Nutrient Intake, Apolipoprotein A5 -1131T>C Polymorphism and Its Relationship with Obesity

M.I.Sari 1* D. I. Sari 2

1 Departement of Biochemistry, Faculty of Medicine, Sumatera Utara University, Medan, Indonesia, Jl. Dr. Mansur Kampuss USU Medan 20155
2 Departemen of Clinical Pathology, Faculty of Medicine, Sumatera Utara University, Medan, Indonesia, Jl. Dr. Mansur Kampuss USU Medan 20155

*muti_dr@yahoo.com

Abstract. Obesity is associated with the development of some of the most prevalent diseases of modern society. The World Health Organization estimates that at least 2.8 million adult die each year as result of being obesity. Nutrient intake is a key environmental factor that may interact with genotype to affect risk of obesity. The aim of study was assess the relation between nutrient intake and apolipoprotein A5 -1131T>C polymorphism with obesity. A cross sectional study has been carried out on 139 subjects. Nutrient intake data was collected by using a 24 hour dietary recall and analyzed by nutrisurvey software. Anthropometric variables were measured and body mass index (BMI). Apolipoprotein A5 -1131T>C polymorphism was visualized with 5% agarose gel after restriction length fragment polymorphism (RFLP) digested with MseI.

Results: Subjects in this study were 55 male and 84 female, with average age 19.20 ± 1.08, 75 had obese and 64 non-obese. Based on the chi square test is found a relationship between total energy intake and protein intake in obese group compared to the non-obese group (p = 0.029, p = 0.006) and no relationship was found in Apolipoprotein A5 -1131T>C polymorphism with obesity.

These findings indicate that nutrient intake no depending with apolipoprotein A5 gene variant to modulate obesity

1. Introduction

Obesity is a condition where there is an excessive accumulation of fat in adipose tissue than necessarily needed in normal body function. Obesity is associated with the development of some of the most prevalent diseases of modern society. Prolonged obesity increases risks of developing metabolic syndrome (MetS), type 2 diabetes and cardiovascular diseases. The incidence and prevalence of obesity are rising around the world. The World Health Organization estimates that at least 2.8 million adult die each year as result of being obesity [1].

The development of obesity is influenced by both genetic and environmental risk factors. Environmental factors as a cause of obesity one of which is nutrient intake. Nutrients are chemicals from food that provides energy for the body. Excessive energy with continuous of high fat, carbohydrate and protein can cause obesity. High energy intake associated with an increase in the storage of body fat [2,3].

Genetic variations lead to differences in response to the needs of specific nutrients and eventually causing obesity or otherwise nutrients may also affect the expression of the human genome, causing obesity [4]. Polymorphism of apolipoprotein A5 (ApoA5) -1131T>C is one genetic variation which interact with nutrition as the originator of obesity. This protein gene works as a regulator of triglycerides which mechanism is unclear. Dolores et al [5] in his study found an association between polymorphisms...
ApoA5 -1131T> C with lipid intake with body mass index (BMI), weight gain and risk of obesity both in the group of men as well as women's groups.

Until now, it has been a lot of research in studying the adequacy of energy intake from macronutrient, gene with incidence obesity. Although these studies provide mixed results, even contradictory so it is unclear what factors mostly affect the cause of the state of obesity.

The aim of the current study was to analyze the relationship intake of macronutrients and apolipoprotein gene polymorphism A5-1131T> C, with the incidence of obesity in students of the Faculty of Medicine, Sumatera Utara University.

2. Method

This study was carried out after ethical approval was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Sumatera Utara University. All participating were informed about the study protocol by written information and thereafter signed informed consent sheets.

This study was a cross sectional study, conducted at the Integrated Laboratory of the Faculty of Medicine, Sumatera Utara University. The subjects are college students aged between 17 and 22 years, from Faculty of Medicine, University of North Sumatera. Nutrient intake data was collected by using a 24 hour dietary recall and analyzed by nutrisurvey software. Measurement of weight and height is done using weight scales and height measuring instrument digital height to the nearest 0.1 kg and 0.1 cm. We used body mass index (BMI) >25 kg/m² or BMI > 95th percentile (according to age and sex of Centre for Disease Control/ CDC Growth Chart) to estimate obesity.

