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Xylooligosaccharides attenuates insulin resistance in obese mice by modification of short-chain fatty acids

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Abstract
Diabetes is a major public health concern worldwide. In our previous study, we reported that XOS supplementations for 12 weeks could successfully attenuated obesity, improved glucose intolerance and fasting blood glucose in mice fed a high fat diet. However, how dietary XOS improved blood glucose remains vague. Therefore, this study was designed to examine the effect of dietary XOS supplementation on insulin resistance in obese mice. Levels of fasting insulin were measured in the blood of mice fed one of the following diets: low-fat diet (LFD), high-fat diet (HFD), HFD plus 5 XOS (LXD) and HFD plus 10 % XOS (HXD). Levels of blood Ghcagons-like peptide 1 (GLP-1) and hepatic Free fatty acids (FFAs) were determined by Mouse ELISA Kit and GC respectively, insulin sensitivity was evaluated by using HOMA2 and QUICKI indexes, and Short-chain fatty acids (SCFAs) in fecal of mice were measured by HPLC.
Compared to the HFD group, the LXD and HXD groups displayed a remarkable attenuation in insulin resistance. This attenuation was accompanied with an increase in levels of the hormone of GLP-1 as well as the production of acetate, propionate, butyrate, and total SCFAs. By contrast, long-chain FFAs in the liver including saturated fatty acids (SFAs), Monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and total FFAs were reduced by XOS supplementations. These results suggest that feeding of XOS can efficiently suppress insulin resistance in the HFD-induced obese mice by enhancing SCFAs production.

Keywords: xylooligosaccharides, obese mice, insulin resistance and short-chain fatty acids

Introduction
Diabetes mellitus is an endocrine disorder that affects more than 100 million people around the world [1]. Over the last few decades, the prevalence of diabetes has increased dramatically. The prevalence was 6.4% in 2010, and by 2030 it is estimated to rise to 7.7%, Diabetes is defined as a metabolic disorder that involves insulin resistance [2]. Hyperglycemia plays a key role in the development of diabetes as well as obesity [3, 4]. Meanwhile, insulin resistance is typical of type 2 diabetes mellitus (T2DM), since insulin plays a major role in maintaining the homeostasis of blood glucose. The reduced glucose absorption from blood to cells is associated with insulin action inhibition, leading to insulin resistance [5]. Interestingly, insulin secretion is potentiating by the intestinal hormone GLP-1 through stimulating the GLP-1 receptor highly expressed on islet β cells, the insulin resistance (IR) of obese and diabetic humans and animals is improved by GLP-1 [6]. What is more, GLP-1 was also playing a crucial role in metabolic functions including appetite repression, gastric emptying, suppression of glucagon secretion, repression of appetite, and the slowdown of gastric emptying slowdown and inhibition of glucagon secretion [7]. Importantly, the formation of gut hormone including GLP-1 hormone is mediated by SCFAs [8]. Overall, natural components applications with healthy properties such as anti-diabetes activity have drawn attention from both consumers and researchers. Numerous dietary supplements and functional foods have been stated to be effective in metabolic disease management including functional oligosaccharides, plant extracts, and fermentation products [9]. Functional oligosaccharides are polysaccharides hydrolytic products. These cannot be digested in the digestive tract but can be used as probiotics [10]. Functional oligosaccharides are regarded as safe (GRAS) food components, they have several favorable health effects, especially the regulation of glucose homeostasis [5].
Xylooligosaccharides (XOS) are xylan hydrolytic products, which extracted from lignocellulosic materials (LCMs). XOS are β-1, 4-linked D-xylose units and XOS are prepared from natural sources [11]. XOS sweetness is nearly half the sweetness of sucrose [12, 13].
XOS have been reported to decrease levels of blood glucose and cholesterol [13], promise the growth of intestinal bacteria, mediate an anti-cancer, and stimulate the immune system effect [14]. In the food industry, XOS are utilized as a soluble dietary fiber, since they not hydrolysis by digestive systems [2].
These characteristics led to assess their application as a sweetener to diabetes mellitus patients that prohibited from consuming large quantities of sucrose. Nevertheless, the evidence about the potential influence of XOS as anti-diabetes is limited. Therefore, the objective of this work was to examine the effect of XOS from corncobs on blood glucose metabolism in HFD treated mice.

