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D-Psicose, a sugar substitute, suppresses body fat deposition by altering networks of inflammatory response and lipid metabolism in C57BL/6J-*ob*/*ob* mice

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ABSTRACT

D-Psicose, a rare sugar, not only has very low caloric value but possesses anti-adipogenic properties. Here, we identified target genes in adipose tissue affected by D-psicose by transcriptomic analysis and provided mechanistic explanations for the anti-adipogenic effect. C57BL/6J *ob*/*ob* mice were fed with a control or 5% D-psicose diet for 12 weeks. D-Psicose decreased final body weight, adipose tissue mass, adipocyte size, and serum total cholesterol levels. We identified 103 differentially expressed genes involved in inflammatory response, molecular transport, and lipid metabolism consequent to D-psicose administration. Genes related to inflammation and adipo/lipogenesis were significantly down-regulated, whereas those associated with β-oxidation were up-regulated by D-psicose. Our data suggest that *Fos*, *Mmp3*, *Fgf21*, and *Abcd2* might be key target genes associated with D-psicose-induced changes in lipid metabolism and subsequent chronic inflammatory responses. D-psicose is thus a promising sugar substitute possessing a direct gene-regulatory function related to the suppression of body fat deposition.

1. Introduction

The increasing prevalence of obesity represents a serious public health concern in both developed and developing countries (Ogden, Carroll, Fryar, & Flegal, 2015; Prentice, 2006). Obesity is characterized by the expansion of adipose tissue mass resulting from an increase in the size and, to a lesser extent, in the number of adipocytes (Bluher, 2009). Adipocyte size is positively related to the expression of adipogenic genes including peroxisome proliferator-activated receptor-gamma (PPAR-γ) and CCAAT/enhancer-binding proteins (C/EBPs) (Farnier et al., 2003; Jernas et al., 2006). Increased adipocyte size is also associated with susceptibility to inflammation, which, in turn, may impair insulin sensitivity and increase lipolysis in adipocytes (Hotamisligil, Shargill, & Spiegelman, 1993; Masoodi, Kuda, Rossmeisl, Flachs, & Kopecky, 2015; Pietilainen et al., 2011; Weisberg et al., 2003; Zhang, Halbleib, Ahmad, Manganiello, & Greenberg, 2002). Obesity-associated chronic low-grade inflammation of adipose tissue leads to abnormal intermediate conditions including hyperinsulinemia, hyperglycemia, and dyslipidemia, resulting in the development of obesity-related metabolic complications (Masoodi et al., 2015; Tchernof & Despres, 2013).

Excess intake of added sugar has been shown to increase visceral fat content and the risk of obesity, metabolic changes, and type II diabetes (Forshee, Anderson, & Storey, 2008; Malik et al., 2010; Stanhope et al., 2009). In an attempt to decrease sugar intake, rare sugars, i.e., monosaccharides and their derivatives that exist in limited quantities in nature, have been recently developed as sugar substitutes. D-Psicose, a C-3 epimer of D-fructose, is approximately 70% as sweet as sucrose but has no calories, and its mass production is possible using immobilized D-tagatose-3-epimerase (Takeshita, Suga, Takada, & Izumori, 2000). D-Psicose is

Abbreviations: *Abcd2*, ATP-binding cassette, sub-family D, member 2; aP2, adipocyte protein 2; C/EBP-α, CCAAT/enhancer-binding protein-alpha; CPT-1, carnitine palmitoyltransferase-1; FAS, fatty acid synthase; FC, fold change; *Fgf21*, fibroblast growth factor 21; *Fos*, FBJ murine osteosarcoma viral oncogene homolog; HSL, hormone-sensitive lipase; IL-6, interleukin-6; LPL, lipoprotein lipase; MCP-1, monocyte chemoattractant protein-1; *Mmp3*, matrix metallopeptidase 3; PPAR-α, peroxisome proliferator-activated receptor-alpha; PPAR-γ, peroxisome proliferator-activated receptor-gamma; qPCR, quantitative polymerase chain reaction; SREBP-1c, sterol regulatory element-binding protein-1c; TNF-α, tumor necrosis factor-alpha.

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absorbed in the small intestine without being metabolized into energy and is not easily fermented in the colon (Iida et al., 2010). Notably, it has been shown that D-psicose possesses hypoglycemic, hypolipidemic, and antioxidant effects (Chung, Oh, & Lee, 2012). In addition, growing evidence from animal and clinical studies suggests that D-psicose contributes to a reduction in body weight and fat accumulation (Chung, Hyun Lee, et al., 2012; Hayashi et al., 2014; Iida et al., 2013; Matsuo et al., 2001b; Ochiai, Nakanishi, Yamada, Iida, & Matsuo, 2013). Furthermore, D-psicose supplementation is reported to lower hepatic lipogenic enzyme activities and abdominal fat accumulation in Wistar rats (Matsuo et al., 2001a). Additionally, a recent study showed that D-psicose favorably influences lipid metabolism and increases 24-h energy expenditure in Sprague-Dawley rats (Nagata, Kanasaki, Tamaru, & Tanaka, 2015). Accordingly, D-psicose appears to be beneficial in controlling body weight and preventing fat accumulation, especially in people with obesity and obesity-related diseases. However, the mechanisms of the anti-obesity and anti-adipogenic effects of D-psicose remain poorly understood.

