Article

β-(1,3)-D-glucan from *Pleurotus ostreatus* correlates with lower plasma IL-6, IL-1β, HOMA-IR, and higher pancreatic beta cell count in High-Fat and High-Fructose Diet (HFFD) rats

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Abstract

Introduction: The increasing consumption of high-fat and high-fructose foods contributes to the increasing prevalence of global obesity. Low-grade chronic inflammation in obesity is a significant risk factor for insulin resistance and type 2 diabetes. Therefore, this study aimed to determine the effect of β-(1,3)-D-glucan from oyster mushroom (Pleurotus ostreatus) extract on rats fed with a high-fat and high-fructose diet.

Design and Methods: This experimental study was conducted on 35 male Sprague-Dawley rats aged eight weeks. The rats were divided into groups given a normal (N) diet, a high-fat and high-fructose diet (HFFD), D1 (HFFD+125 mg/kg BW β-glucan), D2 (HFFD+250 mg/kg BW β glucan), and D3 (HFFD+375 mg/kg BW β-glucan) with an intervention of 14 weeks. IL-6 and IL-1β levels were measured by the ELISA method, while HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) was calculated by the fasting insulin (ng/mL) x fasting blood glucose (mg/dL)/405 formula. Pancreatic beta-cell counts were measured by hematoxylin and eosin (H&E) staining.

Results: The results showed no differences in IL-6 and IL-1β between the treatment groups. However, there were significant differences in HOMA-IR and pancreatic beta-cell counts between groups. There were negative correlations between the dose of β-glucan and IL-6, IL-1β, and HOMA-IR levels. Also, there was a positive correlation between the dose of β-glucan and the number of pancreatic beta cells.

Conclusions: Administration of β-(1,3)-D-glucan from oyster mushroom (Pleurotus ostreatus) extract prevented hyperglycemia and insulin resistance, also reduced inflammation in rats fed with HFFD regardless of weight gain.

Introduction

Obesity is a significant global health issue that triggers insulin resistance, the beginning of non-communicable diseases such as type 2 diabetes mellitus.[1,2] In Indonesia, the prevalence of adult obesity increased from 10.5% in 2007 to 14.8% in 2013 and 21.8% in 2018. This condition was accompanied by an increase in diabetes mellitus based on the physician's diagnosis, from 1.5% in 2013 to 2% in 2018. Furthermore, diabetes mellitus diagnosis based on blood glucose testing increased from 6.9% in 2013 to 8.5% in 2018.[3] Changes in people's consumption patterns to high-fat and high-fructose foods and drinks increase the prevalence of obesity.[4] Fat tissue accumulation in overweight and obese conditions causes low-grade chronic inflammation by releasing cytokines such as TNF-α, IL-6, and IL-1β.[5,6] Low-grade chronic inflammation is a significant risk factor for insulin resistance and type-2 diabetes mellitus. In this case, elevated IL-6 and IL-1β increase insulin resistance and type 2 diabetes risk.[2,7,8]

Prevention and treatment of metabolic syndrome is a strategy under development by developing functional food. An example is a well-known mushroom consumed for 3000 years and used in traditional Chinese and East Asian medicine.[9,10] For their therapeutic effects, some of the best well-known mushrooms are *Ganoderma lucidum* (Lingzhi mushroom) and *Lentinula edodes* (Shiitake Mushroom). A study showed that adding shiitake mushrooms into a high-fat diet mixture could reduce plasma triglyceride (TG) levels, inhibit weight gain, and reduce fat deposition in rats.[11] In Indonesia, one mushroom popularly cultivated is a white oyster mushroom (*Pleurotus ostreatus*), consumed as an alternative for protein and a source of fiber.[12] White oyster mushrooms have several bioactive components, such as β-glucan, a polysaccharide group composed of D-glucose molecules bound to β-(1,3)- and (1,6)-D-glucan.[13] β-glucan from oats have been explored earlier, which has been proven to improve insulin sensitivity and HOMA-IR and lowers blood glucose, HbA1C levels, and body weight in mice fed with a high-fat diet.[14,15]

Several species from the *Pleurotus* genus have been explored, including *Pleurotus citrinopileatus*, *Pleurotus Sajor-caju*, *Pleurotus tuber-regium*, and *Pleurotus ostreatus,* both *in vitro* and *in vivo*. They show positive effects on weight loss, prevent hyper-