Materials and Methods

Materials

Mice samples including mice blood and fecal of mice were collected from mice fed one of the following diets; low-fat diet (LFD), high-fat diet (HFD), HFD plus 5 XOS (LXD), and HFD plus 10% XOS (HXD) [15]. Other chemicals, standards, and kits were procured from Sigma-Aldrich (St. Louis, MO, USA)

Glucose hormones measurements

Glucose levels in the blood were detected by using a glucometer. After sacrifice, the Plasma was collected by centrifuged blood with present EDTA as an anticoagulant for 15 min at 1000 xg at 5 ℃. After collection, it was stored at -80 °C. Insulin, and ghcagons-like pepfide 1 were determined using Mouse Insulin ELISA Kit, and Mouse ghcagons-like pepfide1 (GLP-1) ELISA Kit respectively.

Insulin resistance and sensitivity

Insulin resistance was assessed by using homeostasis model assessment (HOMA-IR) [fasting insulin (μU/mL) × fasting glucose [(mM/L)/22.5] [5]. Whereas, insulin sensitivity was measured by using quantitative insulin sensitivity check index (QUICKI) (1/log insulin (mU/L) + log glucose (mg/dL)) and homeostasis model assessment-2 (HOMA2) index using an online-based calculator on the Diabetes Trials Unit of the University of Oxford website (https://www.dtu.ox.ac.uk/homacalculator/), the β-cell function was calculated based on the HOMA index (HOMA-% β-cells (HOMA-%B) [16].

Determination free fatty acids in mice liver

Liver samples were grounded and then weighted into 50 ml of the falcon and 15 ml of chloroform-methanol (2:1) mixture and 3ml saline was added, after homogenization and centrifugation, the lower layer was collected and dried by nitrogen, and then internal standard (Heptadecanoic acid) was added.

After dried by nitrogen, fatty acids were converted to fatty acid methyl esters via methylation. Fatty acid methyl esters (FAME) were analyzed in GC equipped with an HP Innowax capillary column and flame ionization detector. Total fatty acids were calculated according to the amount of internal standard added to the samples before methylation [17].

Measurement of Short-Chain Fatty Acids (SCFAs)

The powder of freeze-dried faeces was vortexed with PBS buffer, after centrifugation, the supernatant was collected, and SCFAs in the supernatant was detected by Agilent 1260 High-Performance Liquid Chromatography equipped with an Aminex HPX-87H column and UV detector at 210 nm. H2SO4 (0.005 N) was used as a mobile phase [18].

Result

Effects of corncobs XOS on hyperglycemia and insulin resistance

The results of our previous study showed that levels of fasting glucose in LXD, and HXD groups were significantly lower than those of HFD [15].

