

## IL-2 LEVEL IN DIABETIC MICE DUE TO OBESITY IS HIGHER THAN THAT IN HEALTHY MICE

Dewi Arni K, Dewi Ratna S

Department of Anatomy and Histology  
Faculty of Medicine, Airlangga University  
Surabaya

### ABSTRAK

*Prevalensi obesitas di dunia tumbuh setiap tahun. Salah satu komplikasi obesitas adalah penurunan respon imun. Mekanisme komplikasi ini belum jelas. Penelitian ini bertujuan mengungkap mekanisme penurunan respon kekebalan pada obesitas yang menjadi diabetes dengan jumlah Interleukin (IL) -2 tingkat sitokin pada tikus. Tiga puluh enam mencit BALB/c jantan, umur 12 minggu, berat badan 25 - 30 g dan sehat, dibagi menjadi 2 kelompok: kelompok kontrol dan kelompok perlakuan. Kelompok kontrol diberi diet standart, sedangkan kelompok perlakuan diberi diet lemak tinggi dengan 22,8% lemak untuk menginduksi diabetes selama sepuluh minggu. Setelah itu kedua kelompok diinduksi dengan histamin intraperitoneal, kemudian darah diambil untuk mengukur tingkat IL-2 dengan ELISA reader. Kadar IL-2 kelompok perlakuan didapatkan lebih tinggi bermakna daripada kontrol ( $p < 0,0001$ ). Kesimpulan: Tidak ada penurunan IL-2 pada diabetes yang menyebabkan obesitas. (FMI 2013;49:33-35)*

**Kata kunci:** obesitas, IL-2

### ABSTRACT

*The prevalence obesity in the world grows up every years. One of obesity complication is the decrease of immune response. Mechanism of this complication has not been clear yet. This research studied mechanism the decrease of immune response in obesity which become diabetes by measuring Interleukin (IL)-2 cytokine level in mice. Thirty six mice BALB/c strain, male, 12 weeks years old, weight 25 – 30 g and healthy were classified into 2 groups : control and treatment groups. The control group had standard diet, while the treatment group had high fat diet with 22.8 % fat to induce diabetes for ten weeks. After that, both groups were induced with histamine intraperitoneally then the blood were taken for measuring level of IL-2 with ELISA reader. The IL-2 level of the treatment was found to be significantly higher than the control ( $p < 0,0001$ ). In conclusion, there is no decrease of IL-2 in diabetic causes obesity. (FMI 2013;49:33-35)*

**Keyword:** Obesity, IL-2

**Correspondence:** Dewi Arni K, Department of Anatomy and Histology, Faculty of Medicine, Airlangga University, Surabaya. Email: arnidewi82@gmail.com

### INTRODUCTION

Obesity is one of the symptoms of the disease in metabolic syndrome. The prevalence of obese people in the world has been mapped in each country and shows an increase each year. Obesity can lead to chronic adverse effects, such as susceptibility to infection. This was proved by Tracey et al. (1971), Falagas and Kompoti (2006), Dowsey and Chong (2008) and Atkinson (2011), that obese individuals are prone to influenza infections, nosocomial infections, and postoperative infections. The vulnerability of these infections, according to Falagas and Kompoti (2006), is because obesity lowers immune response by secreted immune mediators. However, the decline in immune response mechanism is still unexplained. Chronic obesity causes leptin resistance by free fatty acid (Adi 2010). Leptin resistance in peripheral organs,

particularly in immune cells may lead to decreased immune response. One of the immune cells that have leptin receptors are lymphocytes. Leptin acts as costimulator on lymphocyte activation antigen bound to secrete cytokines IL-2 (Purwanto 2007). This study tries to observe the relationship between immune response and decreased leptin resistance by measuring the levels of cytokines IL-2 in obese mice that obesity has become diabetic. Conditions of diabetes may be a marker of leptin resistance.

Mice may become obese by administration of a high fat diet. In averagely 10 week administration, high fat diets causes diabetes. The mechanism can be through free fatty acid that causes leptin resistance in beta pancreas cell, thereby lowering insulin secretion (Vieira et al 2012). Cytokine IL-2 is also known as T-cell growth factor, as these proteins play a role in proliferation,

differentiation and cell defense lymphocytes (Abbas & Lichtman 2004). When leptin resistance occurs in lymphocytes, it may decrease IL-2 secretion at the time of infection.

