0.37 (183%), $\boldsymbol{P} < 0.001$; control *vs.* obesity, 2.09 ± 1.32 (100%) *vs*. 3.85 ± 0.63 (184%), $\boldsymbol{P} < 0.001$). The degree of IGFBP-3 proteolysis was correlated with weight ($r = 0.599$, $\boldsymbol{P} < 0.001$), height ($r = 0.241$, $\boldsymbol{P} < 0.001$), ALT ($r = 0.220$, $\boldsymbol{P} < 0.05$), fasting glucose ($r = 0.295$, $\boldsymbol{P} = 0.001$), fasting insulin ($r = 0.307$, $\boldsymbol{P} = 0.001$), BMI ($r = 0.651$,

P < 0.001), waist circumference (*r* = 0.608, ***P***= 0.001), and HOMA-IR (*r* = 0.313, ***P*** <0.001).

Conclusion: The degree of IGFBP-3 proteolysis was increased in overweight and obese subjects and positively correlated with parameters associated with insulin resistance. These results suggest that the proteolysis of IGFBP-3 may be involved in the pathogenesis of insulin resistance and IGFBP-3 fragments could be considered as surrogate markers of insulin resistance in obesity.

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Keywords: obesity, IGFBP-3, proteolytic fragment, insulin resistance, adolescents

The correlation of insulin-like growth factor binding protein-3 proteolysis with insulin resistance in obese adolescents

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I. INTRODUCTION

Obesity is now accepted as a major health problem worldwide. In particular, the prevalence of obesity in children and adolescents is significantly increasing at a rapid rate[1-2]. In adults, metabolic syndrome, defined by the clustering of cardiovascular risk factors, including obesity, insulin resistance, dyslipidemia, and hypertension, is associated with an increased risk for cardiovascular disease and type 2 diabetes. Metabolic syndrome can occur in childhood and is associated with the severity of obesity in varying levels[3-4]. Furthermore, the cluster of cardiovascular risk factors related to metabolic syndrome persists from childhood to young adulthood[5]. Among risk factors, insulin resistance plays a major role in the pathogenesis of metabolic

syndrome and type 2 diabetes[6]. The mechanisms responsible for insulin resistance in obesity can be explained by a number of hypotheses proposing the following causes: defects in insulin-stimulated glucose transport in skeletal muscle by elevated free fatty acids; increased intramyocellular/intrahepatic fatty acid metabolites; increased fat delivery to muscle/liver; defects in mitochondrial fatty acid oxidation; and variable cytokines secreted by adipocytes that affect insulin sensitivity and lead to generalized inflammation[7]. However, the exact mechanisms responsible for insulin resistance in obesity have yet to be elucidated.

Insulin-like growth factor binding proteins (IGFBPs) are components of the insulin-like growth factor (IGF) signaling system, which is comprised of the IGF-I, IGF-II, and insulin ligands, and a family of transmembrane receptors including the insulin, IGF-I, and IGF-II receptors. The six classical IGFBPs (IGFBP-1 to -6) bind IGF-I and IGF-II, but not insulin, with high affinity. The major functions of IGFBPs are to transport IGFs, prolong their half-lives and regulate the availability of free IGFs for interactions with IGF receptors, thereby modulating the effects of IGFs on cell proliferation, differentiation, metabolic activity increase, and cell survival[8]. Additionally, six IGFBPs have been reported to exert IGF/IGF-I receptor-independent actions[9]. Recently, the metabolic-related roles of IGFBPs to regulate the glucose homeostasis by modulating the insulin and IGF systems have been proposed[10, 11]. IGFBP-3 inhibits insulin-stimulated glucose uptake in adipocytes, human omental adipose tissue, and in Sprague-Dawley rats[12, 13]. In addition, transgenic mice

overexpressing IGFBP-3 developed insulin resistance, showing fasting hyperglycemia, impaired glucose tolerance, and reduced glucose uptake in muscle and liver[14]. These findings suggest that IGFBP-3 acts as an insulin antagonist. The possibility of the effect of IGFBP-3 on insulin action that mediated through central nervous system has been proposed, showing that intracerebroventricular infusion of IGFBP-3 significantly impairs insulin action in the rat liver[15]. However, the mechanisms responsible for insulin resistance by IGFBP-3 remain poorly understood. One possible mechanism in insulin resistance by IGFBPs focused on defects in insulin binding to its receptors, thereby resulting in defects of the post-receptor signal processing can be addressed. Yamanaka *et al*.[16] demonstrated that IGFBP-7/mac25, a member of the IGFBP superfamily that binds to IGFs specifically with low affinity, is a high-affinity insulin-binding protein, showing that IGFBP-7 blocks insulin binding to the insulin receptor and thereby inhibits insulin actions such as the autophosphorylation of the insulin receptor β subunit and the phosphorylation of insulin receptor substrate (IRS). Another study reported that IGFBP-3 fragments derived from proteolysis retain weak IGF binding, show specific insulin binding and inhibition of insulin receptor autophosphorylation[17]. These studies suggest that proteolytic fragments of IGFBP-3 or low-affinity binding IGFBPs induce insulin resistance by increasing the affinity of IGFBP-3 for insulin, thereby inhibiting binding to insulin receptor. Alterations in proteolytic cleavage of IGFBP-3 have been reported in several physiological and pathological conditions, including cancer, type 1

and 2 diabetes, burn injuries, and surgery, suggesting that catabolic states increase degradation of circulating IGFBP-3[18-23]. In this study, we hypothesized that protelytic fragments of IGFBP-3 observed in obesity induces insulin resistance. Herein, we have evaluated the degree of IGFBP-3 proteolysis in the sera of obese adolescents and have demonstrated that the degree of IGFBP-3 proteolysis correlates with variables associated with insulin resistance.