In the present study, we investigated the effect of dietary D-psicose substitution for sucrose on adipose tissue fat deposition in C57BL/6J-*ob/ob* mice, an accepted model of obesity and related syndromes (Montague et al., 1997). To better elucidate the molecular mechanisms mediated by D-psicose in adipose tissue, we further identified target genes associated with the anti-adipogenic activity of D-psicose using transcriptomics.

2. Materials and methods

2.1. Animals and study design

Six-week-old male C57BL/6J-*ob/ob* mice were purchased from Central Laboratory Animal, Inc. (Seoul, Republic of Korea) and maintained in air-conditioned quarters with a 12-h light/dark cycle. After acclimatization to a chow diet for one week, the mice were randomly assigned to receive either the AIN-93G diet (Control) or the 5% D-psicose diet (D-Psicose) (n = 15 per dietary group). The D-psicose diet was prepared by replacing sucrose in the control diet with powdered D-psicose of 99.83% purity (Samyang Genex Corporation, Daejeon, Republic of Korea). The detailed compositions of the diets are presented in Table 1. The diets used in this study were designed to be isocaloric to demonstrate anti-adipogenic effects of D-psicose. Both diets contained 3896 kcal (64.9% w/w carbohydrate, 18.8% w/w protein, and 16.4% w/w fat), and the missing caloric content in the D-psicose group was adjusted by substituting corn starch with fiber in the control group. The experimental diets and water were provided *ad libitum* for 12 weeks. Body weight and food intake were recorded twice a week. All care, maintenance, and treatment protocols were approved by the Institutional Animal Care and Use Committee of Sookmyung Women's University (SM-IAUC-2013-0917-033). During the experiment, 9 mice (n = 7 for the control group; n = 2 for the D-Psicose group) were lost owing to the onset of diabetic complications. At necropsy, blood and white adipose tissue samples (n = 8 for the control group; n = 13 for the D-Psicose group) were collected. Serum was separated by centrifuging whole blood at 650*g* for 20 min and stored at −80 °C until analysis. The white adipose tissues (mesenteric, perirenal, and epididymal) were excised immediately, rinsed, weighed, frozen on dry ice, and stored at −80 °C until analysis.

2.2. Serum lipid profile measurements

Serum concentrations of triacylglycerol, non-esterified fatty acids, and total cholesterol were determined using commercial assay kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan) according to the manufacture's protocols. The serum concentrations of high-density lipoprotein (HDL)-cholesterol and low-density lipoprotein (LDL)-cholesterol and HDL/LDL-cholesterol ratio were measured using a colorimetric/fluorometric assay kit (BioVision, Inc., Milpitas, CA, USA).

Table 1
Composition of the experimental diets (g/kg)[a].

Ingredients	Control	D-Psicose
Cornstarch	397.5	445
Dextrin	132	132
Sucrose	100	50
D-Psicose	–	50
Fiber	50	2.5
Casein	200	200
L-Cysteine	3	3
Corn oil	70	70
Mineral mix [b]	35	35
Vitamin mix [b]	10	10
Choline bitartrate	2.5	2.5
t-butylhydroquinone	0.014	0.014
Total (g)	1000	1000
Carbohydrates, % energy	64.9	64.9
Protein, % energy	18.8	18.8
Fat, % energy	16.4	16.4
Total energy, kcal/kg	3896	3896

[a] Diets were prepared according to the AIN-93G diet.
[b] Mineral and vitamin mixtures were prepared according to the AIN-93G diet.

2.3. Histological and immunohistological analyses

Following the fixation of adipose tissue with 10% buffered formalin solution, tissues were dehydrated in ethanol and then embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin for histologic analysis. For adipocyte size measurements, the H&E stained sections were scanned on an Aperio ScanScope AT and viewed on Aperio Image Scope (Leica Biosystems, Buffalo Grove, IL, USA). The longest diameter of each fat cell was measured from four mice per dietary group and the adipocyte volume was determined as the cube of the diameter. For immunofluorescence analysis, adipose tissue sections were deparaffinized, rehydrated, and labeled with rat monoclonal anti-F4/80 antibody (1:200, Bio-Rad Laboratories, Inc., Hercules, CA, USA) followed by incubation with FITC-conjugated goat anti-rat IgG antibody (1:250, Abcam, Cambridge, MA, USA). All images were obtained with an INFINITY3-3UR camera (Lumenera, Ottawa, ON, Canada) on a BX43 microscope (Olympus, Tokyo, Japan).