Significance for public health

Changes in people's consumption patterns to high-fat and high-fructose food contribute to obesity and diabetes. White oyster mushroom has been widely consumed as alternative protein and dietary fiber. This in-vivo animal model study could be references for product development of β-glucan as nutraceuticals or white oyster mushrooms to prevent obesity and type 2 diabetes.

glycemia and insulinemia, reduce gene expression of transcription factor IL-6 and IL-1β, and improve glucose tolerance.[16–19] The increasing cultivation and consumption of oyster mushrooms in Indonesia have not been studied based on their benefits. This has limited the data on the benefits of β-glucan bioactive compounds from oyster mushrooms to prevent and treat metabolic syndrome. Therefore, this study aimed to investigate the effect of β-glucan from oyster mushroom (*Pleurotus ostreatus*) extract on IL-6, IL-1β, and HOMA-IR levels in rats fed with High-Fat and High-Fructose Diet (HFFD).

Design and Methods

Animals and diet

Our study was conducted using a Post Test Only Controlled Group Design. A sample of 35 male Sprague-Dawley (SD) rats aged five weeks were obtained from the animal resource center The National of Drug and Food Control, Jakarta-Indonesia. They were given three weeks of acclimatization to their new environment, with ad libitum access to food and water. Furthermore, all rats were fed a normal diet of modified AIN-93M during the acclimatization period. They were divided into five groups based on the diet and the dose of β-glucan given, as shown in Table 1.

The normal and high-fat diet pellet was based on the AIN-93M formula with modified ingredients amount.[20] In this study, the normal diet contained 4.1 kcal/grams, with 7% fat, while the high-fat diet contained 5.46 kcal/grams, with 36.95% fat. Fructose solution in 30% concentration was made by dissolving 300 grams fructose powder for each 1 L of water. The β-glucan from the oyster mushroom extract was produced based on the previous study.[21] All experimental procedures have been approved by The Research Ethics Committee of the Faculty of Medicine, Universitas Brawijaya, Indonesia (136/EC/KEPK/07/2020).

Food intake, body weight, and body composition

Animals were weighed weekly in the fourteen-week intervention period. Food intake was measured by weighing the total food (g) provided to the rats and subtracting the remaining food (g) in the cage after 24 hours. After the intervention, the rats were sacrificed by ketamine + xylazine (0.1 mL/100 g BW) anesthetic agent after fasting for eight hours. Anthropometric measurement was conducted by weighing, measuring the body (naso-anal) length, and abdominal circumference. The Lee index was calculated by dividing the cubicle root of the weight (g) by the naso-anal length (mm) multiplied by 1000.[22]

Sample collection and parameters analysis

The plasma samples were prepared for parameters analysis by collecting blood through the heart using syringes. The samples were directly inserted into a vacutainer containing ethylene diamine tetraacetic acid (EDTA) anticoagulant. The blood samples were centrifuged for 10 minutes at 10,000 rpm. Moreover, the plasma was aliquoted into several tubes using a micropipette. Plasma IL-6, IL-1β, and insulin levels were performed by Enzyme-linked immunosorbent assay (ELISA) following the manufacturer's protocol (Elabscience Rat IL-6 Cat No: E-EL-R1005; BT-Laboratory Rat IL-1β Cat No: E0119Ra; Elabscience Rat Insulin Cat No: E-EL-R3034). The pancreas was also obtained and preserved in 10% neutral buffered formaldehyde to examine pancreatic beta cell numbers. A histology examination was conducted by hematoxylin and eosin (H&E) staining and scanning with Olympus dot slide microscope at x400 magnification. The number of the pancreatic beta-cell is a count from Langerhans Island in 10 visual fields, assisted by OlyVia software.[23] Fasting blood glucose (FBG) was measured using a glucometer (AUTOCHECK).[24] Lastly, HOMA-IR index was calculated according to the formula fasting insulin (ng/mL) x fasting blood glucose (mg/dL)/405.[25]

Statistical analysis

Food intake, anthropometric parameters, HOMA-IR, plasma IL-6, IL-1β, and insulin levels were presented as mean and standard errors. Statistical analysis was performed using SPSS software (SPSS Inc version 25.0, Chicago, Ill, USA). Moreover, body weight gain, abdominal circumference, Lee Index, an intake of total energy, protein and carbohydrate, fasting blood glucose, plasma IL-6, and pancreatic beta-cell number were analyzed using a One-way ANOVA test. This was followed by a post hoc Tukey test for multiple comparisons. Food and fat intake, plasma insulin, plasma IL-1β, and HOMA-IR level were analyzed using the Kruskal Wallis test. It was followed by a post hoc Mann-Whitney U test to check different comparisons among the groups. The correlations between a dose of β-glucan given and fasting blood glucose, plasma insulin, plasma IL-1β, HOMA-IR, and pancreatic beta-cell number were analyzed using Spearman Correlation Test. The differences and correlations were considered significant when p-value <0.05.