DNA was extracted from buffy coats peripheral blood leukocyte. ApoA5 polymorphism genotyping is done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR product using 5´-CCCAGGAACCTGGAGCGAATT-3´ (Forward) and 5´-TTCAAGCAGAGGGAAGCTGTA-3´ (Reverse). The 50 µl PCR reaction was done on ice with 1 U Go Taq® Green Master Mix, 0.2 mM primers, nuclease free water, 200 µMdNTP, 5 µlbuffer (500 mM KCl, 14 mM MgCl₂, 10 mMTris-HCl, pH 9.0). The reaction was done in thermal cycler preheated to 95°C. The initial denaturation was done at 96°C for 300 secs, followed by denaturation at 95°C for 30 secs, annealing at 55°C for 30 secs, extension at 72°C for 30 secs, and final extension at 72°C for 300 secs for 32 cycles. The 396bp amplicons were then visualized with 5% agarose gel. Amplicon digestion was then done using MseI restriction enzyme incubated at 65°C for 2 hours. The digested DNA (TT: 20bp, 105bp, 271bp; TC: 20bp, 105bp, 271bp, 291bp; CC: 105bp, 291bp) was then visualized using 5% agarose gel using gel documentation system.

All the data were obtained, clustered and statistically analyzed analyzed univariately, bivariately and multivariately use SPSS program version 14.

3. Results and Discussion

3.1. Characteristics of research subjects

In this study, of 139 students who meet the inclusion and exclusion criteria obtained, an average age of 19.20 years old, which consist of 55 male subjects and 84 female subjects, 75 people are obese and 64 were non obese. Characteristics of variable measurement results of this study can be seen in Table 1.
Table 1. Variable characteristic

| Variable                      | N, Mean, SD | Min, Max |
|-------------------------------|-------------|----------|
| Age (year)                    | 19.20±1.08  | 16, 20   |
| Sex                           |             |          |
| Male n(%)                     | 55(40)      |          |
| Female n(%)                   | 84(60)      |          |
| Anthropometry                 |             |          |
| Body Weight (kg)              | 69.91±20.27 | 40, 164  |
| Height (m)                    | 1.62±1.05   | 97, 186  |
| BMI (kg/m²)                   | 26.26±5.81  | 16.41, 44.37 |
| Obese n(%)                    | 75 (54)     |          |
| Non Obese n(%)                | 64 (46)     |          |
| Nutrition Intake              |             |          |
| Energy (kcal/day)             | 1424.84±608.44 | 392.20, 3765.50 |
| Carbohydrate (g /day)         | 155.18±102.80 | 35.80, 1147.00 |
| Protein (g /day)              | 57.27±30.04  | 14.60, 321.30 |
| Lipid (g/day)                 | 63.60±30.24  | 6.3, 177.30 |
| Cholesterol (mg)              | 259.59±165.05 | 16.00, 891.40 |
| SFA (g/day)                   | 30.14±16.06  | 3.80, 88.50 |
| MUFA (g/day)                  | 14.66±6.79   | 0.6, 42.30 |
| PUVA (g/day)                  | 13.45±10.77  | 0.6, 74.60 |
| ApoA5 -1131T>C               |             |          |
| TT n (%)                      | 70 (51%)    |          |
| CC n (%)                      | 68 (49%)    |          |

From the table above it can be seen that the average intake of energy, carbohydrates, lipids subject of this study is lower than the supposed needs (energy, carbohydrate, protein and fat needed for adolescent males aged 16-18 years = 2675 kcal, 368 g, 66 g, 89 g; age 19-29 years = 2725 kcal, 375 g, 62 g, 91 g, adolescent girls aged 16-18 years = 2125 kcal, 292 g, 59 g, 71 g; age 19-29 years = 2250 kcal, 309 g, 756 g, 56 g). [6]

This result may be due to several circumstances such as time of data collection which is represented by the data of nutrient intake one day prior to the study, many of the subjects undergo fasting so that the value of nutrition in consumption does not portray the actual nutritional intake of the state during this time. It can be seen from the range of the lowest and highest energy values in each type of nutrition is very far away. This research uses more women subjects than men. Slim ideal body shape is a dream for teenagers of this age, so that young women generally perform stringent regulation in the number of daily nutritional intake[7]

The average value of cholesterol, saturated fatty acids (Saturated Fatty Acid / SFA), the unsaturated fatty acids (Monounsaturated Fatty Acid / MUFA, Polysaturated Fatty Acid / PUVA) intake on the subjects is adequate, adequate, lower and lower. (Each limits recommended intake is 300mg, and <10%; >15%; >6% from the total energy required) [6]

3.2. Relations of nutrient intake sufficiency level between obese group and non-obese group
Based on Recommended Dietary Allowances (RDA), nutritional intake above can be grouped into three categories:
1. Lower (<80% RDA)
2. Adequate (80-110% RDA)
3. Higher (> 110% RDA)

