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THE EFFECT OF PUMPKIN ON GLP-1 AND HOMA-β IN HYPERCHOLESTEROLEMIC RATS

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Abstract

Background and aim: *High fat and fructose diet may impair β cell function through oxidative stress that is induced by subsequent hypercholesterolemia. The β cell function is usually quantified by homeostatic model assessment beta-cell function (HOMA-β). Oxidative stress may be decreased by β-carotene from pumpkin (Cucurbita maxima). This study aimed to evaluate the effects of pumpkin powder on glucagon-like peptide-1 (GLP-1) level and HOMA-β in rats with high fat and fructose diet.* **Material and method:** *A total 25 rats were administered a high fat and fructose diet during 25 days. They were divided into five groups 1) normal, 2) hypercholesterolemic rats 3) hypercholesterolemic rats with 0.16 g pumpkin/200g bodyweight (BW); 4) hypercholesterolemic rats with 0.32 g pumpkin /200 g BW, and 5) hypercholesterolemic rats with 0.64 g of pumpkin/200 g BW. The lipid levels were measured before and after administration of pumpkin for 4 weeks, and at the end of the study, GLP-1 level and HOMA-β were analyzed.* **Results:** *Administration of pumpkin to the rats on a high fat and fructose diet reduced total cholesterol, triglyceride, and increased HDL levels. Changes were positively correlated with the amount of pumpkin intake. The decrease of cholesterol levels was positively associated with GLP-1 level, and negatively correlated with HOMA-β* **Conclusions:** *This study suggested that pumpkin can improve the HOMA-β and decrease GLP-1 levels, possibly by reducing cholesterol levels.*

key words: *hypercholesterolemia, pumpkin, lipid profile, GLP-1, HOMA-β*

Background and aims

High fat and fructose diet may impair β cell function through hypercholesterolemia [1,2]. Cholesterol homeostasis is important for an adequate insulin secretory performance of β cells. Cholesterol may act in the exocytosis of insulin secretory vesicles of the β cells [1]. Cholesterol alteration affects glucose metabolism involving glucokinase, and excess cellular cholesterol was shown to be directly linked to reduced glucose-stimulated insulin secretion from pancreatic β cells; normal secretion could be restored by cholesterol depletion [3]. In addition, the accumulation of cholesterol in β cells, caused by defective high-density lipoprotein-cholesterol (HDL-c) with reduced cholesterol efflux, was shown to induce

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hyperglycemia, impaired insulin secretion, and β cell apoptosis [1].

The ratio of total cholesterol to HDL-c, and triglycerides levels are positively correlated with glucagon-like peptide-1 (GLP-1) levels, and percent change in GLP-1 was shown to positively correlate with the percent change in total cholesterol and triglycerides in humans [4]. GLP-1 has a number of protective effects on β cells, including a reduction in apoptosis and enhancement of β cell proliferation and neogenesis [5]. GLP-1 is an incretin hormone that facilitates the glucose-dependent release of insulin from the pancreatic β cells, and is involved in energy homeostasis [4]. GLP-1 results in improvement of β cell health in a glucose-dependent manner (post-prandial hyperglycemia) and suppression of glucagon (fasting hyperglycemia) [6]. The GLP-1 level was positively correlated with insulin level, homeostatic model assessment insulin resistance (HOMA-IR), homeostatic model assessment beta-cell function (HOMA-β), and percent gynoid fat, and negatively correlated with HDL-c in overweight/obese individuals [4].

In addition, hypercholesterolemia promotes production of reactive oxygen species (ROS) in hypercholesterolemia-induced hepatotoxicity female rats [7]. Oxidative stress contributes to downregulation of GLP-1R expression in the pancreas, which may result in disruption of the signaling process that involves GLP-1 [8,9]. According to Silva et al. [10], giving β carotene to rats that received cholesterol-enriched diet lowers serum cholesterol and increases cholesterol excretion.

β carotene is found in some vegetables and fruits such as pumpkin (*Cucurbita maxima*). The content of β carotene in pumpkin is 17.92 Retinol Equivalents (RE/g) [11]. Several studies showed that pumpkin powder has antidiabetic and antihypercholesterolemic effects [12]. Sedigheh et al. [13] suggested that pumpkin powder can reduce glucose level, improve lipid profile and beta cell function in alloxan-induced diabetic rats.

This study aimed to evaluate the effects of pumpkin powder on GLP-1 levels and HOMA β in rats with high fat and fructose diet.

