Sex Differences in Neuropeptide Y Serum, But Not in Fat Intake and Body Mass Index

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Abstract

Background: One's appetite has a role in controlling food intake and maintaining energy balance, but its effect on body metabolism related to obesity is still questionable. The purpose of this study was to determine the levels of neuropeptide Y in healthy people and to see differences in gender and anthropometric parameters. The hypothesis of this study was that there would be differences in neuropeptide Y levels in groups with gender and anthropometric parameter differences. **Methods:** This study was a cross-sectional study involving 62 study subjects, male and female, who did not have chronic diseases or metabolic disorders. This research was conducted from April to September 2020. The parameters examined in this study were neuropeptide Y levels and anthropometric parameters. The statistical analysis performed was the Mann-Whitney test to see the differences between groups. **Results:** The mean age of the research subjects was 40.48 ± 10.85 years, with the same ethnic distribution. The distribution of men and women was more women than men. Based on anthropometric examination, it was found that obesity nutritional status was more common in the female group than in the male group; however, serum neuropeptide Y levels were found to be significantly different between male and female groups (male group was higher). **Conclusions:** The study found significant differences in serum neuropeptide Y levels in male and female groups.

Keywords: obesity; appetite; neuropeptide; orexigenic; fat; energy; gender

Background

Motivation and appetite are important phenomena in human life to maintain energy balance and body weight [1, 2]. Increased appetite temporarily or permanently leads to weight gain and thus the risk of obesity is greater [3, 4]. The hypothalamus as the center of hunger and fullness produces neuropeptide Y as a potent anabolic peptide that increases appetite and reduces energy consumption [1, 5, 6].

Previous studies have shown no difference in neuropeptide levels in men and women, but some have shown different results [7-9]. One study linked metabolic parameters, such as body mass index and blood pressure, and found no differences in neuropeptide Y levels between the sexes, which may have been due to a complex mechanism that still needs further research [10, 11].

Neuropeptide Y is a key orexigenic neuropeptide that regulates adiposity by reducing energy storage and utilization [12-14]. Fasting conditions or low caloric intake stimulate neuropeptide Y expression, which increases appetite [12, 13]. Neuropeptides are expressed mostly in the hypothalamus and in peripheral tissues such as white fat tissue and bone cells, which indicates that there are several other physiological functions apart from maintaining energy balance [12, 15, 16].

After reviewing the relevant research, it is clear that the function of neuropeptide Y research is related to the accumulation and mobilization of lipids, as. These regulate peroxisome proliferator-activated receptor gamma 2 and hormone sensitive lipase (HSL) [16, 17]. If neuropeptide Y levels are reduced, the factors that cause imbalance age-related adipose tissue metabolism increases, such as decreased increased inflammation, decreased de novo lipogenesis in visceral fat, and decreased thermogenic activity in subcutaneous fat [14, 17]. Rat studies suggest that low and high levels of neuropeptide Y are sex dependent; however, studies in humans do not find the same results. Low levels of neuropeptide Y did not affect adiposity in mouse studies, whereas some studies have found it humans while some have not [17-19]. For this reason, it is interesting to investigate whether there is a difference in neuropeptide Y levels in the associated factors in male and female groups.

This study aimed to determine whether there were differences in neuropeptide Y levels in certain groups, namely social factors (age and sex) and metabolic parameters (body mass index, energy intake, and fat intake). This study determined the associated factors that will form the basis for future research. This study also provided further information regarding the role of neuropeptide Y on obesity.

Methods

Study design

This study included a cross-sectional design that took sociodemographic data (i.e., age, ethnicity, sex, occupation, history of father, and mother fat), anthropometric examinations (i.e., body mass index and abdominal circumference), examination of daily food intake (i.e., energy, intake fat, protein intake, and carbohydrate intake per day), and serum neuropeptide Y levels in the study subjects.

All research subjects were collected from the same area, namely in areas far from urban areas (i.e., Dusun III, Simpang Dolok Village, Datuk Lima Puluh District, Batu Bara District, and North Sumatra, Indonesia). We avoided subjects from urban areas because they were have a diverse diet. This research was conducted from April to September 2020, after the COVID-19 pandemic had spread across the globe, yet the study location has not had any COVID-19 cases. During the data collection period, it was also carried out by implementing strict health protocols.

