RESEARCH ARTICLE

Association Between Free Fatty Acid (FFA) and Insulin Resistance.

The Role of Inflammation (Adiponectin and high sensivity C reactive Protein/hs-CRP) and Stress Oxidative (Superoxide Dismutase/SOD) in Obese Non-Diabetic Individual

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Abstract

BACKGROUND: Obesity is highly related to insulin resistance, therefore, the increased number of obesity is followed by the increased prevalence of type 2 Diabetes Mellitus. Obesity is associated with increased FFA. Increased FFA in the body can stimulate the increase of reactive oxygen species (ROS) in muscle, liver and endothelial cells. The increase of ROS would lead to insulin resistance (IR) and increased proinflamatory protein. FFA plays an important role in IR by inhibiting muscle glucose transport and oxidation via effects on serin/threonine phosphorylation of IRS-1. The aim of this study was discover the existence of SOD, hs-CRP, and adiponectin levels towards the occurrence of insulin resistance which was caused by elevated level of FFA and to discover the interaction between SOD, hs-CRP and adiponectin in non diabetic obese adult male.

METHOD: This was observational study with cross sectional design. There were 65 obese male non diabetic subjects and 45 non obese male non diabetic subjects who met the criteria. In this study, measurements were done on body mass index (BMI), fasting glucose, insulin, adiponectin, hs-CRP and SOD. Obese was defined as BMI > 25 kg/m^2, normal weight was defined as BMI 18,5-23 kg/m^2 and Insulin Resistance was defined as HOMA-IR > 1.

RESULT: This study showed that Hypoadiponectinemia condition, decreased SOD level and high level of hs-CRP is associated with insulin resistance in obese non diabetic subject. Adiponectin and SOD were correlated negatively with insulin resistance in obese non diabetic (Adiponectin r=-0,455, p<0,001 and SOD r=-0,262, p=0,003), hs-CRP was positively correlated with insulin resistance in obese non diabetic (r=0,592, p<0,001). FFA levels was increased in obese insulin resistance compared with non obese non insulin resistance.The Odds Ratio of Adiponectin, hs-CRP and SOD in this study was analyzed by logistic binary. The OR for SOD 3,6 (p=0,001), hs-CRP 9,1 (p<0,001) and Adiponectin 7,2 (p<0,001).

CONCLUSION: This study suggested that FFA levels increased in obese insulin resistance as compared with non obese non insulin resistance. Hypoadiponectinemia, decreased SOD and elevated hs-CRP were associated with insulin resistance in obese non diabetic subjects.

KEYWORDS: Obesity, Insulin Resistance, FFA, SOD, hsCRP, adiponectin.

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Introduction

Obesity is highly related to insulin resistance, therefore, the increased number of obesity is associated with excess prevalence of type 2 Diabetes Mellitus (7,9). Over nutrition and decrease in physical activity, lead to increased concentrations of FFA and glucose in cells. This condition will result in oxidative stress. Increased oxidative stress condition will cause endothelial dysfunction, insulin resistance in adipocyte and muscle and altered insulin secretion. These circumstances can trigger the occurrence of metabolic syndrome, cardiovascular disease and diabetes. Free Fatty Acids (FFAs) are adipokine secreted from adipocyte. FFA is now recognized as a major link factor between obesity and insulin resistance or type 2 diabetes. In obese non diabetic individuals, high plasma FFA levels is the cause of insulin resistance whereas in patients with type 2 DM, FFA is partly responsible for the development of insulin resistance. FFA plays an important role in insulin resistance by inhibiting muscle glucose transport and oxidation via effects on serin/threonine phosphorylation of IRS-1 (1,3,14,15).

Recently, adipocyte is known to have endocrinologic function which actively secretes proteins and cytokines (adipokines). Adiponectin, one of the adipokine, has been shown to influence inflammatory process. Obesity is not only a chronic inflammatory state but also associated with oxidative stress indicated by decreased superoxide dismutase (SOD). Inflammation is source of oxidative stress, which also implicated in the development of atherosclerosis. An increased intracellular FFA, leads to reduce GLUT4 translocation to the plasma membrane, resulting in resistence to insulin-stimulated glucose up take in muscle and adipose tissue. Besides FFA increased either by increasing intracellular ROS or diacyl glycerol (DAG) will result in PKC activation, and cause an increase in proinflamatory and proatherogenic protein (4,5,6,8,10).

