

ЭПИЗОДИЧЕСКАЯ УГЛЕВОДНАЯ НАГРУЗКА АССОЦИИРУЕТСЯ С УСИЛЕНИЕМ АПОПТОЗА В ОСТРОВКАХ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ, А НЕ С ЭКСПРЕССИЕЙ PANCREATIC DUODENAL HOMEBOX-1 (PDX-1) У МЫШЕЙ

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BACKGROUND: Several caloric restriction studies revealed good for diabetes prevention. However, prevalence of it seems rising yearly. It needs alternative technique thus people can choose suitable way for them.

AIM: To determine the effect of glucose diet intermittently on pancreatic duodenal homeobox-1 (PDX-1), apoptosis in pancreatic islets, and pancreatic islets area.

MATERIALS AND METHODS: Balb/c mice were divided into five groups. Control group was given standard diet. The Continuous group was given standard diet and added with 7.4% calories continuously. The 1x, 2x, and 3x intermittent groups were given standard diet and added 7.4% calories for 1x, 2x, and 3x/week respectively. The 7.4% calorie addition was a glucose solution by oral gavage and ad libitum for 8 weeks.

RESULTS: There was a significantly difference on apoptosis density ($p=0.043$), but not in PDX-1. The islets Int2x and Int3x groups showed a significant decrease than control group ($p=0.048$). Insulin serum levels increased significantly in Continuous group compared to control group ($p=0.04$). In addition, the insulin serum level of 1x and 3x intermittent groups were significantly lower than Continuous group ($p<0.05$). Pre-post blood glucose levels on treatment groups decreased significantly compared to control group ($p=0.012$).

CONCLUSIONS: Continuous and 1-3x/week intermittent addition of 7.4% calories of glucose for 8 weeks indicate a compensation mechanism for maintaining homeostasis, such as increase insulin serum level and seem to initiate the changes of morphologic-biomolecular (mainly apoptosis density in islets). The better mode is 1x/week of additional calories. However it needs further exploration to find out other influenced factors for these mechanism discovery.

KEYWORDS: PDX-1; apoptosis; pancreatic area; glucose, insulin

INCREASED APOPTOSIS, BUT NOT PANCREATIC DUODENAL HOMEBOX-1 EXPRESSION IN PANCREATIC ISLETS IS ASSOCIATED WITH INTERMITTENT GLUCOSE LOADS IN MICE

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ВВЕДЕНИЕ. Ранее проведенные исследования показали, что ограничение суточного потребления калорий является хорошим способом профилактики сахарного диабета (СД) 2 типа. Тем не менее из года в год наблюдается уверенная тенденция к росту заболеваемости СД. Таким образом, необходима разработка новых способов профилактики нарушения углеводного обмена.

ЦЕЛЬ. Данное исследование направлено на определение влияния диеты с высоким содержанием глюкозы на pancreatic duodenal homeobox-1 (PDX-1), апоптоз островков поджелудочной железы и площадь островков поджелудочной железы.

МЕТОДЫ. Мыши Balb/c были разделены на пять групп. Группе контроля (Контроль) давали стандартную диету. В группе продолжительного приема (П) давали стандартную диету с увеличением суточной калорийности на 7,4% постоянно. Группы Инт1, Инт2 и Инт3 получали стандартную диету с добавлением 7,4% калорий 1, 2 и 3 раза в неделю соответственно. В качестве вещества, увеличивавшего суточную калорийность на 7,4%, использовался раствор глюкозы, вводимый мышам перорально в течение 8 нед.

РЕЗУЛЬТАТЫ. В ходе исследования была выявлена значительная разница в активности апоптоза ($p=0,043$), но не экспрессии PDX-1. Площадь островков поджелудочной железы в группах Инт2 и Инт3 уменьшилась значительно больше, чем в группе контроля ($p=0,048$). Уровни инсулина сыворотки крови более значительно увеличились в группе



П по сравнению с контролем ($p=0,04$). Кроме того, уровень инсулина в группах Инт1 и Инт3 был значительно ниже, чем в группе П ($p<0,05$).

ВЫВОДЫ. Постоянное и 1-3-недельное эпизодическое добавление 7,4% калорий с раствором глюкозы в течение 8 нед приводило к активации механизма компенсации для поддержания гомеостаза, который обуславливался повышением уровня инсулина и изменением морфологических и биомолекулярных параметров (в основном интенсивности апоптоза в островках поджелудочной железы). Наиболее адекватный режим – получение дополнительных калорий 1 раз в неделю. Для выявления других факторов, участвующих в данном процессе, необходимо проведение дальнейших исследований.