II. MATERIALS AND METHODS

1. Subjects

197 study subjects aged 12 to 13 years old were selected among adolescents living in Seoul who visited a health-screening center (The National Medical Center, Seoul, Korea) for medical examinations. The 93 boys and 104 girls were classified into the following three groups by Body Mass Index (BMI): obesity group (BMI $\geq$ 95th), overweight group (85th $\leq$ BMI < 95th), and control group (BMI < 85th). The BMI percentiles for age and gender according to 2007 Korea Growth Charts for assessment of obesity were utilized. At the time of study enrollment no subject had either a chronic disease or acute illness, was taking medications for any condition, or had a history of smoking. The Hospital Ethnics Committee reviewed and approved the study, and written informed consent was obtained from guardians and study subjects.

2. Anthropometric Measurements

Clinical and physical examinations were performed by the same physician. For anthropometric measurements, all subjects were dressed in light clothing without shoes. Weight was measured to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm using an automatic height-weight scale. Blood pressure (BP) was measured three times in the sitting position on the right arm using an automatic BP recorder. BMI was calculated as the weight in kilograms divided by the height in meters squared. Waist circumference was measured at the midpoint between the iliac crest and the lower border of the rib cage[24].

3. Blood Samples Analyses

All subjects were fasted for more than 12 hours. Whole blood samples were obtained by venipuncture, and the blood was collected in glass tubes. All blood samples were centrifuged at 4000 rpm for 4 minutes at room temperature, and extracted sera were used for biochemical and hormonal assay within 8 hours, as described below. The remaining sera were immediately stored at -80℃ until analysis. Western immunoblot assay was performed as described below.

A. Biochemical and hormonal analyses

Level of fasting serum glucose, insulin, total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured, and levels of low-density

lipoprotein (LDL) cholesterol were calculated using the following formula: LDL-cholesterol = total cholesterol – HDL-cholesterol – (triglyceride/5). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were also measured. High sensitivity C-reactive protein (hs-CRP) concentrations were measured by IMMAGE assay (IMMAGE CRPH, Beckman Coulter, CA, USA), and fasting insulin levels were measured by electroluminescence immunoassay (Roche, Indianapolis, IN, USA). Insulin resistance was estimated using the homeostatic model assessment of insulin resistance (HOMA-IR) and calculated using the following formula: HOMA-IR =[fasting insulin (μIU/mL) x fasting glucose (mg/dL)/18]/22.5[25].

B. Western immunoblotting analyses

Sera were size-fractionated by 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis under non-reducing condition. Size-fractionated proteins were electrotransferred onto Hybond-ECL nitrocellulose membranes (Amersham Pharmacia, Arlington, VA). Membranes were blocked in 5% nonfat dry milk in tris-buffered saline tween20 (TBST), and incubated with primary antibodies (goat anti-IGFBP-3 antibody) diluted in TBST for 2 hours at room temperature or overnight at 4^{o}C. Membranes were washed in TBST and then incubated with horseradish peroxidase-conjugated secondary antibodies (Southern Biotechnology Associates, Birmingham, AL, USA), diluted 1:3000, for 1 hour at room temperature. Immunoreactive proteins were detected using Renaissance Western Blot Chemiluminescence reagents (New England Nuclear, Boston, MA, USA). The optical

densities of the IGFBP-3 fragments were measured using the Bio-Rad XR Plus imaging densitometer (Bio-Rad, Melville, NY, USA). The intact form of IGFBP-3 is represented by a 41-39 kDa doublet band. IGFBP-3 proteolysis was defined as the absorbance of the sum of IGFBP-3 fragments with molecular weights lower than the intact form, relative to the total density in the lane. All analyses were performed in duplicate.

4. Statistical Analyses

To determine the statistical differences in clinical characteristics among the three groups, we used one-way analysis of variance (ANOVA). Pearson's correlation coefficients were calculated to evaluate the relationship between the degree of IGFBP-3 proteolysis and clinical features, insulin resistance (HOMA-IR), adiposity parameters (e.g., BMI and waist circumference), fasting insulin levels, fasting glucose levels and insulin resistance. Results were expressed as the mean ± SD. Results were considered significant when $P < 0.05$. All statistical analyses were performed with SAS 9.13 service pack3 (SAS Institute, Cary, NC, USA).

III. RESULTS

1. Clinical Characteristics of Subjects

Comparisons of clinical features in all groups are given in Table 1. Mean chronological age was 12.2 ± 0.36 years old in all subjects. Gender ratio did not differ among the three groups. Height was significantly increased in the obesity group (control *vs*. overweight, 156.9 ± 7.1 *vs*. 157.9 ± 7.2, ***P*** = NS; overweight *vs*. obesity, 157.9 ± 7.2 *vs*. 160 ± 5.7, ***P*** < 0.01; control *vs*. obesity, 156.9 ± 7.1 *vs*. 160 ± 5.7, ***P*** < 0.01). The obesity group also had significantly higher weight, BMI, waist circumference, systolic BP, and diastolic BP than controls (respectively, ***P*** < 0.01).

