Pediatric RESEARCH

POPULATION STUDY ARTICLE

Hypertriglyceridemia is associated with impaired fasting glucose in normal-weight children

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BACKGROUND: Previous studies have suggested that elevated triglyceride levels may precede the appearance of glucose metabolic disturbances in adults; nonetheless, this hypothesis has not been tested in children. Hence, we evaluated whether hypertriglyceridemia is associated with impaired fasting glucose (IFG) in normal-weight children.
METHODS: Normal-weight healthy children aged 7–15 years were enrolled in a population-based cross-sectional population study and allocated into groups with and without hypertriglyceridemia. Hypertriglyceridemia was defined by serum triglyceride levels ≥100 and ≥130 mg/dL for children aged 7–9 and 10–15 years, respectively, and IFG by fasting plasma glucose levels ≥100 and <126 mg/dL.
RESULTS: A total of 1453 children with average age of 11.3 ± 2.4 years were enrolled in the study and allocated into the groups with ($n = 172$) and without ($n = 1281$) hypertriglyceridemia. In the overall population, the prevalence of hypertriglyceridemia and IFG was 11.8% and 11.2%, respectively. The logistic regression analysis adjusted by age, gender, BMI, waist circumference, and insulin levels showed that hypertriglyceridemia is associated with IFG in children aged 10–15 years (odds ratio (OR) = 1.67; 95% confidence interval (CI): 1.02–2.77, $p = 0.04$) but not in those aged 7–9 years (OR = 1.48; 95% CI: 0.39–5.58, $p = 0.55$).
CONCLUSION: Hypertriglyceridemia is associated with IFG in normal-weight children aged 10–15 years, but not in those aged 7–9 years.

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INTRODUCTION

It has been reported that development of dyslipidemia during childhood increases the risk of type 2 diabetes and cardiovascular disease in adulthood.[1,2] With this regard, although metabolic disturbances are more prevalent in obesity condition, also they have been described in normal-weight children and adolescents.[3]

Regarding disorders in lipid metabolism, the elevated triglycerides, a common class of dyslipidemia, are involved in the decrease of insulin sensitivity in non-diabetic individuals[4] and have been associated with high insulin levels and insulin resistance in obese adolescents.[5]

Results of a follow-up study conducted in adults showed that hypertriglyceridemia is an independent risk factor for the development of glucose metabolic disorders in adults.[6] These findings suggest that elevated triglyceride levels may precede the appearance of glucose metabolic disturbances in apparently healthy subjects; nonetheless, this hypothesis has not been tested in children. Hence, we evaluated whether hypertriglyceridemia is associated with impaired fasting glucose (IFG) in normal-weight children.

METHODS

After the approval of protocol by the Mexican Social Security Institute Research Committee and the Faculty of Medicine of the University of San Luis Potosí, in accordance with the ethical principles of the Declaration of Helsinki, and obtaining the written informed consent from at least one of each participant's parents and the informed assent from the participants, a cohort study aimed to evaluate risk factors involved in the developing of metabolic disturbances in children was carried out. In this study, using data from the above-mentioned cohort, we performed a cross-sectional analysis.

The sampling strategy was described previously;[7] in summary, elementary and junior high schools from San Luis Potosí and Durango cities were randomly selected and then, using school attendance listing, children were randomly selected and invited to participate.

Eligible participants were normal-weight children aged 7–15 years who, according to the fasting triglyceride concentrations, were allocated into the groups with and without hypertriglyceridemia. In addition, participants were stratified according to age groups of 7–9 and 10–15 years.

Smoking, alcohol intake, pregnancy, overweight, obesity, previous diagnosis of acute or chronic illness, or any kind of medical treatment were exclusion criteria.