КЛЮЧЕВЫЕ СЛОВА: PDX-1; апоптоз; поджелудочная железа; глюкоза; инсулин

BACKGROUND

The number of diabetes mellitus (DM) patients estimated in 2035 will be 592 million [1]. Such as in Indonesia, prevalence of diabetes tend to increase every year [2]. In several big countries, the prevalence is also increase [3,4]. High glucose levels become one of the symptoms in DM disease.

There are several risk factors for DM, one of them is the diet that includes the type of food, the amount of food, and the meal schedule. Based on the amount of food, there are many studies with calorie restriction for DM prevention as well as for improving the condition of DM. However, now, the number of calories consumed in each year shows an increase globally [5,6]. Apparently, this excessive calorie intake has not given the vigilance in the community.

The addition of 100-150 Kcal/person/day calories and if the daily calories is about 2000 Kcal, thus the addition is about 5-7.5% calories/day has been known to increase the risk of DM [7]. As mentioned above, people tend to eat much, therefore calories increase. It has been known that subjects with high-calorie diet continuously will stimulate beta cells to synthesize more insulin until fatigue, and subsequently damage the beta cells, which further trigger DM. In vitro studies of erythrocytes with continuous glucose exposure also showed injured cells which increase in erytrosis after 72 hours [8].

As note before, for DM prevention, there has been a well known diet regimen, caloric restriction. However, several people may think caloric restriction is hard to do, thus it needs other alternative diet, such as high calories diet intermittently. With additional calories intermittently, which means people are allowed to eat much more calories in certain days of the week, once a week or two and three a week.

Intermittent additional calories including glucose load is expected can stimulate several transcription factors in beta cells for adaptation mechanism, like PDX-1 (pancreatic duodenal homeobox-1). PDX-1 is known as the first transcription factor identified in the pancreas at embryonal phase and still expressed in adult [9–11]. PDX-1 is also known to play a pivotal role in beta cell growth and adult beta-cell function [12]. The decreased on PDX1 expression due to gene mutation was found in maturity onset of diabetes in the young or MODY. In addition, there is an abnormality of PDX-1 expression on DM type 2 [13–15]. Beside it, apoptosis is also important to evaluate for anticipating cell damage.

Virtually, the research about intermittent glucose load was conducted, but in vitro in INS-1 cells to provide the effect changes in blood glucose levels fluctuation

[16]. In that study, there was an increase in apoptosis and increase intracellular free radical in INS-1 cells. Yet, in vitro is useful as a model for assessing cellular mechanisms, the actual effects in the body (in vivo) may have different responses, because there are several signals mechanism in the body. If there is a fluctuation of blood glucose level in vitro, it might damage cells, then how cells response if it performs by in vivo.

AIM

This study aims to determine the effect of intermittent addition of high glucose diet on glucose regulation (blood glucose and serum insulin level), PDX-1, and apoptosis in pancreatic islets. It is foreseen to know the body's response to the addition of calories intermittently and provide a limit of the amount of calories that could still be tolerated by the body.

METHODS

Animals

This treatment has been approved by Animal Care and Use Committee, Faculty of Veterinary, Universitas Airlangga.

Eight week-old healthy male Balb/c mice were acclimatized for 2 weeks. The number of study groups was divided into five with sample size of each group was four. The group consisted of a control group (C) had standard diet, continuous group (Conti) got standard diet plus daily glucose solution as 7.4% calories addition, 1x intermittent (Int1x) group (standard diet plus glucose solution as 7.4% calories addition 1x/week), Int2x group (standard diet plus glucose solution as 7.4% calories addition 2x/week), and Int3x group (standard diet plus glucose solution as 7.4% calories addition 3x/week). The treatment duration was 8 weeks.

The standard diet used was pellets made by the Faculty of Veterinary of Universitas Airlangga that derived from fish powder (23%), soybean powder (6%), rice bran (10%), rice (31.5%), corn (20%), wheat flour (5%), minerals (2%), sugar cane molasses (2%), and multivitamins (0.5%). The number of calories was about 2732.61 Calories/kg. Glucose powder was 100% dextrose made in Xingmao (China) and mineral water is used as a solvent.

The addition diet of 7.4% calories was 0.5cc oral galvage of 0.3gram/cc glucose solution. Also, all mice, except the control group, were given a 0.05gram/cc glucose solution ad libitum according to frequency every week of groups [17].