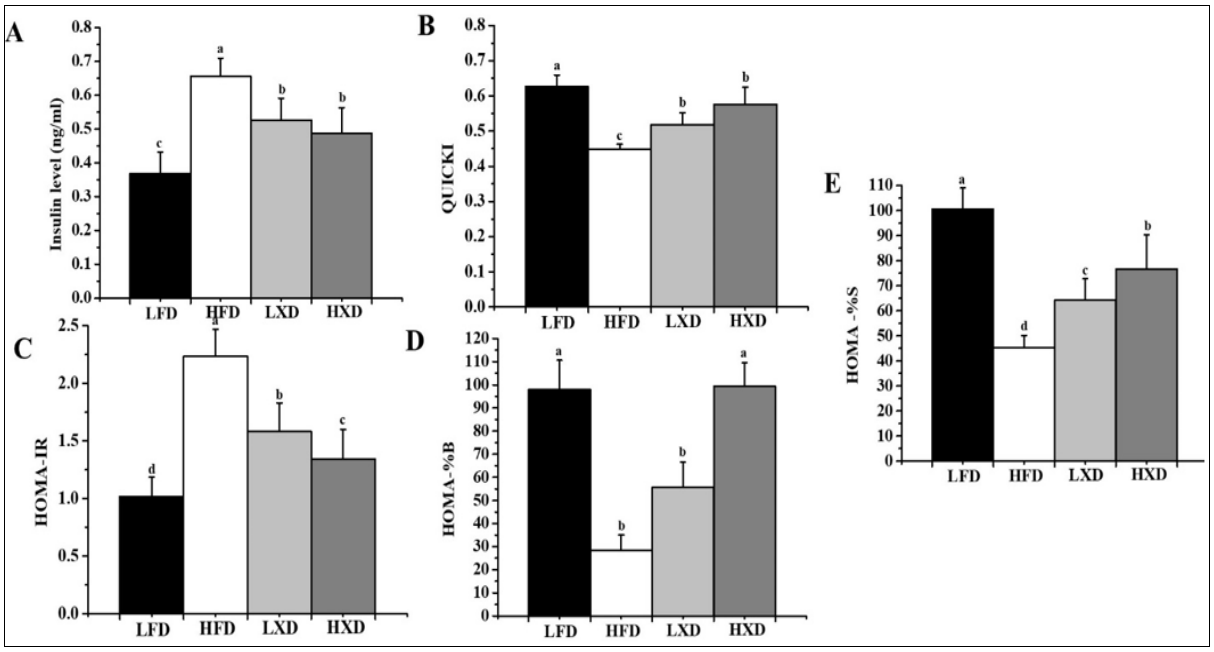

Fig 1: Effect of XOS on diabetes syndromes. A, B, C, D, E and F for insulin level, QUICKI index, HOMA-IR, HOMA-%B, and HOMA-S% respectively. LFD: mice fed LFD diet; HFD: mice fed HFD diet; LXD: mice fed LXD diet; HXD: mice fed HXD diet.

Based on fasting glucose and insulin results, the insulin resistance index was calculated by using the homeostasis model assessment (HOMA) index and quantitative insulin sensitivity check index (QUICKI).

The HOMA index significantly increased in the HFD group compared to the LFD group. In contrast, QUICKI was reduced (Fig.1B, C).

Interestingly, 12 weeks of consumption of XOS had significantly reduced the HOMA index. Besides, XOS group mice showed improved insulin sensitivity values, since, HOMA index was diminished, while the QUICKI index was increased by XOS addition (Fig.1B, C). Furthermore, the β-cell function was improved by XOS intervention (Fig.1D, E).