Table 1. Clinical and anthropometric characteristics of subjects

	Control	Overweight	Obesity	***P*** value
n (M:F)	100 (49:51)	41 (19:21)	56 (25:31)	NS
Age (years)	12.2 ± 0.4	12.2 ± 0.4	12.2 ± 0.3	NS
Weight (kg)	47.2 ± 6.9	61.0 ± 6.9	69.7 ± 7.7	[a]
Height (cm)	156.9 ± 7.1	157.9 ± 7.2	160 ± 5.7	[b]
BMI (kg/m^2)	19.1 ± 1.9	24.4 ± 1.0	27.2 ± 1.8	[a]
Waist circumference (cm)	68.5 ± 6.2	80.8 ± 5.6	87.9 ± 7.0	[a]
Systolic BP (mmHg)	101.2 ± 10.8	107.9 ± 10.6	114.8 ± 10.7	[a]
Diastolic BP (mmHg)	63.6 ± 6.4	65.6 ± 7.5	69.9 ± 7.8	[c]

Abbreviations: NS, not significant; BMI, body mass index; BP, blood pressure. Values are expressed as mean ± SD. Each group was classified by the BMI percentile according to 2007 Korea Growth Charts. [a], control *vs*. overweight; overweight *vs*. obesity; control *vs*. obesity; respectively, $\boldsymbol{P} < 0.01$. [b], control *vs*. obesity ($\boldsymbol{P} < 0.01$), and overweight *vs*. obesity ($\boldsymbol{P} < 0.01$). [c], control *vs*. overweight ($\boldsymbol{P} < 0.01$), and control *vs*. obesity ($\boldsymbol{P} < 0.01$).

2. Biochemical and Hormonal Assays

Biochemical and hormonal variables are summarized in Table 2. There was no significant difference in the total cholesterol levels among the groups. However, HDL-cholesterol levels were markedly decreased in the obesity group (control *vs*. overweight, 53.1 ± 13.3 mg/dL *vs*. 53.6 ± 11.4 mg/ dL, ***P*** = NS; control *vs*. obesity, 53.1 ± 13.3 mg/dL *vs*. 46.5 ± 10.6 mg/dL, ***P*** < 0.01; overweight *vs*. obesity, 53.6 ± 11.4 mg/ dL *vs*. 46.5 ± 10.6 mg/dL, ***P*** < 0.01). The obesity group had much higher levels of triglycerides (control *vs*. obesity, 82.9 ± 37.6 mg/dL *vs*. 103.2 ± 36.8 mg/dL, ***P*** < 0.01), LDL-cholesterol (control *vs*. obesity, 75.9 ± 24.2 mg/dL *vs*. 84.7 ± 26.5 mg/dL, ***P*** < 0.05), and ALT (control *vs*. obesity, 11.4 ± 21.4 IU/L *vs*. 20.9 ± 20.8 IU/L, ***P*** < 0.01) than the control group. However, hs-CRP levels were not significantly different in this study (control *vs*. obesity, 0.07 ± 0.13 mg/dL *vs*. 0.11 ± 0.11 mg/dL, ***P*** = 0.059).

Fasting insulin levels were differed significantly in the obesity group compared with other groups (control *vs*. overweight, 8.0 ± 4.0 μIU/mL *vs*. 12.9 ± 8.7 μIU/mL, ***P*** < 0.001; overweight *vs*. obesity, 12.9 ± 8.7 μIU/mL *vs*. 16.9 ± 9.5 μIU/mL, ***P*** < 0.001; control *vs*. obesity, 8.0 ± 4.0 μIU/mL *vs*. 16.9 ± 9.5 μIU/mL, ***P*** < 0.001). The levels of fasting glucose were increased in the overweight and obesity groups compared to controls (***P*** < 0.001) but were not significantly different between each other. HOMA-IR was increased similarly (control *vs*. overweight, 1.5 ± 0.8 *vs*. 2.9 ± 3.2,***P*** < 0.001; control *vs*. obesity, 1.5 ± 0.8 *vs*. 3.6±2.2; ***P*** < 0.001) as shown in Table 3.

Table 2. Biochemical characteristics of subjects

	Control	Overweight	Obesity	*P* value
Total cholesterol (mg/dL)	145.4 ± 32.3	158.6 ± 29.0	150.3 ± 34.0	NS
HDL-cholesterol (mg/dL)	53.1 ± 13.3	53.6 ± 11.4	46.5 ± 10.6	[d]
Triglycerides (mg/dL)	82.9 ± 37.6	110.9 ± 47.7	103.2 ± 36.8	[e]
LDL-cholesterol (mg/dL)	75.9 ± 24.2	82.9 ± 23.3	84.7 ± 26.5	[f]
AST (IU/L)	21.8 ± 12.6	22.9 ± 10.3	26.0 ± 17.6	NS
ALT (IU/L)	11.4 ± 21.4	17.2 ± 2.7	20.9 ± 20.8	[d]
hs-CRP (mg/dL)	0.07 ± 0.13	0.07 ± 0.13	0.11 ± 0.11	NS

Abbreviations: HDL, high-density lipoprotein; LDL; low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; hs-CRP, high sensitivity C-reactive protein. Values are expressed as mean ± SD. [d], control *vs*. obesity ($P < 0.01$), and overweight *vs*. obesity ($P < 0.01$). [e], control *vs*. overweight ($P < 0.01$), and control *vs*. obesity ($P < 0.01$). [f], control *vs*. overweright ($P < 0.05$), and control *vs*. obesity ($P < 0.05$). *P* values were calculated by one-way ANOVA.

