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Clinical Investigation

The Relationship between Preheparin Lipoprotein Lipase and Metabolic Derangements in Obese Japanese Children

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Abstract. The aim of this study was to clarify the relationship between preheparin lipoprotein lipase (LPL) and derangements of metabolic status in obese Japanese children. We examined 102 obese children (55 boys and 47 girls; mean age 10.9 yr). Anthropometry, blood pressure and levels of liver transaminases, serum lipids and lipoproteins, uric acid, fasting blood glucose (FBG), serum insulin, LPL, leptin and adiponectin were measured. The subjects were divided into the metabolic syndrome (MS) and non-MS groups. The levels of LPL were compared between these groups. Statistical analysis showed that the LPL levels were significantly lower in the MS group compared with the non-MS group, with the levels decreasing progressively as the number of MS components increased. We conclude that LPL levels decrease also in obese Japanese children with a deteriorated metabolic status in the same way as in adults.

Key words: lipoprotein lipase, metabolic syndrome, obese children

Introduction

Obesity in children has become a serious public health problem similar to that found in the adult population. It is well known that obesity increases the risk of complications such as diabetes mellitus and cardiovascular disease in adults. Although obese children have lower incidences of these complications than adults, it is obvious that preventing the development of obesity and its concomitant medical problems is a useful method for decreasing severe health problems in adulthood. For this reason, it is absolutely imperative to initiate aggressive medical interventions in obese children, especially those with a higher risk of developing metabolic abnormalities or complications such as diabetes mellitus or hypertension.

Lipoprotein lipase (LPL) is a key enzyme synthesized in adipose, cardiac and skeletal muscle cells. It hydrolyzes triglyceride (TG) in chylomicrons and very low-density lipoproteins and is regulated by insulin (1, 2). LPL is bound to heparan sulfate proteoglycan (HSPG) on vascular endothelial cells. So, the LPL levels have previously been measured after injection

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of heparin to cleave LPL from HSPG. However, the adequacy of this postheparin LPL measurement remains uncertain. One of the reason for this is that heparin also releases hepatic lipase (HL) from hepatic sinusoid capillaries, resulting in a significant contribution of HL to the total postheparin lipolytic activity (3). Recent reports demonstrated that in nonheparinized plasma, a small part of LPL is associated with circulating lipoproteins (4), and preheparin LPL had a positive correlation with postheparin lipolytic activity (5). Subsequently, the clinical significance of measuring preheparin LPL has been investigated, and it has become apparent that it is useful for obtaining pathophysiologic information about metabolic disorders or acute inflammation (6).

Several researchers have reported that LPL may be a marker of insulin resistance and thereby reflect the risk of developing diabetes mellitus and cardiovascular disease in adulthood (7–12). However, the relationship between LPL and serious metabolic status has remained largely unstudied in children. The purpose of this study was therefore to determine whether preheparin LPL is a marker of deteriorating metabolic status in obese Japanese children.

Subjects

We examined 102 obese children (55 boys and 47 girls; mean age 10.9 yr, range 6.8–14.7 yr) who lived in Niigata Prefecture, Japan and received regular medical examinations in conjunction with "The Prevention of Cardio- and Cerebrovascular Diseases in Childhood" program. The Division of Pediatrics, Niigata University Graduate School of Medical and Dental Sciences and the school health divisions of local governments in Niigata Prefecture undertake this program every year. All subjects were more than 20% overweight based on age- and sex-specified body weights for height (percent relative body weight [%RBW]) and had a body fat percentage >25% for boys or >30% for girls aged <11 yr or >35% for girls aged ≥11 yr. No subjects had known endocrine disorders or diabetes. The anthropometric measurements and blood examinations were performed after informed consent was obtained from the parents or guardians of all the subjects. The Ethics Committee of the Niigata University Graduate School of Medical and Dental Sciences approved this study.

Methods

Height was measured by a portable stadiometer to the nearest 1 mm, weight was measured by a digital scale to the nearest 0.1 kg and %RBW was calculated based on the standard body weight of Japanese children published in 1990 by the Ministry of Education, Science and Culture of Japan. Waist and hip circumferences were measured to the nearest 1 mm. Systolic (SBP) and diastolic blood pressures (DBP) were measured in triplicate in the right arm, with the subjects seated quietly, using an automated sphygmomanometer (Dinamap Model 8104; Critikon Inc., Tampa, FL, USA). The third measurement was used in the statistical analyses. Body composition was measured using an InBody 3.0 multi frequency bioelectrical impedance analyzer (Biospace, Seoul, Korea). Abdominal fat thickness was estimated by ultrasonography (Model SSA-250A; Toshiba Corp., Tokyo, Japan) (13) with the subjects in the supine position and the linear-array probe kept perpendicular to the skin on the upper medial aspect of the abdomen. A longitudinal scan was then performed from the xiphoid process to the navel along the linea alba. Scanning was performed at the optimal position, with the surface of the liver being kept almost parallel to the skin, by requesting that the subjects hold their breath. The probe was applied lightly to the skin in order to avoid compression of the fat layer. Maximum preperitoneal fat thickness (Pmax) and minimum subcutaneous fat thickness (Smin) were measured directly from the screen using electronic calipers

(14).