Patients and Methods

This was an observational study with cross sectional design. Sixty five (65) obese male non diabetic subjects and 45 non obese male non diabetic subjects aged 30-50 years, met the criteria. Participants signed informed consent to have personal data such as height, weight, waist circumference, blood pressure, physical activity, alcohol, smoking and drugs. Subjects fasted for 10-12 hours. Obese was defined as BMI > 25 kg/m^2, non obese was defined as BMI 18,5-23 kg/m^2 and Insulin Resistance was defined as HOMA-IR > 1. Obese subjects had HOMA-IR above 2.

Assays of Biochemical Markers

All venous blood specimens were drawn and the serum was immediately separated by centrifugation and stored at -20°C until measurement. Serum Adiponectin levels were measured by sensitive enzyme immunoassay using recombinant human adiponectin. (Sekisui Medical Co, Tokyo, Japan). Serum hsCRP were measured by immunochemiluminescence using the kits from Diagnostic Product Corporation (DPC). Serum FFA was measured by sensitive immunometric assay using the kits from Roche. SOD measurements requires special treatment of the cells to lyse erythrocytes before SOD measurement. SOD was measured by colorimetric using the kits from Randox. All assays were performed according to manufacturer's instruction. For each run of these biochemical markers, controls were included in the assay and all results were within the acceptable range. These biochemical markers were assessed in Prodia Clinical Laboratory.

Statistical Analysis

Statistical analysis was performed with SPSS for windows ver 11.5. Univariat analysis was performed to calculate mean, maximum and minimum value also Standard Deviation (SD). T test was used to compare all factors between obese non diabetes and non obese non diabetes. Bivariate and partial correlation analysis were used to analyze the correlation of all factors. Results were considered significant if $P \leq 0.05$

Results

1. **Normality using the Kolmogorov-Smirnov test, the result obtain distribution data has a normal distribution.**

2. **SOD levels in non obese non diabetic and obese non diabetic subjects.**
   This study showed that mean fasting SOD level was 1050 ± 173.18 U/g Hb in obese and 1164 ± 135.87 U/g Hb in non obese subject (p=0.001), as shown in Figure 1 (A). We found significant difference in SOD levels in obese subjects and non obese subjects. There was correlation between SOD and BMI (r=0.262; P=0.003), as shown in Figure 2 (A).

3. **FFA levels in non obese non diabetic and obese non diabetic subjects**
   This study showed that mean fasting FFA level was 0.61 ± 0.44 mmol/L in obese and 0.50 ± 0.17 mmol/L in non obese subject (p=0.108), as shown in Figure 1 (B). We found no significant difference in FFA levels in obese subjects and non obese subjects. There was no correlation between FFA and BMI (r=0.162; P=0.07) as shown in figure 2 (B).

4. **Adiponectin levels in non obese non diabetic and obese non diabetic subjects**
   This study showed that mean fasting Adiponectin level was 3.47 ± 1.31 ng/mL in obese and 5.57 ± 1.88 ng/mL in non obese subject (p<0.000), as shown in Figure 1 (C). There was a correlation between Adiponectin and BMI (r=0.445; P<0.001), as shown in Figure 2 (C).

5. **hs-CRP levels in non obese non diabetic and obese non diabetic subjects**
   This study showed that mean fasting hs-CRP level was 3.94 ± 3.68 ng/L in obese and 0.99 ± 0.63 ng/L in non obese subject (p<0.000), as shown in Figure 1 (D). There was a strong correlation between hsCRP and BMI (r=0.592; P<0.001), as shown in Figure 2 (D).