Results and Discussions

After 14 weeks of intervention, the results showed a significant difference in weight gain between the normal and HFFD groups. The lowest Lee Index scores were found in groups D1 and D3, and no significant difference in the rats' abdominal circumference. The measurement of food intake showed significant differences in the intake of fat and carbohydrates, as shown in Table 2. Furthermore, there were no significant differences in groups' plasma IL-6 and IL-1β levels. Significant differences were found in fasting blood glucose levels (p = 0.000), insulin levels (p = 0.05), HOMA-IR (p = 0.005), and pancreatic beta-cell count (p = 0.000) between groups. The lowest blood glucose and HOMA-IR levels and the highest number of pancreatic beta cells were in group D2, as shown in Figures 1 and 2.

The Spearman test results showed a negative correlation between β-glucan administration and levels of IL-6 (P = 0.032; r = -0.406), IL-1β (p = 0.018; r = -0.443), insulin (p = 0.025; r = -0.423), and HOMA-IR (p = 0.039; r = -0.392). However, a positive correlation was found between β-glucan administration and pan-

Table 1. Animal grouping and treatment.

Group	Treatment
Normal	Normal diet
HFFD (High-Fat and Fructose Diet)	High-fat diet + 30% fructose solution (HFFD)
D1	HFFD + 125 mg/kgBW β-glucan
D2	HFFD + 250 mg/kgBW β-glucan
D3	HFFD + 375 mg/kgBW β-glucan

Table 2. Anthropometric and food intake characteristics.

Parameter	N	HFFD	D1	D2	D3	P-value
Anthropometric						
Body Weight Gain (g)	137.7 ± 52.5[a]	209.0 ± 16.8[b]	174.7 ± 23.9[ab]	188.4 ± 38.0[ab]	177.3 ± 53.2[ab]	0.034*
Abdominal Circumference (cm)	18.6± 2.0	19.1 ± 0.9	19.3 ± 1.6	19.3 ± 1.1	19.0 ± 1.9	NS
Lee Index	298.7± 7.6[ab]	291.3 ± 4.9[a]	288.8 ± 9.4[a]	308.2 ± 6.6[b]	288.1 ± 7.1[a]	0.000**
Food Intake						
Feed intake (g)	18.93± 3.1[a]	11.43 ± 0.8[b]	10.96 ± 1.1[b]	12.49 ± 2.1[b]	11.01 ± 1.9[b]	0.001**
Energy intake (kcal)	81.46 ± 9.4	94.77 ± 5.5	87.70 ± 7.7	92.35 ± 12.6	82.70 ± 12.6	NS
Protein intake (g)	2.80 ± 0.3	3.00 ± 0.2	2.46 ± 0.3	2.80 ± 0.5	2.47 ± 0.4	NS
Fat intake (g)	0.54 ± 0.1[a]	2.56 ± 0.2b	2.46 ± 0.3[b]	2.80 ± 0.5[b]	2.47 ± 0.4[b]	0.001**
Carbohydrate intake (g)	10.89 ± 1.3[ab]	12.03 ± 1.2[b]	10.77 ± 1.3[ab]	11.90 ± 1.4[b]	9.49 ± 1.4[a]	0.007*

*One Way Anova Test. *Kruskal Wallis Test. NS: non-significant. A significant difference if p<0.05. Post Hoc Test showed a significant difference between groups marked by different annotation (a,b,c). N: Normal diet. HFFD: High-fat diet + 30% fructose solution. D1: HFFD + 125 mg/kgBW β-glucan. D2: HFFD + 250 mg/kgBW β-glucan.. D3: HFFD + 375 mg/kgBW β-glucan.