Effects of corncobs XOS on the gut hormone of GLP-1

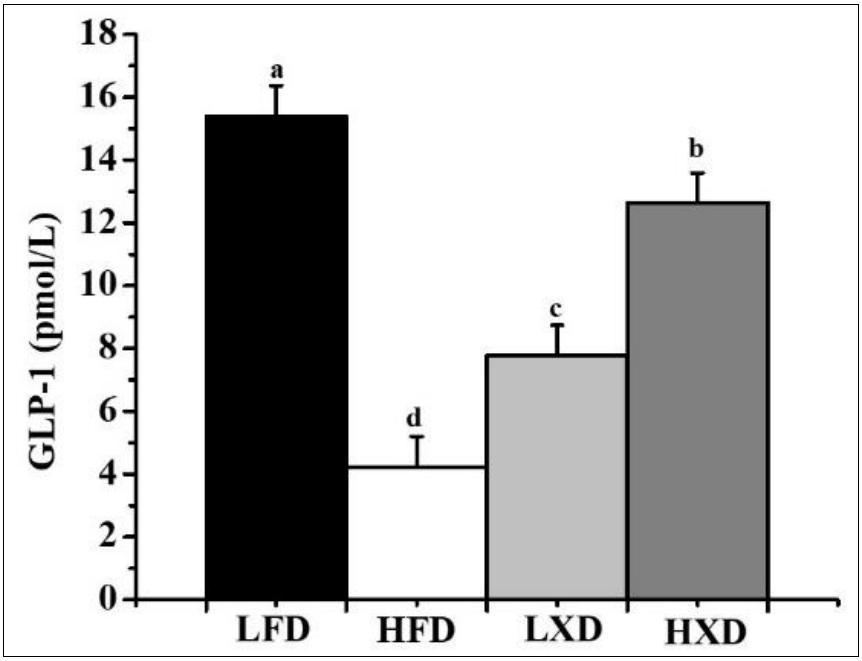

Fig 2: Effect of XOS on ghcagons-like pepfide 1 hormone (GLP-1). LFD: mice fed LFD diet; HFD: mice fed HFD diet; LXD: mice fed LXD diet; HXD: mice fed HXD diet.

The concentration of GLP-1 in the plasma remarkably lower in the HFD group compared to that in the LFD, LXD, and HXD groups, However, after 12 weeks of treatment, the LXD group showed significantly higher levels of GLP-1. Moreover, this hormone improved further in the HXD group (Fig.2). This result clearly displayed that XOS intake can effectively alleviate hyperglycemia induced by HFD via increasing GLP-1 level.

Effects of corncobs XOS on Hepatic FFAs

Table 1: Effect of XOS on Hepatic Free fatty acids (FFAs), saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and total FFAs. LFD: mice fed LFD diet; HFD: mice fed HFD diet; LXD: mice fed LXD diet; HXD: mice fed HXD diet.

Fatty acid	LFD	HFD	LXD	HXD	P-value
C10:0	0.132±0.063 ^{a}	0.373 ± 0.2^{a}	0.254 ± 0.15 ^{a}	0.206 ± 0.1 ^{a}	0.263
C12:0	0.58±0.15 ^{d}	1.593±0.25 ^{a}	1.002±0.2 ^{b}	0.843 ± 0.064 ^{cd}	0.001
C16:0	2.759±0.15 ^{d}	5.096±0.20 ^{c}	9.692±0.15 ^{a}	6.516±0.30 ^{b}	0.001
C18:0	1.966±0.20 ^{c}	11.256±0.15 ^{a}	2.513±0.20 ^{b}	2.199±0.07 ^{c}	＜0.001
C20:0	0.109±001 ^{b}	0.345±0.15 ^{a}	0.263±0.05 ^{a}	0.06±0.01 ^{b}	0.007
C22:0	0.575±0.20 ^{b}	3.927±0.20 ^{a}	0.922±0.15 ^{b}	0.582±0.20 ^{b}	＜0.001
C23:0	0.272±0.15 ^{b}	1.132±0.15 ^{a}	0.29±0.15 ^{b}	0.188±0.15 ^{b}	＜0.001
C24:0	5.084±0.20 ^{c}	10.478±0.15 ^{a}	6.767±0.15 ^{b}	5.212±0.02 ^{c}	＜0.001
Total SFAs	11.48±1.11 ^{d}	34.204±1.45 ^{a}	21.707±1.2 ^{b}	15.527±0.41 ^{c}	＜0.001
C14:1n-5	0.915±0.05 ^{c}	1.877±0.05 ^{a}	1.556±0.05 ^{b}	1.483±0.05 ^{b}	＜0.001
C16:1n-9	0.173±0.05 ^{c}	1.641±0.05 ^{a}	0.43±0.05 ^{b}	0.333±0.1 ^{b}	＜0.001
C18:1n-9	0.016±0.005 ^{c}	0.611±0.1 ^{a}	0.369±0.02 ^{b}	0.289±0.006 ^{b}	＜0.001
C20:1n-9	0.059±0.005 ^{c}	0.904±0.05 ^{a}	0.21±0.05 ^{b}	0.266±0.009 ^{b}	＜0.001
Total MUFAs	1.164 ±0.09 ^{d}	5.034±0.05 ^{a}	2.567±0.07 ^{b}	2.373±0.05 ^{c}	＜0.001
C18:2n-6	0.575±0.01 ^{a}	0.362±0.01 ^{ab}	0.304±0.25 ^{b}	0.361±0.02 ^{b}	0.115
C18:3n-3	0.448±0.04 ^{a}	0.385±0.01 ^{a}	0.39±0.02 ^{a}	0.37±0.02 ^{a}	0.96
C22:1n-9	0.879 ±0.05 ^{c}	4.466±0.1 ^{a}	1.489±0.15 ^{b}	1.314±0.05 ^{b}	＜0.001
C24:1n-9	4.553 ±0.15 ^{c}	8.679±0.1 ^{a}	5.174±0.1 ^{b}	4.657±0.08 ^{c}	＜0.001
C20:2n-6	0.882 ±0.05 ^{d}	11.003±0.1 ^{a}	1.54±0.1 ^{b}	1.223±0.1 ^{c}	＜0.001
C20:3n-6	3.18 ±0.1 ^{d}	7.1±0.1 ^{a}	3.826±0.05 ^{b}	3.477±0.06 ^{c}	＜0.001
C20:3n-3	1.081 ±0.1 ^{c}	2.655±0.1 ^{a}	2.583±0.1 ^{a}	2.215±0.002 ^{b}	＜0.001
Total PUFAs	11.601±0.06 ^{d}	34.653±0.51 ^{a}	15.308±0.75 ^{b}	13.62±0.24 ^{c}	＜0.001
Total FAs	24.246±1.08 ^{d}	73.89±1.91 ^{a}	39.583±1.87 ^{b}	31.522±0.67 ^{c}	＜0.001

