

High levels of interleukin-6 and triglycerides and low levels of soluble leptin receptors as risk factors for leptin resistance in obese adolescents



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ABSTRACT

Introduction: Obesity is a nutritional disorder that can affect all ages, including teenagers. Obesity can affect gut microbiota and cause increased intestinal permeability. This process leads to interleukin-6 and TNF- α production which can increase leptin levels. The soluble leptin receptor (sLR) is a leptin receptor in the plasma that transports leptin to the hypothalamus. Low sLR numbers are associated with high leptin levels in obesity. Obesity in hyperleptinemia conditions indicates leptin resistance (defects in leptin receptors) which causes hyperphagia and reduces energy expenditure. This study was conducted to prove high TG and IL-6 serum levels and low sLR levels in obese adolescents who have a higher risk of developing leptin resistance than normal adolescents.

Methods: This research is a case-control study. The subjects underwent an assessment including food recall, anthropometric status, and lipid profile. The food recall assessment was obtained by means of a questionnaire given to parents. Blood sampling and laboratory examination are carried out by the Prodia[®] Clinical Laboratory. Data analysis was performed by computer, using the Independent t-test, Mann-Whitney test, Chi-Square test, and analysis of the ROC curve.

Results: ROC curve analysis obtained the optimal cut points for TG and IL-6 levels, respectively 101.5 mg/dL (sensitivity 82.1%, specificity 89.7%) and 11.95 pg/mL (sensitivity 84.6%, specificity of 80%), with area under the curve (AUC) values of 0.96 and 0.89, respectively. ROC curve analysis obtained the optimal cut point for sLR levels of 19.3 ng/mL (sensitivity 64.1%, specificity 71.8%) with an AUC value of 0.77. Multivariate analysis using logistic regression found a significant relationship between TG and IL-6 levels on the incidence of hyperleptinemia.

Conclusion: This study reinforces the theory of chronic low-grade inflammation and proved that inflammation and high TG levels play a role in hyperleptinemia in obese adolescents.

Keywords: adolescent, interleukin-6, leptin, obese, triglycerides.

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INTRODUCTION

Obesity is a nutritional disorder that has become a global health problem to date. Obesity can affect all ages, including teenagers. Differences in intestinal microbiota between obese and lean individuals have been identified. In the intestine of obese children, the composition of Firmicutes was found to be dominant. This condition causes an increase in intestinal permeability which can also be directly caused by consuming fatty foods. Accumulation of fat in body tissues and changes in the gastrointestinal microbiota in obesity lead to chronic low-grade inflammation. The bad impact of

chronic low-grade inflammation is the occurrence of leptin resistance which will have a bad impact into adulthood.¹

Obesity can affect all individuals at all ages, but increases significantly in children and adolescents. In 2010, 43 million children were obese, 35 million of whom were in developing countries. The prevalence of obesity increased from 4.2% in 1990 to 6.7% in 2010 and is expected to be 9.1% in 2020.^{2,3} In Indonesia, the prevalence of obesity in children increased from 9.5% in 2007 to 11.7% in 2010.⁴ More than half of obesity that occurs in childhood and adolescents will continue to be obese in adulthood with various adverse effects

on health.⁵ Obesity is associated with an increased incidence of various chronic diseases, such as metabolic syndrome, diabetes, cardiovascular disease, cancer, respiratory disease, and so on. Children and adolescents who are obese may be accompanied by psychological disorders and social prejudice which will largely affect the quality of life.⁶

Obesity involves the process of enlargement of cells in adipose tissue which results in increased activity of M1 macrophages, CD8 T cells and increased IFN- γ resulting in increased pro-inflammatory adipokines, such as: leptin, TNF- α , IL-6, and other cytokines.

