Lipid Profile in Obese Children with and without Insulin Resistance

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Abstract

Obesity is increasing nowadays including in children and there is lack of information on the pathophysiology and their complication such as insulin resistance and dyslipidemia. This study was aimed to investigate the comparison of lipid profile in obese children with and without insulin resistance. A crossectional study held at an elementary school in Denpasar, Bali from August until September 2015. Independent t-test, Mann-Whitney test, and Multivariate analysis of covariance (MANCOVA) were performed to compare lipid profile in obese children with and without insulin resistance. A P-value less than 0.05 was considered statistically significant. A total of 50 obese children were analyzed. In this study, we found 12 subjects (24%) with insulin resistance. Nonsignificant associations were found between insulin resistance with total cholesterol, HDL, LDL, and ratio of HDL/LDL. A significant association was found between insulin resistance and triglyceride level after adjusted age and sex. There was a significant mean difference of triglycerides in the insulin resistance and noninsulin resistance groups in obese children.

Contents

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1. Introduction

The prevalence of childhood obesity in the world has increased dramatically over the past three decades and is considered by World Health Organization (WHO) to be one of the most serious public health problems of the 21st century.[1-3] Worldwide, the prevalence of overweight and obesity has increased among children and adolescents in developing countries, reached 8.1% (7.7–8.6) to 12.9% (12.3–13.5) in 2013 for boys and 8.4% (8.1–8.8) to 13.4% (13.0–13.9) in girls.[1] Data from the United States shows that 17% of children are obese.[2] Prevalence of childhood obesity in Indonesia is 19.7% in boy and 32.9% in a girl.[4]

The presence of obesity is associated with significant adverse effects on health including metabolic, endocrinology, cardiovascular, gastrointestinal, pulmonary, neurologic, psychiatric, hematologic, and skeletal complications, and development of some types of malignancies. All of these unfavorable effects lead up to a shortened lifespan. Studies strongly suggest that vascular, histopathological and metabolic changes begin in the childhood period. Development of metabolic complications associated with obesity during childhood track into adulthood and increases the risk for type 2 diabetes, dyslipidemia, and early cardiovascular disease.[5]

Symptoms of dyslipidemia usually appear in the fourth decade of life, but the development of atherosclerosis is known to begin at earlier ages and related to dyslipidemia. In autopsy studies, fatty lines which are early sign of atherosclerosis can be found even at the age of 2 years old and proportional to body mass index (BMI), serum total cholesterol (TC), *trygliceride* (TG), low density lipoprotein (LDL), and inversely proportional to high density lipoprotein (HDL). Insulin resistance (IR) that is commonly found in obese adult as a component of metabolic syndrome usually persists in normoglycemia or hyperglycemia subjects.[6]

Recent studies suggest that there is a correlation between IR and high lipid level and lipoprotein. Visceral fat that closed to hepatic portal vasculature is responsible for the positive association between dyslipidemia, hyperinsulinemia, and glucose intolerance. Both, hyperlipidemia and insulin resistance are related to cardiovascular disease and type 2 diabetes mellitus in the adult.[7-10] Until recently, there are no studies about IR in obese children in Indonesia. Correlation of IR and dyslipidemia in obese children is unclear. The objective of this study was to comparative lipid profile in obese children with or without insulin resistance.

2. Research Method

This study was an analytic study using a cross-sectional design, held at Nutrition and Metabolic Disease Sub Division, Department of Child Health, Medical School, Udayana University, Denpasar. Study subjects were obese elementary school children in elementary school V, VI, and Cipta Darma Denpasar during August until September 2015. Eligible patients were chosen by consecutive sampling method. The sample size was counted by single proportion formula with α 0.05 and precision 10%. Based on the previous study by Riskerdas the proportion of obese children in Indonesia is 32.9%, this will lead to minimum estimated sample size of 50 subjects. This study was performed after obtaining permission from Ethics Committee of Medical School, Sanglah Hospital, Denpasar. The inclusion criteria were children aged 6 to 11 years old that diagnosed as obese during anthropometry screening. The exclusion criteria were patients with malignancy, having steroid therapy, having diabetes therapy, or parents refused to sign written informed consent. Independent t-test and Mann-Whitney test were performed to compare lipid profile in obese children with and without insulin resistance. A P-value less than 0.05 was considered statistically significant.