Participants

The included research subjects were healthy men and women aged 20-60 years old. Exclusion criteria were pregnant women, nursing mothers, impaired kidney and liver function, chronic disease, or other metabolic disorders. The process of finding research subjects came by appealing to the local community health center, as well as spreading word of mouth, wherein the subjects came voluntarily. When we were selecting research subjects, 96 research subjects were enrolled but only 62 research subjects passed the exclusion criteria.

The research subjects had also read the explanation about the research and were willing to take part in the research by signing the informed consent form. This research procedure and protocol was approved by the Research Ethics Committee of the University of North Sumatra, Indonesia, with the certificate number: No. 61 / KEP / USU / 2020.

Anthropometric examination

Anthropometry included height (to the nearest 0.5 cm), weight (to the nearest 0.1 kg), and body mass index (calculated as kg/m^2). Categorized body mass index (BMI) was based on Asia Pacific, which were <18.5 kg/m^2 classified as underweight, 18.5-22.9 kg/m^2 classified as normoweight, 23-24.9 kg/m^2 classified as overweight/at risk, 25-29.9 kg/m^2 classified as obese I, and >30 kg/m^2 classified as obese II.[20]

We examined waist circumference using a standardized measuring tape in centimeters. The category of abdominal circumference was different between men and women. For men, less than 90 cm was classified as normal and more than 90 cm was classified as central obesity. For women, less than 80 cm was classified as normal and more than or equal to 80 cm was classified as central obesity. [20]

Nutrient intake assessment

Assessment of nutrient intake was based on food recall for two days (one day for weekday and one day for weekend), including energy, protein intake, fat intake, carbohydrate intake, and percentage of fulfilment according to the Indonesian Recommended Dietary Allowances (RDA) 2019. Calculation were conducted using the Nutrisurvey application (2005), which included Indonesian foods.

The following categorizations were determined: for calorie intake, <2500 calories per day was low and ≥2500 calories per day was normal; for protein intake, <60 grams per day was low and ≥60 grams per day was normal; for carbohydrate intake, <400 grams per day was low and ≥400 grams per day was normal; for fat intake, <65 grams per day was low and ≥65 grams per day was normal; for fiber intake, <25 grams per day was low and ≥25 grams per day was normal[21].

Neuropeptide Y examination

This examination was conducted by drawing blood serum from research subjects and performing centrifugation with 2000-3000 revolutions per minute (RPM) for 20 minutes. Furthermore, we carried out blood serum tests to check neuropeptide Y levels using the Human Neuropeptide Y Enzyme-link Immunosorbent Assay (ELISA) kit (Thermo Fisher Scientific brand, Waltham, MA, USA) (Bioassay Technology Laboratory, Shanghai, China).

Statistical analysis

Data were analyzed using version 11.5 of the IBM-SPSS statistical program (IBM Corp., Chicago, IL). Categorical variables were expressed as percentages. Normally distributed continuous variables were expressed as mean ± SD, whereas non-normally distributed continuous variables were expressed as median (minimum-maximum). To compare the two groups, the independent T statistic test was used if the distribution was normal and the Mann–Whitney test was used if data were not normally distributed.

Results

Baseline characteristics of the study population

This oldest population of research subjects were aged 41-50 years old (35.5%) and youngest population were aged 20-30 years old (19.4%). Ethnic groups, namely the Indonesian Malay and Batak tribes, were evenly divided into the two groups. These two tribes are the largest ethnic group found in North Sumatra, Indonesia (**Table 1**).

Table 1 shows that the majority of subjects were women (56.5 vs. 43.5%), and the most common occupation was housewives (37.1%). The study was located at a rural area so that the occupation of most adult women were housewives. Most males in this study were laborers (27.5%), which is a notable financial job in this region.

The history of obesity from patients' parents was also asked to see if there was a relationship to obesity in adulthood. Most patients did not have parents with obesity (83.9 vs. 72.6%) (**Table 1**). The analysis stated that there was no significant relationship between the incidence of obesity and a history of obesity in the father ($p = 0.738$) and mother ($p = 0.581$).

