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ORIGINAL

Elevated glucose level at 30 minutes during an oral glucose tolerance test in obese adolescents: a new disorder of glucose tolerance

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Abstract. We observed glucose levels >140 mg/dL measured at 30 minutes (min) during an oral glucose tolerance test (OGTT) in some obese patients. We aimed to investigate the significance of this finding by comparing lipid profiles, insulin resistance indices, and systemic inflammatory mediators between obese adolescents with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and elevated glucose levels at 30 min. The study involved 80 obese (body mass index >95th percentile for age and sex) adolescents (48 female, 32 male) between 11 and 16 years of age. Depending on OGTT results, patients were divided into NGT and IGT groups. The third group was recruited from the NGT group as having glucose levels > 140 mg/dL at 30 minutes. Lipid profiles, [interleukin-6 (IL-6)], neopterin, and lipoprotein associated phospholipase A_2 (Lp-PLA_2)] were assessed. Neopterin and Lp-PLA_2 levels were significantly higher in obese adolescents with elevated glucose levels at 30 min. compared with those in both NGT and IGT groups (p=0.013, and 0.004, respectively). In these adolescents, IL-6 levels were significantly higher only than the NGT group (p=0.01). In logistic regression analysis, IL-6, neopterin and Lp-PLA_2 levels were detected to be related to high blood glucose levels at 30 min (OR 1.11, p = 0.01; OR 9.03, p=0.013; OR 1.01, p=0.004 respectively). Obese adolescents with elevated glucose levels at 30 min. demonstrated higher inflammatory mediators levels, which were atherosclerotic indicators, than obese adolescents with NGT and IGT. These results suggest that glucose levels >140 mg/dL measured at 30 min during an OGTT may be a new disorder of glucose tolerance in obesity.

Key words: Obesity, Adolescents, Systemic inflammatory mediators, Lp-PLA_2, Interleukin-6

OBESITY is associated with insulin resistance and risk of impaired glucose homeostasis (IGH). In obese children, impaired glucose homeostasis, including impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and type 2 diabetes (T2DM), is increasing [1, 2]. Recently, it has been suggested that the oral glucose tolerance test (OGTT) might be a reliable method in identifying obese children who were at high risk for IGT and T2DM [3, 4]. IGH is associated with various abnormalities, including hypertension [5], intra-abdominal (central or visceral) adiposity [6], and lipid abnormalities [7]. It is reported in previous studies that visceral adiposity was a significant prospective risk factor for coronary heart diseases in adult men [6].

Obesity has been associated with higher inflammatory mediators levels in both children and adults [8,

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Abbreviations: IGH, Impaired glucose homeostasis; IFG, Impaired fasting glucose; IGT, Impaired glucose tolerance; OGTT, Oral glucose tolerance test; T2DM, Type 2 diabetes Mellitus; CRP, C-reactive protein; TNF-α, Tumor necrosis factor alpha; IL-6, Interluekin-6; Lp-PLA_2, Lipoprotein-associated phospholipase A_2, LDL-C, Low density lipoprotein-cholesterol; HbA1c, Glycated hemoglobin; TC, Total cholesterol; TG, Triglyceride; HDL-C, High-density lipoprotein-cholesterol; ApoA, Apolipoprotein AI; ApoB, Apolipoprotein B100; BMI, Body Mass Index; BMI SDS, BMI standard deviation scores; IDF, International Federation of Diabetes; NGT, Normal glucose tolerance; HOMA-IR, Homeostasis model assessment; OR, Odds ratio; WHR, Waist to hip ratio

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9]. It is postulated that increased macrophage accumulation in white adipose tissue, particularly in the visceral depots, promotes synthesis of inflammatory mediators [10]. In the literature, there are numerous studies demonstrating increased C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α) and interluekin-6 (IL-6) levels in obese children [11]. The role of inflammation and immune activation in atherogenesis is well established [12]. Elevated neopterin levels produced by human monocyte- derived macrophages and dendritic cells, provide information about patients at risk for myocardial infarction [13, 14]. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is considered to be a novel inflammatory marker. Moreover, in adults, elevated Lp-PLA_2 is accepted as a cardiovascular risk marker independent of traditional risk factors such as increased low density lipoprotein cholesterol (LDL-C) and CRP [15]. However, studies in obese children regarding neopterin and Lp-PLA_2 levels are few in numbers [16, 17]. Persistent inflammation in obese children may increase the risks of metabolic and cardiovascular events later in life [11].