Definitions

On the basis of the National Cholesterol Education Program, hypertriglyceridemia was defined by fasting serum triglyceride levels ≥100 mg/dL (1.1 mmol/L) and ≥130 mg/dL (1.5 mmol/L) for children aged 7–9 and 10–15 years, respectively.[8]

IFG was considered by fasting plasma glucose ≥100 (5.6 mmol/L) and <126 mg/dL (7.0 mmol/L).[9]

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Table 1. Characteristics of the study population according to gender and age

	7–9 Years		*p* Value	10–15 Years		*p* Value
	Girls	Boys		Girls	Boys	
N	172	182		584	515	
Age, years	8.1 ± 0.8	8.0 ± 0.7	0.55	12.4 ± 1.6	12.3 ± 1.6	0.07
Body mass index, kg/m^2	15.9 ± 1.2	16.0 ± 1.0	0.40	19.2 ± 2.1	18.5 ± 1.9	<0.001
Waist circumference, cm	59.0 ± 5.5	59.4 ± 4.9	0.51	70.2 ± 8.1	69.1 ± 7.8	0.02
Systolic blood pressure, mmHg	92.0 ± 12.9	89.8 ± 12.2	0.10	102.2 ± 12.6	100.0 ± 14.0	0.005
Diastolic blood pressure, mmHg	56.8 ± 11.3	56.0 ± 11.2	0.54	61.8 ± 10.9	59.9 ± 11.0	0.005
Fasting glucose, mg/dL	84.7 ± 8.9	85.9 ± 9.6	0.21	88.7 ± 9.9	88.9 ± 10.1	0.77
HDL-cholesterol, mg/dL	48.0 ± 13.3	51.0 ± 11.7	0.02	49.1 ± 13.2	50.4 ± 13.4	0.11
Triglycerides, mg/dL	82.0 ± 36.7	70.6 ± 22.9	<0.001	86.9 ± 39.5	80.6 ± 36.0	0.002
Fasting insulin, μIU/mL	7.1 ± 5.1	5.9 ± 3.0	0.01	9.9 ± 6.7	8.2 ± 4.2	<0.001

$N = 1453$
Values are mean ± standard deviation

Normal weight was defined by body mass index (BMI) between the 15th and 85th percentiles for age and gender.[10]

Measurements

In the standing position, after 8–10 h of fasting conditions, the weight and height were measured with participants in light clothing and without shoes. Weight and height were measured using a fixed scale with stadiometer (Tanita TBF-215, Tokyo, Japan). Waist circumference (WC) was measured to the nearest centimeter with a flexible steel tape while the participants were in standing position. The two anatomical landmarks used to determine tape placement were midway between the lowest portion of the rib cage and the superior border of the iliac crest (laterally) and the umbilicus (anteriorly). The BMI was calculated as weight (kilograms) divided by height (meters) squared.

Blood pressure was measured with the child seated and according to the recommendations of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.[11]

Assays

A whole-blood sample was collected after 8–10 h overnight fasting. The fasting period was confirmed by direct and independent interview with both the parents and participants in the study. Plasma glucose concentrations were measured using the glucose oxidase method (Sigma Diagnostics, St Louis, MO), with an intra-assay variation of 2.4% and an inter-assay variation of 3.8%. Triglycerides were enzymatically measured using spectrophotometric methods. HDL-C fraction was obtained after precipitation by a phosphotungstic reagent. The intra- and inter-assay coefficients of variation were 1.9 and 3.7% for triglycerides, and 1.5 and 3.1% for HDL-C. Insulin levels were determined by microparticle enzyme immunoassay (Abbott AxSYM System, Chicago, IL) with intra- and inter-assay variation coefficients of 4.1 and 6.2%.[7]

Statistical analyses

Numerical data are reported as mean ± standard deviation and categorical variables as proportions. Differences between the groups were estimated using the Student's *t*-test for comparison of normally distributed quantitative variables (Mann–Whitney *U*-test for skewed data), and χ^2-test for qualitative variables.

Unadjusted logistic regression analysis was performed to evaluate the odds ratio (OR) between hypertriglyceridemia (independent variable) and IFG (dependent variables). An additional logistic regression analysis adjusted by age, gender, BMI, WC, and insulin was conducted in order to control the potential confounders.