3. Results and Analysis

A total of 50 children were studied from August to September 2015. There were no subjects that excluded from the study. Among subjects 33 (66%) were boys and 17 (39%) were girls, giving a male: female ratio of 2: 1. All subjects were obese children according to WHO criteria. All subjects had normal blood fasting glucose, all subjects were having normal lipid profiles and none of them showed clinical signs

like acanthosis nigricans, skin tags, striae, acne, or hirsutism. There were 12 (24%) children diagnosed with insulin resistance, most of them were boys (67%). Characteristics of subjects were shown in Table 1.

Table 1
Subjects Characteristics

Variables	RI (N=12)	Non RI (N=38)	Total
Sex, male, n (%)	8 (66.67%)	25 (65.79%)	33 (66%)
Age (years), mean (SD)	10.07 (1.31)	8.64 (1.40)	8.98 (1.49)
BMI for Age (z score), mean (SD)	3.30 (0.92)	2.93(0.73)	3.02 (0.78)
Total cholesterol (mg/dL), mean (SD)	168.83 (27.37)	160.26 (27.56)	162.32 (27.48)
LDL cholesterol (mg/dL), mean (SD)	106.17 (23.98)	102..13 (24.30)	103.10 (24.04)
HDL cholesterol (mg/dL), mean (SD)	41.00 (6.90)	43.95 (8.32)	43.24 (8.03)
Triglyceride (mg/dL), mean (SD)	156.42 (94.96)	92.92 (45.62)	108.16 (65.92)

Bivariate analysis is performed using an independent t-test to analyze the association of total cholesterol, HDL, LDL, and the ratio of HDL/LDL with insulin resistance due to a normal distribution, while triglyceride association with insulin resistance was analyzed using Mann-Whitney test due to abnormal distribution (Table 2). There was no significant association between total cholesterol, HDL, LDL, and the ratio of HDL/LDL level with insulin resistance found in our study, but there was a significant association between triglyceride and insulin resistance.

Table 2
Bivariate Analysis for Profile Lipid and Insulin Resistance

Variable	Group	Level	Mean difference (95% CI)	P
Total cholesterol (mg/dL), mean (SD)	IR Non IR	168.83 (27.37) 160.26 (27.56)	8.57 (-10.6 to 27.6)	0.357*
Triglyceride (mg/dL), median (min-max)	IR Non-IR	121.00 (46.00-338.00) 83.00 (46.00-227.00)	- -	0.036**
LDL (mg/dL), mean (SD)	IR Non IR	106.17 (23.98) 102.13 (2.43)	4.03 (-12.65 to 20.72)	0.618*
HDL (mg/dL), mean (SD)	IR Non IR	41.00 (6.90) 43.95 (1.05)	2.94 (-7.94 to 2.04)	0.234*
Ratio of total chol:LDL, mean (SD)	IR Non IR	4.23 (1.05) 3.74 (0.82)	0.48 (-0.2 to 1.18)	0.167*

n: number, SD: standard deviation, CI: confidence interval, *independent t-test, **Mann-Whitney test

We found consistent results there was a significant different level of triglyceride in obese children with and without insulin resistance after adjusted by using multivariate analysis of covariance (MANCOVA) with p = 0.003

Obesity in children has reached alarming proportions in the United States. Increasing overweight and obesity has been noted since surveys were begun in 1963, but the rate of increase has been accelerating over the last 2 decades.[6] The prevalence of childhood obesity has nearly tripled since the 1970s and is recognized as a serious public health concern.[11] Obesity tends to track over time, and the increase in overweight among young children is of significant concern.[12] The prevalence of obesity in children has reported an increase from 4.2% in 1990 to 6.7% in 2010 and is estimated to be 9.1% in 2020, while in Denpasar prevalence increase from 11% in 2002 to 21.7% in 2010.[13] A study in 2013 in primary school

children shows the prevalence of obesity is 15% in Denpasar, 21% in urban areas and 5% in rural areas.[14]