At the end of treatment, the mice were sacrificed by intraperitoneal anesthesia. The anesthesia cocktail

Table 1. Semiquantitatively scale of IRS

A (positive immunoreactive cells)	B (color intensity score of immunoreactive cells)
Skor 0 : 0%	Skor 0 : no color reaction
Skor 1 : <10%	Skor 1 : color intensity low
Skor 2 : 11% - 50%	Skor 2 : color intensity moderate
Skor 3 : 51% - 80%	Skor 3 : color intensity high
Skor 4 : >80%	

Notes: Multiply positive immunoreactive cell percentage scores (A) with color intensity score at immunoreactive cells (B). $IRS=A \times B$.

contained of 1 ml ketamine (100 mg/ml), 0.5 ml xylazine (20 mg/ml), 0.3 ml acepromazine (10 mg/ml), and 8.2 ml of sterile water. The intraperitoneal anesthesia dose was 0.05–0.1 ml/10g body weight.

After the mice were sedated, surgery was performed to remove pancreatic organ. Histologic preparations then set by hematoxylin-eosin staining on pancreatic organs to calculate the pancreatic islets area (in microsquare) and immunohistochemical staining (IHC) for PDX-1 expression and tunnel assay for apoptosis.

PDX-1 expression and PDX-1 density

IHC (immunohistochemistry) slides were made to determine the expression of PDX-1. The PDX-1 expression measurements were performed by calculating the average number of beta cells in 5 fields of view, which gave a positive reaction to PDX1 Antibody (Bioss, bs0923R) with 400x enlargement light microscope (Nikon eclipse E100) equipped with DS Fi2 300 megapixel digital camera.

The PDX-1 expression in each sample was assessed semiquantitatively according to the modified Remmele method [18], in which the Remmele's Immuno Reactive Score (IRS) Index was the result of a multiplication of positive immunoreactive cell percentage scores with a color intensity score at immunoreactive cells (table 1). The positive immunoreactive cells will be yellowish colored to chromogenic brown, while the negative cells will be green or blue colored in accordance with the counterstain dye used.

Apoptosis and apoptosis density

Slides by the method of tunnel assay with the apoptotic kit (TACS DtD DAB Trevigen) were made to detect DNA fragmentation in apoptosis. Furthermore, apoptosis was measured by calculating the average number of beta cells in 5 fields of view which gave a positive reaction. Apoptosis in each sample was assessed semiquantitatively according to the Remmele method (table 1).



Fig. 2. Pancreatic islets (arrow) area with hematoxylin eosin staining Microscope Nikon eclipse Ci, enlarged 400x, Optilab Viewer 2.2, Image raster 3.0

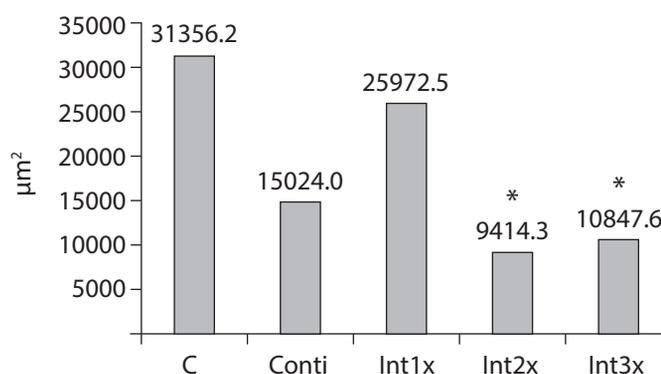


Fig. 1. Pancreatic islets area (mm²)

*: significantly different ($p < 0.05$) with the control group

Pancreatic islets area

The area of pancreatic islets (μm^2) was calculated on Image Raster 3 software, with hematoxylin-eosin staining. Measurement of area was the average of five pancreatic islets.

Changes in random blood glucose levels

Blood glucose samples were blood drops from the tail, which taken at the beginning and end of treatment. It measured by an Accucheck Active glucometer. The difference between blood glucose levels after and before treatment was referred to as a change in blood glucose levels.

Random insulin serum

Intracardial blood collection was performed to measure the serum insulin levels. Afterwards, the blood was centrifuged and the serum was taken for further examination by Elisa (BT laboratory).

Statistic analysis

Normality test was performed to determine whether the data distribution was normal or not. If the distribution was normal, then it was continued with anova test and if the result was significant, it would be followed by post-hoc test. If the data was not normally distributed, it would be followed by kruskal-wallis test and if the result was significant, then it would be tested by mann-whitney. The data was displayed in graphical figures and the tables contained the mean and standard of deviation.

