COMPARISON OF LEVELS LEPTIN IN VISCERAL AND NON VISCERAL OBESITY

Yulina Dwi Hastuty
Department of Midwifery , Poltekkes Kemenkes Medan

Abstract

This study aims to determine the ratio between the levels of leptin, visceral and non-visceral obesity. To achieve the goal, examination and laboratory studies include measurement of height, weight, waist and pelvis as well as the levels of leptin. The study population was all persons suspected of obesity obtained by accidental sampling. Analysis of the results using SPSS with significance level of p <0.05. The results showed that the percentage of obesity is more prevalent in women than in men and obese highest age group was found in the age group 31-40 years. Leptin levels did not differ between visceral obesity with non-visceral obesity (p> 0.05) and leptin levels were higher in women than men (p <0.05).

Key words: leptin, visceral obesity, non-visceral obesity

Introduction

Obesity is a global problem in developed countries and developing countries. The prevalence of obesity is increasing in recent years and has led to serious health problems. Globally, at least 2.8 million deaths each year linked to weight gain and obesity which 300,000 occur in the United States and 350,000 in Southeast Asia (Rahmouni et al, 2005: WHO / SEARO, 2011). Based on data from the Noncommunicable Disease in South-East Asia Region in 2008 the prevalence of individuals with a BMI ≥ 25 kg / m2 increased from 2.7% to 8.9% in Bangladesh, 1.6% to 10% in Nepal and 11% to 15% in India, while in Indonesia percentage reached 16% in men and 25% in women (WHO / SEARO, 2011). For the North Sumatra region data obtained from the Regional Health Research (Riskesda) in 2007 showed the percentage reached 11.9% overweight and 13.5% obese. In 2010 the percentage of overweight in males 10.9% and 12.8% in women, while the percentage of obese 9.4% in males and 17.4% in women (Riskesda, 2010)

Increasing the number of individuals with obesity is bad for health, considering obesity is a chronic disease that is polygenic or monogenic which may result in some circumstances or pathological dysfunction (Klein & Romijn, 2008). Some things that can influence obesity, including genetic factors, food intake, neuroendocrine mechanisms, social, cultural and lifestyle (Librantoro, 2007). In Indonesia, lifestyle changes that lead to Westernization causes changes in diet refers to a diet high in calories, fat and cholesterol that have an impact on the increased risk of obesity (DirKes, 2009).

Obesity is defined as a condition there is an excessive accumulation of body fat, According to the standard body mass index (BMI), BMI> 25 kg / m2 categorized as obese (Asia-Pacific, 2000). Normally the excess fat will be stored layer of subcutaneous, but due to malfunctioning or damage the layer of accumulated visceral fat (Ibrahim, 2009). Fat distribution in different places have implications for morbidity (Flier, 2006; Ibrahim, 2009). Abdominal and intra-abdominal fat has greater significance than the fat that is distributed in the lower extremities or the whole body (Flier, 2006). Obesity is also a factor presdiposisi occurrence of hypertension, dyslipidemia, diabetes, cardiovascular disease, renal failure and inflammatory responses (Bravo, 2006). Prospective studies using anthropometric measurements found that visceral obesity is closely linked with hypertension, diabetes and cardiovascular disease (Tchernof, 2007).

Obesity can be divided into abdominal or visceral obesity and obesity periper or non-visceral (Wajchenberg, 2000; Klein & Romijn, 2008), which distinguishes them is that visceral fat had the glucocorticoid receptor and androgen more, metabolism is more active, more sensitive to lipolysis and more resistant to insulin. Visceral adipose tissue (VAT) has a greater capacity to produce Free Fatty Acid (FFA), increases glucose and more sensitive to adrenergic stimulation (Ibrahim, 2009).

Today has been much studied matters related to obesity, including the causes of obesity and the resulting risks. Leptin is a hormone that is considered to play a role in causing obesity. Leptin was first discovered in 1994 on obese mice (gen ob / ob), is a 16 kDa peptide produced largely by adipose tissue that acts as a major regulator in the regulation of energy balance and body weight (Friedman, 1998).