The relationship between nutrient intake based on the percent nutritional adequacy on obese and non-obese can be seen in table 2
Table 2. Nutrient intake level in obese and non-obese

|                      | Obese  | Non obese | p   |
|----------------------|--------|-----------|-----|
| Energy (kcals/day)   | Lower  | 50        | 37  | 0.029 |
|                      | Adequate | 20      | 14  |
|                      | Higher  | 5        | 13  |
| Carbohydrate (g/day) | Lower  | 63       | 52  | 0.753 |
|                      | Adequate | 11      | 10  |
|                      | Higher  | 1        | 2   |
| Protein (g/day)      | Lower  | 21       | 11  | 0.006 |
|                      | Adequate | 23      | 37  |
|                      | Higher  | 31       | 16  |
| Lipid (g/day)        | Lower  | 41       | 27  | 0.301 |
|                      | Adequate | 16      | 18  |
|                      | Higher  | 18       | 19  |

Based on the chi square test is found an association between the intake of energy and protein intake in obese group compared to the non-obese group (p = 0.029, p = 0.006). In contrast there was no association between the intake of carbohydrates and lipids in obese compared to the non-obese group (p = 0.753, p = 0.301).

Their relationship with the energy intake of obese group than the non-obese group in this study are consistent with research from Iftikar et al [8]. The results are consistent with research from Simatupang [9] which states that energy intake > 2056.1 kcal / day as much as 83.7% showed significant effect with the incidence of obesity.

Humans need energy for sustaining life, supporting growth and physical activity. Energy was derived from carbohydrates, fats, and proteins in food stuffs. Excess energy from consuming these foods will be stored as body fat, causing weight gain. Research shows that a relatively small excess energy, which is 2% every day continuously for one year can increase the weight as much as 2 kg. The more energy that is not used it will increase more and more fat tissue in the body[10].

The relationship between protein intake in obese group compared to the non-obese group in this study, consistent with previous studies conducted by Dolores et al [3]. The results are consistent with the theory which states that if the excessive intake of protein occurs, amino acids will undergo deamination process. Nitrogen is removed from the body, the carbon atom chains converted into acetyl ko A as precursor forming triglycerides by lipogenesis process and will be stored as body fat [11]. It has been common in the public perception that the intake of protein is not a contributing factor to obesity, so the diet composition of nutrients in order to avoid obesity or weight loss, protein intake is rarely avoided. In certain groups are often found in high-protein nutrition composition and eliminates carbohydrates and fats in the diet to avoid obesity. Teens prefer to avoid carbohydrates and fats because they know that high carbohydrates are not good for body shape [7].

This perception enables the present study to find an association between the intake of protein, but no relationship was found between intake of carbohydrates and fats in the obese group compared to non-obese group.

Research conducted by Rizka et al [12] is consistent with the results of this study, that no relationship was found between carbohydrate and fat intake with the incidence of obesity in adolescents in the Catholic High School Cendrawasih Makasar, and the same results on the students in junior Christian
Eben Haezar 1 Manado [13] this suggests that obesity is not only caused by fats or carbohydrates contribution to the total energy alone, but from the intake of other nutrients such as protein intake

3.3. Polymorphism apoA5 -1131T> C and obesity

Relationship of ApoA5 -1131T> C Polymorphism can be seen in Table 3

Table 3. Apolipoprotein A -1131T>C polymorphism in obese and non obese

|        | Obese | Non obese | p       |
|--------|-------|-----------|---------|
| TT     | 39    | 32        | 0.874   |
| CC     | 36    | 32        |         |

In this study, no relationship was found in apoA5 -1131T> C polymorphism with obesity. These results are consistent with research conducted by Carmen et al [14] who did not find a relationship between polymorphisms apoA5 -1131T> C with obesity. Many studies have shown a relationship of polymorphism of apoA5 -1131T> C with obesity such as research by Domínguez- et al [15]. But in the studies the relationship between polymorphisms of apoA5 -1131T> C with obesity is known to occur only if there are interactions between genes and lipid intake [5]. It is known that the relationship between lipid intake with obesity in this study was not found. This allows the same circumstances encountered on polymorphism of apoA5 -1131T> C (p> 0.05)

Apo A5 is one of the strongest regulators of plasma triglyceride concentrations, nevertheless, its mechanisms of action are poorly known. Since its discovery, it has been stated that ApoA5 as a key which regulates triglycerides [16]. Research that has been done previously shown that genes ApoA5 more often associated with triglycerides. It has been observed that transgenic ApoA5 humans to rats had lower triglycerides and taking ApoA5 genes in mice have increased levels of triglycerides [17]. ApoA5 gene polymorphism -1131T> C cause deterioration ApoA5 ability to regulate the transport of triglycerides. Elevated levels of triglycerides modulate obesity

3.4. Most dominant risk factors against incidence of obesity

To see the risk factors for obesity, multivariate analysis of the factors causing obesity were performed. The risk factors which were analyzed in this study is the intake of energy and intake of protein.

Table 4. Effect of energy intake and protein intake against obesity

| No | Variable       | B   | p    |
|----|----------------|-----|------|
| 1  | Energy intake  | 0.159| 0.016|
| 2  | Protein intake | 0.840| 0.171|

From the analysis above, the p-value <0.05 were obtained for variable energy intake. Energy intake is the most appropriate modeling in this study.