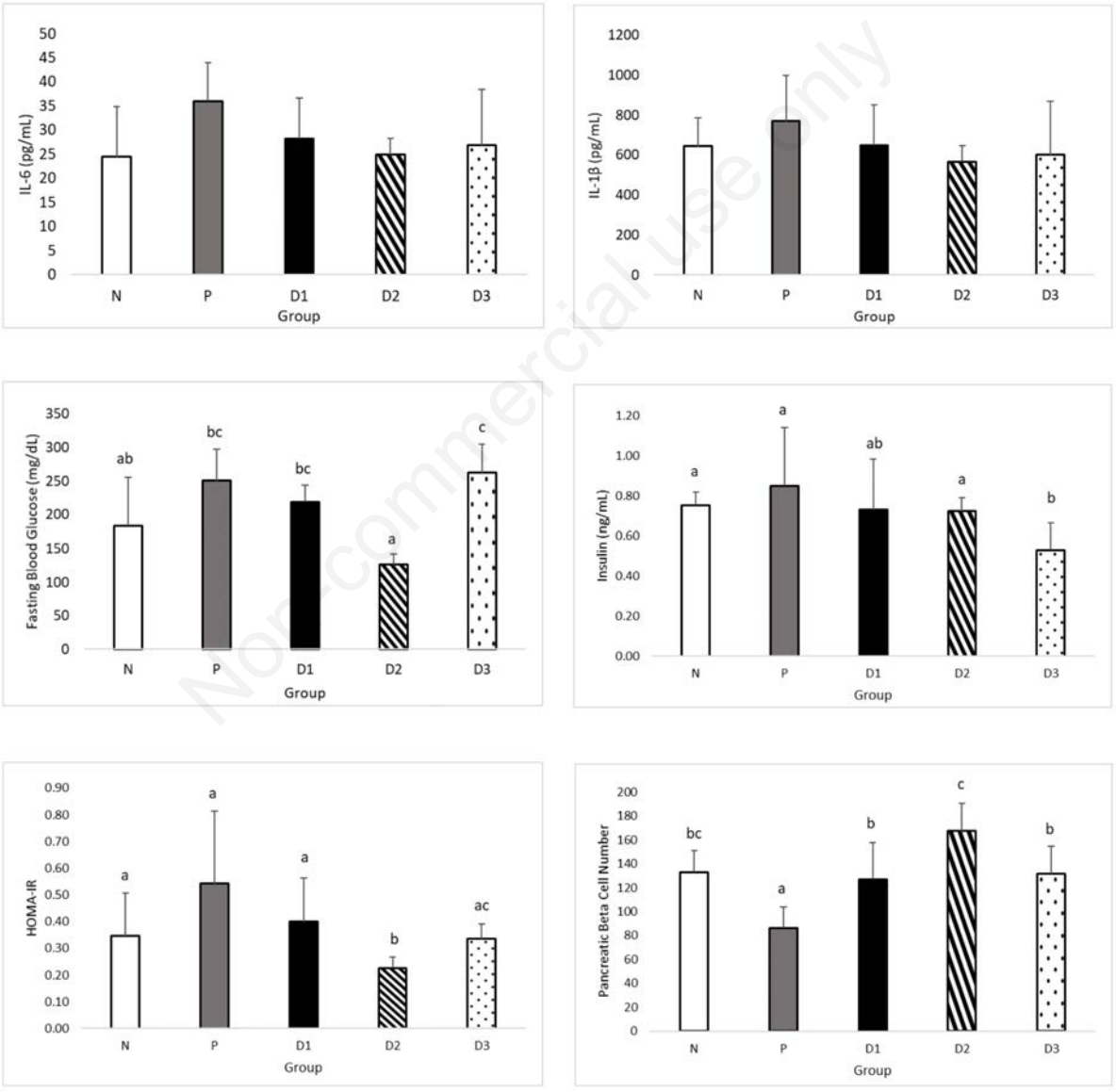

Figure 1. The Comparison of Levels of IL-6, IL-1β, FBG, Insulin, HOMA-IR, and Pancreatic Beta Cell Count between Groups. N: Normal diet, HFFD: High-fat diet + 30% fructose solution, D1: HFFD + 125 mg/kgBW β-glucan, D2: HFFD + 250 mg/kgBW β-glucan, D3: HFFD + 375 mg/kgBW β-glucan Post Hoc Test showed a significant difference between groups marked by different annotation [(a,b,c)].

creatic beta cell count ($p = 0.002$; $r = 0.558$), as shown in Figure 3. A positive correlation was also found between IL-6 levels and plasma IL-1β levels ($p = 0.003$; $r = 0.533$).