Birth weight was obtained from maternal and child health handbooks, and then the current weight–to–birth weight ratio [WBWR: current weight (kg)/birth weight (kg)]) was calculated.

Blood samples were collected from the subjects after an overnight fast for measurement of the levels of serum liver enzymes, lipids, lipoproteins, uric acid, fasting blood glucose (FBG), hemoglobin A1c (HbA1c), fasting serum insulin, leptin, adiponectin and lipoprotein lipase (LPL) in preheparin serum. The homeostasis model assessment-insulin resistance index (HOMA-R) was calculated as FBG [mg/dl] × serum insulin [μU/ml]/405.

Statistical Analysis

Metabolic classification of the children

The children were divided into the two groups, those with metabolic syndrome (MS) and those without MS (MS group and non-MS group, respectively). We used the criteria of MS for Japanese children proposed by the study group of the Ministry of Health, Labour and Welfare of Japan (15): 1) waist circumference ≥80 cm; 2) serum triglyceride (TG) levels ≥120 mg/dl or high-density lipoprotein cholesterol (HDL-C) levels <40 mg/dl; 3) FBG levels ≥100 mg/dl; and 4) SBP ≥125 mmHg or DBP ≥70 mmHg. A diagnosis of MS was made in children who complied with criterion 1 and had at least two of the other criteria (2 to 4).

Age, hip circumference, body fat percentage, SBP, DBP, birth weight, T-Chol (total cholesterol), LDL-C (low-density lipoprotein cholesterol) and HbA1c were normally distributed and were expressed as a range, median, mean and SD. The other parameters were not normally distributed and were expressed as a range, median and mean.

Stepwise multiple regression analysis was used to examine the influence of height, weight, %RBW, age and gender on LPL levels. LPL levels, height, weight and %RBW were log-transformed before analysis.

The relationships between anthropometric measurements, birth weight, WBWR, metabolism-related parameters and LPL levels were analyzed using Spearman's rank correlation coefficients.

Tukey-Kramer honestly significant difference test was used to examine differences in LPL levels between the MS and non-MS groups. LPL levels were log-transformed before analysis, and the results were expressed as means ± SD for the both original and log-transformed values.

We analyzed the relationship between LPL and total number of MS components (number of MS criteria) included in the definition criteria. Insulin and adiponectin levels were also analyzed for comparison. Each value was log-transformed before analysis by the Tukey-Kramer honestly significant difference test. The total number of MS criteria ranged from 0 in the G0 group to 4 in the G4 group. Because there was only one subject in G4 group, G3 and G4 were grouped together and analyzed as one group (G3-4).

All statistical analyses were carried out using JMP Ver. 8.0.2 (SAS Institute Inc., Cary, NC, USA). Probability (p) values <0.05 were considered statistically significant in all the analyses.

Results

The clinical characteristics of the subjects are summarized in Table 1. All anthropometric measurements and metabolism-related laboratory data are expressed independent of gender. The median LPL level was 59.0 ng/ml.

Table 2 summarizes the results of the stepwise multiple regression analyses and confirms the relationship between the LPL levels and height, weight, %RBW, age and gender. The results demonstrated that age correlated negatively with the LPL levels.

Table 3 summarizes the relationships between anthropometric measurements, birth

Table 1 Clinical characteristics of the subjects (n=102; boys 55, girls 47)