2.4. Microarray analysis

Total RNA from adipose tissue samples was isolated using TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA) and purified using RNeasy columns (Qiagen, Valencia, CA, USA) according to the manufacturers' protocols. After processing with DNase digestion, RNA was analyzed on a Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), a microfluidics-based platform, for sizing, quantification, and quality assessment of the RNA. The RNA Integrity Number (RIN) score was generated using Agilent software. For microarray analysis, the RIN scores for all of the samples were $\geqslant$7. The Illumina TotalPrepTM-96 RNA Amplification Kit (Ambion, Austin, TX, USA) was used to yield biotinylated, amplified cRNA according to the manufacturer's protocol. The cRNA yield was quantified using an ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

A total of 750 ng of purified biotinylated cRNA for each sample was hybridized onto the Illumina MouseRef-8 v2 Expression BeadChip (Illumina, Inc., San Diego, CA, USA) which targets

approximately 25,600 annotated RefSeq transcripts over 19,100 genes. After washing and staining, each BeadChip was scanned with the Illumina BeadArray Reader according to the manufacturer's instructions. All data analysis and visualization of differentially expressed genes were conducted using R version 2.15.3 (www.r-project.org). Raw data were extracted using GenomeStudio version 2011.1 (Illumina, Gene Expression Module version 1.9.0), and probe signal values were transformed logarithmically and normalized by the quantile method. For comparison between the two dietary groups, an independent *t*-test using a false discovery rate Benjamini and Hochberg multiple testing correction with a *p*-value cut-off of 0.05 was performed.

2.5. Network analysis

The gene expression network analysis was generated using QIAGEN's Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, www.qiagen.com/ingenuity) to identify regulatory relationships between differentially expressed genes in response to D-psicose supplementation. Genes with a fold change (FC) of >1.5 or <−1.5 and $p < 0.05$ were compared with the Ingenuity Knowledge Base including biological interaction data on more than 19,600 human and 15,100 mouse genes. The right-tailed Fisher's exact test was used to calculate the *p*-value in determining the probability that each biological function and/or disease assigned to a specific network was due to chance alone.

2.6. Real-time quantitative polymerase chain reaction (qPCR) analysis

Template RNA isolated from adipose tissue was reverse-transcribed using a cDNA Synthesis kit (PhileKorea Technology, Seoul, Republic of Korea). Real-time qPCR was performed on a 7500 Fast Real Time PCR system (Applied Biosystems, Foster City, CA, USA) using a QuantiMix SYBR kit (PhileKorea Technology). The primer sequences for the selected genes are presented in Table S1. The PCR conditions were 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. All signals were normalized to mRNA levels of β-actin as an internal control. Relative gene expression data were analyzed using the comparative threshold (Ct) method (Livak & Schmittgen, 2001).

2.7. Statistical analysis

Differences between the two dietary groups were analyzed using the Student's *t*-test function of SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). For adipocyte size, comparison between the two dietary groups was performed using the Wilcoxon rank-sum test. The results were considered statistically significant when two-tailed $p < 0.05$.

3. Results

3.1. Food intake, body weight, and serum lipid concentrations

The food intake and initial body weight did not significantly differ between the two dietary groups, whereas the final body weight of obese mice fed the D-psicose-supplemented diet was lower compared with that of the control group ($p = 0.019$) (Table 2). The obese mice fed a diet supplemented with 5% D-psicose exhibited lower total cholesterol ($p = 0.039$) and LDL-cholesterol ($p = 0.050$) concentrations and higher HDL/LDL-cholesterol ratio ($p = 0.042$) compared with those of the control group (Table 2). Serum concentrations of triacylglycerol, non-esterified fatty acids, and HDL-cholesterol were not significantly different between groups (Table 2).

Table 2
Food intake, body weight, serum lipid concentrations, and white adipose tissue weights in C57BL/6J-*ob/ob* mice fed a control or 5% D-psicose-supplemented diet for 12 weeks.

	Control	D-Psicose
Food intake (g/day)	4.74 ± 0.39	4.17 ± 0.40
Body weight (g)		
Initial body weight	39.67 ± 1.40	38.06 ± 1.26
Final body weight	55.77 ± 1.48	50.99 ± 1.01*
Serum lipid concentrations		
Triacylglycerol (mg/dL)	108.13 ± 3.65	111.78 ± 5.84
Non-esterified fatty acids (mEq/L)	1.03 ± 0.10	0.90 ± 0.10
Total cholesterol (mg/dL)	376.93 ± 29.46	293.41 ± 22.18*
HDL-cholesterol (mg/dL)	118.16 ± 13.14	113.49 ± 3.94
LDL-cholesterol (mg/dL)	247.39 ± 39.65	168.45 ± 17.33
HDL/LDL-cholesterol	0.56 ± 0.09	0.73 ± 0.06*
White adipose tissue weight (% of body weight)		
Mesenteric fat	3.06 ± 0.10	2.60 ± 0.11*
Perirenal fat	7.01 ± 0.25	6.16 ± 0.20*
Epididymal fat	5.25 ± 0.18	5.15 ± 0.18
Total	15.31 ± 0.33	13.91 ± 0.28*

Data are expressed as the mean ± SEM (n = 8 for the control diet group; n = 13 for the D-psicose diet group).
* $p < 0.05$, statistically significant compared with the control diet.