In our clinical practice, we have observed that some obese adolescents had glucose levels >140 mg/dL at 30 minutes during an OGTT. The aim of this study was to evaluate clinical significance of elevated glucose levels at 30 minutes during OGTT in obese adolescents. Therefore, we compared lipid profiles, insulin resistance, and systemic inflammatory mediators which are atherosclerotic indicators, of these adolescents with those of obese adolescents with normal and impaired glucose tolerance.

Subjects and Methods

Study design and methods

Eighty obese adolescents (48 girls and 32 boys; mean age 13.61 ± 2.07 years; mean body mass index (BMI) 30.24 ± 5.70 kg/m^2) at puberty were included in this study. All adolescents were recruited from the Pediatric Endocrinology and Metabolism outpatient unit of the hospital.

After the overnight fasting, venous blood samples were drawn to assess glucose, insulin, glycated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), apolipoprotein AI (Apo A), apolipoprotein B100 (Apo B), fibrinogen, CRP, IL- 6, neopterin levels and Lp-PLA_2 activities. In all subjects, thyroid function tests, diurnal cortisol levels and basal adrenocorticotropic hormone levels were assessed to exclude hypothyroidism and hypercortisolism. All participants were at puberty, so they were referred to as "adolescents". The pubertal stages were determined according to Marshall and Tanner [18]. The study was approved by the Scientific Ethics Committee of Celal Bayar University, Medical School. An informed written consent was obtained from the parents of all children who underwent OGTT and other biochemical investigations without any incentive or compensation.

Anthropometric measurements

Weight was measured to the nearest 0.5 kg using a balance beam scale and height was measured to the nearest 0.1 cm with a manual height board. The calculated Body Mass Index (BMI; kg/m^2) was used as an index of relative weight. BMI standard deviation scores (SDS) were calculated and BMI percentiles were evaluated for age and gender according to national standarts. The SDS represented the number of standard deviations above or below the mean value of the considered population which is based on standardized tables for children and adolescents.

Oral glucose tolerance test (OGTT)

A standard OGTT at 8:00 was performed in all obese adolescents to establish their glucose tolerance. After the overnight fasting, an antecubital intravenous catheter was inserted for blood sampling and its patency was maintained by slow infusion of normal saline. A flavored glucose solution at a dose of 1.75g/kg body weight (up to a maximum of 75 g) was given orally and blood samples were obtained for glucose and insulin measurements every 30 minutes for 120 minutes.

Definitions

Obesity was defined as having a percentile of BMI ≥ 95^{th} for age and sex [19]. Based on OGTT results, 80 participants were divided into three groups. First and second groups were defined according to International Federation of Diabetes (IDF) criteria. The first group (normal glucose tolerance, NGT) was defined as having fasting glucose levels < 100 mg/dL and 2-hour glucose levels < 140 mg/dL. The second group (IGT) was defined as having fasting glucose levels < 100 mg/dL and 2-hour glucose levels between 140-199 mg/dL [20, 21]. Finally, the third group (blood glucose (BG)>140 mg/dL at 30 minutes) was recruited from

the NGT group as having glucose levels > 140 mg/dL at 30 minutes. Insulin sensitivity was evaluated by both the Matsuda index (ISI $_{MATSUDA}$) and [22], calculated as 10,000 per square root of (fasting glucose [mg/dL] x fasting insulin [μIU/mL]) x (mean glucose x mean insulin during OGTT) and homeostasis model assessment (HOMA-IR) = [(fast. insulin (μIU/mL) × fast. glucose (mg/dL)/405] [23]. Insulinogenic index (ΔI30/ΔG30) was calculated as I_{30}-I_0/G_{30}-G_0, as a measure of insulin secretion at 30 min in OGTT [24].

Biochemical analysis

All of biochemical analyses were performed on the sample collection day, except for IL-6, neopterin and Lp-PLA_2 measurements, for which fasting serum samples were stored at - 80 °C until the time of analysis. Glucose levels were measured with hexokinase (Beckman Coulter, USA) and insulin levels were measured with immunchemiluminescence (Siemens, USA) methods on autoanalyzers. The fasting values of HbA1c, TC, TG, HDL- C, Apo A, Apo B and CRP were determined by commercially available kits (Beckman Coulter, USA). Plasma fibrinogen levels were measured by Clauss method on a coagulometer (Beckman Coulter, USA). LDL-C was calculated by the Friedewald equation [25].