Data were analyzed using the statistical package SPSS version 15.0 (SPSS Inc., Chicago IL). A 95% confidence interval (95% CI) or p value <0.05 defined statistical significance.

RESULTS

A total of 1453 participants, 756 (52.0%) girls and 697 (48.0%) boys, with average age of 11.3 ± 2.4 years were enrolled in the study and allocated into groups with ($n = 172$) and without ($n = 1281$) hypertriglyceridemia.

Girls aged 7–9 years showed higher triglyceride and fasting insulin levels and lower HDL-C concentration than boys, whereas in the age's stratum of 10–15 years, the girls had higher BMI, WC, systolic and diastolic blood pressure, triglycerides, and fasting insulin levels than boys (Table 1).

Participants with hypertriglyceridemia showed higher fasting insulin levels as well as lower HDL-C concentration compared with the control group. In the age's stratum of 10–15 years, participants with hypertriglyceridemia exhibited higher BMI and WC than those with normal triglycerides, whereas in the age stratum of 7–9 years there were a higher proportion of girls in the hypertriglyceridemic group compared with the controls (Table 2).

In the overall population, the prevalence of hypertriglyceridemia and IFG was 11.8% and 11.2%, respectively. In children aged of 7–9 years, the frequency of hypertriglyceridemia and IFG was 12.7% and 5.3%, whereas in the participants aged 10–15 years was 11.5% and 13.1%, respectively.

The unadjusted logistic regression analysis showed that hypertriglyceridemia is significantly associated with IFG in the overall population (OR = 1.60; 95% CI: 1.02–2.51, $p = 0.03$) as well as in the participants aged 10–15 years (OR = 1.70; 95% CI: 1.05–2.76, $p = 0.03$), but not the group aged 7–9 years (OR = 1.30; 95% CI: 0.36–4.68, $p = 0.68$); association that remained significant in the logistic regression analysis adjusted by age, gender, BMI, WC, and insulin levels in both, the overall population (OR = 1.68; 95% CI: 1.06–2.68, $p = 0.02$) and participants aged 10–15 years (OR = 1.67; 95% CI: 1.02–2.77, $p = 0.04$).

DISCUSSION

The findings of our study showed that hypertriglyceridemia is associated with IFG in the normal-weight children aged 10–15 years but not in those aged 7–9 years.

It has been proposed that elevated triglyceride levels are an early metabolic abnormality that precedes the development of insulin resistance, impaired glucose tolerance, and type 2

Table 2. Characteristics of the study population stratified by age

	Overall population		7–9 Years		10–15 Years	
	HTG	No HTG	HTG	No HTG	HTG	No HTG
N	172	1281	45	309	127	972
Age, years	11.0 ± 2.2	11.3 ± 2.4	8.0 ± 0.8	8.1 ± 0.8	12.1 ± 1.4	12.4 ± 1.7
Girls, *n* (%)	102 (59.3)	654 (51.0)	30 (66.6)	142 (45.9)[a]	72 (56.6)	512 (52.6)
Body mass index, kg/m^2	18.6 ± 2.3	18.1 ± 2.2[a]	16.0 ± 1.3	15.9 ± 1.1	19.5 ± 1.9	18.8 ± 2.0[a]
Waist circumference, cm	68.4 ± 9.0	67.0 ± 8.6[a]	60.4 ± 5.8	59.1 ± 5.1	71.3 ± 8.2	69.5 ± 8.0[a]
Systolic blood pressure, mmHg	99.1 ± 13.9	98.6 ± 13.9	93.6 ± 12.6	90.4 ± 12.5	101.0 ± 14.0	101.2 ± 13.2
Diastolic blood pressure, mmHg	60.2 ± 11.0	59.7 ± 11.2	56.5 ± 10.1	56.4 ± 11.4	61.5 ± 11.0	60.8 ± 11.0
Fasting glucose, mg/dL	88.7 ± 11.1	87.9 ± 9.8	85.1 ± 10.9	85.4 ± 9.0	90.0 ± 10.9	88.7 ± 9.9
HDL-cholesterol, mg/dL	43.9 ± 13.0	50.4 ± 12.9[a]	43.9 ± 11.1	50.3 ± 12.5[a]	43.9 ± 13.7	50.4 ± 13.1[a]
Triglycerides, mg/dL	155.5 ± 37.5	72.2 ± 22.5[a]	134.9 ± 41.2	67.6 ± 16.6[a]	162.8 ± 33.3	73.6 ± 23.9[a]
Fasting insulin, μIU/mL	11.0 ± 7.6	8.1 ± 5.1[a]	7.4 ± 3.7	6.3 ± 4.3[a]	12.3 ± 8.3	8.7 ± 5.2[a]