## MATERIALS AND METHODS

A total of 36 mice were selected from the population of mice in Animal Unit, Biochemistry Laboratory, Faculty of Medicine, Airlangga University, with criteria of BALB/c strain, male, aged 12 weeks, weight 25-30 g and healthy. Mice were grouped by simple random sampling into two groups, the control and treatment group. The control group received a standard diet, while the treatment group received a high fat diet with a composition of 22.8% fat to induce diabetes mellitus for 10 weeks. Fasting blood glucose (FBG) was recorded in both groups before and after 10 weeks of treatment. Furthermore, both groups were induced by histamine intraperitoneally at a dose of 10 mg/20 g BW to induce immune response. Mice were sacrificed after 8 hours to take blood to measure serum levels of IL-2 by ELISA reader.

## RESULTS

Table 1. Frequency Distribution of Fasting Blood Sugar (GDP) in Mice after 10 Weeks of Treatment Diet

Groups	Characteristics	Mean	Median
Control (n=18)	GDP (mg/dl)	72.22±7.80	72.00±7.80
Treatment (n=18)	GDP (mg/dl)	156.56±8.91	154.00±8.91

Based on Table 1, the mean of fasting blood sugar in control group mean was  $72.22 \pm 7.80$  mg/dl, and that in treatment group was  $156.56 \pm 8.91$  mg/dl. This represents an increase in fasting blood sugar in mice treated with high fat diet in a composition of 22.8% lard of 125 mg/dl. The research data was identical to the study of Wu et al (2006), which proved that the administration of high lard diet composition of 35.2% over 20 weeks induced diabetes in mice with fasting blood glucose levels of  $169 \pm 5$  mg/dl.

Levels of cytokines IL-2 in treatment group was averagely  $349.25 \pm 64.67$  pg/dl, whereas in control group  $37.75 \pm 15.14$  pg/dl. This suggests that there are differences in the levels of IL-2 in controls and treatment, which is supported by statistical analysis ( $p < 0.0001$ ). Whereas, levels of cytokines IL-2 in treatment group was higher by 312.19 pg/dl or by 89.39% compared to that in control group. These results are

identical with that of the research by Venkatramana (1998) on levels of cytokines IL-2 were observed in runners who were given high fat diets with different percentages without induction of histamine. The results of the study by Venkatramana found that levels of cytokines IL-2 increased by a corresponding increase in the percentage of fat in the high fat diet given even without induction of histamine (Table 2).

Table 2. Frequency distribution of levels of interleukin (IL) -2 serum Mice

Groups	Kadar IL-2 (pg/dl)	
	Mean	Median
Control (n=18)	37.06±12.37	38.00±12.37
Treatment (n=16)	349.25±64.67	332.00±64.67

## DISCUSSION

Abbas et al (2007), Jutel et al (2005) and Purwanto (2007) mentions that the leptin has receptor on lymphocytes and acts as costimulator of cell activation in lymphocytes in secreting IL-2. Leptin resistance that occurs in obesity and diabetes decrease the number of lymphocytes so that blood levels of IL-2 decreased as well. Karlsson et al (2010) investigated the relationship between obesity and severity of H1N1 influenza that affects mice. They found that there was a decrease in memory T cell proliferation indicated by decreased levels of IFN gamma, causing death in 25% of mice and 25% had abnormalities in the lung. Milner and Beck (2012) explains that obesity that becomes diabetic has changes in nutritional status and the metabolism becomes immunodeficient. Bastard et al (2006) found that there is a relationship between leptin and inflammatory processes in childhood obesity. Leptin has receptors in adipose tissue macrophage cells, which is similar to the structure of its receptor class 1 cytokine receptor family. Leptin binding to cells can activate macrophages resulting in the production of TNF gamma increase (Bastard et al 2006). This is likely to occur in adipose tissue lymphocytes. Activation can also be induced by leptin that increased levels of IL-2, but this needs further research.

Above findings are in contrasts with the results of our study, which found that levels of IL-2 diabetic mice became obese has increased compared to healthy controls. This possibility can be explained by the research by Kalupahana et al. (2011). Kalupahana study was conducted on mice given low-fat diet and a high fat diet, and found an increase in inflammatory gene expression of toll-like receptor-1, thrombospondin-1, CD59a antigen in gonadal adipose tissue of high fat diet

group. This leads to inflammation in adipose tissue, characterized by the infiltration and activation of macrophages and lymphocytes. Lymphocyte activation causes the secretion of cytokines IL-2, while the infiltration of macrophages and its activation cause secretion of TNF alpha (Kalupahana et al. 2011).

## CONCLUSION

Levels of IL-2 in diabetic mice with obesity due to the administration of a high fat diet for 10 weeks is higher than that in healthy mice, which may be caused by a process of inflammation in adipose tissue. Decreased immune response in obesity can be further investigated with other parameters.

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