Table 3. Insulin resistance of subjects

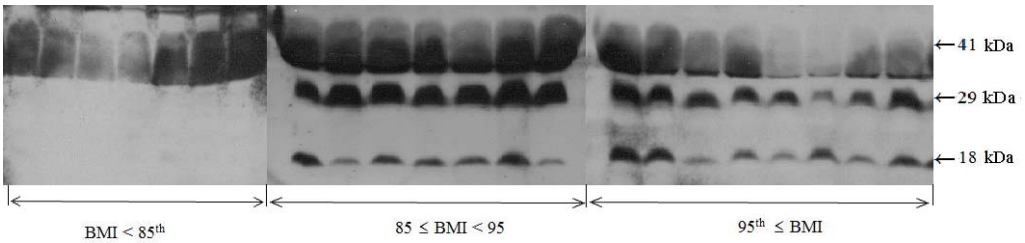
	Control	Overweight	Obesity	*P* value
Fasting glucose (mg/dL)	75.8 ± 11.3	86.3 ± 12.5	85.3 ± 7.2	[g]
Fasting insulin (μIU/Ml)	8.0 ± 4.0	12.9 ± 8.7	16.9 ± 9.5	[h]
HOMA-IR	1.5 ± 0.8	2.9 ± 3.2	3.6 ± 2.2	[g]

Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance. HOMA-IR was calculated using the following formula: HOMA-IR = [fasting insulin (μIU/mL) x fasting glucose (mg/dL)/18]/22.5. [g], control *vs*. overweight (***P*** < 0.001), and control *vs*. obesity (***P*** < 0.001). [h], control *vs*. overweight, overweight *vs*. obesity, and control *vs*. obesity, respectively; ***P*** < 0.001.

3. **Western Blot Analyses**

There were predominant expressions of the IGFBP-3 proteolytic fragmented forms (29-kDa, 18-kDa) in the obesity and overweight groups, but non-expression or timid fragments were shown in controls (Fig. 1A.). The proteolytic densities of the IGFBP-3 fragments were significantly increased in the obesity and overweight groups (control *vs*. overweight, 2.09 ± 1.32 *vs*. 3.83 ± 0.37, ***P*** < 0.001; control *vs*. obesity, 2.09 ± 1.32 *vs*. 3.85 ± 0.63, ***P*** < 0.001) (Fig. 1B). ***P*** values were calculated by one-way ANOVA.

A.



B.

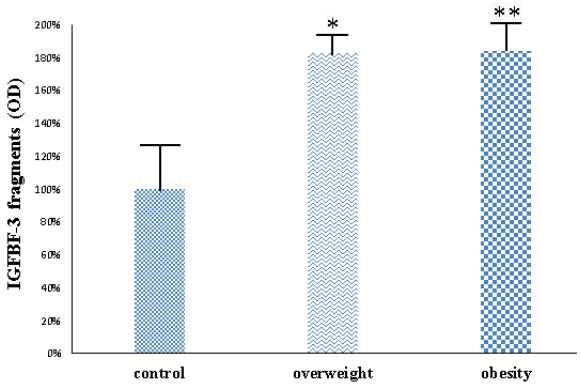


Fig. 1. Increased IGFBP-3 proteolysis in obese adolescents. (A) The IGFBP-3 proteolytic fragments were detected by Western blot assay. (B) Optical density was significantly increased in the obesity and overweight groups. *, control *vs*. overweight, 2.09 ± 1.32 (100%) *vs*. 3.83 ± 0.37 (183%), $\boldsymbol{P} < 0.001$; **, control *vs*. obesity, 2.09 ± 1.32 (100%) *vs*. 3.85 ± 0.63 (184%), $\boldsymbol{P} < 0.001$).

4. Correlation of IGFBP-3 proteolysis with Clinical Variables

The degree of IGFBP-3 proteolytic fragment density was positively correlated with adiposity parameters such as waist circumference (r = 0.608, $\boldsymbol{P}$ < 0.001), BMI (r= 0.4651, $\boldsymbol{P}$ < 0.001) as well as levels of ALT (r = 0.220, $\boldsymbol{P}$ = 0.014), fasting glucose (r = 0.295, $\boldsymbol{P}$ = 0.001), fasting insulin (r = 0.307, $\boldsymbol{P}$ = 0.001), and insulin resistance index (HOMA-IR) (r = 0.313, $\boldsymbol{P}$ < 0.001). $\boldsymbol{P}$ values were calculated by Pearson's correlation coefficient (Fig. 2.).