The fasting serum concentrations of IL-6, neopterin and Lp-PLA_2 were determined by use of ELISA (DIA Source, Belgium, DRG Instruments GmbH, Germany and dia Dexus, Inc. USA, respectively). The lowest detection limits were approximately 2 pg/mL for IL-6, 0.2 ng/mL for neopterin, and 0.34 ng/mL for Lp-PLA_2.

Statistical analysis

Statistical Package for Social Sciences for Windows statistical software version 16.0 was used. Data distribution was analyzed by using the Kolmogorov–Smirnov test. Anthropometric and biochemical parameters were compared by Kruskal–Wallis analysis among the three groups. Mann-Whitney-U test was used to compare the two groups. Pearson's or Spearman's correlation coefficients were used to quantify the univariate associations among variables. Two logistic regression models were used to identify predictors of high glucose levels measured at 30 min. during OGTT. First model was calculated for NGT and groups which had glucose levels >140 mg/dL at 30 min. The second one was calculated for NGT and IGT groups. IL-6, neopterin, Lp-PLA_2, CRP and fibrinogen were independent variables for both models. Data were expressed as mean±SD. Level of significance for differences was considered at $p < 0.05$.

Results

Mean age of obese adolescents was 13.61 ± 2.07 years. Of these patients 40.0% (n=32) were male, 60.0% (n=48) were female. The female to male ratios in NGT, IGT and the group which had increased glucose levels at 30 min. were 15:10, 17:9 and 16:13, respectively. There was no significant difference in age between three groups (p=0.228) (Table 1).

Glucose and insulin levels at fasting, 30 th, 60 th, 90 th, 120 th minutes during OGTT for all groups are shown in Fig. 1. Insulin levels at 90 and 120 minutes during OGTT in obese with IGT were significantly higher

Table 1 Anthropometric parameters of obese adolescents with NGT, IGT, and elevated glucose level at 30 min during OGTT.

Anthropometric parameters	NGT (n= 25)	IGT (n= 26)	BG>140 mg/dL at 30 min. (n:29)	p
Age (years)	12.99±2.32	14.01±2.05	13.78±1.78	0.228
Height SDS	0.21±1.17	0.54±0.72	-0.14±1.27	0.119
BMI (kg/m^2)	30.09±5.6	30.41±5.0	30.28±6.43	0.816
BMI SDS	4.66±1.86	4.36±2.08	4.48±1.72	0.660
Waist (cm)	97.16±12.48	98.1±11.17	98.7±14.19	0.882
Hip (cm)	106.92±12.08	106.0±10.1	105.51±2.86	0.819
Waist/Hip	0.90±0.4	0.91±0.06	0.93±0.05	0.225

Kruskal-Wallis, Data are expressed as mean ± SD. p<0.05 is significant.