4. Conclusions

In this study, it is found that the intake of energy and protein is associated with obesity, where energy intake is the most appropriate modeling in this study. These findings indicate that nutrient intake no depending with Apolipoprotein A5 gene variant to modulate obesity
Acknowledgments
The authors gratefully acknowledge that the present research is supported by Ministry of Research and Technology and Higher Education Republic of Indonesia. The support is under the research grant DIPA USU of Year 2016.

References
[1] World Health Organization-South-East Asia Regional Office (WHO-SEARO) 2011 Noncommunicable diseases in the South-East Asia Region: Situation and response WHO Library Cataloguing-in-Publication data. India: World Health Organization, Regional Office for South-East Asia.
[2] Pauline M, Emmett and Louise R Jones 2015 Diet, growth, and obesity development throughout childhood in the Avon Longitudinal Study of Parents and Children Nutrition Reviews VR Vol. 73(S3) pp 175–206
[3] Dolores M, Wolongevicz, Lei Z, Michael JP, Ruth WK, PK Newby, Ralph B, D’Agostino and Barbara EMullen 2010 Diet quality and obesity in women: the Framingham Nutrition Studies. British Journal of Nutrition 103 pp 1223–1229
[4] Stover, P.J., Caudill M.A. 2008 Genetic and epigenetic contributions to human nutrition and health: Managing genome-diet interactions. J. Am. Diet. Assoc., 108 pp 1480–1487
[5] D. Corella, C.-Q. Lai, K. L. Tucker, J. M. Ordovas 2007 APOA5 gene variation modulates the effects of dietary fat intake on body mass index and obesity risk in the Framingham Heart Study J Mol Med 85:119–128
[6] Ministry of Health Decree No. 75 Year 2013, The Nutritional Adequacy for Indonesian
[7] Michael L Booth, Rachael L Wilkenfeld, Deanna L Pagnini 2008 Perceptions of adolescents on overweight and obesity: The weight of opinion study. Journal of Paediatrics and Child Health 44 pp 248–252
[8] Iftikhar Alam, Anis Larbi, Graham Pawelec and Parvez I Paracha 2011 Relationship between anthropometric variables and nutrient intake in apparently healthy male elderly individuals: A study from Pakistan. Nutrition Journal, 10:111
[9] Romauli M S, Ida Y, Zuraidah N 2008 The influence of pattern of consumption, physical activity, heredity and risk factor which is dominant to be the incident of obesity in the student of private Primary Schools in Medan Baru sub district, Medan. USU Repository
[10] Read, R. S. and Antigone, K. B. 2007. Overweight and Obesity. St Leonard : Allen and Unwin.
[11] Robert K. Murray , Peter J. Kennelly , David A. Bender , Kathleen M. Botham , P. Anthony Weil , Victor W. Rodwell. 2012 March Metabolism of Proteins and Amino Acids in Harper’s Illustrated Biochemistry (29th Edition) Tata McGraw – Hill Education
[12] Rizka Ruhul Afalah, Rahayu Indiasari, Yustini 2014 Dietary Relationship with Incidence of Obesity in Teenager at Cendrawasih Katolik Senior High School http://repository.unhas.ac.id/handle/123456789/10878
[13] Feby Musralianti, A. J. M Ratu, Wulan P. J Kaunang 2016 The relationship between the physical activity and dietary habits with the obesity incidence in student in Christian Junior High School of 1 Eben Haezaz Manado. PHARMA CON Jurnal Ilmiah Farmasi – UNSRAT Vol. 5 No. 2 MEI ISSN 2302 – 2493
[14] Carmen Sanchez-Morena, Jose M.Ordovas, Caren E.Smith, Juan C. Baraza, Yu-Chi-Lee and Marta Garaulet 2011 ApoA5 gene variation interacts with dietary fat intake to modulate obesity and circulating triglycerides in Mediterranean population. The Journal of Nutrition; Mar; 141, 3pg. 380
[15] Dominguez-Reyes et al 2015 Interaction of dietary fat intake with APOA2,
[16] APOA5 and LEPR polymorphisms and its relationship with obesity and dyslipidemia in young subjects Lipids in Health and Disease 14:106
[17] Garelnabi, M., Lor, K., Jin, J., Chai, F., Santanam, N. 2013. The paradox of ApoA5 modulation of triglycerides : Evidences from clinical and basic research. Clinical Biochemistry, 46, 12-19.
[18] Li, Y. Y., et al. (2011). Association of apolipoprotein A5 gene polymorphisms and serum lipid levels. Nutrition, Metabolism and Cardiovascular Diseases, 21, 947-956.