3.2. Adipose tissue weight and adipocyte size

D-Psicose supplementation significantly decreased the weight of white adipose tissues including mesenteric, perirenal, and epididymal fat compared with those of the control group ($p = 0.006$) (Table 2). The D-psicose-supplemented diet group also showed lower weights of mesenteric ($p = 0.008$) and perirenal ($p = 0.021$) fat compared with the control group, whereas epididymal fat weight did not significantly differ between groups (Table 2). Furthermore, adipocyte size was significantly reduced in obese mice fed the D-psicose-supplemented diet ($p < 0.001$) (Fig. 1).

3.3. Effect of D-psicose on genome-wide gene expression profiles in the adipose tissue of obese mice

To better understand the altered serum lipid profile and decreased adipose tissue weight and adipocyte size in response to D-psicose supplementation, we next performed genome-wide analysis of gene expression in the adipose tissue of C57BL/6J-*ob/ob* mice using the Illumina MouseRef-8 platform. We identified 64 down-regulated and 39 up-regulated genes affected by D-psicose supplementation. The list of genes differentially expressed in response to D-psicose supplementation is presented in Table S2. From these genes, we further analyzed 103 differentially expressed genes using IPA for network analysis to identify significant networks and functions associated with D-psicose supplementation. The top 5 networks with the highest scores and their associated top functions are shown in Table 3. These networks were associated with inflammatory response (Network 1), molecular transport (Network 2), and lipid metabolism (Networks 3 and 4). The list of D-psicose-responsive genes associated with inflammatory response, molecular transport, and lipid metabolism is presented in Table S3.

Network 1 was the most significant network with the highest score and was mainly composed of down-regulated genes associated with inflammatory response, cellular development, and hematopoiesis (Table 3; Fig. 2a). The IPA upstream regulator analysis predicted that altered expression of *Cd44* would lead to the inhibition of *Adam8* (ADAM metallopeptidase domain 8), *Adgre1* (adhesion G protein-coupled receptor E1, also known as *Emr1*), *Cd36*, *Col3a1* (collagen, type III, alpha 1), *Mmp3* (matrix

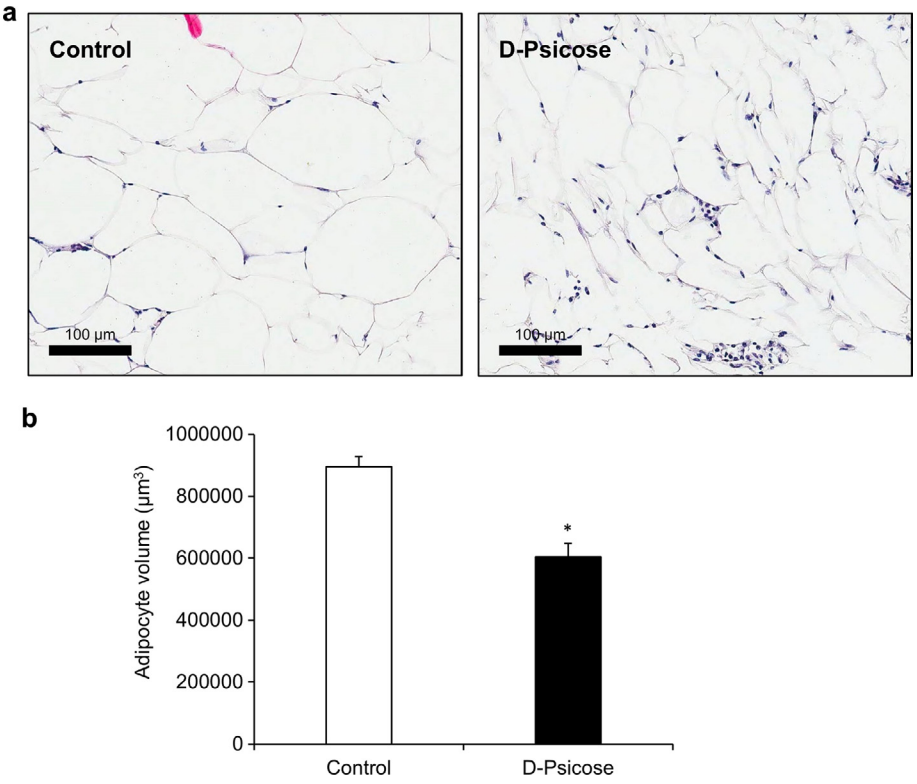

Fig. 1. Effects of D-psicose supplementation on adipocyte morphology and size in C57BL/6J-*ob/ob* mice. Representative histological sections of white adipose tissue (a) and relative adipocyte size in histological sections (b) were evaluated. Adipose tissue sections obtained at the end of 12 weeks were stained with hematoxylin and eosin (magnification × 400). Each bar represents the mean ± SEM (n = 4 per dietary group). $^{*}p < 0.001$ compared with the control diet.

Table 3
Top networks of genes differentially expressed in the adipose tissue of C57BL/6J-*ob/ob* mice in response to D-psicose supplementation.