These pro-inflammatory adipokines cause chronic low-grade inflammation, insulin resistance, dyslipidemia, non-alcoholic fatty liver disease, and other metabolic syndrome diseases.⁶

Recently, differences in intestinal microbiota have been identified between obese and lean individuals. Identification of the gut of obese children found changes in the composition of the microbiota with Firmicutes becoming dominant. This condition causes an increase in intestinal permeability.⁷ Increased intestinal permeability can be directly caused by consuming fatty foods.⁸ Fat also affects intestinal permeability through activation of mast cells in the intestinal mucosa. The secretion of mediators by mast cells, such as TNF- α , IL-1 β , IL-4, and IL-13, as well as tryptase via receptor-2 activation by protease affect the permeability of the intestinal mucosa.⁷

Increased intestinal permeability causes LPS translocation, so that serum LPS levels increase. The binding of LPS and unesterified fatty acids to the Toll like receptor (TLR) 4 initiates the inflammatory cascade and release of the cytokines IL-6 and TNF- α from macrophages, adipocytes, and the liver, and stimulates a condition called metabolic endotoxemia. Interleukin-6 and TNF- α act as paracrine regulators which can increase leptin levels.⁹⁻¹¹

Leptin is an adipocyte-derived signaling molecule, which acts on leptin receptors in the hypothalamus to reduce food intake and increase energy expenditure.¹² Most of the serum leptin will increase in obesity and is positively related to body mass index (BMI), percentage of body fat, and fat mass. The high level of leptin in obesity is not in line with the working effect it produces. This suggests that there are other mechanisms that affect the action of leptin, so that obesity still occurs.¹³

The soluble leptin receptor (sLR) is a leptin receptor in the plasma that transports leptin to the hypothalamus. The study in adults by Ogier et al. (2002) found that the sLR was higher in lean people than in obese people.¹⁴ Low sLR numbers are associated with high leptin levels in obesity. This relationship suggests reflex downregulation of leptin receptors by high leptin levels.¹⁴

Obesity in hyperleptinemia conditions indicates leptin resistance (defects in leptin receptors) which causes hyperphagia and reduces energy expenditure.¹² Apart from the low sLR, leptin resistance is thought to occur due to the failure of circulating leptin to cross the blood-brain barrier. Triglycerides inhibit leptin transport according to levels.¹⁵ Another mechanism is thought to be due to the mechanism of inhibition of the leptin b receptor (LRb) signal cascade in the hypothalamus. This is related to 2 regulatory inhibitory molecules, namely the suppressor of cytokine signaling-3 (SOCS3) and protein tyrosine phosphatase (PTP1B). Under conditions of high circulating leptin levels (such as obesity), increased SOCS3 expression largely offsets the increased expression of LRb signaling processes. SOCS3 expression is induced by proinflammatory mediators and cytokines, such as IL-6, TNF- α , fatty acids, and other fats, as well as those that activate counter regulatory signals such as corticosteroids.¹⁶

Research related to TG, IL-6, leptin, sLR levels or the ratio of leptin/sLR levels separately in obese adults has been published relatively, but regarding TG, IL-6 levels, there is no complete sLR level in obese adolescents. This study was conducted to prove high TG and IL-6 serum levels and low sLR levels in obese adolescents who have a higher risk of developing leptin resistance than normal adolescents.

METHODS

This research is a case-control study with the same sample size as the control sample. The research was conducted in four junior high schools in the Denpasar city area, selected by purposive sampling. The research started from January 2022 to April 2022. Cases were obese adolescents with BMI > 95th percentile and leptin resistance who attended junior high school (12–15 years old) who met the selection criteria. Controls were non-obese adolescents with BMI < 85th percentile who met the selection criteria, matched in terms of sex and age. Exclusion criteria for cases and controls were subjects who were obese due to genetic disorders, suffered from congenital disorders, were suffering from

acute or chronic infections, received long-term drug therapy and those that affected lipid levels such as statin drugs, steroids, gemfibrozil, were receiving antimicrobial therapy, immunosuppressive therapy, long-term glucocorticoid therapy, prebiotic, probiotic, or synbiotic therapy and in immunocompromised conditions.