RESULTS

Pancreatic islets area

The control group had the largest pancreatic islets and showed significant differences with the Int2x and Int3x groups (fig. 1 and 2).

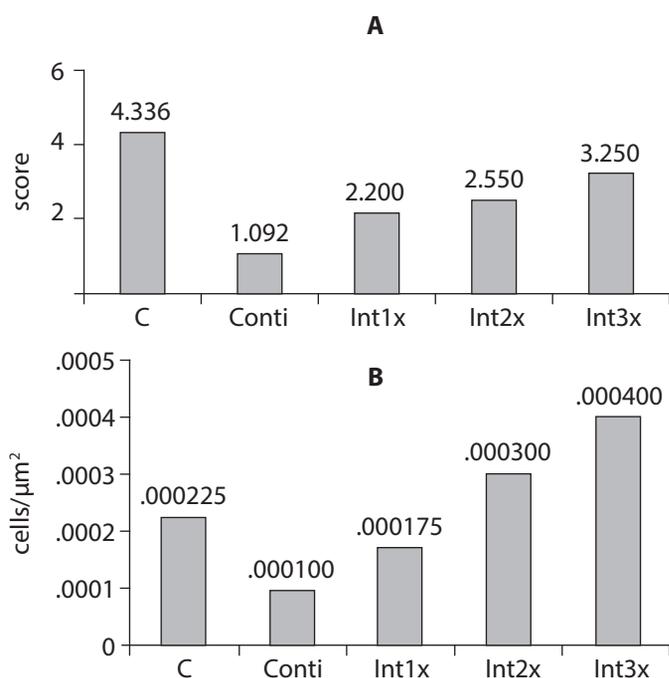


Fig. 3. PDX-1 expression (A) and PDX-1 density (B) on pancreatic islets after treatment. No significantly different among groups ($p \geq 0.05$).

PDX-1 expression and PDX-1 density of pancreatic islets

The PDX-1 expression showed no difference, however there was a decrease of PDX-1 in Conti. While, int3x showed higher expression of PDX-1 than other treatment groups (fig. 3 and 4).

Apoptosis and apoptosis density of pancreatic islets

The number of cells that providing an apoptotic cells on pancreatic islets showed no significant differences among groups. Nevertheless, from the average of expression, the control group showed the lowest apoptosis while the highest apoptosis showed in continuous group (fig. 5 and 6).

In apoptotic density, there was a significant difference ($p < 0.05$), and interestingly, Int1x group showed the lowest rate of apoptosis density.

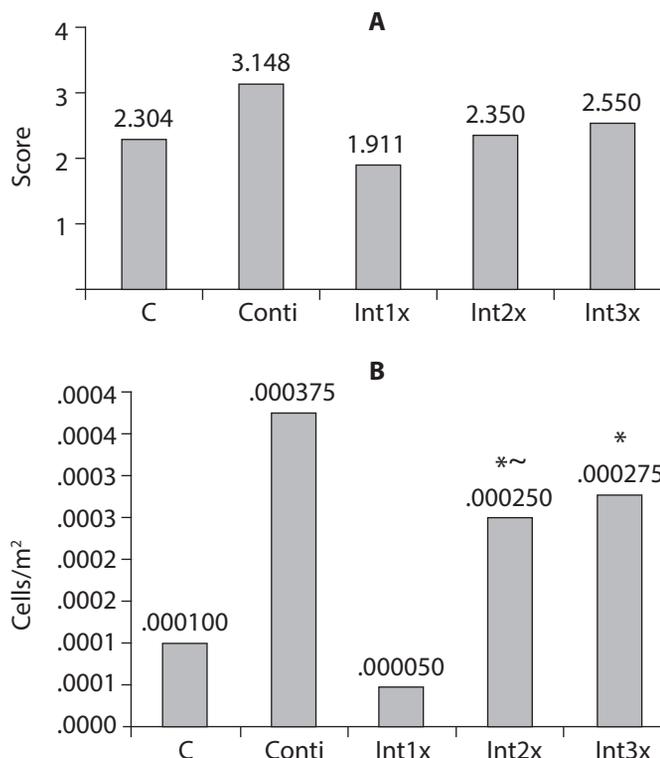


Fig. 5. Apoptosis and apoptosis density of pancreatic islets. * significantly different with the control group ($p < 0.05$). ~ significantly different with Int1x group ($p < 0.05$).