No	Top functions	Score[a]	Focus Genes[b]	Genes in network
1	Inflammatory Response, Cellular Development, Hematopoiesis	39	22	***Adam8****, ***Adam12***, ***Adgre1***, *Akt*, *Alpha catenin*, ***Atf3***, *Bcr (complex)*, ***Blnk***, *Cd3e*, ***Cd9***, ***Cd36***, ***Cd44***, ***Col3a1***, *Erk*, *ERK1/2*, ***Fos***, *IgM*, *Il12 (family)*, ***Il7r***, *Immunoglobulin*, *Jnk*, ***Lat2***, ***Lum***, ***Lyz***, ***Ms4a6a****, *Nfkb (complex)*, *p38 MAPK*, *PI3K (complex)*, ***Pik3r1***, ***Rab7b***, ***Rgs1***, ***Satb1***, ***Tlr8***, ***Tlr13***, ***Tpt1***
2	Molecular Transport, Cellular Development, Cellular Growth and Proliferation	25	16	***Abcd2***, ***Aldh1a1***, *Aqp3*, *Aqp8*, ***Ar***, *Asap1*, ***Cma1****, ***Cpa3****, ***Cux2***, ***Dpp4***, ***Ebf1***, ***Ehd3***, ***Fos***, *Gsr*, ***Gsta4***, *Gstm1*, *Hipk3*, ***Hoxb4***, *Hsd17b10*, *Lgals4*, ***Map1b***, *Mapk8*, ***Mmp3****, *Nat6*, *Ndst2*, *Nsdhl*, *Ntf4*, *Por*, *Ppara*, ***Ripk4***, ***Slc2a3***, *Stat5b*, *Tnf*, *Xpa*, *Yy1*
3	Lipid Metabolism, Small Molecule Biochemistry, Molecular Transport	21	14	*Acadvl*, *Acox1*, ***Adam8****, ***Anxa8***, ***Ces1***, *Col18a1*, ***Dpp4***, *Ehhadh*, ***Emp3***, *Esr2*, *Fabp3*, ***Ffar4***, *G6pd*, ***Gsta3***, *H2afy*, *Htt*, *Lep*, ***Prodh***, *Mki67*, *Ncoa6*, *Pawr*, ***Ppp1r3c***, ***Pxmp2***, ***Qpct***, ***Slc5a7***, *Sptlc2*, *Srsf2*, ***Thrsp***, ***Timp1***, *Timp2*, *Timp3*, *Tp53*, *Tpm2*, *Trib3*, *Trpv1*
4	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry	19	13	*ADRB*, *Apoc2*, ***Ca3***, ***Cd68***, ***Cd163***, *Ctnnb1*, ***Ctsk***, *Cycs*, *F2r*, *Gnas*, ***Gpr137b***, *HK2*, ***H2-DMb***, *Hmgn3*, *Il13*, ***Lilrb4****, *Lipa*, *Lrp6*, *Mal*, *Mc4r*, *Me1*, ***Mup1 (includes others)****, *Myh6*, *Nr1h*, *Oma1*, ***Ppargc1a***, *Proinsulin*, ***Ptges***, ***Pvalb***, *Rbp4*, *Rhox5*, *Slc13a1*, ***Timp4***, *Tlr4*, ***Trem2***
5	Hematological System Development and Function, Tissue Morphology, Cellular Development	15	11	***Anpep***, *Atf4*, *Btla*, ***Cd84***, *Cd244*, *CD8a*, ***Chst8***, *Def6*, *Erap1*, *Fcgr1a*, ***Fgf21***, ***Gpnmb****, ***Gm10499***, ***HPX***, *Ifng*, *IgG*, *IgG1*, *Igll1*, *Il1r*, *Il17a*, *Il23a*, *Il2rg*, *Il36g*, *Itch*, ***Lcp1***, *Nfat5*, *Nfil3*, ***Plcd1***, *Plp1*, ***Slc6a6***, ***Stc2***, *Tapbp*, *Tcirg1*, *Tnfsf13b*, *Traf3ip2*

An asterisk (*) indicates that a given gene is represented in the microarray set with multiple identifiers.
[a] The score indicates the likelihood of the Focus Genes in a network being found together owing to random chance.
[b] The Focus Genes (bold) indicate the uploaded genes of interest for which information is available in the Ingenuity Knowledge Base.

metallopeptidase 3), and *Tlr8 (toll-like receptor 8)* in response to D-psicose supplementation in the adipose tissue of obese mice (p = 5.69E−05) (Fig. 2b). Network 2 was associated with molecular transport, cellular development, and cellular growth and proliferation, whereas genes in Networks 3 and 4 were associated with lipid metabolism, small molecule biochemistry, and molecular transport (Table 3). We also validated the microarray-based expression results of selected top network genes associated with inflammation and lipid metabolism by real-time qPCR. The mRNA expression of all of the selected genes was consistent with the microarray results; in particular, according to the array analysis, in the D-psicose-supplemented diet group, there was a decrease in the mRNA expression of down-regulated genes including *Adam12* (ADAM metallopeptidase domain 12, FC −1.58), *Adgre1* (FC −1.54), *Cd36* (FC −1.73), *Cd44* (FC −1.56), *Fos* (FBJ murine osteosarcoma viral oncogene homolog, FC −1.68), *Il7r* (interleukin 7 receptor, FC −1.64), *Tlr8* (FC −1.51), *Mmp3* (FC −1.84), *Cd68* (FC −1.58), and *Fgf21* (fibroblast growth factor 21, FC −1.55), whereas there was an increase in the mRNA expression of up-regulated genes including *Abcd2* (ATP-binding cassette, sub-family D (ALD),