$N = 1453$
Values are mean ± standard deviation
HTG hypertriglyceridemia
[a]$p < 0.05$ intragroup

diabetes.[12,13] In this context, the elevated concentrations of triglyceride levels and free fatty acids leads to peripheral insulin resistance[14,15] by increasing hepatic and intramyocellular triglyceride content.[16] The above mentioned suggests that chronically elevated triglyceride levels may play an important role in the pathogenesis of insulin resistance and type 2 diabetes.

In order to control the potential effect of hormonal changes that lead to developing transient insulin resistance, a physiological condition during puberty,[17] we stratified the target population in children aged 7–9 and 10–15 years and used the suggested cutoff point by the National Cholesterol Education Program to define hypertriglyceridemia according to age for both children aged 7–9 and 10–15 years.[8] Regarding the potential role that puberty exerts on triglyceride levels, results are inconsistent with some studies showing that puberty is associated with the increase of triglyceride levels,[18,19] while others found no association.[20] In this regard, our results revealed that the mean of triglyceride levels in children aged 7–9 and 10–15 years was similar (76.1 ± 30.9 and 84.0 ± 38.0 mg/dL, respectively), finding that suggests a minimal influence of puberty on triglyceride levels.

In addition, because in the age group of 10–15 years we found high BMI, WC, insulin levels, and lower HDL-C concentrations, an additional analysis adjusted for such variables was conducted in order to control the main confounders.

Given that the target population was in normal weight, our findings suggest that hypertriglyceridemia might be linked to the pathophysiology of the phenotype metabolically obese normal-weight children, a subset of individuals characterized by the presence of metabolic disorders irrespective of obesity.[21] Thus, hypertriglyceridemia along with elevated insulin levels and low HDL-C concentrations could be related with the presence of the above-mentioned phenotype. Moreover, it has been indicated that anthropometric measurements of adiposity including the BMI and WC do not fully explain the presence of metabolic abnormalities during puberty;[22] undoubtedly, further research is mandatory in this field.

Interestingly, hypertriglyceridemia was associated with IFG in children aged 10–15 years but not in those aged 7–9 years, inconsistency that could be related to the low frequency of IFG (5.3%) in the age's stratum of 7–9 years that could be related with the lack of statistical power. Nonetheless, participants with hypertriglyceridemia in both aged groups exhibited higher fasting insulin levels compared with the control group, suggesting that hypertriglyceridemia may be an early biomarker of the risk of glucose metabolic disorders and cardiovascular disease in adulthood.

To the best of our knowledge, there are no previous investigations exploring the association between hypertriglyceridemia and IFG in pediatric population. Although some cohort studies have reported that elevated triglyceride concentrations are an independent risk factor for IFG in adults,[6,23,24] the underlying mechanism of hypertriglyceridemia for the development of IFG has not been fully understood. It has been described that triglycerides may affect the insulin-signaling pathways resulting in peripheral insulin resistance by suppression of the insulin receptor and the tyrosine phosphorylation of their substrates.[25–27] Furthermore, the evidence supports a probable causal role of triglycerides on lipid signaling of β-cells that results in increased insulin release through the activation of islet lipases, activation of protein kinase C, and changes in the membrane of β-cells.[28–30] Also, results of clinical trials have revealed that reducing triglyceride levels can decrease insulin resistance,[31,32] finding that indicates a unidirectional mechanism of triglycerides on insulin concentrations.[33] However, whether hypertriglyceridemia is cause or consequence of glucose metabolic disorders is a phenomenon that still remains to be clarified. Therefore, further epidemiological studies are required to elucidate the potential role of elevated triglycerides in the pathophysiology of IFG.