Samples for cases were obese adolescents with BMI \geq 95th percentile based on the 2000 CDC with leptin serum levels of 7000 pg/mL in males and >15000 pg/ml in females.¹⁷ The sample for control was non-obese adolescents with BMI < 85th percentile based on the 2000 CDC.¹⁸ In the sample calculation, it was found that the sample size for each group was 36 subjects, so that the overall sample size in this study was 72 (36 samples for cases and 36 samples for controls). Subjects were recruited by consecutive sampling. Subjects and parents were given an explanation of the aims and procedures of the research, including the potential benefits and risks. Parents/guardians of subjects who meet the inclusion criteria will be given an explanation about this study. If the parents/guardians agree, then they are asked to sign the informed consent. Then, subjects underwent an assessment including food recall, anthropometric status, and lipid profile. The food recall assessment was obtained by means of a questionnaire given to parents.

Food ratings were obtained during the previous three days to calculate total calories, protein, fat, and carbohydrates. Anthropometric measurements included weight, height, and body mass index (BMI) and were plotted using the CDC 2000 chart. Blood samples were then taken to measure triglyceride, IL-6, leptin and sLR levels. Blood sampling and laboratory examination are carried out by the Prodia® Clinical Laboratory.

Data analysis was performed by computer. Categorical data are presented in percentage form, normally distributed numerical data are presented in mean and standard deviation (SD), while non-normally distributed numerical data are presented in median and range (minimum-maximum). Analysis of the normality of the distribution of numerical data was determined by the Kolmogorov-Smirnov test. The bivariate test for

differences in the means of variables with normal distribution between the independent and dependent variables uses the Independent t-test, if the data is not normally distributed, the bivariate test uses the Mann-Whitney test. Bivariate test the difference in the proportion of categorical variables using the Chi-Square test. TG, IL-6 and sLR levels on a numerical scale related to the incidence of leptin-resistant obesity were tested by descriptive analysis of the ROC curve with the output being the optimal cut point (sensitivity and specificity) and AUC (area under the curve). The bivariate test of optimal cut-off points for TG, IL-6 and sLR levels on the incidence of hyperleptinemia in obesity uses the Chi-Square test with the final result being the odds ratio (OR) with a 95% confidence interval (95% CI). Calculation of the effects of several control variables and analysis simultaneously of all dependent variables using a multivariate logistic regression test. The end result of logistic regression is the odds ratio (OR) with a 95% confidence interval (95% CI). The significance level used in this study was $p < 0.05$

RESULTS

This research was conducted for 4 months, from January to April 2022 at Denpasar City Middle School, taking subjects who met the inclusion and exclusion criteria. The research was conducted in 4 junior high schools, starting with socialization. Some of the socialization is done at school, others from house to house. The number of students contacted per telephone was 141 people, 120 people who were willing to take part in counseling about obesity in adolescents. Students who clinically appear to be obese or not, are advised to take part in the study and be given an informed consent form, which will then be submitted to their respective parents for approval. As many as 95 of them stated that they were willing to include their children in the study. After carrying out physical examinations and anthropometric measurements of these 95 students, it was found that the BMI of 7 students was between the 85-95th percentile, so they were not included in the study. Furthermore, of the 88 students who signed an informed consent, 8 students were not present during the

laboratory examination, and 2 students were sick. This study found cases were obese adolescents with leptin resistance and controls were non-obese adolescents, 39 subjects each.

The characteristics of the 78 study subjects had a median age of 15 years (range, 12-15 years) with most of the subjects being women. The female gender in each case and control group was the same, namely 21 people (58.3%). There were statistically significant differences in the characteristic variables between the two groups, except for age, sex and TB. The characteristics of the research subjects are shown in [Table 1](#).