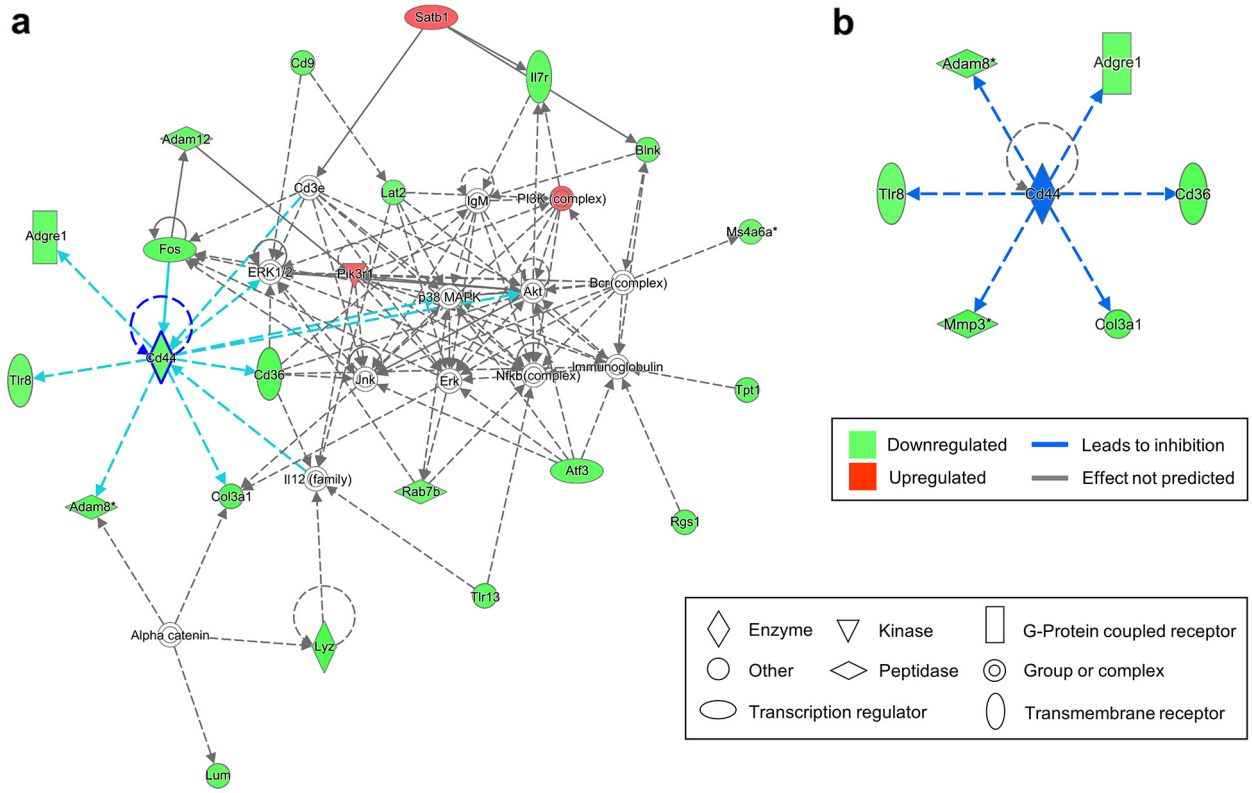

Fig. 2. Network analysis of genes differentially expressed in the adipose tissue in response to D-psicose supplementation in C57BL/6J-*ob*/*ob* mice. The most significant network was associated with inflammatory response, cellular development, and hematopoiesis (a). Upstream regulator analysis predicted that the change in CD44 expression would lead to the inhibition of several genes including *Adgre1*, *Cd36*, *Mmp3*, and *Tlr8* in response to D-psicose supplementation (b). Genes are represented as nodes with different shapes. The solid and dotted arrows indicate direct and indirect interactions, respectively. Genes in uncolored notes were not identified as differentially expressed in this study. An asterisk indicates that a given gene is represented in the microarray set with multiple identifiers.

member 2, FC 1.62) and *Ffar4* (free fatty acid receptor 4, FC 1.54) (Table 4).