Table 4. Correlation of the degree of IGFBP-3 proteolysis with clinical variables

	OD of IGFBP-3 Proteolysis	*P* value
	Pearson's correlation coefficient(*r*)	
Height	0.241	0.007
Weight	0.599	< 0.001
Waist circumference	0.608	< 0.001
BMI	0.651	< 0.001
ALT	0.220	0.145
Fasting glucose	0.295	0.001
Fasting insulin	0.307	0.001
HOMA-IR	0.313	< 0.001

A. B.

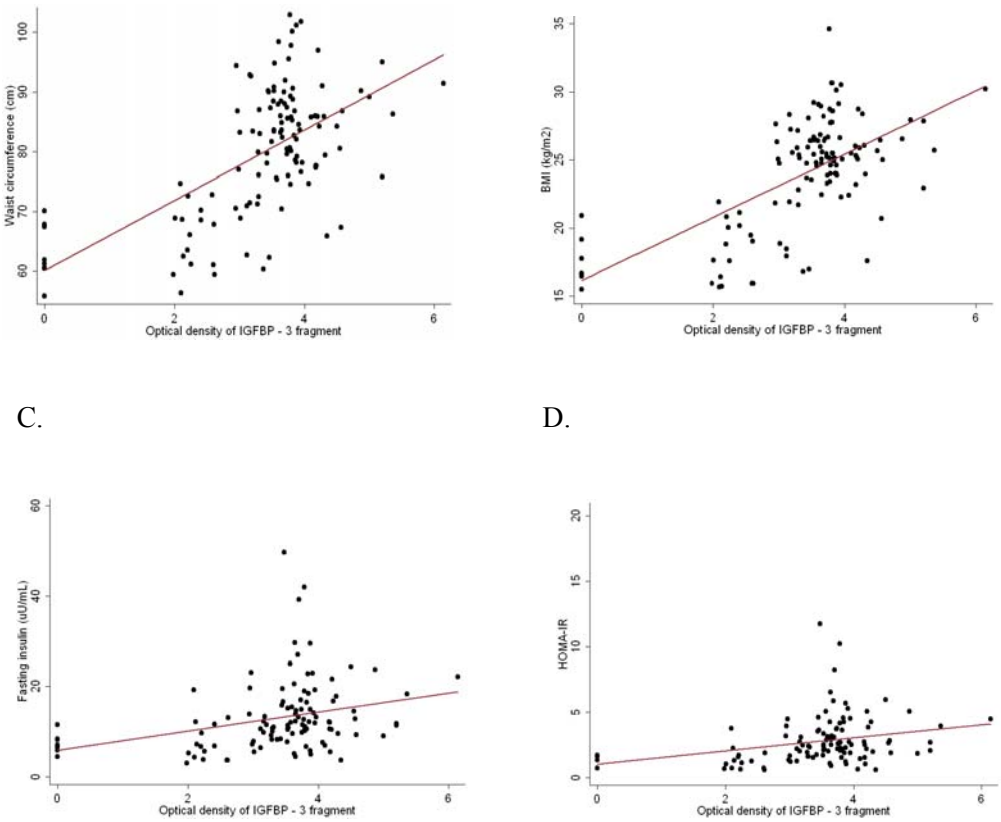


Fig. 2. Relationship between IGFBP-3 proteolysis and (A) waist circumference (r = 0.608, $\boldsymbol{P} < 0.001$), (B) BMI (r= 0.4651, $\boldsymbol{P} < 0.001$), (C) fasting insulin (r = 0.307, $\boldsymbol{P}$ = 0.001), and (D) HOMA-IR (r = 0.313, $\boldsymbol{P} < 0.001$) were calculated by Pearson's correlation coefficient.

IV. DISCUSSION

In this study, we have demonstrated that IGFBP-3 proteolysis is increased in subjects with overweight and obesity compared to normal control. The first identification of protease for IGFBP-3 has been reported in pregnancy sera[18, 19]. After then, the proteolytic degradation of IGFBP-3 has been reported in several catabolic conditions, including cancer, type 1 and type 2 diabetes, Turner syndrome, burn injury, surgery, and arthritis[20-23]. Proteolytic cleavage of IGFBPs has been accepted as the predominant mechanism for the regulation of IGF bioavailability in the serum as well as pericellular environment. Proteases found in a variety of biological fluids including serum, seminal plasma, urine, cerebrospinal, synovial, interstitial, and peritoneal fluids can degrade IGFBP-1-6 into fragments that greatly reduce affinity for IGF-I and IGF-II, thereby increasing the concentration of free IGF and allowing IGF to bind to and activate the IGF receptor[22]. Therefore, IGFBP proteolysis directly regulates IGF receptor signaling and indirectly modulates cell survival, mitogenesis, and differentiation[23]. The rate of IGFBP proteolysis is most likely regulated by multiple mechanisms[22]. Changes in concentrations of IGFBP-directed proteases, such as serine, cysteine, aspartic preoteases, metalloproteases, cathepsins, and/or their inhibitors can directly modulate the rate of IGFBP degradation. Structural modification of IGFBPs by post-translational modifications, such as phosphorylation and glycosylation, can also regulate the rate of IGFBP proteolysis by restricting protease access to the midregion of IGFBP-3. In addition, non-covalent modification

of IGFBPs, such as ligand binding and interaction with extracellular matrix, has been reported to regulate the rate of proteolysis of IGFBPs. Fowlkes *et al.*[26] reported that proteolysis of IGFBP-3, -4, and -5 can be inhibited by intact IGFBPs or fragments derived from IGFBPs, which contain heparin-binding domains.