The final anthropometric measurements showed no significant difference in the rats' abdominal circumference between groups. The weight gain was inconsistent with the given dose, with group D2 having the highest weight gain. The results of the Lee Index in the group given the highest dose of β-glucan (375 mg/kg BW) showed the lowest value compared to other groups. These results are consistent with previous studies that showed no effect on weight changes in diabetic rats fed on a high-fat diet and STZ induction fed with oat β-glucan, oat starch, or whole oats.[26] However, the results contradict another study, which stated that administering *Pleurotus sajor-caju* mushroom extract in rats fed with a high-fat diet could inhibit weight gain.[19] In this study, the highest intake of carbohydrates, including fructose solution, was found in the HFFD group, and the lowest was in the D3 group. Previous studies showed that the fluid intake of rats given β-glucan was lower than the positive control group that experienced polydipsia.[26] The group of rats fed with HFFD experienced symptoms of polydipsia through increased intake of fructose solution, increasing carbohydrate intake than the rats given β-glucan. A decrease in carbohydrate intake lowers the food efficiency ratio and energy balance. The lower total carbohydrate intake is related to inhibiting the adipogenesis process. This indicates the group given the highest dose of β-glucan had the lowest Lee Index.[19] However, there was no significant difference in energy intake between groups. These results contradict previous studies, which showed that administering β-glucan from oats and *Pleurotus sajor-caju* extract could reduce energy intake.[19,27] The energy density of normal feed and HFFD used in this study has a small difference. The difference in intake is more influenced by feed composition and consumption of fructose solution.

Plasma IL-6 and IL-1β levels decreased in the group given β-glucan from the oyster mushroom extract. The correlation test results showed a negative correlation between β-glucan dose and plasma IL-6 and IL-1β levels. These results support previous studies that concentrates from *Pleurotus ostreatus* could suppress IL-6 secretion in LPS-exposed mice, while *Pleurotus tuber-regium* extract could inhibit the release of IL-6 in LPS-induced cell lines.[16,17] Another study on β-glucan from oat sources stated that giving a β-glucan intervention to rats with a high-fat diet reduced plasma IL-6 and IL-1β levels.[26] Moreover, several in-vitro studies showed that β-glucan from sources such as *Pleurotus sajor-caju*, *Ganoderma lucidum*, and *Poria cocos* could suppress the production of IL-1β and TNF-α.[28,29] This study found that β-glucan reduces pro-inflammatory cytokine levels, indicated by a significant negative correlation between the dose of β-glucan administered and plasma levels of IL-6 and IL-1β. The increase in pro-inflammatory cytokines in obesity and metabolic syndrome may occur by activating the transcription factor NF-kB. Further increases in circulating cytokine levels increase the risk of insulin resistance and type 2 diabetes. The mechanism of β-glucan in activating the anti-inflammatory pathway is not fully understood. However, β-glucan molecules can be recognized as pathogen-associated molecular patterns (PAMP) that bind to receptors such as dectin 1, complement receptor 3 (CR3), or toll-like receptors (TLRs).[30] The interaction between β-glucan and TLR inhibits the activation of transcription factors NF-kB and AP-1, suppressing cytokine production and providing anti-inflammatory effects.[16,31] This study did not determine the process that explains the anti-inflammatory mechanism of β-glucan from the oyster mushroom extract.

This study showed significant differences between groups regarding fasting blood glucose, insulin, and HOMA-IR levels, and pancreatic beta-cell counts. Administering β-glucan from oyster mushroom extract (Pleurotus ostreatus) could reduce Fasting Blood Glucose (FBG), insulin, and HOMA-IR levels and increase pancreatic beta cells. These results support previous studies, which stated that administering oyster mushroom powder and extract could reduce FBG levels and improve pancreatic beta cells in dia-