Changes in body weight

Body weight values before (BW pre) and after (BW post) treatment was presented in table 2. It illustrated that BW post had significantly difference among group ($p = 0.039$). Continue and Int2x groups had lower significantly difference values between control group, and Int2x groups also had lower significantly difference values between Int1x group.

Weight changes that obtained from the difference in weight before and after the treatment. Weight gain occurred in all groups, but the continuous diet group showed the lowest (fig. 7 and tab. 2).



Fig. 4. Expression of PDX-1 of Pancreatic islets cells (arrow). Microscope Nikon eclipse Ci, enlarged 400x, Optilab Viewer 2.2, Image raster 3.0.



Fig. 6. Apoptosis cells of pancreatic islets (arrow), tunnel assay. Microscope Nikon eclipse Ci, enlarged 400x, Optilab Viewer 2.2, Image raster 3.0.

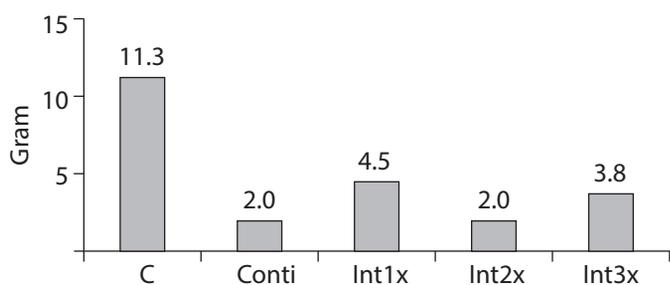


Fig. 7. Changes in body weight (gram) was the difference in body weight after and before the treatment
No significantly different among groups ($p \geq 0.05$)

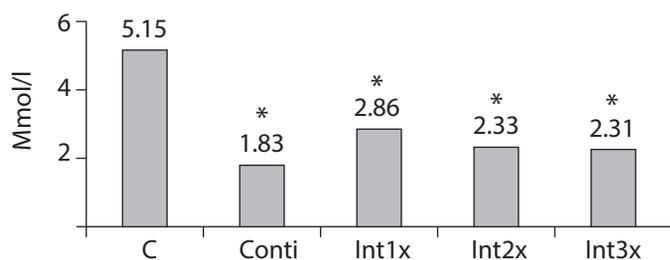


Fig. 8. Changes in random blood glucose levels
*: significantly different with the control group ($p < 0.05$)

Changes in random blood glucose levels

Random blood glucose level before (BG pre) and after (BG post) treatment showed significant difference among groups (tab. 2). Regarding the before treatment difference, it assumed that at the start of treatment, blood glucose level was already in different state. Hence, we did deep analyzed for the changing of their level. Nevertheless, blood level after treatment had trend to increase.

Random blood glucose levels were examined before and after the treatment, the difference was to show blood glucose response due to addition of calorie intake for 8 weeks.

Figure 8 and table 2 were showed that changes on blood glucose levels in the treatment groups were lower and significantly different with the control group ($p < 0.05$). However, there was no significant difference among the treatment groups.

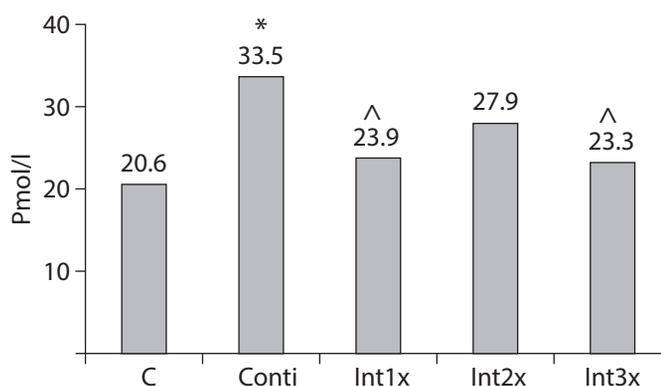


Fig. 9. Insulin serum levels after treatment (Pmol/l)

*: significantly different with the control group ($p < 0.05$)
^: significantly different with the Conti group ($p < 0.05$)

Random insulin serum levels

The results of insulin levels (fig. 9 and tab. 2) showed that insulin serum levels in the control group was the lowest and continuous group had the highest levels ($p = 0.004$). The entire intermittent groups did not differ significantly with the control group. Int1x and Int3x had a significant difference with Conti.