Characteristic data on sex differences

This study compared age and anthropometric data between men and women, aiming to see if there were differences between these two groups. This study showed that no significant differences were found between men and women in terms of age. In both groups, most patients were in the 41-50 age group (**Table 2**).

An anthropometric examination did not show any differences between the two groups of men and women. The obese group was more common in the female group than in the male group, but there was no significant difference (**Table 2**). **Table 2** also shows that there was no abdominal circumference difference between men and women, yet the percentage of abdominal circumference exceeded the normal limit more in the female group (54.3%).

In **Table 3**, based on food intake for two days, there was no difference between men and women, except in total energy ($p = 0.019$), wherein the average energy intake for women is higher than that of men. The intake of fat, protein, and carbohydrates also showed that the intake of women was higher than men but did not show a significant difference.

Table 4 shows the differences in neuropeptide Y levels in several parameters related to neuropeptide Y activity as orexigenic. The mean neuropeptide Y levels of all study subjects were 277.81±289.31 ng / L, with the minimum value being 71.5 ng / L. The median value was 116 ng / L and the maximum value was 981 ng / L. The data were not normally distributed. The neuropeptide Y level with the 5th percentile was 78.2 ng / L; the 10th percentile was 81.69 ng /

L; the 25th percentile was 90.48 ng / L; the 50th percentile was 116 ng / L; the 75th percentile was 428 ng / L; the 90th percentile was 872.1 ng / L; and the 95th percentile was 974.55 ng / L. The range of neuropeptide Y levels in this study was 91.2 to 422 ng / L. **Table 4** shows that the differences in neuropeptide Y levels were only found in the male and female groups. This study did not show any significant differences between the obese or non-obese groups, nor the high and low fat intake groups.

Based on the results of this study, it can be seen that neuropeptide Y showed significant differences in the male and female groups, yet not in the obese or fat intake groups. This difference requires further discussion.

Discussion

Motivation and appetite are the basis for regulating energy balance and weight, and can determine whether a person is obese or not [13, 14, 17]. Obesity is caused by consuming energy that exceeds basic needs; however, becoming obese is also influenced by several factors apart from high serum neuropeptide Y levels, including the availability of available food ingredients and diet. Residents in rural areas who are far from a high-fat diet will naturally consume the food available to them. This differs from urban areas that have more diverse foods.

High energy intake can be attributed to consuming too much saturated fat from red meat, animal fat, palm oil, or deep fried foods. High-fat food ingredients are found in fast food, which is generally found in urban areas. However, in locations far from urban areas, excess energy intake generally comes from high carbohydrate consumption, which consists of rice, noodles, or sweet potatoes. Excess glucose is stored in the form of fatty acids. The role of neuropeptide Y does not seem to focus solely on high fat intake, but, in our study, it appeared that higher levels of neuropeptide Y were found in the male group.

Neuropeptide Y is a orthigenic peptide that plays an important role in life extension. It is also restricted by calories and the mechanism is less clear.[17] Low neuropeptide Y levels combined with calorie restriction lead to high mortality associated with lipolysis and lipodystrophy. Low levels of neuropeptide Y cause active lipolysis and thermogenic signals, i.e., Beta3 adrenergic receptors and lipase sensitive hormones in white adipose tissue. The end result is a negative energy balance.[12, 17] The role of neuropeptide Y includes regulating lipid metabolic homeostasis and survival via lipolysis and thermogenesis control pathways in a negative balance.[12, 13, 22]

Neuropeptide Y is an appetite stimulator that increases fat collection through insulin and corticosteroid secretion, increases hepatic glucose utilization, reduces muscle glucose, increases glucose utilization by white fat tissue, activates brown fat tissue metabolism, decreases sympathetic activity, increases parasympathetic activity, and decreases thermogenesis[2, 3, 14, 23]. Neurons containing neuropeptide Y become active during negative energy balance conditions such as hunger, dietary restrictions, breastfeeding, physical exercise, and uncontrolled diabetes [4, 24, 25]. Neuropeptide Y provides changes in eating behavior, but the role of neuropeptide Y receptors and their expression also have an effect on increasing appetite.[26, 27] Seven subtypes of neuropeptide Y receptors, namely Y_1 to Y_7, are associated with stimulating appetites.