	Range	Median	Mean	SD
Age (yr)	6.8–14.7	11.0	10.9	2.0
Height (cm)	120.0–186.1	144.7	144.3	
Weight (kg)	31.5–132.0	53.4	56.9	
%RBW (%)	31.6–93.3	47.1	50.1	
Waist circumference (cm)	65.0–125.0	82.0	84.0	
Hip circumference (cm)	68.5–118.0	87.5	88.7	9.8
Body fat percentage (%)	26.1–49.2	38.6	38.1	4.1
Smin (mm)	3.8–29.0	12.1	12.9	
Pmax (mm)	2.4–21.4	11.9	12.0	
SBP (mmHg)	87–148	113.0	113.9	11.6
DBP (mmHg)	37–73	57.5	55.8	7.8
Birth weight (g)	1,850–4,678	3,215.5	3,214	506.2
WBWR	9.4–40.7	16.3	18.0	
ALT (IU/l)	7–292	24.0	35.5	
T-Chol (mg/dl)	121–264	184.0	184.3	29.5
HDL-C (mg/dl)	36–87	52.5	54.6	
LDL-C (mg/dl)	41–191	113.6	117.0	26.9
TG (mg/dl)	26–242	89.5	97.1	
FBG (mg/dl)	71–102	85.0	84.6	
UA (mg/dl)	3.2–10.8	5.1	5.3	
HbA1c (%)	4.4–5.7	5.0	5.0	0.2
Serum insulin (μU/ml)	2.9–51.1	11.9	14.3	
Leptin (ng/ml)	2.7–52.3	15.1	16.9	
Adiponectin (μg/ml)	2.8–20.4	8.0	8.5	
LPL (ng/ml)	33.4–105.0	59.0	62.1	
HOMA-R	0.6–12.7	2.5	3.0	

%RBW, percent relative body weight; Pmax, maximum preperitoneal fat thickness; Smin, minimum subcutaneous fat thickness; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBWR, current weight-to-birth weight ratio; ALT, alanine aminotransferase; T-Chol, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; FBG, fasting blood glucose; UA, uric acid; HbA1c, hemoglobin A1c; LPL, lipoprotein lipase; HOMA-R, homeostasis model assessment-insulin resistance (FBG [mg/dl] × serum insulin [μU/ml]/405).

Table 2 Stepwise multiple regression analysis of LPL levels showing the relationship with height, weight, %RBW, age and gender

	r	*F*	
Height	NO	0.001	R^2=0.062
Weight	NO	0.156	
%RBW	NO	1.048	
Age	–0.016	7.620	p<0.01
Gender	NO	0.959	

NO, not obtained. The abbreviations are the same as in Table 1. Height, weight, %RBW and LPL levels were log-transformed before analysis.

weight, WBWR, metabolism-related laboratory data, HOMA-R and LPL levels. The LPL levels correlated positively with adiponectin and negatively with WBWR and serum insulin. There were no significant correlations observed between any of the other parameters and the LPL levels.

The data summarized in Table 4 confirms the relationships between the LPL levels and metabolic status. There were significant differences in the LPL levels between the MS

Table 3 Spearman's rank correlation coefficients between LPL and anthropometric measurements, metabolism-related laboratory data and HOMA-R

	r	p		*r*	p
Weight	–0.27	<0.01	ALT	–0.13	NS
%RBW	–0.15	NS	T-Chol	–0.19	NS
Waist circumference	–0.27	<0.01	HDL-C	0.18	NS
Hip circumferences	–0.24	<0.05	LDL-C	–0.28	<0.005
Body fat percentage	–0.14	NS	TG	–0.25	<0.05
Smin	–0.17	NS	FBG	0.07	NS
Pmax	–0.21	<0.05	UA	–0.25	<0.01
SBP	0.05	NS	HbA1c	0.14	NS
DBP	0.03	NS	Serum insulin	–0.31	<0.005
Birth weight	0.11	NS	leptin	–0.13	NS
WBWR	–0.33	<0.001	adiponectin	0.31	<0.005
			HOMA-R	–0.27	<0.01

Abbreviations are the same as in Table 1.

Table 4 Serum LPL Levels grouped according to metabolic status

	Non-MS (n=94)	MS (n=8)	p
log (LPL)	1.783 ± 0.122	1.685 ± 0.113	
Mean	60.7	48.4	<0.05
Mean-1SD-Mean+1SD	45.9–80.4	37.3–62.8	

MS, metabolic syndrome; Other abbreviations are the same as in Table 1.

and non-MS groups.

Table 5 shows the LPL, insulin and adiponectin levels of the subjects divided into four groups according to their total number of MS components. LPL and insulin showed significant differences between these subgroups, while there were no statistical differences in adiponectin between the G0–G3-4 groups. The LPL levels became progressively lower as the total number of MS components increased.

Discussion

In our study, multiple regression analyses examining the influence of physical attributes, age and gender on the LPL levels revealed a negative correlation between the LPL levels and age, although the correlation coefficient of this relationship was considered too small to be statistically significant (R^2=0.062). Furthermore, it was previously shown that the LPL levels were not influenced by either gender or age in adults (16), and therefore, in our study, we analyzed all subjects together without considering age and gender.