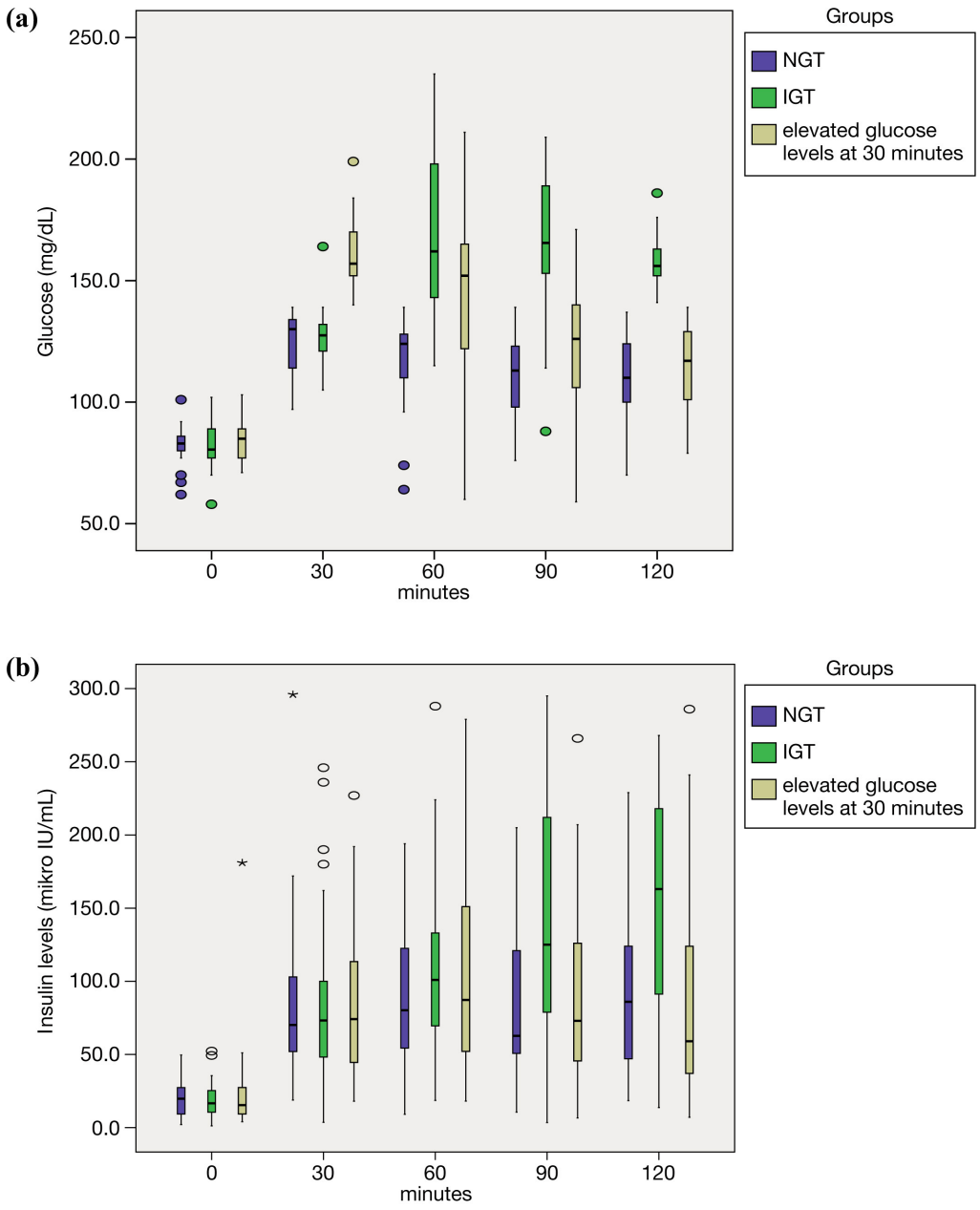


Fig. 1 Glucose (a) and insulin (b) levels at fasting, 30th, 60th, 90th , 120th minutes during OGTT for all groups. Insulin levels at 90 and 120 minutes during OGTT in obese with IGT were significantly higher than those in other two groups (p<0.05). Each box-plot represents the median and the 25th and 75th centiles.

than those in other two groups (p<0.05). No significant difference was found in insulin levels at 30 min among the three groups (90.0 ± 61.2 µIU/mL in NGT, 85.7 ± 66.4 µIU/mL in IGT and 86.8 ± 53.4 µIU/mL in subjects with elevated glucose levels at 30 min, p>0.05).

Anthropometric characteristics are shown in Table 1. Biochemical parameters are given in Table 2. No significant difference was observed between the three groups in any of the parameters except the insulinogenic indices calculated as ΔI30/ΔG30. The lowest value of ΔI30/ΔG30 was obtained from obese patients with elevated glucose levels at 30 min compared to the other groups. The insulinogenic index differs significantly only between obese patients with NGT and elevated glucose levels at 30 min (p<0.01) (Table 2). When systemic inflammatory mediators levels were

Table 2 Biochemical parameters of obese adolescents with NGT, IGT, and elevated glucose level at 30 min. during OGTT

Biochemical parameters	NGT (n=25)	IGT (n=26)	BG>140 mg/dL at 30 min. (n=29)	*p*
TG (mg/dL)	129.45±117.7	105.34±42.0	102.03±62.65	0.711
TC (mg/dL)	166.96±33.54	166.65±29.9	169.27±34.11	0.985
HDL-C (mg/dL)	38.76±10.57	36.38±10.29	36.93±9.5	0.587
LDL-C (mg/dL)	106.26±25.04	105.03±29.7	111.93±27.76	0.706
Apo-A (mg/dL)	122.4±13.66	119.5±14.72	119.03±16.98	0.474
Apo B (mg/dL)	78.44±16.03	84.19±14.91	82.55±15.39	0.398
HbA1c (%)	5.49±0.33	5.47±0.36	5.5±0.36	0.982
HOMA-IR	1.44±0.5	1.46±0.5	1.58±0.5	0.507
$ISI_{MATSUDA}$	3.99±2.51	3.03±2.04	3.73±2.38	0.312
ΔI30/ΔG30	2.16±2.65	1.48±1.46	0.88±0.85	0.042*

Kruskal-Wallis, Data are expressed as mean ± SD. *$p<0.05$ is significant.