The main function of leptin is to provide a signal of energy stores in the body to the central nervous system so that the brain can make adjustments required to balance energy intake and expenditure (Friedman & Halaas, 1998; Enriori, 2006). Leptin levels decreased within 12 hours after starvation or during fasting and increased after several days of consuming a lot of food (Klein & Romijn, 2008). As a control of the energy balance in humans, leptin is an anti-obesity hormone that is based on the hypothesis that high leptin levels will prevent the occurrence of obesity (Bravo et al, 2006). Unfortunately this does not happen, most obese individuals have higher levels of leptin, but does not stimulate the expected loss of fat mass (Myers, 2008; Oswal, 2010).

Some researchers have found that higher leptin levels in obese people compared to people with normal weight (Considine, 1996). Leptin levels are also found to be higher in women with hypertension compared to non-hypertensive and leptin levels were higher in pre-menopausal women compared to postmenopausal (Khokhar, Et al, 2010). Leptin suppress gene expression acetil Karboxylase CoA, fatty acid synthesis and lipid synthesis, biochemical reactions that contribute to the accumulation of lipids (Fruhbeck, 2001: William, 2002: Turner, 2006) Although there has been much research on leptin, but until now has not been widely studied how the comparison levels leptin between visceral and non-visceral obesity.

METHOD

1. Equipment and Materials

Tools used: meter, microtaise, 96-Wells Microplate with anti-human leptin, Micropipettes, Multichannel pipettes, pipette and tips, Elisa test kits, syringes 5 cc, centrifuge, incubator, test tubes, silicon tubes containing EDTA, tissue, handscoon, measuring cup 100 ml, software for data analysis ELISA.

Materials used: plasma samples, wash buffer concentrate, standard (recombinant human leptin), Sodium Azide 0:09%, deionized water (distilled water), buffer concentrations, leptin detection antibody (Biotinylated anti-human leptin), HRP-Streptavidin concentrate, Tetramethylbenzidine (TBM) one-step substrate reagents, Stop Solution (sulfuric acid).

2. Working procedures

a. Data collection

Study subjects who meet the criteria for obesity, requested willingness to follow the research, and then made a personal anamnesis, previous medical history and physical examination. All subjects who entered the inclusion criteria are required to complete a research agreement. The whole subject of further sampled measured BB, TB, pelvic and waist circumference were then categorized into or non-visceral visceral obesity.

b. Measurements Weight (BB) and Height (TB)

Weighing performed by the weighing stand (platform beam balance scale) which has been calibrated prior to the accuracy of 100 grams. Measurements carried out by way of a subject standing upright on the scales and then figure appointed needle (scale) scales read as a result (in kg).

Height measurement is done by using a measuring instrument upright (microtaise) up to 0.1 cm accuracy. Measurements were made with upright, face facing straight ahead without wearing footwear, the result is read in cm.

c. Waist Circumference Measurements (LP) and Pelvic Ring (LPA)

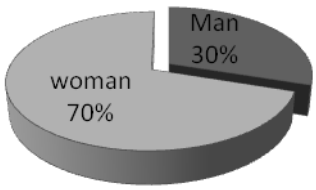
Waist circumference was measured in an upright position and quiet. Shirt or obstructions removed measurement. Place the tape measure on the top edge Crista illiaca dextra. Measuring tape looped around the abdominal wall as high as Crista illiaca. Make sure the measuring tape is not too tight leather pressing and parallel to the floor. Measurements were made at the end of a normal expiration. Read waist circumference in centimeters. Circumference measurements of the pelvis (LPA) was performed using a measuring tape in a standing position and breathe as usual. Measured by circling the pelvis at the point of maximum protrusion buttocks. Results expressed in centimeters.