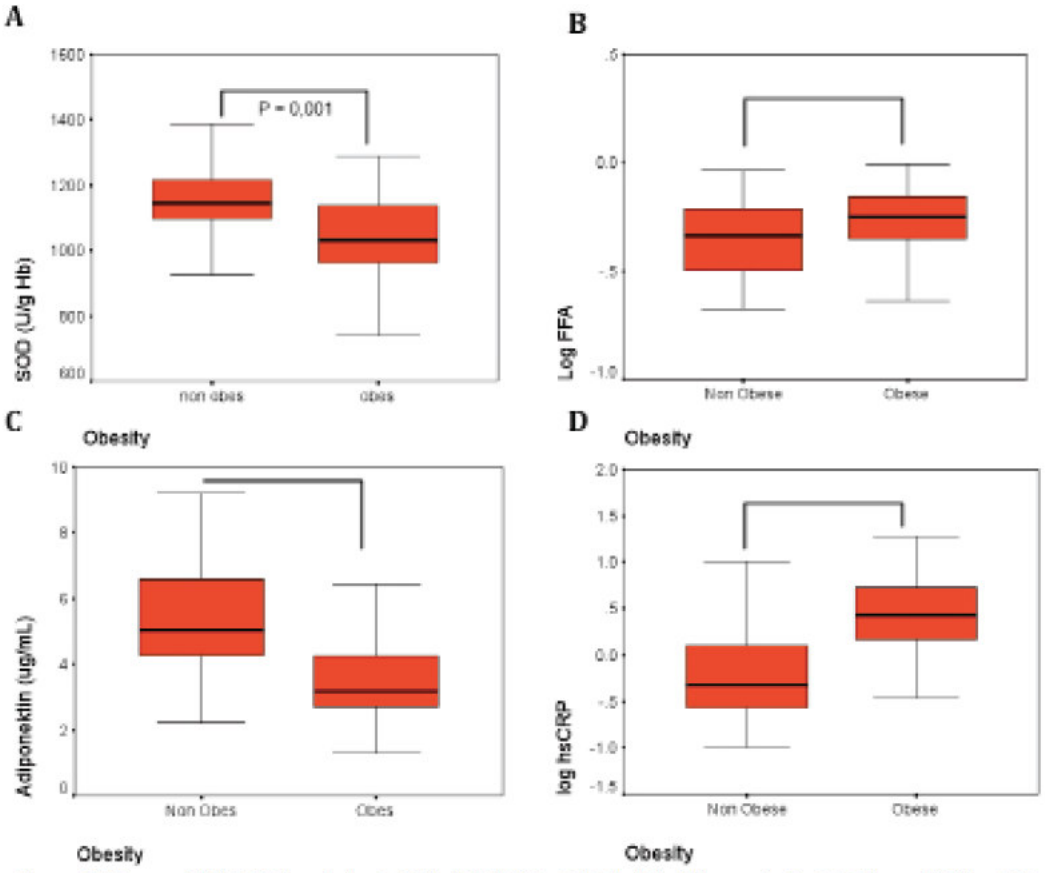

Figure 1. Means of (A) SOD levels (p=0.001), (B) FFA (p=0.108), (C) Adiponectin (p=0.000), and (D) hsCRP in non obese and obese subjects (p<0.000).