Material and method

Experimental animals

Twenty five (25) male Sprague-Dawley (SD) rats (180-200 g, 8 weeks) were obtained from the National Agency of Drugs and Foods Controls (Jakarta, Indonesia). They were housed individually in cages and maintained under standard condition (12:12-h light/dark cycle and 22-25°C room temperature). They were acclimatized, for 5 days, by a semipurified diet formula for rats by Wostmann [14] with slight modification (whole wheat flour was subtituted by cornstarch). The diet consisted of 24% casein, 0.30% DL-methionine, 61% cornstarch, 1% vitamin mix, 3.5% mineral mix, 0.2% choline chloride, 5% alpha cell, and 5% corn oil (Percentages in diet composition means total % in 100 g diet). Then, the rats were divided into five groups: 1) normal rats (N); 2) hypercholesterolemic rats (HyperCh); 3) hypercholesterolemic rats with 0.16 g of pumpkin powder/200g body weight (BW) (P.0.16); 4) hypercholesterolemic rats with 0.32 g of pumpkin powder/200g BW (P.0.32), 5) hypercholesterolemic rats with 0.64 g of pumpkin powder/200g BW (P.0.64). The rats in groups 2, 3, 4, and 5 were given a high fat and fructose diet for a period of 25 days to induce hypercholesterolemia. The high fat and fructose diet was prepared according to Tranchida et al. [15] and Ble-Castillo et al. [16], with a slight modification. This diet contained casein 25%, cornstarch 30%, vitamin and mineral mix 1.5%, saturated fat 36.3%, corn oil 1%, and fructose

6.2%. The rats were considered as hypercholesterolemic, if they had serum cholesterol > 200 mg/dL. Before and after the administration of pumpkin powder via gavage for 4 weeks, the cholesterol, triglyceride, and HDL-c level were measured. Pumpkin powder was prepared using freeze drying method. At the end of the study, GLP-1 level and HOMA-β were measured. The study was approved by Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Universitas Gadjah Mada.

Biochemical analysis

Serum cholesterol, triglyceride, and HDL-c were enzymatically analyzed using a commercial kit (DyaSis®, Holzheim, Germany). Enzyme Linked Immunosorbant Assay (ELISA) method was used to measure GLP-1 level (Sigma-Aldrich®, USA). The HOMA beta was calculated using following formula 20 × fasting insulin (μIU/ml)/fasting glucose (mmol/ml) − 3.5 [17].

Statistical analysis

All values are presented as mean ± standard error. One way ANOVA was used to analyze the differences in cholesterol, triglyceride, HDL-c, GLP-1 levels, and HOMA beta between the groups. Paired t-test was used to evaluate cholesterol, triglyceride, and HDL-c levels before and after administration of pumpkin powder. Tukey's honestly significant difference (HSD) and Games-Howell were used as pos hoc tests. Differences were considered statistically significant at $p<0.05$.

Results

The effect of pumpkin powder intake on cholesterol levels in rats is given in Table 1. The decrease of total cholesterol was positively correlated with the amount of pumpkin powder intake, with the largest reduction in hypercholesterolemic rats with 0.64 g pumpkin powder /200g BW (p=<0.001).

Table 1. Serum cholesterol level in rats before and after administration of pumpkin

Group	Serum cholesterol (mg/dL)		Mean Difference 95% CI	p
	Before administration	After administration		
Normal	108.13 ± 3.38**	110.27 ± 3.28†	2.14 (-4.20 – 0.07)	0.045
HyperCH	298.02 ± 2.98	297.95 ± 2.85*	-0.08 (-2.40 – 2.55)	0.936
HyperCH+ P.0.16	275.16 ± 7.65	241.30 ± 2.85*†§	-33.87 (31.18 – 36.56)	<0.001
HyperCH + P.0.32	263.52 ± 3.43	165.41 ± 2.49*†¶	-98.11 (94.26 – 101.96)	<0.001
HyperCH+ P.0.64	271.65 ± 2.42	140.32 ± 2.15*†±	-131.32 (129.47 – 133.18)	<0.001
p	<0.001	<0.001		

Values are presented as mean ± S.E (n=5). **Significant difference versus hypercholesteroleic group and hypercholesterolemic with pumpkin powder groups (p<0.05). †Significant difference versus hypercholesterolemic group (p<0.05). *Significant difference versus normal group. (p<0.05). §,¶, ± Significant difference between pumpkin groups (p<0.05), according to ANOVA followed by Games-Howell test. HyperCH: Hypercholesterolemic

The high fat-fructose diet led to an increase in triglyceride level and a decrease of HDL cholesterol level as shown in Tables 2 and 3. Intake of pumpkin powder improved this changes, with the largest decline in triglyceride level evidenced in hypercholesterolemic rats with 0.64 g pumpkin/200g BW (Table 2), for which the highest increase of HDL-c level was also noticed (Table 3).