Determination of Cut-Off Points and Bivariate Analysis of the Incidence of Hyperleptinemia in Obesity

[Figure 1](#) shows the ROC curve of TG and IL-6 levels on the incidence of obesity hyperleptinemia. ROC curve analysis obtained the optimal cut points for TG and IL-6 levels, respectively 101.5 mg/dL (sensitivity 82.1%, specificity 89.7%) and 11.95 pg/mL (sensitivity 84.6%, specificity of 80%), with area under the curve (AUC) values of 0.96 and 0.89, respectively.

Table 1. Baseline Characteristics

Variables	Obese (N=39)	Non-Obese (N=39)	Nilai p
Age (year), median (mix-max)	15 (12-15)	15 (12-15)	1.000 ^a
Gender, n (%)			1.000 ^b
Boys	15 (41.7)	15 (41.7)	
Girl	21 (58.3)	21 (58.3)	
Weight (kg), mean (SD)	79.63 (9.65)	48.72 (9.29)	0.001 ^c
Height (cm), median (mix-max)	160 (146-170)	160 (132-173)	0.64 ^a
BMI (kg/m ²), median (mix-max)	30.4 (25.8-42.18)	19.33 (14.28-23.8)	0.001 ^a
TG (mg/dL), median (mix-max)	132 (97-359)	91 (70-104)	0.001 ^a
IL-6 (pg/mL), median (mix-max)	16.9 (5-56)	9.9 (2.3-17.2)	0.001 ^a
Leptin (pg/mL), mean (SD)	52,378.74 (16,558.24)	30,475.15 (6,934.62)	0.001 ^c
sLR (ng/mL), mean (SD)	17.57 (2.75)	20.27 (2.16)	0.001 ^c
PAQ-A score, median (mix-max)	1.79 (1-2.41)	2.01 (1.33-3.97)	0.001 ^a
Daily intake, median (mix-max)			
Calories (kcal/hari)	2,839.1 (2,291.4-3,178)	1,874.2 (1,540-2,790.7)	0.001 ^a
Carbohydrate (g/hari)	414.2 (275-473.1)	206.69 (169.42-362.98)	0.001 ^a
Protein (g/hari)	93.2 (62.2-111.6)	81.75 (45-103)	0.11 ^a
Fat (g/hari)	76.5 (63-109.6)	55 (49-80.3)	0.001 ^a
Adequacy of calories based on RDA			
≥ RDA, n (%)	31 (86.1)	13 (36.1)	0.001 ^b

^aMann-Whitney Test, ^bChi-square Test, ^cIndependent T-Test

BMI= Body Mass Index, TG = Triglycerides, IL-6= interleukin-6, sLR= soluble leptin receptor, PAQ-A= Physical Activity Questionnaire for Adolescents, RDA = Recommended Dietary Allowance, SD = Standard Deviation.

Figure 2 shows the ROC curve of sLR levels for non-obese events. ROC curve analysis obtained the optimal cut point for SLR levels of 19.3 ng/mL (sensitivity 64.1%, specificity 71.8%) with an AUC value of 0.77.

Bivariate Analysis of the Effect of Control Variables

Based on the optimal cut point value for each level obtained through ROC analysis,

a bivariate test was carried out on the incidence of obesity as shown in Table 2.

Multivariate Analysis of Effects of Control Variables

Multivariate analysis using logistic regression is shown in Table 3. There is a significant relationship between TG and IL-6 levels on the incidence of hyperleptinemia.

DISCUSSION

ROC curve of TG levels on the incidence of hyperleptinemia in obesity

There are abnormalities in blood fat levels in obese people, namely increased levels of cholesterol and TG in the body. The composition of carbohydrates and obesity are factors that influence the increase in TG in the blood. Another mechanism for increasing TG in obesity can also be due to