Dietary XOS significantly influenced the liver fatty acid profile (Table 1). To be specific, XOS reduced total liver fatty acids by 46 and 57% for 5 and 10 % XOS respectively. This was characterized by a proportional decrease in SFAs, MUFAs, and PUFAs by 36-60%. Dietary XOS significantly decreased the liver of all individual FFAs by 17-88% except C10:0, C16:0, C18:2n-6, and C18:3n-3 did not affect by XOS addition.

Effects of corncobs XOS on fecal SCFAs

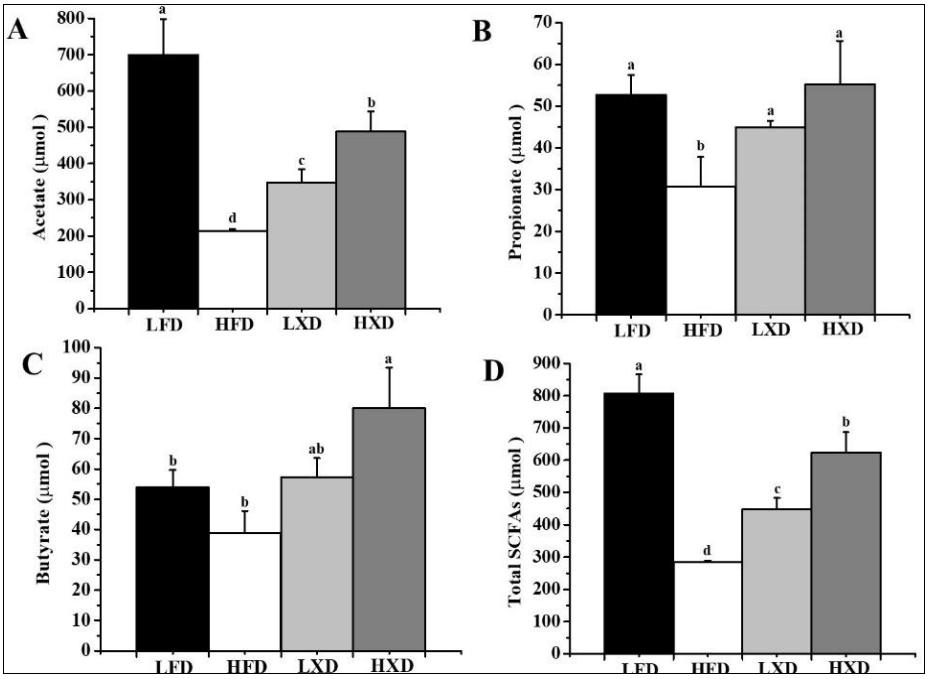

Fig 3: Effect of XOS on short chain fatty acids (SCFAs) production. A, B, C and D, for acetate, propionate, butyrate and total SCFAs respectively. LFD: mice fed LFD diet; HFD: mice fed HFD diet; LXD: mice fed LXD diet; HXD: mice fed HXD diet.