Neuropeptide Y changes one's energy balance in a positive direction by increasing food intake, limiting energy expenditure, and reducing thermogenesis in brown fat tissue [25, 28]. In parallel with this process, neuropeptide Y also facilitates fat storage in white fat tissue through increased insulin activity [2, 18, 25]. However, in this study, there are various factors that affect increased food intake. Based on our analysis, high levels of neuropeptide Y in serum did not show a direct relationship to obesity and fat intake. The condition of food shortages can be a

direct cause of this, considering that most research subjects are as laborers and housewives who live in rural areas.

Although the age of patients in this study was predominantly older (41–60), the total calorie intake was still low. The female group showed more obesity than the male group, and the female group also had larger abdominal circumferences. For women with hormonal influences, the combination of food availability and inactivity caused greater obesity than men, even though the serum neuropeptide Y levels were found to be significantly lower than men. This shows several factors that greatly influence the incidence of obesity in a person. In addition, there were hereditary factors that influenced obesity, but this study showed that, when a person entered adulthood, there were multifactorial occurrences that increased their likelihood of becoming obese. High appetite also caused neuropeptide Y to be released by the hypothalamus[29].

The history of obese parents did not show an association with patient obesity, although most patients did not have obese parents [30, 31]. Family history of obesity or metabolic disease played an important role, especially in childhood, as it was associated with obesity [32, 33]. Multifactorial lifestyles that provide an availability to consume high-fat or high-carbohydrate foods, encourage a lack of physical activity, breakfast habits, or genetic polymorphisms, were independent of high and low levels of neuropeptide Y released by the hypothalamus [32, 34-36]. All conditions are related to obesity and mostly impacted children entering adulthood. Various processes can make a person obese, including the role of neuropeptide Y.

In this study, women had lower levels of neuropeptide Y than men. Fat intake was unrelated to these findings. Previous studies suggested that women had lower expressions of neuropeptide Y than men in non-stressed states, especially when their brains were associated with stress. Further, estrogen levels play a potential role in its regulation [11, 37-39]. Another

possibility for appetite control is related to neuropeptide Y, which regulates depressive-like behavior. The effects of anxiety, however, are more common in men [37, 40, 41]. Therefore, high levels of neuropeptide Y affect appetite if intake is not excessive.

Gender plays an important role in the development of stress-induced psychological disorders [37]. Stress causes more traumatic disorders in men than in women. However, for other types of neuropsychiatric disorders, women have twice the vulnerability than men, such as post-traumatic stress disorder, depression, anxiety, and anorexia nervosa.[37] This also triggers the possibility that, in this study, there was no visible increases in body weight or high fat intake in the male group despite higher levels of neuropeptide Y in women.

This study has limitations. First, research subjects do not easily remember what they ate or drank on a given day and some of the subjects are afraid to report what they have consumed, especially in the male group. Future research should attempt to obtain more accurate data. That said, our findings provide important insights into the role of neuropeptide Y in the occurrence of obesity, especially in sex differences. This requires further research on the role of neuropeptide Y in men to overcome obesity.

Conclusions

The study found that there were significant differences in serum neuropeptide Y levels in the male and female groups, with neuropeptide Y levels being higher in men than in women.

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Authors contributions

Dina Keumala Sari (DKS):Conceptualization, Data curation, methodoly, and funding acquisition. M. Ichwan (MI): Formal analysis, Project administration, Resources, Investigation.

Dewi Masyithah (DM): Software, Supervision, and Validation. Ridha Dharmajaya (RD): Validation, Writing-original draft and review-editing. All authors have read and approved the final manuscript. None of the authors report a conflict of interest related to this study.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

All participants knowledgeably consented to participate in this study. Procedures and protocols used were approved by the Universitas Sumatera Utara Ethical Committee, No. 61/KEP/USU/2020.

Competing interests

The authors declare that they have no competing interests.

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Table 1. Socio-demographic data of all subjects.