In this study, the LPL levels showed significant negative correlations with WBWR and serum insulin levels and a positive correlation with adiponectin. As there were no subjects with diabetes mellitus or apparent elevations in FBG in our study, the serum insulin levels were considered to directly reflect insulin resistance. The results indicate that the LPL levels may also be an indicator of insulin resistance in obese children.

In overweight subjects, it is known that degradation of adiponectin and its receptor induces oxidant stress (17, 18), resulting in

Table 5 Serum LPL, insulin and adiponectin levels grouped according to the number of MS components

	G0 (n=31)	G1 (n=41)	G2 (n=22)	G3-4 (n=8)
LPL				
log (LPL)	1.819 ± 0.120*	1.768 ± 0.124	1.761 ± 0.115	1.685 ± 0.113
Mean	65.9	58.7	57.6	48.4
Mean-1SD-Mean+1SD	50.0–86.8	44.1–78.1	44.2–75.1	37.3–62.8
Insulin				
log(insulin)	0.893 ± 0.200‡ §*	1.099 ± 0.203*	1.219 ± 0.198	1.375 ± 0.273
Mean	7.8	12.5	16.5	23.7
Mean-1SD-Mean+1SD	4.9–12.4	7.9–20.0	10.5–26.1	12.6–44.4
Adiponectin				
log(adiponectin)	0.953 ± 0.181	0.904 ± 0.167	0.853 ± 0.148	0.799 ± 0.119
Mean	9.0	8.0	7.1	6.3
Mean-1SD-Mean+1SD	5.9–13.6	5.4–11.8	5.1–10.0	4.8–8.3

‡ $p<0.05$ versus the value of the G1 group. § $p<0.05$ versus the value of the G2 group. * $p<0.05$ versus the value of the G3-4 group. Analyzed using the Tukey-Kramer honestly significant difference test. The abbreviations are the same as in Table 1.

infiltration of macrophages into adipose tissue and increased levels of tumor necrosis factor-α, free fatty acids and interleukin-6, followed by increased insulin resistance (19, 20). As the LPL levels are regulated by insulin (1, 2), such metabolic conditions would be expected to result in lower LPL levels. The positive correlation we observed between the LPL levels and adiponectin indicates that the same sequence of changes may also lead to decreased LPL levels in obese children.

Our results demonstrated that there was a stronger correlation between LPL and WBWR than either current weight or birth weight alone. We have reported previously that lower birth weight and current overweight status increased the risk of MS (21, 22). There is also evidence that the LPL levels are decreased in adults with MS (23). Taken together, these results suggest that the LPL levels may decrease in children with MS probably mediated by the development of insulin resistance.

On the basis of these results, we decided to compare the LPL levels in children divided into the MS and non-MS groups. The LPL levels were significantly lower in the MS groups, suggesting that the LPL levels were lower in obese children with more severe metabolic conditions and associated complications.

To further our investigations, we compared LPL, insulin and adiponectin levels in subjects grouped according to the number of MS components they possessed. LPL showed a progressive decrease in levels as the number of components increased and significant differences between G0 and G3-4. Insulin also showed significant differences between Group G0 and Groups G1 to G3-4 and between Groups G1 and G3-4.

In adults, it has been reported that the LPL levels are significantly lower in subjects with type 2 diabetes mellitus than in healthy controls and show a negative correlation with HbA1c (23). There is also evidence that lower LPL levels are associated with an increased risk of cardiovascular disease (11). Diabetes mellitus and cardiovascular disease are major health problems in adulthood, and therefore, measurements of the LPL levels would have clinical use for predicting the development of these conditions. Additionally,

it has previously been reported that body weight loss induced an increase in the expression of LDL-C receptor and LPL followed by a decrease in the TG and LDL-C levels in adults (24). Although further investigation is required, LPL may also be a clinical indicator of effective medical interventions.

Obesity in childhood usually occurs without obvious symptoms and has a lower incidence of complications. However, Morrison *et al.* reported that MS children had an increased risk of also having the syndrome or developing type 2 diabetes mellitus in adulthood (odds ratio: 9.4 and 11.5, respectively) (25). Therefore, detecting deteriorated metabolic condition in obese children is important. We conclude that LPL levels may also decrease in obese children as a consequence of insulin resistance caused by visceral fat accumulation, with the magnitude of the decrease being dependent on the severity of the metabolic abnormalities. For this reason, LPL may be usable to estimate the metabolic conditions of obese children as in adults.

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