d. Blood sampling
Performed after the patient fasted for 10 hours. Sampling was done by taking venous blood 3 ml and put into tubes containing EDTA as an anticoagulant. The blood that has been taken centrifuged for 10 minutes at a speed of 2000 rpm then separated between serum and plasma and transferred into a tube that has been labeled. These specimens stored at -20C until examination. e. Leptin levels Measurement
1. Preparation regensia, standards and samples.
All regensia and samples were taken to a place with room temperature (18-25˚c) before use. Buffer concentrate is diluted 5-fold with distilled water. Wash concentrate in the form of crystals warmed to room temperature and stirred until larur. Dilute 20 ml of Wash Buffer concentrate with distilled water to produce 400 ml wash buffer. Detection antibody concentrate was prepared by adding 100 mL of buffer concentrate into a vial containing the detection antibody. Detection antibody concentrate is diluted 80-fold with a buffer concentrate. HRP-Streptavidin concentrate is diluted 8000 times with the buffer concentrate. Make 220 ng / ml standard by adding 800 ml 0:09% sodium azide into the vial C which contains recombinant human leptin. Then slowly stirred to dissolve. To prepare 400 pg / ml stock standard solution add 2 mL vial leptin standard of C into a test tube with 1098 mL sodium azide 0:09%. 8 pieces provide a test tube. Pipette 300 mL of 0.09% sodium azide added to each test tube. 1 tube only contains sodium azide 0:09% and not added anything. To make serial dilutions Enter 200 mL standard solution in the tube 2 then stir until evenly distributed. Take 200 mL of solution in a tube inserted into the tube 2 and 3 and stir until evenly distributed, take 200 mL of solution in a tube inserted into the tubes 3 and 4, and so on until the tube 8
2. The examination procedure
All regensia and the sample is placed at room temperature. Enter 100 mL standard and sample into the well that has been provided. Close well and incubated for 2.5 hours at room temperature or overnight at 4C with shaking slowly. Throw away liquid and washing with a wash buffer and then dry with tisue. Add 100 mL Biotinylated antibody in each well. Incubation for 1 hour at room temperature. Discard the liquid and washing with a wash buffer. Add 100 mL streptavidin solution in each well. Incubation for 45 min at room temperature. Discard the liquid and washing with a wash buffer. Add 100 mL of TMB substrate to each well. Incubation 30 minutes at room temperature and dark. Add 50 mL stop solution to each well. Results immediately read on Elisa reader using a wavelength of 450 nm (RayBiotech 2011).
3. Analysis of the results by ELISA using the data analysis software to obtain the value / concentration levels of leptin were examined.

DATA ANALYSIS
All data obtained are recorded and tabulated. The data obtained were processed statistically by using SPSS. To compare leptin levels between the study group used independent t test if the data were normally distributed both groups or the Mann Whitney test if data distribution is not normal. Significant differences determined by the value of $p < 0.05$.

RESULTS AND DISCUSSION
A. Sample Characteristics Research

Of the total study sample obtained percentage of obese men is 30% while 70% of women are obese (figure 1).



The data obtained from the World Health Organization Noncommunicable diseases in the south-east Asian region in 2011 states that the incidence of obesity is greater in women than in men.Figure 1. Distribution of the sample by gender
Data from 2010 shows that the percentage Riskesda year overweight in males 10.9% and 12.8% in women, while the percentage of obese 9.4% in males and 17.4% in women.

Some studies suggest an association between the sexes with obesity. Sandjaya (2005) found the prevalence of obesity is greater in women than men. Budiman (1997) states that over nutrition and obesity are more common in women than men, ie, 29.1% and 50.1% in females, whereas in males at 19.5% and 1.7%. The same was found in the study Kodyat et al (1996) found that the prevalence

of obesity in women is almost double than the group of men (14.7% in women and 7.4% in men).

Sandjaya 2005 revealed several factors that may be related to the high percentage of obesity in women, among others are: the consumption of fatty foods is probably more often than men, sports activity that is rarely done, marital status, where married women tend to gain weight bodies at a later date and the use of hormonal contraceptives tools such as: implants, pills, and injections can cause side effects of weight gain

Use of hormonal contraceptives have a risk of 2.05 times more likely to be obese than non-hormonal contraceptives tool.

Distribution of age groups of research subjects appear in table 1 of this been below

Table 1. Distribution of sample by age group

Age group (year)	Amount	Percentase
21-30	7	17.5
31-40	23	57.5
41-50	10	25
Amount	40	100

The sample age range is 21-50 years, the number of 31-40-year age group obtained most that 23 people (57.5%), followed by 41-50 year age group of 10 people (25%) and the 21-30 age group that is 7 people (17.5%). Sandjaya and Sudigdo in 2005 found that most obese sample age group was 21% in the age group 45-49 years. In the study Kodyat, et al. (1996) the percentage of obesity is highest in the age group 41-55 years. Riskesda data in 2010 showed that the percentage of obesity is highest in the age group 40-44 years in both men and women. By age group, the tendency to obesity is more common with increasing age of the sample and the decline began to occur at the age of 50 years or more.