Characteristics	n(%)	Mean±SD
Age (years)		40.48±10.85
20-30	12(19.4)	
31-40	15(24.2)	
41-50	22(35.5)	
51-60	13(20.9)	
Ethnics		
Indonesian Malay	31(50)	
Bataknese	31(50)	
Gender		
Male	27(43.5)	
Female	35(56.5)	
Occupation		
Housewife	23(37.1)	
Labour	17(27.5)	
Farmer	9(14.5)	

Government staff	8(12.9)
Student	5(8)
Father's obesity history	
No	52(83.9)
Yes	10(16.1)
Mother's obesity history	
No	45(72.6)
Yes	17(27.4)

Notes:
Age data presented in mean and standard deviation
Others presented in number of the subject and percentage

Table 2. Characteristic data of the subjects based on anthropometry status

Characteristics of anthropometry status	Male (mean±SD)	Female (mean±SD)	*p*
Age (years)	41.89±10.98	39.40±10.78	0.375
Categorized, n(%):			
20-30	4(6.5)	8(12.9)	0.501
31-40	6(9.7)	9(14.5)	
41-50	9(14.5)	13(21)	
51-60	8(12.9)	5(8.1)	
Body mass index (kg/m^2)	28.66±16.31	25.15±4.12	0.793
Categorized, n(%):			
Underweight	2 (3.2)	1(1.6)	0.727
Normal	10(16.1)	13(21)	
Overweight	2(3.2)	5(8.1)	
Obese	13(21.0)	16(25.8)	
Waist circumference (cm)	83.67±10.3	82.6±11.92	0.713
Categorized for male, n(%):			

<90 cm	22(81.5)	
>90 cm	5(18.5)	
Categorized for female, n(%):		
<80 cm		16(45.7)
>80 cm		19(54.3)

Notes:
Numeric data were presented in mean and standard deviation
Categorical data were presented in number of the subject and percentage
The analysis test for age and abdominal circumference used independent t test
Mann Whitney test was used to analyze body mass index

Table 3. Characteristic data of the subjects based on food intake

Characteristics of food intake	Male (mean±SD)	Female (mean±SD)	*p*
Energy intake	1105.32±577.77	1597.22±939.89	0.019*
Categorized, n(%):			
Less than 2500 kal/day	23(37.1)	34(54.8)	0.086
More than 2500 kal/day	4(6.5)	1(1.6)	
Fat intake	39.21±25.56	44.13±41.42	0.809
Categorized, n(%):			
Less than 65 gram/day	4(6.5)	6(9.7)	0.805
More than 65 gram/day	23(37.1)	29(46.8)	
Protein intake	44.91±18.48	56.10±29.73	0.056
Categorized, n(%):			
Less than 60 gram/day	18(29)	29(46.8)	0.12
More than 60 gram/day	9(14.5)	6(9.7)	
Carbohdrate intake	39.21±25.56	44.13±41.42	0.809
Categorized, n(%):			
Less than 400 gram/day	27(100)	27(100)	-

More than 400 gram/day	-	-

Notes:
*Significant: p<0.005
Numeric data were presented in mean and standard deviation
Categorical data were presented in number of the subject and percentage
The analysis test for age and abdominal circumference used independent t test
Mann Whitney test was used to analyze body mass index

Tabel 4. Difference in mean of Neuropeptide Y in groups

Neuropeptida serum level (ng/L)	n(%)	Median (minimum-maximum)	Mean ± SD	*p*
In:				
Male group	27(43.5)	121(71.5-981)	348.37±330.09	0.036*
Female group	35(56.5)	100(71.5-938)	223.38±244.62	
In:				
Non-obese group	33(53.2)	117(71.5-981)	278.18±301.11	0.447
Obese group	29(46.8)	116(77.8-981)	277.39±52.1	
In:				
Low fat intake group	52(83.9)	116(71.5-981)	270.18±279.98	0.66
High fat intake group	10(16.1)	118.5(80.5-981)	317.45±347.85	

Notes:
Data reprensted in mean±standard deviation (SD)
Using Mann-Whitney test
*: significant; $p<0.05$

Approval Sheet After Explanation (Informed Consent)

My Informed Consent Sheet signed below,

Name :

Age :

Gender :

Has been clearly explained by the researcher about the study "Sex Differences in Neuropeptide Y Serum, But Not in Fat Intake and Body Mass Index", so I hereby voluntarily and without coercion stated :

☐ Willing to be included in the study.

Such is this statement to be used as necessary.

Sincerely,

_____