3.4. Effects of D-psicose on expression of genes involved in inflammation and lipid metabolism and macrophage infiltration in adipose tissue of obese mice

To further investigate the molecular mechanism underlying the effects of D-psicose in adipose tissue metabolism, we determined the mRNA expression of genes relevant to inflammation and lipid metabolism, which were the top networks influenced by D-psicose supplementation, in the adipose tissue of obese mice by real-time qPCR. Adipose tissue expression of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), representative cytokines in obesity-related inflammation (Fried, Bunkin, & Greenberg, 1998; Hotamisligil et al., 1993), were reduced in obese mice fed the D-psicose-supplemented diet compared with those in the control group (TNF-α, $p = 0.007$; IL-6, $p = 0.032$) (Table 5). D-Psicose supplementation also decreased the expression of monocyte chemoattractant protein-1 (MCP-1), an important chemokine for macrophage infiltration into adipose tissue (Kamei et al., 2006) ($p = 0.019$) (Table 5). In addition, immunofluorescence analysis demonstrated that expression of F4/80, a macrophage marker encoded by *Emr1*, was significantly reduced in the D-psicose diet group compared with the control diet group (Fig. 3). These results were in parallel with the down-regulated inflammatory response observed in the network analysis (Table 3; Fig. 2).

The mRNA expression levels of PPAR-α, PPAR-γ, C/EBP-α, sterol regulatory element-binding protein-1c (SREBP-1c), adipocyte protein 2 (aP2), and lipoprotein lipase (LPL), which are associated with adipogenesis (adipocyte differentiation), were significantly reduced in the D-psicose-supplemented diet group (PPAR-α, $p = 0.019$; PPAR-γ, $p = 0.035$; C/EBP-α, $p = 0.029$; SREBP-1c, $p = 0.033$; aP2, $p = 0.029$; LPL, $p = 0.004$) (Table 5). Expression of lipogenic gene fatty acid synthase (FAS), which is regulated by SREBP-1c, was also significantly decreased in response to D-psicose supplementation ($p = 0.031$) (Table 5), whereas expression of hormone-sensitive lipase (HSL) and carnitine palmitoyltransferase-1 (CPT-1), which are involved in lipolysis and β–oxidation, respectively, was significantly increased in the D-psicose group compared with the control group (HSL, $p = 0.027$; CPT-1, $p = 0.030$) (Table 5). These results indicated that the D-psicose-supplemented diet was associated with decreased adipo/lipogenesis and increased β–oxidation.

4. Discussion

In the present study, D-psicose supplementation decreased final body weight, adipose tissue weight, and adipocyte size, even though food intake did not differ between the groups. These results suggest that D-psicose contributes to reduction of body weight and fat accumulation as reported in previous studies (Chung, Lee, et al., 2012; Hayashi et al., 2014; Iida et al., 2013; Matsuo et al., 2001a, 2001b; Ochiai et al., 2013). In addition, we demonstrated that D-psicose supplementation improved the serum lipid profile by reducing the concentrations of total cholesterol and LDL-cholesterol and by increasing the HDL/LDL-cholesterol ratio, and it decreased the expression of the macrophage marker F4/80. These findings are consistent with previous evidence that adipocyte size is an important predictor for abnormal intermediate conditions

Table 4
mRNA expression of selected top network genes in the adipose tissue of C57BL/6J-*ob*/*ob* mice.

Network No	Gene	Description	Microarray Fold change (D-Psicose vs. Control)	Real-time qPCR Relative fold change[a]	
				Control	D-Psicose
1	*Adam12*	ADAM metallopeptidase domain 12	−1.58	1.00 ± 0.03	0.67 ± 0.07***
	Adgre1	Adhesion G protein-coupled receptor E1	−1.54	1.28 ± 0.33	0.53 ± 0.08*
	Cd36	CD36 molecule	−1.73	1.01 ± 0.05	0.87 ± 0.03*
	Cd44	CD44 molecule	−1.56	0.97 ± 0.05	0.76 ± 0.05**
	Fos	FBJ murine osteosarcoma viral oncogene homolog	−1.68	0.99 ± 0.07	0.65 ± 0.06**
	Il7r	Interleukin 7 receptor	−1.64	1.21 ± 0.31	0.36 ± 0.05*
	Tlr8	Toll-like receptor 8	−1.51	1.12 ± 0.22	0.40 ± 0.05*
2	*Abcd2*	ATP-binding cassette, sub-family D, member 2	1.62	1.03 ± 0.11	1.43 ± 0.11*
	Mmp3	Matrix metallopeptidase 3	−1.84	1.03 ± 0.10	0.59 ± 0.06***
3	*Ffar4*	Free fatty acid receptor 4	1.54	0.92 ± 0.05	1.49 ± 0.18*
4	*Cd68*	CD68 molecule	−1.58	1.02 ± 0.08	0.76 ± 0.03**
5	*Fgf21*	Fibroblast growth factor 21	−1.55	1.08 ± 0.17	0.45 ± 0.06**

Data are expressed as the mean ± SEM (n = 8 for the control diet group; n = 13 for the D-psicose diet group).
[a] Results were normalized to β–actin mRNA expression.
* p < 0.05 statistically significant compared with the control diet.
** p < 0.01 statistically significant compared with the control diet.
*** p < 0.001 statistically significant compared with the control diet.

Table 5
Effects of D-psicose supplementation on adipose tissue mRNA expression of genes associated with inflammation and lipid metabolism in C57BL/6J-*ob*/*ob* mice.