The supplementation of XOS was correlated with increases in levels of fecal acetate, propionate, and butyrate, and total SCFAs. Compared with the HCD group, XOS consumption increased acetate, propionate, and butyrate and total SCFAs by 38, 31, 32, 37% respectively with 5% XOS and by 56, 44, 51, and 54 with 10% XOS (Fig. 3).

Discussion

The consumption of long-term HFD caused insulin resistance and hyperglycosemia as indicated by increased blood glucose, and HOMA index. Metabolic stressors, such as high-fat diets, is reportedly connected with the elevation of insulin resistance, diabetes, and obesity in humans [19]. To explore the functional potential administration of XOS as a lifestyle approach for glucose metabolism regulation**,** we explored XOS effects on insulin resistance in HFD-fed mice. The results have shown that with XOS administration for 12 weeks, the insulin resistance was significantly moderated in HFD-fed mice. Furthermore, the HOMA index and QUICKI index were improved by XOS addition. Consistently, it has been shown that the intake of wheat bran XOS at 5% for 8 weeks led to decrease levels of fasting blood glucose in rats fed a high-fat diet [20]. In streptozotocin-induced diabetic rats, 10% of XOS supplementation for 5 weeks improved serum glucose elevation [13]. In type 2 diabetes mellitus patients, supplementation of XOS for 8 weeks could moderate the blood glucose [21]. Therefore, the present study and these two reports suggest that XOS are beneficial to diabetic patients in attenuating hyperglycemia severity. Connection with this, Antidiabetic effects of several dietary fibers in animal modules included up to 10% have been stated without any adverse effects [1]. Therefore, we used the 5% and 10% levels of XOS in our study.

GLP-1 stimulates biosynthesis and secretion of insulin from the pancreas, as well as it may stimulate uptake of glucose in cultured muscle cells, thus, it increases glucose use [22]. GLP-1 maintains β cell hemostasis via β cell proliferation promotion and suppression of β cell death [23, 24, 25]. Furthermore, GLP-1 exerts several biological actions independent of islet β cells such as glucagon secretion suppression [7]. In our study, the level of GLP-1 was increased by XOS addition, interestingly. Abnormal hepatic FFAs contents also are perceived as crucial phenomena to assess the progression of T2DM and obesity in human patients [2]. Since the higher FFAs levels have been associated with insulin resistance through inhibition of glycogen synthesis and insulin-stimulated glucose uptake [26]. Supporting with this, XOS in this study could decrease hepatic FFAs contents in HFD mice.

SCFAs are defined as dietary fiber end metabolites in the large intestine, are formed by gut flora. 5 SCFAs have been stated to have several health benefits including anti-insulin resistance [27]. In this connection, this study found that XOS supplementation could enhance fecal SCFAs production including acetate, propionate, and butyrate (Fig. 3). The mechanism associated with the alleviation of insulin resistance by XOS is probably mediated by improving SCFAs production. It established that SCFAs inhibited the weight gain of C57BL/6N mice through their effect on the formation of gut hormone [8]. Since they have stimulated gut hormones [28]. Thus, the increase of plasm GLP-1 levels in the XOS diet-fed mic was due to the effect of XOS on SCFAs production. Moreover, earlier research has stated a key role for SCFAs in modulating glucose homeostasis and decreasing lipid accumulation [28]. This study established that the boosted SCFAs in XOS-containing diet significantly improved insulin resistance. Overall, the supplement of XOS may drives redistribution of gut microbiome produced SCFAs to motivate the release of gut GLP-1 resulting in diminished insulin resistance. In summary, the current results found that preparations of XOS are favorable for improving the metabolic parameters of diabetes in obese mice.