Several factors can influence obesity include genetic factors, food intake, neuroendocrine mechanisms, social, cultural and lifestyle (Librantoro, 2007).

B. Comparison of leptin levels between visceral and non-visceral obesity

In Figure 1 we can see the difference in leptin levels between groups of non-visceral obesity with visceral obesity group. Mean value ± standard deviation leptin levels in the group of non-visceral obesity is 9:37 ± 28 712 pg / ml, while in the group of visceral obesity is 26 344 ± 24.1 pg / ml.

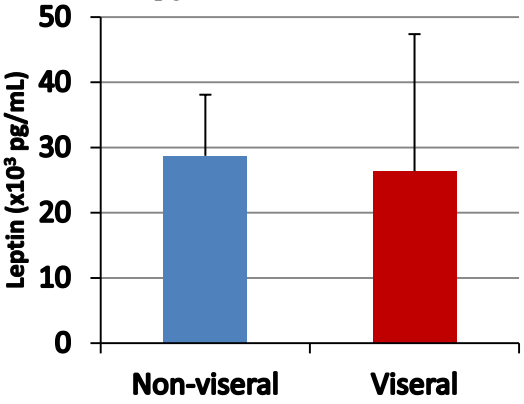


Figure 1. Comparison of leptin levels between visceral and non-visceral obesity (p> 0.05)

Leptin levels between visceral and non-visceral groups were not significantly different (p> 0.05). Previous research by Considine in 1996 found the average value of leptin in the obese group was 31 301 ± 24.1 pg / ml. Soegondo et al, 2004 to get the average of leptin in men with central obesity 9710 pg / ml while Ma et al Awdah 2004 and 2009 to get almost the same results with the average of 23 150 pg / ml and 24 580 pg / ml in normal obese women. Khokhar et al in 2010 found average levels of leptin in obese women was 40 930 ± 17.3 pg / ml. Librantoro et al 2007 mentioned by previous studies in several countries mean leptin levels normally range 1000-12000 pg / ml.

This suggests that leptin plasma levels varied with the variation of the race. The results showed no significant difference between the average levels of leptin in the group with non-visceral visceral obesity. It is caused by the more leptin associated with the accumulation of body fat and not the region where the fat is located. Visceral obesity is not solely describe the fat content in the body but rather shows the region of the fat is located. because visceral obesity is the accumulation of fat in the visceral layer contained in the abdominal cavity. Leptin is a hormone secreted by fat cells in proportion to body fat storage (Friedman, 1998) so that the concentration of circulating leptin is parallel to the BMI, body fat percentage and total body fat weight (Considine, 1996). Almeida in 2006 found that serum leptin concentrations correlated with the size of obesity as a BMI or body fat percentage. Besides the production of leptin in adipose tissue under the regulations

of nutritional, hormonal and neural (Fruhbeck, 2001).

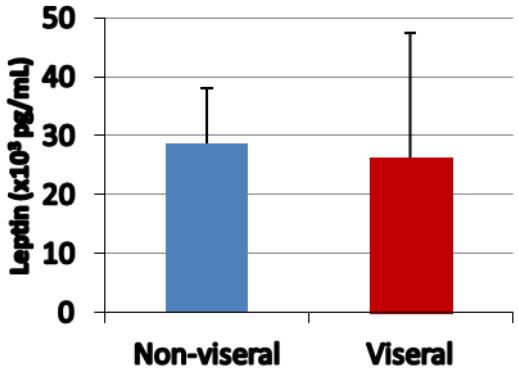


Figure 2 shows a comparison between the levels of leptin, visceral obesity and non visceral obesity by sex.

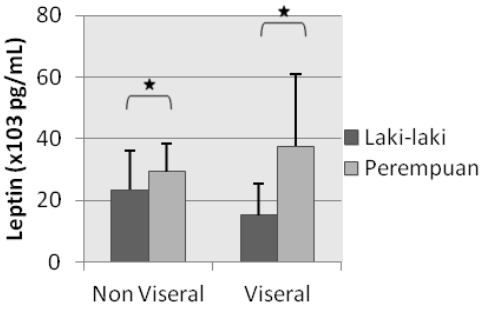


Figure 2. Comparison of the mean leptin levels between men and women in the group of visceral obesity and non-visceral obesity group ($p < 0.05$).