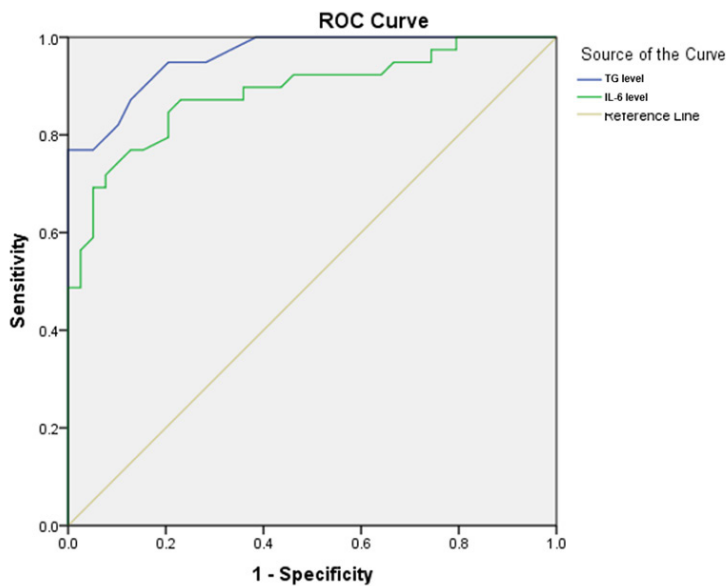


Figure 1. ROC Curve of TG and IL-6 Level.

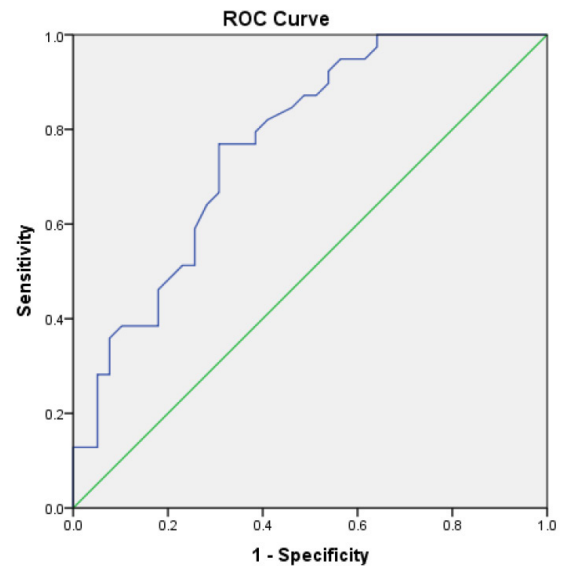


Figure 2. ROC Curve of sLR Level

Table 2. Bivariat Analysis

Variables	Obese N (%)	Non-Obese N (%)	OR (95%CI)	P value
TG \geq 101.5 mg/dL	33 (42.3)	4 (5.1)	48.13 (12.46-185.93)	0.001
IL-6 \geq 11.95 pg/mL	36 (46.2)	6 (7.7)	66 (15.26-285.38)	0.001
sLR $<$ 19.3 ng/mL	28 (35.9)	14 (17.9)	4.5 (1.75-11.83)	0.001

TG = Triglycerides, IL-6= interleukin-6, sLR= soluble leptin receptor

Table 3. Multivariat Analysis

Variables	Obese N (%)	Non-Obese N (%)	OR (95%CI)	P value
TG \geq 101.5mg/dL	33 (42.3)	4 (5.1)	46.9 (1.44-1,526)	0.03
IL-6 \geq 11.95 pg/mL	36 (46.2)	6 (7.7)	30.91 (1.03-927.1)	0.04
sLR $<$ 19.3 ng/mL	28 (35.9)	14 (17.9)	68.62 (0.55-8,591.7)	0.08
PAQ-A score light acitivity	37 (47.4)	29 (37.2)	33 (0.22-5,028.4)	0.17
Total caloris $>$ RDA	35 (44.9)	5 (6.4)	1.36 (0.02-51.8)	0.69
Total carbohydrate $>$ RDA	35 (44.9)	5 (6.4)	757.44 (0.67-856,250)	0.06
Total fat $>$ RDA	21 (26.9)	1 (1.3)	5.51 (0.18-168.21)	0.33