Table 2. Serum triglycerides level in rats before and after administration of pumpkin.

Group	Serum triglyceride (mg/dL)		Mean Difference 95% CI	p
	Before administration	After admnistration		
Normal	71.94 ± 1.12**	72.87 ± 1.11†	0.93 (0.29 – 1.57)	0.016
HyperCH	134.50 ± 1.82	136.44 ± 1.31*	1.93 (0.21 – 3.65)	0.036
HyperCH + P.0.16	131.14 ± 5.06	125.09 ± 3.50*	–6.05 (0.13 – 11.94)	0.047
HyperCH + P.0.32	142.42 ± 3.15	115.35 ± 2.28*†	–27.07 (24.50 – 29.64)	<0.001
HyperCH + P. 0.64	142.71 ± 2.90	91.35 ± 1.54*†§	–51.37 (47.35 - 55.38)	<0.001
p	<0.001	<0.001		

Values are presented as mean ± S.E (n=5). **Significant difference versus hypercholesterolemic group and hypercholesterolemic with pumpkin powder group (p<0.05). †Significant difference versus hypercholesterolemic group (p<0.05). *Significant difference versus normal group. (p<0.05). §Significant difference between pumpkin groups (p<0.05), according to ANOVA followed by Games-Howell test.

Table 3. Serum HDL-c level in rats before and after administration of pumpkin.

Group	Serum HDLc (mg/dL)		Mean Difference 95% CI	p
	Before administration	After administration		
Normal	58.96 ± 0.80**	54.16 ± 1.06†	4.78 (3.98 - 3.62)	<0.001
HyperCH	18.81 ± 0.82	17.12 ± 0.74*	-1.68 (1.13-2.24)	0.001
HyperCH + P.0.16	23.11 ± 0.67	23.03 ± 0.80*†§	-0.07 (-3.21 – 3.35)	0.953
HyperCH + P.0.32	21.51 ± 0.80	29.73 ± 1.03*†¶	8.22 (5.03 – 11.40)	0.002
HyperCH + P. 0.64	18.80 ± 1.03	36.89 ± 0.84*†¶	18. 08 (14.20 – 21.97)	<0.001
P	<0.001	<0.001		

Values are presented as mean ± S.E (n=5). **Significance difference versus hypercholesterolemic group and hypercholesterolemic with pumpkin powder group (p<0.05). †Significant difference versus hypercholesterolemic group (p<0.05). *Significant difference versus normal group. (p<0.05). §,¶, ± Significant difference between pumpkin groups (p<0.05), according to ANOVA followed by Tukey HSD (Honest Significant Difference) test.

The administration of pumpkin to the rats with high fat and fructose diet tended to reduce GLP-1 levels, and increase HOMA-β. The HOMA- β in hypercholesterolemic rats with 0.62 g pumpkin/200g BW was found significantly different from hypercholesterolemic rats without pumpkin intake (p=0.013), as shown in Table 4.

Table 4. Comparison of GLP-1 levels and HOMA-β in the five groups at the end of study.

	Normal	HyperCH	Pumpkin powder		
			0.16	0.32	0.62
GLP-1 (pg/mL)	213.3 ± 27.3	235.8 ± 30.5	221.7 ± 30.2	198.5 ± 16.1	190.7 ± 22.1
HOMA beta	74.1 ± 12.8*	25.2 ± 5.3	31.3 ± 6.0	36.1 ± 3.8	50.2 ± 3.5**

Values are presented as mean ± S.E (n=5). *Significant difference versus hypercholesterolemic, pumpkin 0.16 g/200 g BW and pumpkin 0.32 g/200 g BW group (p<0.05). **Significant difference versus HFD group (p<0.05), according to ANOVA test followed by Tukey HSD test.

Figure 1 reflects the reduction of cholesterol and triglyceride levels in rats with high fat and fructose diet after pumpkin administration. Although reduction of cholesterol was not related with the GLP-1 level (p=0.219; r=0.337), it did correlate with elevation of HOMA-β (p=0.029; r= -0.563) according to Pearson test.