There are conflicting reports about the effect of obesity on IGFBP-3 proteolysis. In contrast to our results, some studies have demonstrated that IGFBP-3 proteolytic activity in obese subjects is same as that in lean control[27, 28]. However, it is evident that IGFBP-3 proteolysis is increased in patients with noninsulin-dependent diabetes mellitus[29, 30]. In our study, the major proteolytic fragment was migrated at 18 kDa, which is compatible to N-terminal IGFBP-3 fragment observed in urine and serum from healthy children[21] and type 1 diabetes with microalbuminuria [31]. Previous studies have demonstrated that recombinant human IGFBP-3, which is an N-terminal IGFBP-3 fragment cleaved by plasmin[17], and recombinant human IGFBP-3[16] binds insulin with high affinity and blocks insulin action. Taken together, there is a possibility that an 18 kDa-IGFBP-3 fragment observed in the obese subjects may inhibit insulin action by interfering insulin to bind to its receptor, thereby inducing insulin resistance.

Increased serum IGFBP-3 proteolytic activity has been demonstrated in clinical conditions of insulin resistance, including severe illness, after surgery, and noninsulin-diabetes mellitus. Bang *et al.*[32] have demonstrated that patients after colo-rectal surgery have increased IGFBP-3 proteolytic activity in serum and reduced

insulin sensitivity, as determined by hyperinsulinemic, normoglycemic clamps, suggesting IGFBP-3 proteolysis may be associated with insulin resistance. Moreover, they have demonstrated that insulin infusion result in a further increase in IGFBP-3 proteolytic activity, suggesting insulin regulates IGFBP-3 proteolytic activity in the postoperative state. Zachrisson *et al*.[33] reported that IGFBP-3 proteolytic activity is increased in pubertal type 1 diabetic boys, proposing that elevated levels of IGFBP-3 proteolytic activity in pubertal diabetics are related to deteriorated glucose homeostasis and that it may be a compensatory mechanism to attenuate the decrease in free IGF-I in order to partly restore insulin sensitivity and glycemic control. In our study, the degree of proteolysis, determined by immunoblotting analysis, is positively correlated with parameters representing insulin resistance, such as waist circumference, body mass index, fasting glucose, fasting insulin, and HOMA-IR. These findings suggest that IGFBP-3 proteolysis may be involved in the pathogenesis of insulin resistance by obesity. Further, more discrete evidences showing that an 18-kDa fragment of IGFBP-3 in obesity binds to insulin, thereby inhibiting insulin action should be elucidated. It also should be determined whether increased level of IGFBP-3 proteolysis induces insulin resistance, or hyperinsulinemia that is a state in insulin resistance, results in an increase of IGFBP-3 proteolysis. The limitation of this study is that we measured the degree of IGFBP-3 proteolysis by only immunoblotting, but not serum IGFBP-3 proteolytic activity assay. Another one is that we did not evaluate the pubertal stages in study subjects, so we can not consider the difference of

insulin sensitivity as pubertal development. However, this effect may be minimal because the study subjects are in same age period.

Therefore, we suggest that IGFBP-3 proteolytic fragments will be surrogate markers for insulin resistance. Further studies and clinical evidences must be obtained to confirm these observations.

V. CONCLUSION

In conclusion, IGFBP-3 proteolytic fragments were significantly increased in the overweight as well as obesity groups compared to normal controls. The degree of IGFBP-3 proteolysis was positively correlated with parameters representing insulin resistance, such as waist circumference, body mass index, fasting glucose, fasting insulin, and HOMA-IR. These results suggest that proteolysis of IGFBP-3 may be involved in the pathogenesis of insulin resistance and IGFBP-3 fragments could be considered as surrogate markers of insulin resistance in obesity.

REFERNCES

1. Kaur H, Hyder ML, Poston WS. Childhood overweight: an expanding problem. Treat Endocrinol 2003;2:375-88.
2. Kim HM, Park J, Kim HS, Kim DH, Park SH. Obesity and cardiovascular risk factors in Korean children and adolescents aged 10-18 years from the Korean National Health and Nutrition Examination Survey, 1998 and 2001. Am J Epidemiol 2006;164:787-93.
3. Park J, Hilmers DC, Mendoza JA, Stuff JE, Liu Y, Nicklas TA. Prevalence of metabolic syndrome and obesity in adolescents aged 12 to 19 years: comparison between the United States and Korea. J Korean Med Sci 2010;25:75-82.
4. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 2004;350:2362-74.
5. Bao W, Srinivasan SR, Wattigney WA, Berenson GS. Persistence of multiple cardiovascular risk clustering related to syndrome X from childhood to young adulthood. The Bogalusa Heart Study. Arch Intern Med 1994;154:1842-7.
6. Reaven GM. The insulin resistance syndrome: definition and dietary approaches to treatment. Annu Rev Nutr 2005;25:391-406.
7. Savage DB, Petersen KF, Shulman GI. Mechanisms of insulin resistance in humans and possible links with inflammation. Hypertension 2005;45:828-33.
8. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 2002;23:824-54.
9. Ingermann AR, Yang YF, Han J, Mikami A, Garza AE, Mohanraj L, FAN l, Idowu M, Ware JL, Kim HS, Lee DY, Oh Y. Identification of a novel cell death receptor mediating IGFBP-3-induced anti-tumor effects in breast and prostate cancer. J Biol Chem 2010;285:30233-46.
10. Zapf J, Hauri C, Futo E, Hussain M, Rutishauser J, Maack CA, Froesch ER. Intravenously injected insulin-like growth factor (IGF) I/IGF binding protein-3 complex exerts insulin-like effects in hypophysectomized, but not in normal rats. J Clin Invest 1995;95:179-86.
11. Ruan W, Lai M. Insulin-like growth factor binding protein: a possible marker for the metabolic syndrome? Acta Diabetol 2010;47:5-14.