Table 3 Systemic inflammatory markers of obese adolescents with NGT, IGT and elevated glucose level at 30 min. during OGTT

Systemic inflammatory markers	NGT (n= 25)	IGT (n= 26)	BG>140 mg/dL at 30 min. (n=29)	*p*
Neopterin (ng/mL)	3.39±0.53	3.67±0.82	4.09±0.84	0.013*
Interleukin-6 (pg/mL)	13.17±6.35	17.3±11.7	30.28±6.43	0.01*
CRP (mg/dL)	3.72±4.51	4.38±5.42	3.82±6.14	0.654
Lp-PLA_2 (ng/mL)	402.20±106.96	440.8±75.6	487.94±84.99	0.004*
Fibrinogen (mg/dL)	341.32±64.34	372.5±66.51	366.76±49.5	0.073

Kruskal-Wallis, Data are expressed as mean ± SD. *$p<0.05$ is significant.

compared (Table 3), obese adolescents with elevated glucose level at 30 min. displayed significantly higher neopterin and Lp-PLA_2 levels in comparison with those in NGT and IGT groups (p=0.004 and p= 0.042 with p=0.002 and p= 0.021, respectively), whereas IL-6 levels in this group was significantly higher only than those in NGT (p=0.003).

In bivariate analysis, BMI positively correlated with fasting insulin levels and HOMA-IR in the whole group (r=0.646 and r=0.631, respectively) ($p<0.05$). Similarly, a positive correlation between BMI and fasting insulin levels were obtained within groups (r=0.532, r=0.517, r=0.651; $p<0.05$). There was a positive correlation between Lp-PLA_2 levels and BMI SDS only in obese adolescents with elevated glucose levels at 30 min (r=0.461; $p<0.05$).

BMI, IL-6, neopterin, fibrinogen, CRP, and Lp-PLA_2 were included in the first logistic regression analysis of NGT and glucose level>140 mg/dL at 30 minutes groups, to predict blood glucose levels at 30 minutes. The statistically significant variables were IL-6, neopterin and Lp-PLA_2. One unit increase in IL-6 level has increased the risk of having an elevated blood glucose level at 30 minutes by 1.11 folds [odds ratio (OR) 1.11 (95% confidence interval, 1.01 to 1.23), p = 0.01]. The same increment was calculated as 9.03 folds for neopterin [OR 9.03, (95% confidence interval 1.77 to 45.97), p=0.013] and 1.01 folds for Lp-PLA_2 [OR 1.01, (95% confidence interval 1.0 to 1.02), p=0.004]. The same independent variables were included in the second logistic regression model for NGT and IGT groups. There was no statistically significant relationship between the variables (Table 4).

Table 4 Logistic regression models for predicting "glucose levels ≥140 mg/dL at 30 min. during an OGTT" and "impaired glucose tolerance" during an OGTT

Model I: ("blood glucose levels>140 mg/dL at 30 min on OGTT" *vs* normal) †		
	p	OR (95% CI)
IL-6	0.02	1.11 (1.01-1.23)
Neopterin	0.008	9.03 (1.77-45.97)
Lp-PLA_2	0.016	1.01 (1.0-1.02)
CRP	0.12	0.84 (0.67-1.05)
Fibrinogen	0.21	1.01 (0.99-1.02)
Constant	0.002	0.000
Model II: ("blood glucose levels>140 mg/dL at 2 hour on OGTT" *vs* normal) †		
IL-6	0.229	1.04 (0.97-1.13)
Neopterin	0.327	1.65 (0.60-4.51)
Lp-PLA_2	0.055	1.00 (1.00-1.01)
CRP	0.705	0.97 (0.85-1.11)
Fibrinogen	0.094	1.00 (0.99-1.02)
Constant	0.011	0.00

†R square=0.069 for Model I, R square=0.083 for Model II

Discussion

Similar to reports from all over the world, the prevalence of obesity is increasing in our country. In our previous study, we have reported the obesity frequency as 16.8% among high school adolescents [26]. Obesity is associated with alterations in glucose metabolism that may be present in adolescents. OGTT is a screening tool necessary to assess the risk of alterations in glucose metabolism in obese adolescents [27, 28]. In our clinic, all adolescents with obesity, defined as having a BMI equal to or greater than the age- and sex-specific cutpoints, undergo 2-h OGTT. During evaluations of OGTT results according to IDF criteria [20], we have observed a different pattern neither NGT nor IGT. Some obese adolescents had glucose levels >140 mg/dL in samples drawn at 30 minutes. This is the first study which has investigated clinical significance of glucose level>140 mg/dL at 30 min during an OGTT by comparing lipid profiles, HOMA-IR and systemic inflammatory mediators levels between obese adolescents with NGT, IGT and elevated glucose levels at 30 min.