Conclusion

In this study, the XOS supplement led to a significant diminution in HFD-induced insulin resistance. The

administration of XOS altered the distribution of gut metabolites, characterized by increase SCFAs including acetate, propionate, and butyrate. The altered SCFA distribution was may attributed to the increase in SCFAs-producing bacteria. Furthermore, the increased SCFAs motivated the release of GLP-1. The other mechanism (s) for the effects of XOS diets on the diabetes module should be assessed. Besides, the assessment application of XOS as a sweetener for diabetic patients is worthwhile.

Acknowledgments
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References

1. Gobinath D, Madhu AN, Prashant G, Srinivasan K, Prapulla SG. Beneficial effect of xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats. British Journal of Nutrition. 2010; 104(01):40-47.
2. Lim E, Lim JY, Kim E, Kim YS, Shin JH, Seok PR *et al*. Xylobiose, an Alternative Sweetener, Ameliorates Diabetes-Related Metabolic Changes by Regulating Hepatic Lipogenesis and miR-122a/33a in db/db Mice. Nutrients. 2016; 8(12):791.
3. Kubota T, Kubota N, Kadowaki T. Imbalanced insulin actions in obesity and type 2 diabetes: key mouse models of insulin signaling pathway. Cell metabolism. 2017; 25(4):797-810.
4. Cannon A, Handelsman Y, Heile M, Shannon M. Burden of illness in type 2 diabetes mellitus. Journal of managed care & specialty pharmacy. 2018; 24(9-a Suppl):S5-S13.
5. Zhu D, Yan Q, Li Y, Liu J, Liu H, Jiang Z *et al*. Effect of Konjac Mannan Oligosaccharides on Glucose Homeostasis via the Improvement of Insulin and Leptin Resistance *In Vitro* and In Vivo, Nutrients. 2019; 11, 1705.
6. Gastaldelli A, Gaggini M, Daniele G, Ciociaro D, Cersosimo E, Tripathy D *et al*. Exenatide improves both hepatic and adipose tissue insulin resistance: a dynamic positron emission tomography study. Hepatology. 2016; 64:2028-2037.
7. Jiang Y, Wang Z, Ma B, Fan L, Yi N, Lu B *et al*. GLP-1 improves adipocyte insulin sensitivity following induction of endoplasmic reticulum stress. Frontiers in pharmacology. 2018; 9:1168.
8. Weitkunat K, Stuhlmann C, Postel A, Rumberger S, Fankhänel M, Woting A *et al*. Short-chain fatty acids and inulin, but not guar gum, prevent diet-induced obesity and insulin resistance through differential mechanisms in mice. Scientific reports. 2017; 7(1):1-13.
9. Adefegha SA. Functional foods and nutraceuticals as dietary intervention in chronic diseases; novel perspectives for health promotion and disease prevention. J Diet Suppl. 2018; 15:977-1009.
10. Kim YA, Keogh JB, Clifton PM. Probiotics, prebiotics, synbiotics and insulin sensitivity. Nutr. Res. Rev. 2018; 31:35-51.
11. Abdo AAA, Zhang C, Lin Y, Kaddour B, Li X, Fan G *et al*. Nutritive sweetener of short-chain xylooligosaccharides improved the foam properties of hen egg white protein via glycosylation. Journal of Food Measurement and Characterization, 2020, 1-8.
12. Lee OS, Rhee IK. The production of xylooligosaccharides with microbial xylanase. Food Nutr. 2001; 6:21-24.
13. Ministry of Food and Drug Safety. Nutrition Labeling Information Legistration; Ministry of Food and Drug Safety: Cheongwon County, Korea, 2016, 1-164.
14. Imaizumi K, Nakatsu Y, Sato M, Sedarnawati Y, Sugano M. Effects of xylooligosaccharides on blood glucose, serum and liver lipids and cecum short-chain fatty acids in diabetic rats. Agric. Biol. Chem. 1991; 55:199-205.
15. Aachary AA, Prapulla SG. Corncob-induced endo-1, 4--D-xylanase of aspergillus oryzae MTCC 5154: production and characterization of xylobiose from glucuronoxylan. Journal of Agricultural and Food Chemistry. 2008; 56:3981-3988.
16. Zhang C, Abdo AAA, Kaddour B, Wu Q, Xin L, Li X *et al*. Xylan-oligosaccharides ameliorate high fat diet induced obesity and glucose intolerance and modulate plasma lipid profile and gut microbiota in mice. Journal of Functional Foods. 2020; 64:103622.
17. Avtanski D, Pavlov V, Tracey K, Poretsky L. Characterization of inflammation and insulin resistance in high fat diet-induced male C57BL/6J mouse model of obesity. Animal Model Exp Med. 2019; 2:252-258.
18. Lei L, Li YM, Wang X, Liu Y, Ma KY, Wang L *et al*. Plasma triacylglycerol-lowering activity of citrus polymethoxylated flavones is mediated by modulating the genes involved in lipid metabolism in hamsters. European Journal of Lipid Science and Technology. 2016; 118(2):147-156.
19. Nagpal R, Wang S, Ahmadi S, Hayes J, Gagliano J, Subashchandrabose S *et al*. Human-origin probiotic cocktail increases short-chain fatty acid production via modulation of mice and human gut microbiome. Sci. Rep. 2018; 8(1):1-15.
20. Tierney AC, Mc Monagle J, Shaw DI, Gulseth HL, Helal O, Saris WH *et al*. Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome-LIPGENE: a European randomized dietary intervention study. International journal of obesity. 2011; 35(6):800-809.
21. Wang J, Cao Y, Wang C, Sun B. Wheat bran xylooligosaccharides improve blood lipid metabolism and antioxidant status in rats fed a high-fat diet. Carbohydrate Polymers. 2011; 86(3):1192-1197.
22. Sheu WH, Lee IT, Chen W, Chan YC. Effects of xylooligosaccharides intype2diabetesmellitus. J. Nutr. Sci. Vitaminol. (Tokyo). 2008; 54:396-401.
23. Green CJ, Henriksen TI, Pedersen BK, Solomon TPJ. Glucagon Like Peptide-1-Induced Glucose Metabolism in Differentiated Human Muscle Satellite Cells Is Attenuated by Hyperglycemia. PLoS ONE. 2012; 7(8):e44284.
24. Kumar M, Hunag Y, Glinka Y, Prud'homme GJ, Wang

