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Effect of Trans Fatty Acid on Glucose Level and Pancreatic β Cell in Rats

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Abstract: A high intake of trans fatty acids can cause inflammation and lipid peroxidation of cell membranes which, in turn, damage cells and tissues. The purpose of the current study was to investigate the effects of dietary trans fatty acids on blood glucose levels and pancreatic β cells. Our randomized pre-post test control group study design included two groups of Wistar rats fed either a diet containing 2% trans fat or a control diet containing no trans fat for 10 d. The results showed that blood glucose levels were not significantly different between the two groups. There was no obvious damage to pancreatic β cells, but the number of normal cells in the treatment group was less than that in control animals.

Key words: Trans fatty acid, blood glucose, pancreatic β cell

INTRODUCTION

Fats are composed of fatty acids and glycerol. Fatty acids consist of two saturated (single bonds) and/or unsaturated fatty acids (double bond) hydrocarbon chains of varying lengths. Chemically, hydrogen atoms on trans fatty acid chains bind hydrogens on the opposite chain, but bind adjacent hydrogens in cis fatty acids. Trans fatty acids were thought to be metabolized similarly to saturated forms due to their conformational similarity (Hunter *et al*., 2010).

Intake of high-fat foods, including trans fatty acids, may have an impact on the formation of free radicals which can induce oxidative stress, inflammatory responses and destruction of pancreatic β cells. In addition to their effect on membranes and blood glucose levels, trans fatty acids are also associated with systemic inflammation (Mozaffarian, 2006). Muller *et al*. (2001) concluded that a high intake of trans fatty acids may increase insulin resistance.

Blood glucose levels are fundamental to the diagnosis of hyperglycemia. An epidemiological study by Salmeron *et al*. (2001) showed that intake of trans fatty acids is a risk factor for type 2 diabetes mellitus. Zapolska-Downar *et al*. (2005) concluded that trans fatty acids induce apoptosis in endothelial cells. Therefore, the objective of the present study was to determine the effect of dietary trans fatty acids on blood glucose levels and pancreatic β cells in rats.

MATERIALS AND METHODS

The current study had a randomized pre-post test control group design and compared two groups of male Wistar rats (200-300 g each) fed a standard diet (control, n = 6) or a diet containing 2% trans fatty acids (treatment, n = 6) for 10 d. Blood glucose levels and pancreatic β cells were examined before and after treatment.

Rats were housed individually in plastic cages with a stainless steel cover and acclimatized for 1 week at room temperature under a 12 h light/12 h dark cycle, 40% minimum relative humidity and distilled water *ad libitum*. Any rats that developed diarrhea characterized by unformed stools, a weight change of >10% and/or that died during the study period were excluded. Animals included in our study were healthy with no anatomical abnormalities, active and had an initial fasting blood glucose level of <110 mg/dL.

After a 12-h fast, blood was sampled from all rats using a microhematocrit and capillary tube. Approximately 5 mL of blood was collected from each rat in collection tubes containing potassium oxalate as an antiglycolytic agent. Plasma was separated by centrifugation (400 x g) for 15 min at 4°C. Plasma glucose was determined spectrophotometrically using a Hitachi 902 automatic analyzer (Hitachi, Tokyo, Japan). Examination of serum glucose levels was done by Prodia Laboratory (Jogjakarta, Indonesia) in accordance with laboratory-based protocols (ISO-15189). After the 10 d treatment period, rats were sacrificed pancreatic tissue removed for histopathological examination. Pancreatic endocrine cells in the islets of Langerhans were observed using hematoxylin and eosin staining. Observation was performed descriptively by calculating the number of β cells.

All breeding phases and experiments conformed to rules outlined by the Integrated Analysis and Research Laboratory and Pathology Laboratory, Faculty of Veterinary Medicine, University of Gajah Mada (Jogjakarta, Indonesia) and were conducted with approval of the Faculty of Public Health Ethics Committee of the University of Indonesia (Depok, Indonesia).

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Results were presented as means±standard deviation of treatment and control groups. A Student's t-test was used for all analyses and $p<0.05$ was considered statistically significant. The data were analyzed using the SPSS software.

RESULTS AND DISCUSSION

Blood glucose level: Although blood glucose levels were not significantly different between the two groups before or after the 10-d experimental feeding period, they tended to be higher in rats fed the high trans fat diet (16.17 mg/dL) versus the control diet (2.5 mg/dL) (Table 1). These results are similar to those reported by Aronis *et al.* (2012) in which trans fatty acid intake did not significantly alter glucose or insulin levels. Glucose is a major regulator of transcription and translation in β cells, an effect that is necessary for long-term maintenance of their highly differentiated state and the secretory requirements imposed by prolonged elevation of glucose (Schuit *et al.*, 2002). Our results showed that glucose levels ranged from 99.00 to 115.17 mg/dL in rats fed the high trans fat diet (Table 1). An increase in blood glucose levels causes an increase in insulin secretion, which increases the transport of glucose into liver, muscle and other cells, thereby reducing blood glucose concentrations back to normal values.