TG = Triglycerides, IL-6= interleukin-6, sLR= soluble leptin receptor, PAQ-A= Physical Activity Questionnaire for Adolescents, RDA = Recommended Dietary Allowance

the fermentation of food polysaccharides that cannot be digested by enzymes causing the formation of SCFAs. This lipogenic substance is then transported to the liver, thereby causing an increase in de novo hepatic lipogenesis. The process of hepatic lipogenesis plays a role in the release of fatty acids and TG from circulating lipoproteins in muscle and adipose tissue.¹⁹ ROC curve analysis in this study, obtained the optimal cut point for TG levels of 101.5 mg/dL (sensitivity 82.1%, specificity 89.7%) with an area under the curve (AUC) value of 0.96 (very Good). The cut-off points for TG levels >101.5 mg/dL was 48.13 times the risk of developing hyperleptinemia in obese adolescents compared to normal adolescents (95% CI 1.44-1.526; $p = 0.001$). Until now, the limit for hypertriglyceridemia is if the TG level is more than 100 mg/dL in children (<10 years) or 130 mg/dL in adolescents (10-19 years). These different cutoff points for TG levels can be caused by ethnic differences, as previously reported.²⁰ Further research is needed to develop specific ethnic boundaries.

Triglycerides (TG) mediate hyperleptinemia by inhibiting leptin transport to the brain by binding to circulating leptin, inhibiting I-Lep uptake to the brain, or directly acting on leptin transporters. The leptin transporter has a regulatory area that is controlled by the TG. Triglycerides inhibit leptin transport according to levels. Disruption of leptin transport affects glycemic control, fatty acid oxidation, and insulin regulation, which in turn has an impact on increasing body fat mass which ends in obesity.^{15,21}

ROC curve of IL-6 levels on the incidence of hyperleptinemia in obesity

Interleukin-6 is a cytokine that has a wide range of functions, such as inflammation, body defense and tissue destruction. Enlargement of cells in adipose tissue results in increased activity of M1 macrophages, CD8 T cells and increased IFN- γ which causes an increase in pro-inflammatory adipokines, one of which is IL-6. These pro-inflammatory adipokines cause chronic low-grade inflammation which in turn causes leptin resistance. Fatty foods can also indirectly

increase IL-6 levels by affecting intestinal permeability through activation of mast cells in the intestinal mucosa. Mast cells are directly related to the regulation of transcellular and paracellular intestinal permeability through the secretion of mediators, such as TNF- α , IL-1 β , IL-4, and IL-13, as well as tryptase through receptor-2 activation by proteases, which will cause LPS translocation.²² The binding of LPS and unesterified fatty acids to the Toll like receptor (TLR)4 initiates the inflammatory cascade and release of the cytokines IL-6 and TNF- α from macrophages, adipocytes, and the liver, and stimulates a condition called metabolic endotoxemia.²³

Apart from adipose tissue, IL-6 producers are immune cells, fibroblasts, endothelial cells, and skeletal muscle cells. The contribution of fat tissue to IL-6 levels is 15-30%. Previous studies have shown that IL-6 production increases sharply in obese children. A study of 203 subjects aged 7-18 years consisting of 115 obese and 88 normal reported significantly increased levels of IL-6 in addition to CRP, fibrinogen and Plasminogen activator inhibitor-1 in obese subjects. Research conducted by Rajendran et al. (2012) reported that in obese subjects who experienced acute myocardial infarction, levels of IL-6, CRP and Leptin were higher which concluded that inflammation played a role in the occurrence of this condition.²⁴ Research by Maria et al. (2021) also reported that levels of IL-6, CRP and leptin were high in obese children, adolescents and adults.²⁵ Warnberg et al.'s research (2004) got different results. The inflammatory markers CRP, IL-6 and TNF- α showed generally higher values in overweight/obese subjects than in those who were not overweight, with only CRP showing a significant difference (means were 0.83 and 1.27 mg/l in the non-overweight and overweight/obese groups, respectively).²⁶