Pediatric obesity is related to an increased risk of metabolic alterations such as inflammation, insulin resistance, glucose intolerance, and hepatic steatosis, as well as to established pathological conditions such as nonalcoholic fatty liver disease, metabolic syndrome, type 2 diabetes, and cardiovascular disease, either at the time or later in life. The scientific evidence demonstrated that chronic low-grade inflammation is the link between obesity and insulin resistance; the main mechanism involved is an increased synthesis of cytokines in adipose tissue and the resident macrophages, which interfere with insulin course and with the expression of genes involved in insulin performance. That inflammation and insulin resistance is the underlying cause of most the pathological complications and obesity-related comorbidities.[8,10] Obesity condition is marked with IL-6, CRP, TNF-α increase with adiponectin and IL-10 decrease that lead into a proinflammation condition that ends as insulin resistance and endothelial dysfunction.[15] An animal study showed that insulin signal abnormality apparently is the main key to insulin resistance.[16], [31]

Insulin resistance can be diagnosed through several examinations, such fasting glucose test, oral glucose tolerance test, intra vena glucose tolerance test, and insulin level examination. The gold standard for diagnosis is hyperinsulinemic-euglycemic clamp and intra vena glucose tolerance test, but these tests are expensive and invasive and not recommended for large epidemiologic study. Diagnosis method that recommended for the epidemiologic study is homeostasis model assessment of insulin resistance (HOMA-IR).[17] In this study, we performed HOMA-IR method to assessed insulin resistance. HOMA-IR can predict insulin resistance from fasting glucose and insulin level. Limitation of this examination is until recently there is no gold standard for cut-off point of diagnosis. Study in America found cut-off point was 3.16 (sensitivity 76%, specificity 66%)[18], study in Chili found 2.6 (sensitivity 59%, specificity 87%)[19], study in China found 2.6 (sensitivity 78%, specificity 67%)[20], study in India found 2.5 (sensitivity >70%, specificity >60%)[21], In this study we used cut off point 2.6 for diagnosing insulin resistance. [32]

In this study, we conducted a study that assessed children with obese aged 6-11 years old. As shown in Table 1, most of our subjects were boys, this was in opposition with Survei Sosial Ekonomi Nasional (Susenas) data which find that there is 4.6% boy and 8% girl obese in urban areas in Indonesia.[13] In our study, insulin resistance was higher in male subjects compared to female subjects (66.67% vs 33.33%, respectively).This result similar to another study that showed male subjects are more susceptible to insulin resistance. This might be related to estrogen in female subjects as protector factor for insulin resistance.[22], [33]

In this study we found that there were 12 subjects had IR (24%) with normal blood sugar. This result is lower compared to the worldwide published number (50-70%)[23] but similar with study in British that showed insulin resistance prevalence was 30% in children under 12 years old in 2003.[24] Obesity is strongly related to cardiovascular disease and insulin resistance in normoglycemia or types 2 diabetes adult subjects in one study. Cardiovascular disease is increasing in accordance with body weight increase. It is apparently due to the strong relationship between adipose tissue and insulin resistance. Body weight reduction also related to insulin decrease and insulin sensitivity increase.[25] This result is in accordance with most studies in children that showed a majority of persons with IR will not develop type 2 diabetes even if IR is strongly related to type 2 diabetes and cardiovascular disorder in later life. There is a genetic background that strongly influences the adequacy of pancreatic β-cell function, and this is influenced by the human leukocyte antigen haplotype. Individual with IR that can compensate trough hyperinsulinemia may escape diabetes but still prone to other complications such early atherosclerosis, hypertension, hypercoagulation, dyslipidemia, fatty liver, and also malignancy. This make IR condition is not benign even if no diabetes or high blood sugar found.[26]

In this study, we found that all subjects had normal TC, LDL, HDL, and TG. Obesity has a negative impact on glucose/insulin axis and lipid metabolism. Insulin secretion rate is significantly increased in obese compared to a normal adult, but there were no differences in insulin clearance or hepatic insulin clearance. Abnormality on glucose metabolism is found very early on obese children, that showed insulin response on food stimulus and maximal glucose uptake is decreased according to higher age and duration of obesity. It can be concluded that first negative impact of obesity is insulin resistance. This explained there was 24% subject to insulin resistance with normal lipid profile since we performed a study on early age. This study

is similar to study in Czech children that found a high incidence of IR in obese children. Dyslipidemia only found in older children in their study (9-17 years) and IR found in 53% obese subjects.[25-28]