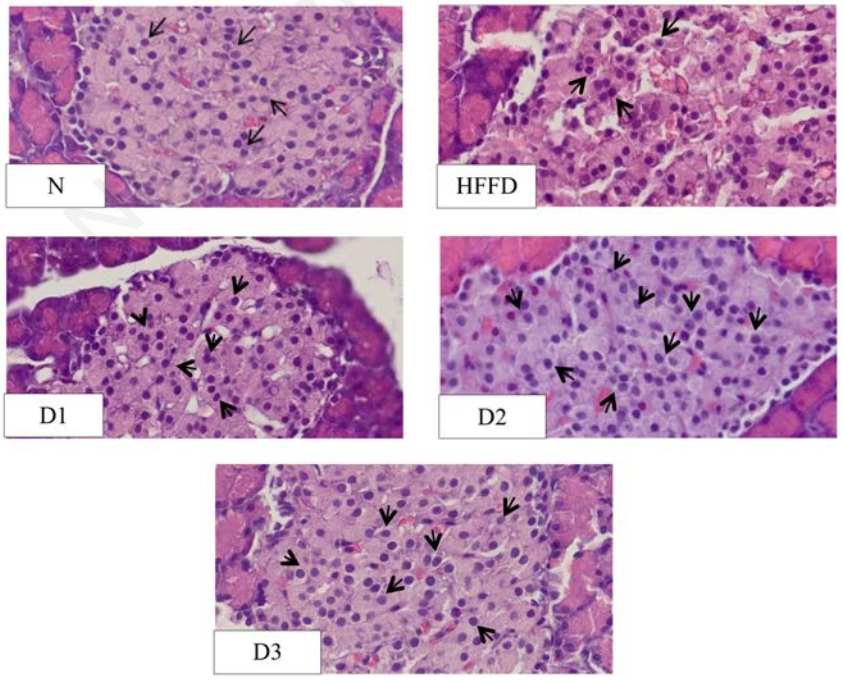

Figure 2. Histological Examination of Pancreatic Beta Cells between Groups. N: Normal diet, HFFD: High-fat diet + 30% fructose solution, D1: HFFD + 125 mg/kgBW β-glucan, D2: HFFD + 250 mg/kgBW β-glucan, D3: HFFD + 375 mg/kgBW β-glucan.

betic rats.[32,33] Another in-vivo study in mice and rats fed with a high-fat diet showed that administering *Pleurotus citrinopileatus* and *Pleurotus tuber-regium* extracts could reduce fasting blood glucose levels.[19,34] Administering β-glucan from sources such as oats and chitin reduces FBG and HOMA-IR levels insulin secretion and repairs pancreatic beta cells.[15,26,35,36] Furthermore, this study found that β-glucan improves glycemic control, insulin resistance, and pancreatic beta cells. This is seen by the negative correlation between β-glucan dose and insulin and HOMA-IR levels and a positive correlation between β-glucan dose and pancreatic beta-cell counts. Several mechanisms concerning the effect of lowering glucose and insulin occur through the ability of soluble fiber (β-glucan) to form a viscous layer on the gastrointestinal tract. This slows gastric emptying, digestion, absorption and reduces nutrient transport to enterocytes.[35,36] Administering Pleurotus ostreatus extract also increased p-AMPK in muscle and adipose tissue. It leads to upregulation of the transcriptional regulator of the GLUT4 gene for increasing glucose uptake, providing an anti-hyperglycemic effect.[33] Moreover, β-glucan works as an antioxidant, protecting against pancreatic beta-cell apoptosis and increasing the production of hematopoietic stem cells (HSCs). This indicates HSCs could differentiate into special fibroblasts and liver, endothelial, and pancreatic cells. β-glucan becomes a potent molecule to improve glucose homeostasis in the body through hypoglycemic effects, improvement of insulin resistance, and decreased apoptosis of pancreatic cells.[15,32,34] Decreased pro-