Some limitations of our study deserve to be mentioned. First, owing to study design, causality between hypertriglyceridemia and IFG cannot be assured. Second, data regarding Tanner stage were not obtained; however, we stratified the target population by age in order to minimize the potential bias related with hormonal activity. Third, fat mass was not measured; however, we used the BMI that has been recognized as a useful indicator of adiposity in children and adolescents.[34,35] Finally, although we did not assess the customary diet, a minimal risk of bias is expected, given that the participants were recruited from the same sociocultural and economic background.

The main strength of the present study was the sampling strategy and the large sample size of normal-weight children.

In conclusion, results of the present study suggest that hypertriglyceridemia is associated with IFG in normal-weight children aged 10–15 years, but not in those aged 7–9 years.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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REFERENCES

1. Zimmet, P. Z., Collins, V. R., Dowse, G. K. & Knight, L. T. Hyperinsulinaemia in youth is a predictor of type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* **35**, 534–541 (1992).
2. Sinaiko, A. R., Donahue, R. P., Jacobs, D. R. Jr & Prineas, R. J. Relation of weight and rate of increase in weight during childhood and adolescence to body size, blood pressure, fasting insulin, and lipids in young adults. The Minneapolis Children's Blood Pressure Study. *Circulation* **99**, 1471–1476 (1999).
3. Guerrero-Romero, F. et al. Biochemical characteristics and risk factors for insulin resistance at different levels of obesity. *Pediatrics* **131**, e1211–e1217 (2013).
4. Phillips, D. I. et al. Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metabolism* **45**, 947–950 (1996).
5. Steinberger, J., Moorehead, C., Katch, V. & Rocchini, A. P. Relationship between insulin resistance and abnormal lipid profile in obese adolescents. *J. Pediatr.* **126** (5 Pt 1), 690–695 (1995) .
6. Guerrero-Romero, F. & Rodríguez-Moran, M. Hypertriglyceridemia is associated with development of metabolic glucose disorders, irrespective of glucose and insulin levels: a 15-year follow-up study. *Eur. J. Intern. Med.* **25**, 265–269 (2014).
7. Rodríguez-Moran, M. et al. Family history of hypertension and cardiovascular risk factors in prepubertal children. *Am. J. Hypertens.* **23**, 299–304 (2010).
8. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics* **128** (Suppl **5**), S213–S256 (2011).
9. Zimmet, P. et al. The metabolic syndrome in children and adolescents—an IDF consensus report. *Pediatr. Diabetes* **8**, 299–306 (2007).
10. Ogden, C. L. et al. Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. *Pediatrics* **109**, 45–60 (2002).
11. Chobanian, A. V. et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension* **42**, 1206–1252 (2003).
12. McPhillips, J. B., Barrett-Connor, E. & Wingard, D. L. Cardiovascular disease risk factors prior to the diagnosis of impaired glucose tolerance and non-insulin-dependent diabetes mellitus in a community of older adults. *Am. J. Epidemiol.* **131**, 443–453 (1990).
13. Mykkänen, L., Kuusisto, J., Pyörälä, K. & Laakso, M. Cardiovascular disease risk factors as predictors of type 2 (non-insulin-dependent) diabetes mellitus in elderly subjects. *Diabetologia* **36**, 553–559 (1993).
14. Søvik, O., Vestergaard, H., Trygstad, O. & Pedersen, O. Studies of insulin resistance in congenital generalized lipodystrophy. *Acta Paediatr. Suppl.* **413**, 29–37 (1996).