These different results are likely because our study compared groups of subjects who were not overweight with obese subjects (excluding overweight subjects). ROC curve analysis in this study found that the optimal cut point for IL-6 was 11.95 pg/mL (sensitivity 84.4%, specificity 80%), with an area under the curve (AUC) value of 0.89 (good). The cut point for

IL-6 levels of 11.95 pg/mL resulted in a 66 times greater risk of developing hyperleptinemia in obese adolescents than normal adolescents (95% CI 15.26-285.38, $p = 0.001$). Hyperleptinemia conditions causing STAT3 signaling by LRB induce expression of inhibitory SOCS3, which interferes with LRB action by binding to Tyr985 and Jak2. Proinflammatory cytokines and mediators, such as IL-6, tumor necrosis factor (TNF)- α , fatty acids and other lipids, and counter regulatory signal activators such as corticosteroids induce the expression of SOCS3 and/or other leptin resistance mediators, resulting in leptin resistance.^{16,27}

ROC sLR curve on the incidence of hyperleptinemia in obesity

Circulating leptin levels are related to adipose tissue mass. Leptin is secreted into the blood circulation to the hypothalamus and acts as an appetite controller. If energy intake exceeds what is needed, the mass of adipose tissue increases, accompanied by an increase in leptin levels in the blood circulation. Leptin will stimulate the anorexigenic center in the hypothalamus to reduce the production of neuropeptide Y (NPY), resulting in a decrease in appetite and food intake. Leptin works through leptin receptors.²⁸ Soluble leptin receptor (sLR) exhibits major leptin-binding activity in blood. Long-chain leptin receptors (LRb) are most abundant in neurons of several nuclei in the hypothalamus, including the arcuate (ARC), dorsomedial (DMH), ventromedial (VMH) and premammillary nuclei responsible for receiving leptin.¹⁶

Decreased levels of sLR found in obesity. Like other biological signaling pathways, leptin regulates its receptors and signaling itself. A low sLR count is associated with high leptin levels and this indicates reflex downregulation of the leptin receptor which can lead to pathological leptin resistance.¹⁴ Research by Sinha et al. (1996) reported that in lean adults, leptin in circulation is mostly in bound form, whereas in obese people, the majority of leptin circulates in free form.²⁹ Plasma soluble leptin receptors act like soluble IL-6 receptors against IL-6 cytokines and increase leptin signaling. Decrease in the number of sLRs found

in obesity causes insufficiency of leptin transport at the cellular level which results in leptin not working or is referred to as leptin resistance.³⁰

Triglycerides also mediate hyperleptinemia by inhibiting leptin transport to the brain. The mechanism of inhibition of the LRB signal cascade in the hypothalamus is related to 2 regulatory inhibitory molecules, namely SOCS3 and PTP1B (protein tyrosine phosphatase 1B). Hyperleptinemia conditions causing STAT3 signaling by LRB induce expression of inhibitory SOCS3, which interferes with LRB action by binding to Tyr985 and Jak2. High circulating leptin concentrations cause accumulated SOCS3 to counteract increased signal processing in LRB. A number of studies have shown that chronically high LRB activation induces self-feedback inhibition, possibly through SOCS3, which effectively limits the efficacy of high leptin in chronic exposure. Expression of SOCS3 and/or other leptin resistance mediators is also induced by proinflammatory mediators and cytokines, such as IL-6, TNF- α , fatty acids and other lipids, and counter regulatory signal activators such as corticosteroids.¹⁶