DISCUSSION

The area of pancreatic islets indicated that the control group had the largest pancreatic islets area and Int2x and Int3x had significant differences with the control group. This result was similar to the study of Petrik et al. (2001) [19] who mentioned that in a neonatal mice who was given a substitute milk with high carbohydrate nutrients, pancreatic islets was dominated by small-sized islets but in a lot of number. We suggested that smaller pancreatic islets area in treatment group could be a transition stage between early and middle stage of adaptation mechanism for glucose regulation in high glucose condition. If duration of treatment continued longer, pancreatic islets area could become larger, because the demand of insulin increased thus pancreatic islet became larger. Our study found interesting result that insulin increased but pancreatic islets area smaller. It might be response of stress, first response was a functional function then followed by morphologic changing. In the

Table 2. The effect of intermitently high glucose diet on changes in body weight, changes in blood glucose levels, and insulin serum levels. Data were presented as mean \pm SD, n=4 in each group

Group	BW pre (gram)	BW post (gram)	BW diff. (gram)	BG pre (mmol/L)	BG post (mmol/L)	BG diff (mmol/L)	SI (Pmol/L)
C	16 \pm 5.2	27 \pm 2.5	11.25 \pm 7.4	2.8 \pm 0.9	8.0	5.2 \pm 1.2	20.6 \pm 2.87
Conti	19 \pm 3.4	21 \pm 5.2*	2.0 \pm 8.3	3.7 \pm 1.0	5.5	1.8 \pm 1.30*	33.5 \pm 4.44*
Int1x	19 \pm 3.0	24 \pm 1.7	4.5 \pm 1.7	3.4 \pm 0.45	6.3	2.9 \pm 0.85*	23.9 \pm 4.96^
Int2x	17 \pm 2.2	19 \pm 2.6*#	2.0 \pm 2.2	3.9 \pm 0.85	6.3	2.3 \pm 0.80*	27.9 \pm 8.10
Int3x	19 \pm 1.4	23 \pm 1.3	3.8 \pm 2.1	5.6 \pm 1.00*	7.9	2.3 \pm 1.71*	23.3 \pm 4.71^

Diff: changes in such variable.
*significantly different with control ($p < 0.05$).
^ significantly different with Conti ($p < 0.05$).
significantly different with Int1x ($p < 0.05$).
~ significantly different with Int3x ($p < 0.05$)

Table 3. The correlation among variables

		PDX-1 dens	Apoptosis	Apoptosis dens	Islets	BW diff	BG diff	SI
PDX-1	Correlation Coefficient	.771**	0.1	0.286	-0.081	-0.235	.449*	0.078
	Sig. (2-tailed)	0.00	0.676	0.221	0.735	0.318	0.047	0.743
PDX-1 dens	Correlation Coefficient		-0.021	.497*	-.620**	-.528*	0.155	0.028
	Sig. (2-tailed)		0.929	0.026	0.004	0.017	0.515	0.907
Apoptosis	Correlation Coefficient			.544*	-0.009	-0.134	0.242	0.333
	Sig. (2-tailed)			0.013	0.97	0.575	0.303	0.151
Apoptosis dens	Correlation Coefficient				-.635**	-.578**	-0.017	0.423
	Sig. (2-tailed)				0.003	0.008	0.943	0.063
Islets	Correlation Coefficient					.624**	0.135	-0.117
	Sig. (2-tailed)					0.003	0.571	0.624
BW diff	Correlation Coefficient						0.064	-0.219
	Sig. (2-tailed)						0.788	0.353
BG diff	Correlation Coefficient							-0.173
	Sig. (2-tailed)							0.465

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

final stage, pancreatic islet will diminish because beta cells fatigue as occurred in DM type 2. Nonetheless, it also could be a similar inflammation process as in DM type 1 which is has smaller pancreatic islets by autoimmune [20]. Beside those, our study discovered that changing in body weight had a positive correlation with pancreatic islets area (table 3). As we have known that pancreatic islets consist of several types of cells and area square of pancreatic islets had no correlation with insulin serum level which reflexed function of one of pancreatic islets, beta cells.

The PDX-1 expression in the control group showed the highest scores of PDX-1 expression but different results when compared to the pancreatic islets area (PDX-1 density). This might be due to percentage of beta cells that express PDX-1 in the control group was relatively fewer, although the number of cells of pancreatic islets was more numerous. If it was associated with insulin levels, it could be assumed that the area of pancreatic islets was not the primary factor, but beta cells which become more active as they stimulated by addition calories diet of glucose.