In this study, we found that there was no significant association between TC, LDL, HDL, and ratio of HDL/LDL with IR, but there was a significant association with trygliceride. These results are similar to another study in adults that showed the relation of IR and trygliceride and cardiac heart disease events in later life (14 years follow up) but not with total cholesterol. Insulin resistance apparently has a direct effect on cardiovascular events with or without hyperlipidemia.[28] Another study in Italy found that HDL and triglyceride correlated with IR, but not with total cholesterol and LDL.[29] In obese subjects, there is evidence that suggests an association of insulin resistance and hepatic accumulation of fat. This is related to a reduced effect of insulin action on adipose tissue that will reduce lipolysis and increased the flux of free fatty acid to the liver. This effect, together with hepatic lipogenesis due to hyperinsulinemia is responsible for triglyceride accumulation in the hepatocytes.[30]

Limitation of the present study is we did not analyze birth weight, pubertal stage, or thyroid status that can contribute to insulin status. We did not gain any information about the metabolic syndrome that strongly related to IR.

4. Conclusion

Recent studies showed that IR was not associated with cholesterol but significantly associated with triglyceride level in obese children. More studies are needed to explore the causality of these relationships. Furthermore, future research needs larger samples and should focus on the association of IR with dyslipidemia and metabolic syndrome and also their relationship to visceral or peripherals fat.
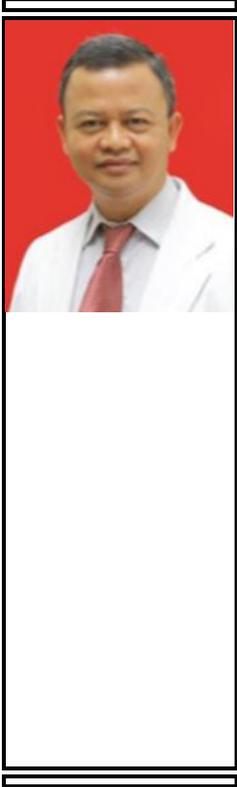
References

1. Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., ... & Abraham, J. P. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The lancet*, *384*(9945), 766-781.
    View in (Google Scholar)

2. Ogden, C. L., Carroll, M. D., Kit, B. K., & Flegal, K. M. (2014). Prevalence of childhood and adult obesity in the United States, 2011-2012. *Jama*, *311*(8), 806-814.
    View in (Google Scholar)

3. Williams, J., Scarborough, P., Matthews, A., Cowburn, G., Foster, C., Roberts, N., & Rayner, M. (2014). A systematic review of the influence of the retail food environment around schools on obesity-related outcomes. *Obesity reviews*, *15*(5), 359-374.
    View in (Google Scholar)

4. Kesehatan, D., & RI, K. K. (2013). Riset kesehatan dasar. *Jakarta: Badan Penelitian dan Pengembangan Kesehatan Departemen Kesehatan Republik Indonesia*.
    View in (Google Scholar)

5. Arslan, N., Erdur, B., & Aydin, A. (2010). Hormones and cytokines in childhood obesity. *Indian pediatrics*, *47*(10), 829-839.
    View in (Google Scholar)

6. García, O. P. (2012). Effect of vitamin A deficiency on the immune response in obesity. *Proceedings of the Nutrition Society*, *71*(2), 290-297.
    View in (Google Scholar)

7. Nicklas, T. A., Baranowski, T., Cullen, K. W., & Berenson, G. (2001). Eating patterns, dietary quality and obesity. *Journal of the American College of Nutrition*, *20*(6), 599-608.
    View in (Google Scholar)

8. Gahagan, S. (2011). Overweight and obesity. *Kliegman RM, Stanton BF, Behrman RE, Geme JWS, Schor NF. Nelson textbook of pediatrics. 19th ed. Philadelphia: Elsevier/Saunders*, 179-188.
    View in (Google Scholar)