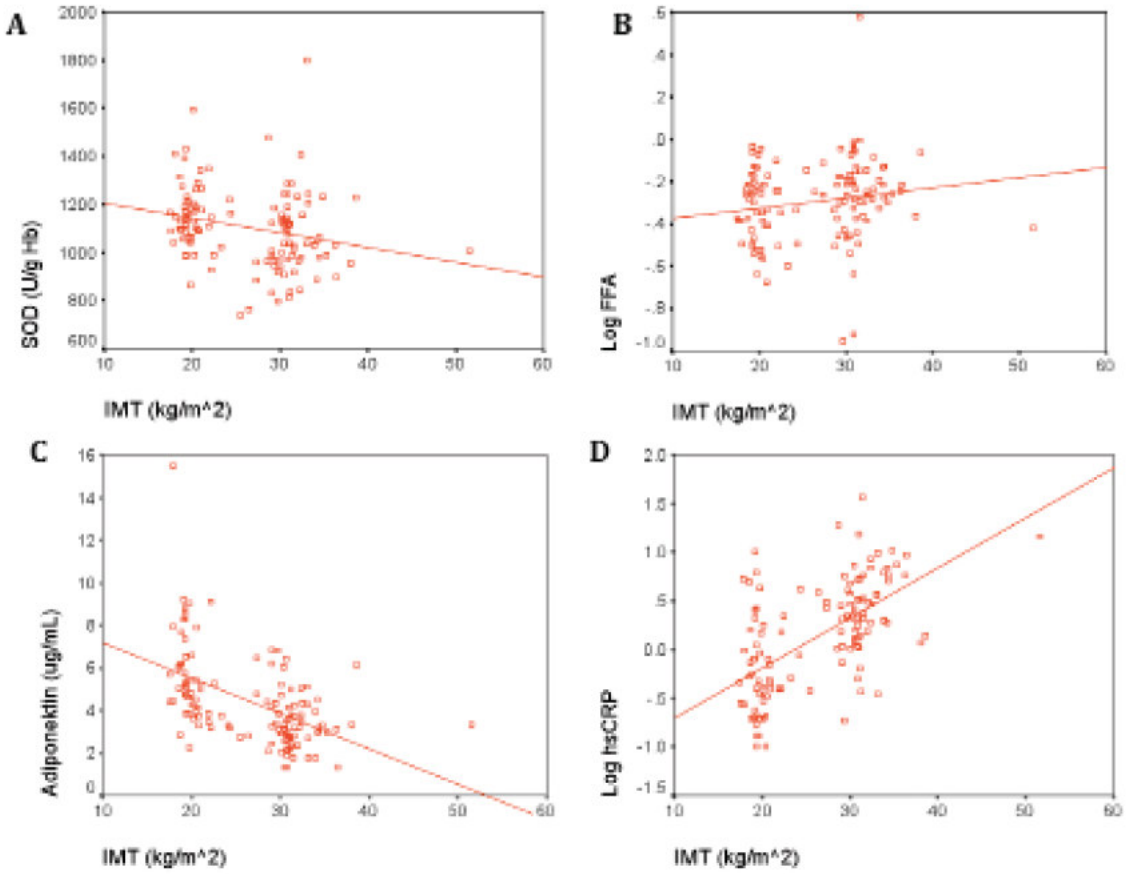

Figure 2. Correlation between (A) SOD, (B) Log FFA, (C) Adiponectin, and (D) hsCRP and BMI in non obese and obese subjects.

6. Analysis of the interaction between SOD, Adiponectin and hsCRP on insulin resistance subject.

Table 1 shows the result of logistic regression analysis of the measured of each parameter

Table 1. Logistic regression analysis of the parameters.

Variable	B	Wald	P	OR	95% CI
SOD	-1.269	11.242	0.001	3.558	1.694–7.471
hsCRP	2.212	27.799	0.000	9.137	4.014–20.803
Adiponectin	-1.973	23.378	0.000	7.196	3.233-16.014

Discussions

Occurence studies support the involvement of FFA in the event of insulin resistance and type 2 Diabetes. FFA levels increased in condition of obesity and type 2 diabetes (1,2,11,13,15). From this study there was no difference or correlation between FFA and BMI of the subjects. The possible explanation was in physiological conditions, levels of FFA after fasting for 10-12 hours were between 0.1-0.5 mmol/L, where as in pathological condition such as obese non diabetic, levels of FFA between 0.6–0.8 mmol/L (12)

Obesity is associated with chronic inflammatory conditions. This is caused by overproduction of TNF-α that would inhibit further secretion of adiponectin and

increased hsCRP production through stimulus production of IL-6 in the liver (1,9,11). This study found a significant difference and negative correlation between adiponectin and BMI. There was a significant difference and positive correlation between hsCRP and BMI in obese & non obese subjects.

Obesity is not only a chronic inflammatory state but is also associated with oxidative stress indicated by decrease in superoxide dismutase (SOD). Inflammation is source of oxidative stress, which also contribute to the development of atherosclerosis (2,9). There are several biomarkers that can be used to assess the condition of stress oxidative indirectly, example using the parameters of Superoxide Dismutase (SOD) dan Glutathion Peroxidase (GPx) which are intracellular antioxidants and also total antioxidant status. In this study, the measurement of SOD was used to assess the oxidative stress condition. There was significant difference of SOD concentration in obese and non obese subjects. There was a negative correlation between SOD and BMI.

The Odds Ratio of Adiponectin, hs-CRP and SOD in this study was analyzed by logistic binary. The OR for SOD was 3,6 (p=0,001), hs-CRP was 9,1 (p<0,001) and Adiponectin was 7,2 (p<0,001). This study showed that the condition of obesity is associated with inflammation, with the evidence that hsCRP concentration was higher and adiponectin concentration was lower in obese subjects than in non obese subjects. The oxidative stress condition that accompanies the obese non diabetes subjects was indicated by the viewer levels of SOD in obese subjects compare with non obese. Limitation of this study was the number of subjects who were recruited either obese and non obese subject. FFA limitation of measurement is the measurement of total fatty acids and the measurement with ELISA method is less sensitive when compared with other method such as the GC-MS.

Conclusions

From this study we concluded that there is increased FFA levels in obese non diabetic compared with non obese non diabetic. Hypoadiponectinemia, decreased SOD and elevated hs-CRP are associated with insulin resistance in obese non diabetic subject.

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