There is a fairly significant difference or significant ($p < 0.05$) between the average value of the levels of leptin in males and females. Mean value ± standard deviation in women visceral obesity group was 37599.73 ± 23387.46 and the group of non-visceral obesity 29293.39 ± 9247.08 while the mean value ± standard deviation leptin levels in men visceral obesity group was 15090.25 ± 10243.03 and the group of non-visceral obesity 23482.80 ± 12453.28

Results of this study found levels of leptin in women is higher than men in both groups both visceral and non-visceral obesity. The results are consistent with several previous studies which state that there are differences in leptin levels in women and men. Shankar et al 2010 found that leptin levels in women is higher than men. Khokhar et al in 2010 found average levels of leptin in obese women was 40 930 ± 17.3 pg / ml. Awdah in 2004 to get the levels of leptin in obese women amounted to 23 150 ± 6.73 pg / ml while Librantoro 2009 found levels of leptin in obese men 8,801 ± 7.9 pg / ml, Ma et al 2009 to obtain the results of leptin levels were higher in women than men (24 580 ± 18.98 and 8,440 ± 7.69), the women were also found almost two-fold higher leptin concentrations (Kratzsch, 2002). The range of normal values of serum leptin in women ie 3877 to 77.273 pg / mL while men from 2205 to 11.149 pg / mL.

This is probably related to hormonal influences where theory is that estrogen and prolactin is a stimulator of the synthesis and secretion of leptin so the more estrogen then leptin levels will be higher. Hormone estrogen and prolactin is a hormone found in women who are otherwise hormone androgen synthesis inhibitor of the more leptin obtained in men.

Figure 3 shows a comparison of the average levels of leptin women between visceral and non-visceral obesity and the average ratio of male leptin levels between visceral and non-visceral obesity.

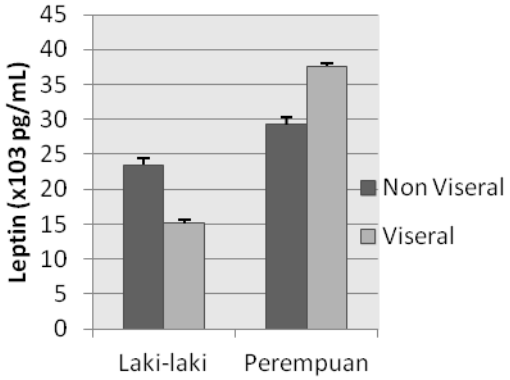


Figure 3 ratio leptin levels between visceral and non-visceral obesity for the female gender and males ($p > 0.05$).

Although average levels of leptin was significantly different between men and women, but when compared to the average levels of leptin in women between the visceral and non-visceral obesity was not significantly different ($p > 0.05$) as well as for the average male leptin levels between visceral and non-visceral obesity no significant differences ($P > 0.05$). These results further strengthen the previous explanation that suggests that leptin is associated with the accumulation of body fat rather than on the region where the fat is located, and the synthesis of leptin is

influenced by nutrition regulation, hormonal and neural (Fruhbeck, 2001).

CONCLUSION

Based on the research results it can be concluded as follows:

1. The prevalence of obesity is greater in women than in men because of differences in food intake, neuroendocrine mechanisms, social, cultural and lifestyle ..
2. The risk of obesity tended to increase with age and will be reduced at age 50 years and older.
3. There is no difference in leptin levels between visceral and non-visceral obesity, because leptin is associated with the accumulation of body fat is not the area where the fat is located.
4. There are differences in leptin levels between women and men. Leptin levels were found to be significantly higher in women than men both in the group of visceral obesity and non visceral group ($p < 0.05$). This is because the synthesis and secretion of leptin also influenced by hormones such as estrogen and prolactin is a hormone that is known to the women as the stimulus secretion of leptin, otherwise known androgen is the male hormone acts as an inhibitor of secretion of leptin.

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