15. Boden, G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* **46**, 3–10 (1997).
16. Cree, M. G. et al. Intramuscular and liver triglycerides are increased in the elderly. *J. Clin. Endocrinol. Metab.* **89**, 3864–3871 (2004).
17. Moran, A. et al. Insulin resistance during puberty: results from clamp studies in 357 children. *Diabetes* **48**, 2039–2044 (1999).
18. Berenson, G. S., Srinivasan, S. R., Cresanta, J. L., Foster, T. A. & Webber, L. S. Dynamic changes of serum lipoproteins in children during adolescence and sexual maturation. *Am. J. Epidemiol.* **113**, 157–170 (1981).
19. Azizi, F. et al. Serum lipid levels inan Iranian population of children and adolescents: Tehran lipid and glucose study. *Eur. J. Epidemiol.* **17**, 281–288 (2001).
20. Pinhas-Hamiel, O., Lerner-Geva, L., Copperman, N. M. & Jacobson, M. S. Lipid and insulin levels in obese children: changes with age and puberty. *Obesity* **15**, 2825–2831 (2007).
21. Guerrero-Romero, F. & Rodríguez-Moran, M. Metabolically obese normal-weight children. *World J. Clin. Pediatr.* **1**, 37–39 (2012).
22. Moran, A. et al. Insulin resistance during puberty: results from clamp studies in 357 children. *Diabetes* **48**, 2039–2044 (1999).
23. Lin, S. X. et al. Does elevated plasma triglyceride level independently predict impaired fasting glucose? The Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care.* **36**, 342–347 (2013).
24. Kametani, T., Koshida, H., Nagaoka, T. & Miyakoshi, H. Hypertriglyceridemia is an independent risk factor for development of impaired fasting glucose and diabetes mellitus: a 9-year longitudinal study in Japanese. *Intern. Med.* **41**, 516–521 (2002).
25. Petersen, K. F. & Shulman, G. I. Etiology of insulin resistance. *Am. J. Med.* **119** (5Suppl 1), S10–S16 (2006).
26. Schinner, S., Scherbaum, W. A., Bornstein, S. R. & Barthel, A. Molecular mechanisms of insulin resistance. *Diabet. Med.* **22**, 674–682 (2005).
27. Grundy, S. M. Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. *Am. J. Cardiol.* **83** (9B), 25F–29F (1999).
28. Nolan, C. J., Madiraju, M. S., Delghingaro-Augusto, V., Peyot, M. L. & Prentki, M. Fatty acid signaling in the beta-cell and insulin secretion. *Diabetes* **55** (Suppl **2**), S16–S23 (2006).
29. Peter-Riesch, B., Fathi, M., Schlegel, W. & Wollheim, C. B. Glucose and carbachol generate 1,2-diacylglycerols by different mechanisms in pancreatic islets. *J. Clin. Invest.* **81**, 1154–1161 (1988).
30. Sheu, L. et al. Regulation of insulin exocytosis by Munc13-1. *J. Biol. Chem.* **278**, 27556–27563 (2003).
31. Tenenbaum, A. et al. Attenuation of progression of insulin resistance in patients with coronary artery disease by bezafibrate. *Arch. Intern. Med.* **166**, 737–741 (2006).
32. Tenenbaum, H. et al. Long-term effect of bezafibrate on pancreatic beta-cell function and insulin resistance in patients with diabetes. *Atherosclerosis* **194**, 265–271 (2007).
33. Han, T. et al. Changes in triglycerides and high-density lipoprotein cholesterol may precede peripheral insulin resistance, with 2-h insulin partially mediating this unidirectional relationship: a prospective cohort study. *Cardiovasc. Diabetol.* **15**, 154 (2016).
34. Himes, J. H. & Dietz, W. H. Guidelines for overweight in adolescent preventive services: recommendations from an expert committee. The Expert Committee on Clinical Guidelines for Overweight in Adolescent Preventive Services. *Am. J. Clin. Nutr.* **59**, 307–316 (1994).
35. Dietz, W. H. & Robinson, T. N. Use of the body mass index (BMI) as a measure of overweight in children and adolescents. *J. Pediatr.* **132**, 191–193 (1998).