9. Birch, L. L., & Fisher, J. O. (1998). Development of eating behaviors among children and adolescents. *Pediatrics*, *101*(Supplement 2), 539-549.
    View in (Google Scholar)

10. López-Alarcón, M., Perichart-Perera, O., Flores-Huerta, S., Inda-Icaza, P., Rodríguez-Cruz, M., Armenta-Álvarez, A., ... & Mayorga-Ochoa, M. (2014). Excessive refined carbohydrates and scarce micronutrients intakes increase inflammatory mediators and insulin resistance in prepubertal and pubertal obese children independently of obesity. *Mediators of inflammation*, *2014*.
    View in (Google Scholar)

11. Slyper, A. H. (2004). The pediatric obesity epidemic: causes and controversies. *The Journal of Clinical Endocrinology & Metabolism*, *89*(6), 2540-2547.
    View in (Google Scholar)

12. O'Connor, T. M., Yang, S. J., & Nicklas, T. A. (2006). Beverage intake among preschool children and its effect on weight status. *Pediatrics*, *118*(4), e1010-e1018.
    View in (Google Scholar)

13. Dewi, M. R., & Sidiartha, G. L. (2013). Prevalensi dan faktor risiko obesitas anak sekolah dasar di daerah urban dan rural. *Medicina*, *44*(1).
View in (Google Scholar)

14. Yussac, M. A. A., Cahyadi, A., Putri, A. C., Dewi, A. S., Khomaini, A., Bardosono, S., & Suarthana, E. (2007). Prevalensi obesitas pada anak usia 4-6 tahun dan hubungannya dengan asupan serta pola makan. *Majalah Kedokteran Indonesia*, *57*(2), 47-53.
View in (Google Scholar)

15. Bastard, J. P., Maachi, M., Lagathu, C., Kim, M. J., Caron, M., Vidal, H., ... & Feve, B. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. *European cytokine network*, *17*(1), 4-12.
View in (Google Scholar)

16. Saad, M. J., Araki, E., Miralpeix, M., Rothenberg, P. L., White, M. F., & Kahn, C. R. (1992). Regulation of insulin receptor substrate-1 in liver and muscle of animal models of insulin resistance. *The Journal of clinical investigation*, *90*(5), 1839-1849.
View in (Google Scholar)

17. Lee, J. M., Okumura, M. J., Davis, M. M., Herman, W. H., & Gurney, J. G. (2006). Prevalence and determinants of insulin resistance among US adolescents: a population-based study. *Diabetes care*, *29*(11), 2427-2432.
View in (Google Scholar)

18. Keskin, M., Kurtoglu, S., Kendirci, M., Atabek, M. E., & Yazici, C. (2005). Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. *Pediatrics*, *115*(4), e500-e503.
View in (Google Scholar)

19. Burrows, R., Correa-Burrows, P., Reyes, M., Blanco, E., Albala, C., & Gahagan, S. (2015). Healthy Chilean adolescents with HOMA-IR≥ 2.6 have increased cardiometabolic risk: association with genetic, biological, and environmental factors. *Journal of diabetes research*, *2015*.
View in (Google Scholar)

20. Yin, J., Li, M., Xu, L., Wang, Y., Cheng, H., Zhao, X., & Mi, J. (2013). Insulin resistance determined by Homeostasis Model Assessment (HOMA) and associations with metabolic syndrome among Chinese children and teenagers. *Diabetology & metabolic syndrome*, *5*(1), 71.
View in (Google Scholar)

21. Singh, Y., Garg, M. K., Tandon, N., & Marwaha, R. K. (2013). A study of insulin resistance by HOMA-IR and its cut-off value to identify metabolic syndrome in urban Indian adolescents. *Journal of clinical research in pediatric endocrinology*, *5*(4), 245.
View in (Google Scholar)

22. Moran, A., Jacobs, D. R., Steinberger, J., Steffen, L. M., Pankow, J. S., Hong, C. P., & Sinaiko, A. R. (2008). Changes in insulin resistance and cardiovascular risk during adolescence: establishment of differential risk in males and females. *Circulation*, *117*(18), 2361-2368.
View in (Google Scholar)