As mentioned above, PDX-1 is a transcription factor that plays an important role in the regulation of beta cell function and beta cell survival, both before birth and after birth [21,22]. The number of beta cells and alpha cells might be indirectly affected by PDX-1. The increased PDX-1 expression could reduce alpha cells population and increase beta cells population during the embryonic phase. In contrast, reduced beta cells and

higher alpha cells, since the transdifferentiation of alpha cells into beta cells could result from impaired PDX-1 expression. Decreased PDX-1 expression occurs in cases of chronic hyperglycemia and dyslipidemia, therefore beta cells did not work properly. One of the underlying mechanism was interference of insulin-like growth factor (IGF) followed by the decreased PDX-1 expression that triggers apoptosis of beta cells [11, 21].

The PDX-1 expression and PDX-1 density among groups in this study did not show a significant difference. When PDX-1 expression and PDX-1 density pattern associated with the amount of insulin secretion, the result showed an inconsistent pattern. This means that besides PDX-1 there were other factors that play a role, in this case, the change of blood glucose has a positive correlation with PDX-1 expression. Another mechanism could be an adaptation process that causes the beta cells to become more active because it has been accustomed by addition intermittently of calories from glucose. Moreover, in mice embryo E.13.5 until birth, there are several transcription factors such as MafA (mammalian transcription factor A) and MafB. MafA also has a role to activate insulin transcription. MafB can influence MafA. The absent of MafB can inhibit MafA and PDX-1 on beta cells function [23]. Thus it needs further study to explore those factors.

Apoptosis showed that there was no significant difference with control. However, the apoptosis and apoptosis density in Conti group was the highest and Int1x was the lowest. The other research conducted

by Yamamoto (20017) [24] stated that apoptosis in β Pdx1;Ins2Akita mice with Ins2Akita mice and controls was not significantly different. In addition, in this study, there could be compensation-protective mechanism, because if apoptosis higher, expression of PDX-1 also higher. Previous study said that beside apoptosis, there was an inflammation mechanism in DM [25]. Hyperglycemia in DM triggers tissue necrosis in limbs [26]. Eventhough several studies revealed the main mechanisme of beta cells death is apoptosis, it is still possibility that ischemic necrosis also occured in beta cells, because there are abundance inflamatory factors released in DM [27, 28].

Pancreatic beta cells (and other cells such as nerve cells, endothelial cells) were non-insulin-dependent cells or insulin-independent cells for glucose uptake. When the blood glucose level rises, uptake glucose will increase beyond what it should be. Furthermore, it can trigger a mechanism of circuit reaction of damage cells, such as increase of intracellular reactive oxygen species (ROS) production that initiates intrinsic pathway apoptosis or mitochondrial pathway [29,30]. Research conducted by Sun et al.(2016) [31] with a high-fat diet showed an increase in apoptosis. In this study, there was a trend that the more frequent of additional calories diet, the more increase of apoptosis.

The highest body weight changes was control group. The body weight changes in the treatment groups were not as much as in the control group. This weight gain was related to mice growth due to the duration of treatment for 8 weeks. The group with an additional 7.4% calories with 1x/week showed the highest increasing in body weight compare to other treatment groups. Continue and Int2x reached the smallest values on body weight changing. Similar studies that use carbohydrates in the form of sugar solution also showed a lower body weight increase compared to the control group [32,33]. Research conducted by Adeyi et al. (2012) [34] showed that the high glycemic index/low-fat diet for 8 weeks also showed a similar results. Then, another research by Xu et al. (2010) [35] on 12-week carbohydrate diet in male wistar rats 3 weeks of age showed that the most weight in sequence was low carbohydrate diet group, normal carbohydrate group, and high carbohydrate diet group. It said that the levels of leptin in the group with low carbohydrates was the highest while the lowest was in the high carbohydrate group. However, other study connected diet intervention with leptin receptor. There was a decrease in leptin receptors in the hypothalamus at the low carbohydrate group. It mentioned that leptin also played a role in glucose metabolism and decreased appetite [36].

In our study, insulin level was higher in treatment group, which had no coresspond to the data of body weight and statistical analysis (tab. 3). It might be correlated with energy metabolism disruption. In physiologic state, insulin inhibits breakdown of fat and promotes synthesis freefatty acids, in consequence, it will induce increase values on body weight. Nevertheles, our result presented lower in body weight. Therefore, it needs more variables to be measured such as insulin receptor and also other hormones i.e leptin and its receptors.