12. Chan SS, Twigg SM, Firth SM, Baxter RC. Insulin-like growth factor binding protein-3 leads to insulin resistance in adipocytes. J Clin Endocrinol Metab 2005;90:6588-95.

13. Kim HS, Ali O, Shim M, Lee KW, Vuguin P, Muzumdar R, Barzilai N, Cohen P. Insulin-like growth factor binding protein-3 induces insulin resistance in adipocytes in vitro and in rats in vivo. Pediatr Res 2007;61:159-64

14. Silha JV, Gui Y, Murphy LJ. Impaired glucose homeostatsis in insulin-like growth factor-binding protein-3-transgenic mice. Am J Physiol Endocrinol Metab 2002;283:E937-45.

15. Muzumdar RH, Ma X, Fishman S, Yang X, Atzmon G, Vuguin P, Einstein FH, Hwang D, Cohen P, Barzilai N. Central and opposing effects of IGF-I and IGF-binding protein-3 on systemic insulin action. Diabetes 2006;55:2788-96.

16. Yamanaka Y, Wilson EM, Rosenfeld RG, Oh Y. Inhibition of insulin receptor activation by insulin-like growth factor binding proteins. J Biol Chem 1997;272:30729-34.

17. Vorwerk P, Yamanaka Y, Spagnoli A, Oh Y, Rosenfeld RG. Insulin and IGF binding by IGFBP-3 fragments derived from proteolysis, baculovirus expression and normal human urine. J Clin Endocrinol Metab 1998;83:1392-5.

18. Hossenlopp P, Segovia B, Lassarre C, Roghani M, Bredon M, Binoux M. Evidence of enzymatic degradation of insulin-like growth factor-binding proteins in the 150K complex during pregnancy. J Clin Endocrinol Metab 1990;71:797-805.

19. Lewitt MS, Scott FP, Clarke NM, Wu T, Sinosich MJ, Baxter RC. Regulation of insulin-like growth factor-binding protein-3 ternary complex formation in pregnancy. J Endocrinol 1998;159:265-74.

20. Davenport ML, Isley WL, Pucilowska JB, Pemberton LB, Lyman B, Underwood LE, Clemmons DR. Insulin-like growth factor-binding protein-3 proteolysis is induced after elective surgery. J Clin Endocrinol Metab 1992;75:590-5.

21. Spagnoli A, Gargosky SE, Spadoni GL, MacGillivray M, Oh Y, Boscherini B, Rosenfeld RG. Characterization of a low molecular mass form of insulin-like growth factor binding protein-3 (17.7 kilodaltons) in urine and serum from healthy children and growth hormone (GH)-deficient patients: relationship with GH therapy. J Clin Endocrinol Metab 1995;80:3668-76.

22. Maile LA, Holly JM. Insulin-like growth factor binding protein (IGFBP) proteolysis: occurrence, identification, role and regulation. Growth Horm IGF Res 1999;9:85-95.

23. Bunn RC, Fowlkes JL. Insulin-like growth factor binding protein proteolysis. Trends Endocrinol Metab 2003;14:176-81.

24. Lee DC, Lee JW, Im JA. Association of serum retinol binding protein 4 and insulin resistance in apparently healthy adolescents. Metabolism 2007;56:327-31

25 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

26. Fowlkes JL, Thrailkill KM, George-Nascimento C, Rosenberg CK, Serra DM. Heparin-binding, highly basic regions within the thyroglobulin type-1 repeat of insulin-like growth factor (IGF)-binding proteins (IGFBPs) -3, -5, and -6 inhibit IGFBP-4 degradation. Endocrinology 1997;138:2280-5.

27. Ballerini MG, Ropelato MG, Domene HM, Pennisi P, Heinrich JJ, Jasper HG. Differential impact of simple childhood obesity on the components of the growth hormone-insulin-like growth factor (IGF)-IGF binding proteins axis. J Pediatr Endocrinol Metab 2004;17:749-57.

28. Ramussen MH, Juul A, Kjems LL, Hilsted J. Effects of short-term caloric restriction on circulating free IGF-I, acid-labile subunit, IGF-binding proteins (IGFBPs)-1-4, and IGFBPs-1-3 protease activity in obese subjects. Eur J Endocrinol 2006;155:575-81.

29. Bang P, Brismar K, Rosenfeld RG, Hall K. Fasting affects serum insulin-like growth factors (IGFs) and IGF-binding proteins differently in patients with noninsulin-dependent diabetes mellitus versus healthy nonobese and obese subjects. J Clin Endocrinol Metab 1994;78:960-7.

30. Lassarre C, Duron F, Binoux M. Use of the ligand immunofunctional assay for human insulin-like growth factor (IGF) binding protein-3 (IGFBP-3) to analyze IGFBP-3 proteolysis and IGF-I bioavailability in hwathy adults, GH-deficient and acromegalic patients, and diabetics. J Clin Endocrinol Metab 2001;86:1942-52.