23. Cruz, M. L., & Goran, M. I. (2004). The metabolic syndrome in children and adolescents. *Current diabetes reports*, *4*(1), 53-62.
View in (Google Scholar)

24. Viner, R. M., Segal, T. Y., Lichtarowicz-Krynska, E., & Hindmarsh, P. (2005). Prevalence of the insulin resistance syndrome in obesity. *Archives of Disease in Childhood*, *90*(1), 10-14.
View in (Google Scholar)

25. Ten, S., & Maclaren, N. (2004). Insulin resistance syndrome in children. *The Journal of Clinical Endocrinology & Metabolism*, *89*(6), 2526-2539.
View in (Google Scholar)

26. Steinberger, J., & Daniels, S. R. (2003). Obesity, insulin resistance, diabetes, and cardiovascular risk in children: an American Heart Association scientific statement from the Atherosclerosis, Hypertension, and Obesity in the Young Committee (Council on Cardiovascular Disease in the Young) and the Diabetes Committee (Council on Nutrition, Physical Activity, and Metabolism). *Circulation*, *107*(10), 1448-1453.
View in (Google Scholar)

27. Pastucha, D., Filipcikova, R., Horáková, D., Radová, L., Marinov, Z., Malincikova, J., ... & Dobiás, M. (2013). The incidence of metabolic syndrome in obese Czech children: the importance of early detection of insulin resistance using homeostatic indexes HOMA-IR and QUICKI. *Physiological research*, *62*(3), 277.
View in (Google Scholar)

28. Robins, S. J., Lyass, A., Zachariah, J. P., Massaro, J. M., & Vasan, R. S. (2011). Insulin resistance and the relationship of a dyslipidemia to coronary heart disease: the Framingham Heart Study. *Arteriosclerosis, thrombosis, and vascular biology*, *31*(5), 1208-1214.
View in (Google Scholar)

29. Klop, B., Elte, J. W. F., & Cabezas, M. C. (2013). Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients*, *5*(4), 1218-1240.
View in (Google Scholar)

30. Chiarelli, F., & Marcovecchio, M. L. (2008). Insulin resistance and obesity in childhood. *European Journal of Endocrinology*, *159*(suppl 1), S67-S74.
View in (Google Scholar)

31. Billaiya, R., Jain, A., Agarwal, R., & Jain, P. (2017). Introduction about Child Health Status in India. *International Journal of Health Sciences (IJHS)*, *1*(1), 12-22.
View in (Google Scholar)

32. Agarwal, R., Jain, P., Ghosh, M. S., & Parihar, K. S. (2017). Importance of Primary Health Care in the Society. *International Journal of Health Sciences (IJHS)*, *1*(1), 6-11.
View in (Google Scholar)

33. Malaiya, S., Shrivastava, A., Prasad, G., & Jain, P. (2017). Impact of Medical Education Trend in Community Development. *International Journal of Health Sciences (IJHS)*, *1*(1), 23-27.
View in (Google Scholar)

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2. **Sidiartha L**, Bakta I, Wiryana I, Sutirtayasa I, Sjarif D. 2017. Eicosapentaenoic acid and docosahexaenoic acid in fish oil capsule supplementation in obese children decreases serum interleukin-6 and hepcidin and improves iron status. Bali Med J, 6(1):97-101.
3. Daisy S, Dyah KW, **Sidiartha IGL,** Suparyata IB, Hartawan NB. 2017. Prevalence and association of cost and hospital malnutrition in Pediatric Intensive Care Unit Sanglah Hospital during 2015. Crit Care Shock, 20:10-16.
4. Cempaka PMVP, **Sidiartha IGL**. 2017. Waist circumference and insulin levels in obese children. Paediatr Indones, 57:194-7.
5. Paryati PV, **Sidiartha IGL**, Windiani IGAT, Adnyana IGANS. 2017. Quality of life among obese and non-obese early adolescents. Paediatr Indones, 57:216-22.
6. Suwitri NPE, **Sidiartha IGL**. Omega-6 and omega-3 fatty acid content ratio of commercial complementary foods. International Journal of Health Sciences (IJHS), v.2, n.1, p.21-8.
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