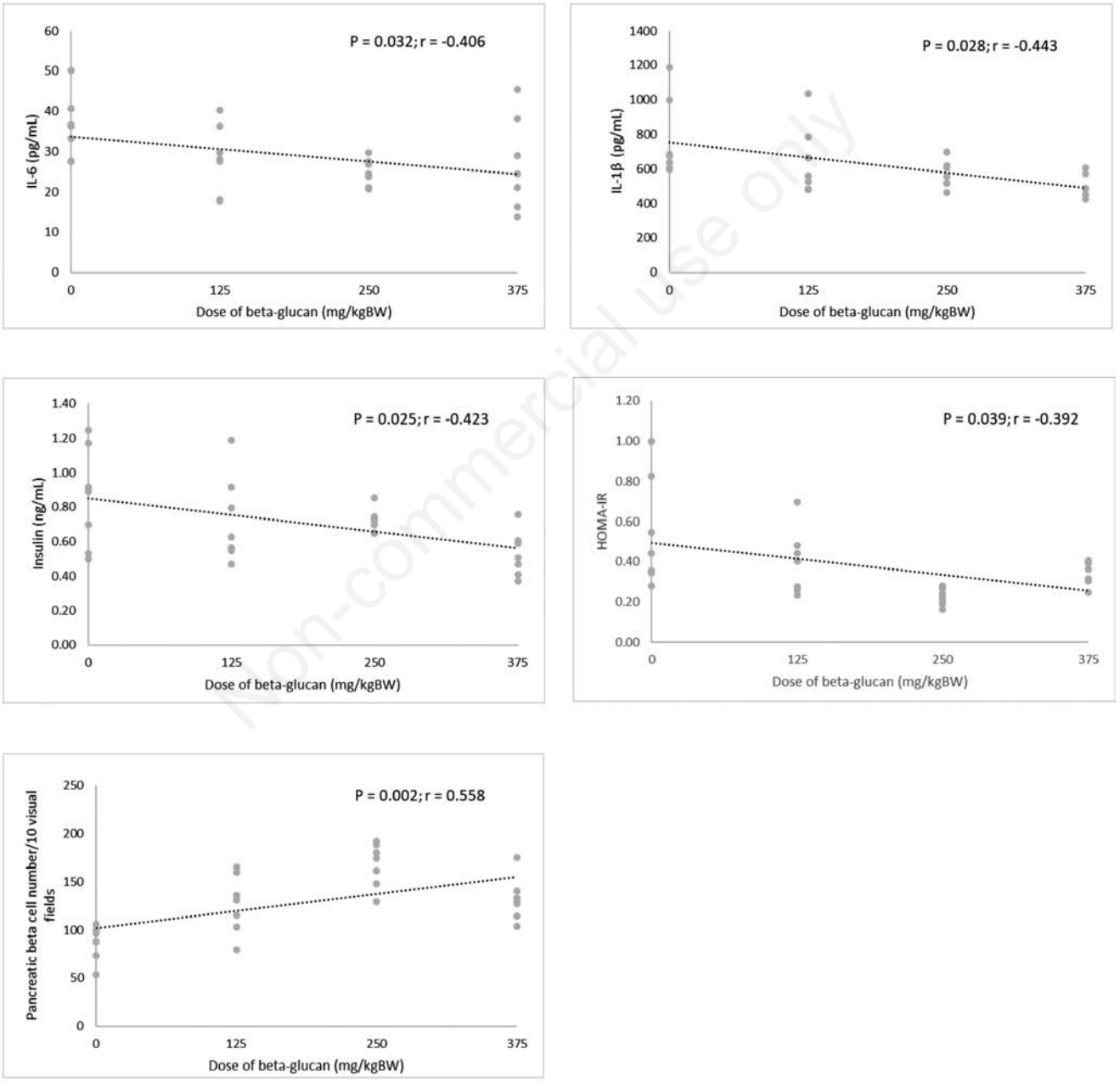

Figure 3. Spearman Correlation Test Results between Dose of β-glucan administration and Levels of IL-6, IL-1β, insulin, HOMA-IR, and pancreatic beta-cell count.

inflammatory cytokines also improved insulin resistance, where low IL-6 and IL-1β increased the expression and sensitivity of insulin receptor substrate, specifically IRS-1. This resulted in increased insulin-mediated glucose uptake into cells.[37,38]

β-glucan has various structural and molecular weight variations, depending on its source and extraction procedure. The β-glucan extracted from the genus *Pleurotus* has a high molecular weight. An example is *Pleurotus tuber-regium,* which contains β-glucan with a molecular weight of 5.76 x 10^4 - 77.4 x 10^4 g/mol, nearly similar with oat β-glucan, with a molecular weight of 15.6 x 10^4 - 68.7 x 10^4 g/mol.[13,39] High molecular weight and viscosity β-glucans have hypocholesterolemic and hypoglycemic effects. In contrast, low molecular weight β-glucans have antioxidant and immunological effects.[39] Studies on the functionality of molecular weight show pros and cons influenced by the source of β-glucan, the amount of daily food intake, and the dose of β-glucan given.[13,39,40] This study did not determine the molecular weight of β-glucan. Therefore, further studies should analyze the molecular weight of *Pleurotus ostreatus* β-glucan and its functionality. The β-glucan structure is also associated with its functionality. It is composed of beta-D-glucose monomer units linked by glycosidic bonds at (1,3), (1,4), or (1,6), with or without branches.[31] Brown algae, oats, and barley contain β-(1,3/1,4)-D-glucan that modulate microbiota, lowering cholesterol and blood glucose levels. Moreover, mushrooms and yeasts contain β-(1,3/1,6)-D-glucan that modulates the immune system and has antimicrobial and anticancer properties. Agrobacterium contains branchless β-(1,3)-D-glucan used as a thickening agent in food processing.[31] The oyster mushroom extract used in this study had a β-(1,3)-D-glucan structure.[21] The result supports another study on oyster mushroom (*Pleurotus ostreatus*) using an alkaline extraction method, which stated that the compound found was β-(1,3)-D-glucan.[41] The study of β-glucan from chitin sources stated that it contains β-(1,3)-D-glucan, which improves blood glucose, triglyceride, and cholesterol levels and glucose tolerance in mice fed on a high-fat diet.[36] Regardless of the structural variation, biological activity is the β-(1,3)-D-glucan core/backbone binding, which improves blood glucose control and dyslipidemic conditions. It also modulates immune responses and gut microbiota and improves obesity conditions.[31] The findings concerning the correlation between differences in branch structure and functionality of β-glucans are still limited. Therefore, further studies should examine the β-glucan structure with the desired therapeutic target.