Inflammation is one of the strongest mechanisms linking increased adiposity and the development of insulin resistance, type 2 diabetes and cardiovascular diseases [27, 28]. In this study, we demonstrated marked increases of IL-6, neopterin and Lp-PLA_2 in obese adolescents with elevated glucose level at 30 min compared to NGT and IGT groups. Similarly, in logistic regression models, we showed that IL-6, neopterin, and Lp-PLA_2 levels were independent predictors for high blood glucose levels at 30 minutes in OGTT. Our results have emphasized significance of high blood glucose levels measured at 30 min. During OGTT. Yeste *et al*. demonstrated that IL-6 levels in glucose-intolerant obese patients were significantly higher compared to normotolerant obese control subjects [29]. Inconsistent with Yeste [29], we found that IL-6 and other inflammatory mediators did not differ between obese subjects with NGT and IGT. These results suggest that in obesity, higher blood glucose levels at 30 minutes result in more increased inflammatory response than IGT. Bardini *et al*. have reported that elevated first hour plasma glucose in subjects with NGT and pre-diabetes were related with subclinical inflammation, high lipid ratios, and insulin resistance in adults. Therefore, they proposed that this finding as a new "marker" for cardiovascular risk [30]. There is no study regarding the relationship between inflammatory mediators and glucose levels>140 mg/dL at 30 min during on OGTT in the literature.

Indeed, subjects with elevated glucose levels at 30 min have normal glucose tolerance. However, they have more risk for inflammation than subjects with IGT according to our results. Even normally handled load of glucose could lead to pro-inflammatory oxidative stress. Insulin secreted to decrease glucose level has an anti-inflammatory effect [31]. Insulin exhibits proinflammatory effects in the long-term period [32]. Defects in early phase insulin secretion may also be related to increased inflammatory mediators. However, our subjects in three groups had high insulin levels at 30 min in OGTT as well, but no significant difference was detected in early phase insulin levels among the three groups. In addition, the insulinogenic index defined as the ratio of the increment in insulin concentration to the increment in glucose concentration ($\Delta I/\Delta G$) was the lowest in subjects with elevated glucose levels at 30 min. In other words, increment of insulin concentration to the increment of glucose concentration was insufficient in these subjects. Obese subjects with NGT had both the highest increment of insulin concentration and the lowest systemic inflammatory mediator levels when compared to other two groups. These results suggest that proinflammatory effects of glucose have been more prominent, because it seems like insulin increment has failed to decrease glucose concentrations in obese with BG>140 mg/dL at 30 min.

Despite the large number of studies assessing inflammatory mediators in obese children and adolescents in the literature [8, 9, 29, 33, 34], there are only few studies regarding inflammatory mediators in obese adolescents with IGT [29, 35]. We have studied five markers of inflammation in obese adolescents. Among them, IL-6, neopterin and Lp-PLA_2 are significantly increased in obese adolescents with the elevated glucose level at 30 min. There is only one study regarding neopterin in obesity, in which lower neopterin concentrations have been reported in juvenile obese patients [17]. We did not compare obese patients to healthy subjects. Despite lack of knowledge of effects of Lp-PLA_2 in obesity, a large number of prospective studies conducted in initially healthy subjects and on patients with manifest atherosclerosis have documented, Lp-PLA_2 activity was associated with increased cardiovascular risk, suggesting a proatherogenic activity [16, 36]. We found elevated Lp-PLA_2 levels and a positive relationship with the degree of obesity in participants with the elevated glucose levels at 30 min. IL-6 is produced by omental and subcutaneous adipose tissues [37]. WC, as anthropometric index was a significant marker of total and abdominal fat independently from BMI percentiles in adolescents [38]. Although subjects with elevated glucose levels at 30 min. had higher IL-6 levels which have been indicated the increased fat than in those with NGT groups, WC did not differ significantly among three groups.