31. Spagnoli A, Chiarelli F, Vorwerk P, Boscherini B, Rosenfeld RG. Evaluation of the components of insulin-like growth factor (IGF)-IGF binding protein (IGFBP) system in adolescents with type 1 diabetes and persistent microalbuminuria: relationship with inceased urinary excretion of IGFBP-3 18 kD N-terminal fragment. Clin Endocrinol 1995;51:587-96.

32. Bang P, Nygren J, Carlsson-Skwirut C, Thorell A, Ljungqvist O. Postoperative induction of insulin-like growth factor binding protein-3 proteolytic activity: relation to insulin and insulin sensitivity. J Clin Endocrinol Metab 1998;83:2509-15.

33. Zachrisson I, Brismar K, Carlsson-Skwirut C, Dahlquist G, Wallensteen M, Bang P. Increased 24 h mean insulin-like growth factor binding protein-3 proteolytic activity in pubertal type 1 diabetic boys. Growth Horm IGF Res 2000;10:324-31.

ABSTRACT (in Korean)

비만 청소년에서 인슐린양성장인자 결합단백-3 의 단백분해와 인슐린저항성과의 연관성

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김기은

목적: 전세계적으로 소아청소년의 비만이 증가하고 있으며, 이로 인해 청소년들의 대사증후군에 대한 유병률 증가가 초래되어 사회경제적 문제를 야기한다. 최근에 비만세포와 동물모델에서 인슐린양성자인자 결합단백-3의 대사와 관련된 역할이 밝혀지고 있으나 임상과 관련된 연구는 충분하지 않은 실정이다. 본 연구는 비만한 청소년에서 인슐린양성장인자 결합단백-3 단백분절이 인슐린저항성과 관련성이 있는지를 알아보고자 하였다.

대상 및 방법: 건강검진을 위해 병원을 방문한 12-13 세 청소년 197 명을 대상으로 성별, 연령별 체질량지수에 따라 정상군 100 명 (남아 49 명, 여아 51 명), 과체중군 41 명 (남아 19 명, 여아 22 명), 비만군 56 명 (남아 25 명, 여아 31 명)으로 구분하였다. 체중, 키, 허리둘레의 신체계측을 하고 혈압을 측정하였다. 혈액검사를 통해 공복시 혈당, 인슐린, hs-CRP, AST, ALT, 혈청지질대사분석을 시행하고, 저밀도콜레스테롤을 산출하였으며, 인슐린저항성의 지표로 HOMA-IR 을 산출하였다. 혈청을 전기영동 후 Western immunoblot 을 시행하여 인슐린양성장인자 결합단백-3 의 29-kDa , 18-kDa 분절을 얻었으며, 이에 대한 흡광도를 측정하였다.

결과: 체중, 키, 허리둘레, 체질량지수, 수축기 혈압, 이완기 혈압, 중성지방, 저밀도콜레스테롤, ALT, 공복혈당, 공복 인슐린이 정상군에 비해 과체중군 또는 비만군에서 증가되었다($P < 0.01$). 고밀도콜레스테롤은 비만군과 과체중군에서 감소되었다($P < 0.01$). HOMA-IR 은 정상군에 비해 과체중군과 비만군에서 의미있는 증가를 보였다. (정상군 *vs.* 과체중군, 1.5±0.8 *vs.* 2.9±3.2, $P < 0.001$; 정상군 *vs.* 비만군, 1.5±0.8 *vs.* 3.6 ± 2.2, $P < 0.001$), 인슐린양성장인자 결합단백-3 의 단백분절 정도도 정상군에 비해 과체중군과 비만군에서 증가되었다 (정상군 *vs.* 과체중군, 2.09 ± 1.32 *vs.* 3.83 ± 0.37, $P < 0.001$; 정상군 *vs.* 비만군, 2.09 ± 1.32 *vs.* 3.85 ± 0.63, $P < 0.001$).

인슐린양성장인자 결합단백-3 의 단백분절 정도는 체중 ($r = 0.599$, $\boldsymbol{P} < 0.001$), 키 ($r = 0.241$, $\boldsymbol{P} < 0.001$), ALT ($r = 0.220$, $\boldsymbol{P} < 0.05$), 공복혈당 ($r = 0.295$, $\boldsymbol{P} = 0.001$), 공복인슐린 ($r = 0.307$, $\boldsymbol{P} = 0.001$), 체질량지수 ($r = 0.651$, $\boldsymbol{P} < 0.001$), 허리둘레 ($r = 0.608$, $\boldsymbol{P} = 0.001$) 와 각각 양의 상관관계를 보였다. 인슐린양성장인자 결합단백-3 의 단백분절 정도가 증가 할수록 인슐린저항성을 반영하는 HOMA-IR 또한 증가하는 양상을 보였다 ($r = 0.313$, $\boldsymbol{P} < 0.001$).

결론: 본 연구를 통하여 청소년, 비만군과 과체중군에서 혈청 인슐린양성장인자 결합단백-3 의 단백분해 분절이 증가되어 있었으며, 분절 정도가 증가 할수록 인슐린저항성이 증가됨을 확인하였다. 이는 인슐린양성장인자 결합단백-3 의 분절이 비만의 인슐린저항성 발병과정에 관여함을 시사하며, 인슐린저항성의 표지자로 이용될 수 있을 것으로 생각된다.

핵심 되는 말: 비만, 인슐린양성장인자 결합단백-3, 단백분해 분절, 인슐린저항성, 청소년.