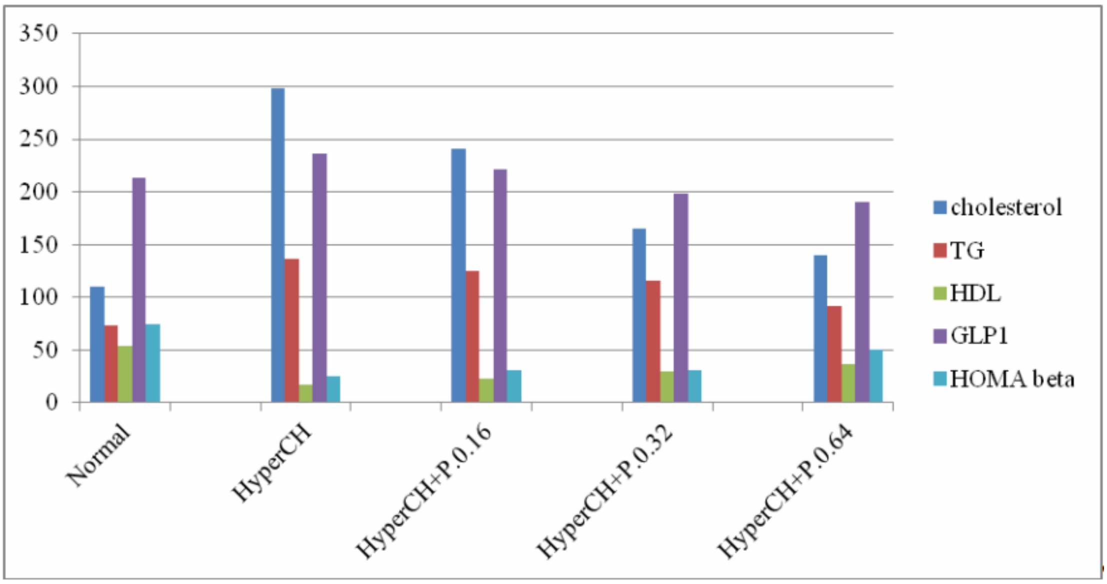

Figure 1. *Comparison of cholesterol, triglyceride, HDL, GLP-1 and HOMA beta in normal, hypercholesterolemic and hypercholesterolemic rats with pumpkin powder.*

Discussion

This study showed that administration of a high fat and fructose diet for 25 days increases cholesterol, and triglyceride levels, and reduces HDL-c. This result is consistent with Dhingra, et al. [2]. After the administration of pumpkin powder, we observed a decrease of cholesterol level in hypercholesterolemic rats followed by the reduction of GLP-1, and increment of HOMA- β. This may be attributed to the antihypercholesterolemic effects of the pumpkin powder as previously reported by Caili et al. [12]. The antihypercholesterolemic effect may be due to β carotene content in the pumpkin. Pumpkin is one of the sources of carotenoid, especially α-carotene and β-carotene [18]. According to Anggrahini et al. [11], the content of β carotene in pumpkin is 17.92 RE/g, but the pumpkin used in this study contained 404.508 µg/g of β carotene. Silva et al. [10] suggested that β carotene can lower serum cholesterol and increase cholesterol excretion in rats that received cholesterol-enriched diet.

As described previously, hypercholesterolmia may induce oxidative stress through increasing ROS [19]. The oxidative stress injures pancreatic β cell and impairs the ability of this cell to produce and secrete insulin [2]. GLP-1, an incretin hormone, is released into circulation in response to food intake and increases insulin secretion via its receptors on the pancreatic β cell [20-22]. Because hypercholesterolemia promotes production of ROS [7], the decreased level of cholesterol in hypercholesterolemic rats after receiving pumpkin will presumably reduce oxidative stress. Oxidative stress was also reported to alter gene expression, including that of glucagon like peptide-1 receptor (GLP-1R) in pancreas. Thus, according to Kaneto & Matsuoka [8] and Xu et al. [9], oxidative stress contributes to the down-regulation of GLP-1R expression in pancreas, which disrupts GLP-1 signaling and the consecutive increase in insulin secretion. Therefore, the increased HOMA-β in hypercholesteroleic rats may be partially explained by the up-regulation of GLP-1R expression on pancreatic beta cells after administration of pumpkin powder that increased insulin secretion and GLP-1 sensitivity. In this study we did not measure low density lipoprotein level and antioxidant status.

Conclusion

This study showed that administration of pumpkin powder to the rats with high fat and fructose diet reduced total cholesterol, triglyceride, and increased HDL and also improved HOMA-β.

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