Although treatment rats were fed a diet high in trans fatty acids (2%), it is possible that 10 d is an insufficient amount of time to induce a significant increment in blood glucose levels in male Wistar rats. A study by Huang *et al.* (2009) also reported that Wistar rats fed a diet containing 4.5% trans fats for 16 weeks did not show significant changes in plasma glucose levels, insulin levels, or the insulin sensitivity index. This result is presumably due to unpredictable metabolic and hormonal factors associated with rats. Rats have endogenous homeostatic mechanisms that keep blood glucose levels within the normal range; liver, extrahepatic tissue and several hormones (insulin, glucagon, cortisol and catecholamine) play important roles in the regulation of blood glucose levels (Rosen and Spiegelman, 2006).

Pancreatic β cell: From histopathological result, we found that the number of normal pancreatic β cells in the treatment group (67.80 cells) was less than that in the control group (74.83 cells), though not significant (Table 2). β Cell numbers increase markedly in the first year of rodent life. A decrease in β cell numbers implies an imbalance between cell death and replication or neogenesis (Rahier *et al.*, 2008). In rodents, β cell replication rates in 12-week-old young-adult mice are highly variable, ranging from 2 to 15% per day. Furthermore, glucose has been shown to increase the rate of β cell proliferation *in vitro* (Kwon *et al.*, 2004) and in mice (Alonso *et al.*, 2007), while insulin resistance has been shown to result in a compensatory increase in β cells (Kulkarni *et al.*, 2004).

Glucose is a major regulator of transcription and translation in β cells, an effect that is necessary for long-term maintenance of their highly differentiated state and the secretory requirements imposed by prolonged elevation of glucose (Schuit *et al.*, 2002). Cellular damage due to severe and/or prolonged exposure to pathological stimuli exceeds the adaptive capacity of the cell (Ferri and Kroemer, 2001). A high intake of trans fatty acids induces pro-oxidative conditions that increase the risk of exogenous free radicals and subsequent damage to cells and tissues. The imbalance between free radicals and antioxidants causes oxidative stress and stimulates inflammatory responses in the vascular endothelium, impairs glucose uptake and metabolism in muscle and adipose tissue, decreases insulin secretion and causes endothelial dysfunction (Zambonin *et al.*, 2006). A study by Estadella *et al.* (2013) showed that trans fatty acids favor a proinflammatory state, leading to insulin resistance. Kusmiyati *et al.* (2015) reported that trans fatty acids can generally cause pancreatic β cell necrosis. β Cell dysfunction without a significant reduction in numbers is sufficient to cause hyperglycemia. However, severe loss of β cells can also result in hyperglycemia and may be accompanied by intrinsic β cell dysfunction (Ferrannini, 2010).

The current study did not find a significant interaction between glucose levels and the number of normal

Table 1: Mean concentration of blood glucose in the treatment and control group

Blood glucose level	N	Mean±SD (mg/dl)	p-value (95% CI)
Before intervention	Treatment group	99.00±10.69	0.246 (-6.184; 21.518)
	Control group	97.33±10.84	
After intervention	Treatment group	115.17±14.30	0.097 (-3.262; 27.928)
	Control group	99.83±9.45	

*Not statistically significant (p>0.05); SD: Standard deviation

Table 2: Mean number of pancreatic β cell in the treatment and control group

Pancreatic β cell 'normal'	N	Mean±SD	p-value (95% CI)
Number of cells	Treatment group	67.80±14.29	0.364 (-7.519;29.452)
	Control group	74.83±11.15	

*Not statistically significant (p>0.05), SD: Standard deviation

pancreatic β cells, but rats with higher glucose levels tended to have fewer normal pancreatic β cells. Ferrannini (2010) stated that hyperglycemia is associated with β cell failure, including cell number and function. A 60% decrease in β cell mass has been reported in type 2 of diabetes in humans (Butler *et al*., 2003). Generally, free fatty acids have complex effects on β cell function. Although the mechanism by which this occurs is still hotly debated, it has been suggested to involve impaired glucose metabolism, reduced insulin biosynthesis and β cell loss (Poitout and Roberston, 2008).

Conclusion: Our experimental findings indicated that eating a 2% trans fat diet for 10 d did not significantly increase blood glucose levels of male Wistar rats. Though the amount of normal pancreatic β cells in the treatment group was less than controls, this change was not significant. Other studies with different doses of trans fats and longer experimental periods may better explain the mechanisms by which dietary trans fatty acids affect blood glucose levels and pancreatic β cells.

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