In our study, lipoprotein levels did not differ between three groups. It should be emphasized that obese adolescents with IGT have marked peripheral insulin resistance, which is mainly at the level of muscle [39]. In previous study, we didn't obtained similar results. In this study, insulin resistance was assessed by both HOMA-IR and $ISI_{MATSUDA}$. Weiss *et al*. demonstrated elevations in fasting insulin levels, and insulin resistance was increased as the obesity stage was increased [40]. Consistent with Weiss *et al*., we found positive correlations between fasting insulin level and HOMA-IR and BMI in all obese adolescents.

In conclusion, obese adolescents with elevated glucose level at 30 min. during OGTT, have higher systemic inflammatory mediator levels even than in those with IGT. In these subjects, insulin increment is insufficient to decrease blood glucose concentrations at 30 th minutes after glucose loading. This finding may be classified as a new alteration of glucose metabolism in obese adolescents, a different status other than IFG and IGT. We have thought that this alteration must be taken into consideration while evaluating OGTT results of obese adolescents. Additionally, in obese children, neopterin and Lp-PLA_2 may be accepted as new markers of systemic inflammation. Further longitudinal studies which will enlighten whether this condition is a transient phenomenon in the same cohort, are required to confirm our results.

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Conflict of Interests

None of the authors had any personal or financial conflicts of interest.

References

1. Goran MI, Ball GD, Cruz ML (2003) Obesity and risk of Type 2 diabetes and cardiovascular disease in children and adolescents. *J Clin Endocrinol Metab* 88: 1417-1427.
2. Gungor N, Hannon T, Libman I, Bacha F, Arslanian S (2005) Type 2 diabetes mellitus in youth: the complete picture to date. *Pediatr Clin North Am* 52:1579-1609.
3. Rocchini A (2002) Childhood obesity and a diabetes epidemic. *N Engl J Med* 346:854-855.
4. Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, Savoye M, Rieger V, Taksali S, Barbetta G, Sherwin RS, Caprio S (2002) Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. *N Engl J Med* 346:802-810.
5. Kashiwabara H, Inaba M, Maruno Y, Morita T, Awata T, Negishi K, Iitaka M, Katayama S (2000) Insulin levels during fasting and the glucose tolerance test and Homa's index predict subsequent development of hypertension. *J Hypertens* 18: 83-88.
6. Fujimoto WY, Bergstrom RW, Boyko EJ, Chen KW, Leonetti DL, Newell-Morris L, Shofer JB, Wahl PW (1999) Visceral adiposity and incident coronary heart disease in Japanese-American men: the 10-year follow-up results of the Seattle Japanese-American Community Diabetes Study. *Diabetes Care* 22: 1808-1812.
7. Lehto S, Ronnemaa T, Pyorala K, Laakso M (2000) Cardiovascular risk factors clustering with endogenous hyperinsulinaemia predict death from coronary heart disease in patients with type II diabetes. *Diabetologia* 43: 148-155.
8. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB (1999). Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 282:2131-2135.
9. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB (2001). Low-Grade systemic inflammation in overweight children. *Pediatrics* 107: e13.
10. Cancello R, Clement K (2006) Is obesity an inflammatory illness? Role of low-grade inflammation and macrophage infiltration in human white adipose tissue. *BJOG* 113:1141-1147.
11. Tam CS, Clement K, Baur LA, Tordjman J (2010) Obesity and low-grade inflammation: a paediatric perspective. *Obes Rev* 11; 118-126.
12. Hansson GK (2005) Mechanisms of disease: inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 352:1685-1626.
13. Murr C, Widner B, Wirleitner B, Fuchs D (2002) Neopterin as a marker for immune system activation. *Curr Drug Metab* 3: 175-187.
14. Avanzas P, Arroyo-Espliguero R, Quiles J, Roy D, Kaski JC (2005) Elevated serum neopterin predicts future adverse cardiac events in patients with chronic stable angina pectoris. *Eur Heart J* 26: 457-463.
15. Zalewski A, Macphee C (2005) Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. *Arterioscler Thromb Vasc Biol* 25:923-931.
16. Nagel G, Rapp K, Preva Wabitsch M, Buchele G, Kroke A, Zollner I, Weiland SK, Koenig W (2008) Prevalence and cluster of cardiometabolic biomarkers in overweight and obese schoolchildren: results from a large survey in southwest Germany. *Clin Chem* 54:317-325.
17. Mangge H, Freytag F, Almer G, Weghuber D, Bauer-Denk C, Fuchs D (2011) Serum neopterin is not increased in obese juveniles. *J Obes* 946795. Epub 2011 Jan 12.
18. Marshall WA, Tanner JM (1970) Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 45:13-23.
19. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. *Br Med J* 320:1240-1243.
20. American Diabetes Association (2004) Diagnosis and classification of diabetes mellitus (Position Statement). *Diabetes Care* 27 (Suppl 1): S5-S10.
21. Amemiya S, Dobashi K, Urakami T, Sugihara S, Ohzeki T, Tajima N (2007) Metabolic syndrome in youths. *Pediatr Diabetes* 8 Suppl 9: 48-54.
22. Matsuda M, DeFronzo RA (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22: 1462-1470.
23. Matthews DR, Hosker JP, Rudenski AS (1985) Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28*:* 412-419.
24. Hanson RL, Pratley RE, Bogardus C, Narayan KM, Roumain JM, Imperatore G, Fagot-Campagna A, Pettitt DJ, Bennett PH, Knowler WC (2000) Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. *Am J Epidemiol* 151: 190-198.
25. Friedwald WT, Levy RI, Frederickson DS (1972) Estimation of the concentration of low-density lipoprotein in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18: 499-502.
26. Ozguven I, Ersoy B, Ozguven A, Dundar PE (2010) Evaluation of nutritional status in Turkish adolescents as related to gender and socioeconomic status. *J Clin Res Pediatr Endocrinol* 2: 111-116.
27. Brufani C, Ciampalini P, Grossi A, Fiori R, Fintini D, Tozzi A, Cappa M, Barbetti F (2010) Glucose tolerance status in 510 children and adolescents attending an obesity clinic in Central Italy. *Pediatr Diabetes* 11: 47-54.
28. Tsay J, Pomeranz C, Hassoun A, Zandieh SO, Rutledge