This study found that rats in the D2 group administered with 250 mg/kg BW β-glucan from oyster mushroom showed the best improvement effects in IL-6, IL-1β, HOMA-IR, and pancreatic beta-cell count. However, this mechanism was not elucidated in this study. Previous studies on the toxicity of β -glucans are still limited, such as giving the β-(1,3/1-6)-D-glucan with doses of 500, 1000, and 2000 mg/kg BW Sprague-Dawley rats for 90 days without side effects on anthropometric and hematological blood parameters.[42] Another study administered β-(1,3/1-4)-D-glucan with doses of 0.7, 3.5, and 7% through a mixture of feed on Wistar rats for 28 days. The results showed no side effects on growth, hematological abnormalities, and organ weight of rats.[43] The levels of IL-6 in the D3 group were +9.9% higher, IL-1β -6.4% lower, HOMA-IR -3.3% higher. Additionally, pancreatic beta cells were -0.7% lower than the normal group, with no significant difference. The β-glucan used in this study has a different β-(1,3)-D-glucan structure from the two previous studies, hence, it is necessary to explore the differences in structure and functionality.

Conclusions

This study showed that administering β-glucan with the structure of β-(1,3)-D-glucan from oyster mushroom (*Pleurotus ostreatus*) extract could reduce the levels of IL-6 IL-1β, FBG, HOMA-IR, and insulin. The administration could increase pancreatic beta-cell counts in HFFD-treated rats, with the best effect reported in the dose of 250 mg/kg BW β-glucan. Furthermore, the administration of β-glucans had a preventive effect on hyperglycemia and insulin resistance in inflammatory rats by inducing high-fat and fructose diets regardless of the weight gain. Further studies should explore the influence of the structure and molecular weight of β-glucan as well as examine its mechanism as an anti-inflammatory, its prevention of hyperglycemia, and reduction of insulin resistance index.

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Key words: β-(1,3)-D-glucan; HOMA-IR; IL-6; IL-1β; pancreatic beta cell.

Acknowledgment: The authors thank the Faculty of Medicine and Institute of Research and Community Services Universitas Brawijaya for their support and motivation during this study.

Contributions: Dian Handayani (DH), Inggita Kusumastuty (IK), and Ema Pristi Yunita (EY) designed and coordinated the study. Alma Maghfirotun Innayah (AI) and Elvira Nur Sa'idah Hariani (EH) conducted the experiments and biological assays. The first author and co-authors prepared and conducted the data analysis and wrote the manuscript. Dian Handayani (DH), Husnul Khotimah (HK), Inggita Kusumastuty (IK), and Ema Pristi Yunita (EY) assisted in data interpretation and contributed to the final manuscript. DH is a corresponding author.

Conflict of Interests: The authors have declared no conflict of interest.

Funding: This study was funded by the Institute of Research and Community Services Universitas Brawijaya (Decree: 437.4/UN10.C10/PN/2020).

Clinical trials: All procedures were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Brawijaya, Indonesia (Ethics Approval Number: 136/EC/KEPK/07/2020).

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Informed consent: Not applicable.

Conference presentation: Part of this paper was presented at the 2nd International Nursing and Health Sciences Symposium that took place at the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.

Received for publication: 5 December 2021.
Accepted for publication: 10 May 2022.

Healthcare in Low-resource Settings 2023; 11(s1):11165
doi:10.4081/hls.2023.11165

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