ROC curve analysis in this study found that the optimal sLR cut point was 1.93 (64.1% sensitivity, 71.8% specificity) with an area under the curve (AUC) value of 0.77 (good). This intersection point resulted in the risk of hyperleptinemia in obese adolescents being 4.5 times greater than normal adolescents (95% CI 1.75-11.83, $p = 0.001$). Considine et al. (1996) explained the postulate that high and long-lasting leptin concentrations in overweight patients result in a leptin resistance effect.³¹ High leptin levels are associated with low SLR levels, this indicates reflex downregulation of the leptin receptor.¹⁴ A decrease in the number of SLRs causes insufficiency of leptin transport at the cellular level which results in leptin not working or is referred to as leptin resistance.³⁰

Control variables TG levels ≥ 100 mg/dL, IL-6 ≥ 11.95 pg/mL, Leptin/sLR ratio ≥ 2 , PAQ-A score of light activity, and total calories after multivariate analysis using logistic regression found a significant relationship between levels of TG, IL-6 and leptin/sLR ratio to the incidence of

obesity successively with RO 15.1 (1.37-166.08; CI 95%); 15.53 (1.28-188.4; CI 95%); 46.99 (4.18-528.23; 95% CI) with each p value < 0.05 .

The effect of the variable PAQ-A score on light activity and total calorie intake exceeding the RDA on the incidence of leptin resistance in obese patients in this study was not significant. The main component of energy expenditure is physical activity, which is about 20-50% of total energy expenditure. Research in developed countries found a relationship between low physical activity and the incidence of obesity. Research by Hemmingsson (2006) showed that physical activity had a good effect on the BMI of the obese respondent group compared to the non-obese group of respondents. The level of strenuous activity has more effect on the BMI of respondents who are obese than low activity levels with obesity.³² This study did not look for whether BMI has an effect on the incidence of leptin resistance.

Regarding daily calorie intake, studies in America and Finland show that groups with high fat intake have a greater risk of increasing body weight than the group with low fat intake with an RO of 1.7. Other research shows that increasing meat consumption will increase the risk of obesity by 1.46 times.³³ This situation is caused by fatty foods having greater energy density and not filling and having a smaller thermogenesis effect than foods that contain lots of protein and carbohydrates. These two studies did not examine whether intake directly affected the incidence of leptin resistance. The PAQ-A score of light activity and total calories on the incidence of leptin resistance in obesity in this study was not significant, possibly due to the impact of regulations implemented during the COVID-19 pandemic, causing activity restrictions and eating patterns that were almost similar to the study subjects.

Previous studies reported the occurrence of a condition of chronic low grade inflammation in children, adolescents and obese adults which is characterized by increased levels of TG and proinflammatory mediator IL-6. Research on obese adults reported the role of proinflammatory mediators IL-6 and TG in the occurrence of leptin resistance. Leptin resistant conditions cause high leptin levels to be followed by low SLR

levels as a result of a downregulation effect. This research is the first study on obese adolescents looking for an integrated relationship between TG levels, IL-6 levels, and sLR levels on the occurrence of hyperleptinemia. This study strengthens the theory of chronic low grade inflammation and demonstrates the role of inflammatory mediators IL-6 and TG in the effect of down regulation (leptin/sLR ratio) in obese adolescents with leptin resistance.

The limitation in this research is that this research was carried out in the COVID-19 pandemic situation, resulting in restrictions on the community including school students. This condition can affect children's activities and eating patterns, so that it can affect the results of the study and serological tests for SARS-Cov2 antibodies are not carried out.

CONCLUSION

This study reinforces the theory of chronic low grade inflammation in obese adolescents which is characterized by high levels of pro-inflammatory cytokines. In addition, this study also proved that inflammation and high TG levels play a role in hyperleptinemia in obese adolescents which causes high leptin levels and low SLR.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared.

FUNDING

This study has no funding from external sources.

ETHICAL STATEMENT

This study received ethical approval from the Research Ethics Commission of the Faculty of Medicine, Udayana University/Sanglah Hospital, Denpasar, with an Ethical Eligibility Letter no: 2650/UN14.2.VII.14LT/2021 dated 23 November 2021.

AUTHOR CONTRIBUTION

All authors contributed equally in this study

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