J, Vogiatzi MG, Oberfield SE, Motaghedi R (2010) Screening markers of impaired glucose tolerance in the obese pediatric population. *Horm Res Paediatr* 73:102-107.

29. Yeste D, Vendrell J, Tomasini R, Broch M, Gussinye M, Megia A, Carrascosa A (2007) Interleukin-6 in obese children and adolescents with and without glucose intolerance. *Diabetes Care* 30:1892-1894.
30. Bardini G, Dicembrini I, Cresci B, Rotella CM (2010) Inflammation Markers and Metabolic Characteristics of Subjects With 1-h Plasma Glucose Levels. *Diabetes Care* 33: 411-413.
31. Dandona P Chaudhuri A, Mohanty P, Ghanim H (2007) Anti-inflammatory effects of insulin. *Curr Opin Clin Nutr Metab Care* 10: 511-517.
32. Iwasaki Y, Nishiyama M, Taguchi T, Asai M, Yoshida M, Kambayashi M, Terada Y, Hashimoto K (2009) Insulin exhibits short-term anti-inflammatory but long-term proinflammatory effects in vitro. *Mol Cell Endocrinol* 298:25-32.
33. Franks PW, Hanson RL, Knowler WC, Sievers ML, Bennett PH, Looker HC (2010) Childhood obesity, other cardiovascular risk factors, and premature death. *N Engl J Med* 362:485-493.
34. Bosy-Westphal A, Geisler C, Onur S, Korth O, Selberg O, Schrezenmeir J,Muller MJ (2006) Value of body fat mass vs anthropometric obesity indices in the assessment of metabolic risk factors. *Int J Obes* 30: 475-483.
35. Skinner AC, Steiner MJ, Henderson FW, Perrin EM (2010) Multiple markers of inflammation and weight status: cross-sectional analyses throughout childhood. *Pediatrics* 125: e801-809.
36. Metzig AM, Schwarzenberg SJ, Fox CK, Deering MM, Nathan BM, Kelly AS (2011) Postprandial Endothelial Function, Inflammation, and Oxidative Stress in Obese Children and Adolescents. *Obesity (Silver Spring)* 19:1279-1283.
37. Fried SK, Bunkin DA, Greenberg AS (1998) Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab* 83: 847-850.
38. Lee S, Bacha F, Gungor N, Arslanian SA (2006) Waist circumference is an independent predictor of insulin resistance in black and white youths. *J Pediatr* 148:188-194.
39. Pascot A, Despres JP, Lemieux I, Bergeron J, Nadeau A, Prud'homme D, Tremblay A, Lemieux S (2000) Contribution of visceral obesity to the deterioration of the metabolic risk profile in men with impaired glucose tolerance. *Diabetologia* 43:1126-1135.
40. Weiss R, Kaufman FR (2008) Metabolic complications of childhood obesity: identifying and mitigating the risk. *Diabetes Care* 31 Suppl 2: S310-316.