Q. Gene therapy of diabetes using a novel GLP-1/IgG1-Fc fusion construct normalizes glucose levels in db/db mice. Gene Ther. 2007; 14:162-172.
25. Soltani N, Kumar M, Glinka Y, Prud'Homme GJ, Wang Q. In vivo expression of GLP-1/IgG-Fc fusion protein enhances beta-cell mass and protects against streptozotocin-induced diabetes. Gene therapy. 2007; 14(12):981-988.
26. Wang Q, Chen K, Liu R, Zhao F, Gupta S, Zhang N *et al*. Novel GLP-1 fusion chimera as potent long acting GLP-1 receptor agonist. PloS one. 2010; 5:e12734.
27. Ni Y, Zhao L, Yu H, Ma X, Bao Y, Rajani C *et al.* Circulating unsaturated fatty acids delineate the metabolic status of obese individuals. E Bio Medicine. 2015; 2(10):1513-1522.
28. Hao W, He Z, Zhu H, Liu J, Kwek E, Zhao Y *et al*. Sea buckthorn seed oil reduces blood cholesterol and modulates gut microbiota. Food & function. 2019; 10(9):5669-5681.
29. Si X, Shang W, Zhou Z, Shui G, Lam SM, Blanchard C *et al*. Gamma-aminobutyric Acid Enriched Rice Bran Diet Attenuates Insulin Resistance and Balances Energy Expenditure via Modification of Gut Microbiota and Short-Chain Fatty Acids. Journal of Agricultural and Food Chemistry. 2018; 66(4):881-890.