The highest changes in blood glucose levels were the control group and all the treatment groups gave a significant differences with the control group. It was unusual result that control group was the highest level on glucose changing. However, if the data of BG pre and BG post (tab. 2) was evaluated, they significantly increased on BG post ($p \geq 0.05$). Several prior research had varied finding, such as high glucose diet on rats which showed lower level of fasting glucose in control group [37]. Whereas other study on mice presented no significantly difference on blood glucose which exposed by high glucose diet [38].

Our result showed different result might be because insulin serum in the control group has the lowest level. The lower insulin levels caused the blood glucose to enter the cells lower as well that affecting higher blood glucose level in the control group. The treatment group had an experience of high blood glucose level and did compesation mechanism for survival.

The additional 7.4% calories in the form of glucose proved to stimulate beta cells to secrete more insulin in the treatment group, mainly at the Conti group, compared to the control group. Several high calories diet studies revealed that serum insulin level reached a higher level compare to the control group [38]. However, there was another question for further research because insulin levels with additional calories of glucose at 2x/week intermittent group had greater levels than at the 3x/week intermittent group.

The addition calories of glucose on the day of the intermittent glucose exposure resulting in an increase acute blood glucose levels, and this increase was different from the other day when there were no additional calories of glucose. Some literatures explained that the high blood glucose levels after meals could induce the secretion of glucagon-like peptide-1 (GLP-1) that secreted by intestinal cells. GLP-1 could optimize further stimulation of insulin secretion and inhibit glucagon secretion by pancreatic islets [21]. More insulin increment in the treatment group might be an adaptation of mechanism when high-dose glucose was given at interminttent methods.

CONCLUSION

Based on the results of this study, the chronic additional 7.4% calories of glucose at 1-3x/week intermittently indicates a compensation mechanism for maintaining homeostasis, such as increase insulin serum level, initiate the changes of morphologic-biomolecular of pancreatic islets (decrease in the area of micromilimeter square of pancreatic islets and apoptosis density increase). PDX-1 density has trivial increase its level in intermittent mode. It seems that 1x/week intermittent addition of glucose calories diet could be tolerated and the 1x/week intermittent addition of it is better than 2-3x/week. Those result has not shown any interference in glucose metabolism yet. It can be seen from blood glucose and insulin levels that still within normal limits. Nevertheles, we suggest that we should be aware of the increment trend on blood glucose and insulin level, and morphology-biomolecular

alteration in pancreatic islets, which need further and complete investigation.

ADDITIONAL INFORMATION

Source of funding. This research is supported by funding from Fundamental Research Program Ministry of Research, Technology and Higher Education, Indonesia.

Conflict of interests. The authors declare no conflicts of interest related to the manuscript.

Authors involvement. L. Herawati - conception and design the experiments, conducted the experiments, interpreted results of experiments, prepared the figures, drafted, edited and revised

manuscript; K.W. Wigati - prepared the figures, edited and revised manuscript; P.S. Rejeki - interpreted results of experiments; W. Widjati - conducted the experiments, interpreted results of experiments; R. Irawan - conception and design the experiments, interpreted results of experiments, edited and revised manuscript.

All authors contributed equally to the review. All authors have read and approve the final version of the manuscript.

Acknowledgements. The authors thank all team members at the embryology laboratory for their technical assistance during the research, Dewita vet, Tia vet, Ronald vet, and team (Faculty of Veterinary, Universitas Airlangga, Surabaya) as technical assistance related to immunohistochemical preparations, and Wahyu Wibowo (Department of Statistics, Institute Teknologi Sepuluh November, Surabaya) for his statistical analysis.

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TO CITE THIS ARTICLE:

Herawati L, Wigati KW, Rejeki PS, Widjiati W, Irawan R. Increased apoptosis, but not pancreatic duodenal homeobox-1 expression in pancreatic islets is associated with intermittent glucose loads in mice. *Diabetes Mellitus*. 2018;21(6):497-505. doi: 10.14341/DM9437

ЦИТИРОВАТЬ:

Herawati L, Wigati KW, Rejeki PS, Widjiati W, Irawan R. Эпизодическая углеводная нагрузка ассоциируется с усилением апоптоза в островках поджелудочной железы, а не с экспрессией pancreatic duodenal homeobox-1 (PDX-1) у мышей // *Сахарный диабет*. — 2018. — Т. 21. — №6. — С. 497-505. doi: 10.14341/DM9437