Pathway	Gene	Relative fold change[a]	
		Control	D-Psicose
Inflammation	TNF-α	1.04 ± 0.12	0.56 ± 0.04**
	IL-6	1.15 ± 0.24	0.59 ± 0.11*
	MCP-1	1.05 ± 0.13	0.72 ± 0.06*
Adipogenesis	PPAR-α	1.11 ± 0.09	0.81 ± 0.05*
	PPAR-γ	1.03 ± 0.08	0.78 ± 0.07*
	C/EBP-α	0.85 ± 0.13	0.44 ± 0.11*
	SREBP-1c	1.07 ± 0.16	0.69 ± 0.08*
	aP2	1.06 ± 0.14	0.64 ± 0.08*
	LPL	1.21 ± 0.27	0.52 ± 0.05**
Lipogenesis	FAS	1.07 ± 0.14	0.77 ± 0.12*
Lipolysis	HSL	1.00 ± 0.07	1.36 ± 0.11*
β–Oxidation	CPT-1	0.81 ± 0.05	0.97 ± 0.03*

Data are expressed as the mean ± SEM (n = 8 for the control diet group; n = 13 for the D-psicose diet group).
[a] Results were normalized to β–actin mRNA expression.
* p < 0.05 statistically significant compared with the control diet.
** p < 0.01 statistically significant compared with the control diet.

including dyslipidemia, which might result from macrophage accumulation in proportion to adipocyte size (Arner et al., 2010; Veilleux, Caron-Jobin, Noel, Laberge, & Tchernof, 2011; Weisberg et al., 2003).

Our results demonstrated that D-psicose likely exerted its anti-adipogenic activity in C57BL/6J-*ob*/*ob* mice by suppressing the expression of genes associated with inflammation and adipo/lipogenesis and increasing β-oxidation-related gene expression. Obesity leads to changes in adipose tissue metabolism and endocrine function, resulting in the increased production of pro-inflammatory molecules associated with the development of obesity-related metabolic complications (Weisberg et al., 2003). In the current study, D-psicose supplementation decreased the mRNA expression of pro-inflammatory genes including TNF-α, IL-6, and MCP-1, which agrees with the reduced expression of F4/80. Adipose tissue expression levels of TNF-α and IL-6 have been found to be associated with insulin resistance followed by development of diabetes (Fried et al., 1998; Hotamisligil et al., 1993; Xu et al., 2003). MCP-1 overexpression in the adipose tissue of obese mice also leads to macrophage recruitment and insulin resistance (Kamei et al., 2006; Sartipy & Loskutoff, 2003). Therefore, the results from this study suggest that D-psicose reduces the expression of adipose tissue-derived pro-inflammatory proteins, possibly improving insulin sensitivity and metabolic homeostasis.

To identify systematic molecular mechanisms involved in the anti-adipogenic activity of D-psicose, we performed a genome-wide functional analysis of the adipose tissue of C57BL/6J-*ob*/*ob* mice in response to different diets. Our transcriptional profiling of adipose tissue followed by network function analysis revealed that D-psicose-responsive genes were associated with inflammatory response, molecular transport, and lipid metabolism. Network 1, the most significant network in response to D-psicose supplementation, consisted primarily of down-regulated genes including *Adam12*, *Adgre1*, *Cd36*, *Cd44*, *Fos*, *Il7r*, and *Tlr8* that were associated with inflammatory response. *Adam12* (ADAM metallopeptidase domain 12), which encodes disintegrin, is involved in the development of adipose tissue. Decreased expression of *Adam12* is associated with resistance to weight gain, primarily owing to an impaired ability to increase adipocyte numbers, indicating defects in adipocyte differentiation (Masaki, Kurisaki, Shirakawa, & Sehara-Fujisawa, 2005). *Adgre1* (adhesion G protein-coupled receptor E1) encodes EMR1 (EGF-like module-containing mucin-like hormone receptor-like 1, also known as F4/80) and is a marker for mature macrophages. Its expression is positively correlated with adipocyte size and body mass (Weisberg et al., 2003), as observed in this study. CD36 promotes the uptake of long-chain fatty acids and contributes to inflammatory responses by enhancing macrophage infiltration into adipose tissue and pro-inflammatory cytokine production (Kuda et al., 2011). *Cd36* knockout mice show impaired fatty acid uptake by adipose tissue, improved insulin sensitivity, and reduced inflammation in adipose tissue (Coburn et al., 2000; Kennedy et al., 2011). In addition, *Cd44* encodes an immune cell receptor that regulates inflammatory responses including the expression of pro-inflammatory cytokines (McKee et al., 1996). CD44 participates in the development of adipose tissue inflammation and insulin resistance in both rodents and humans, and CD44 deficiency improves these symptoms (Kodama et al., 2012). A recent study reported that CD44 expression was positively correlated with the expression of CD68 and IL-6 in human adipose tissue (Liu et al., 2015), similar to the present findings. FOS (FBJ murine osteosarcoma viral oncogene homolog) is an important transcription factor for adipocyte differentiation, and reduced FOS expression inhibits adipocyte development (Knebel et al., 2013; Xiao et al., 2011). IL7R (interleukin 7 receptor) plays a critical role in T cell lymphopoiesis and

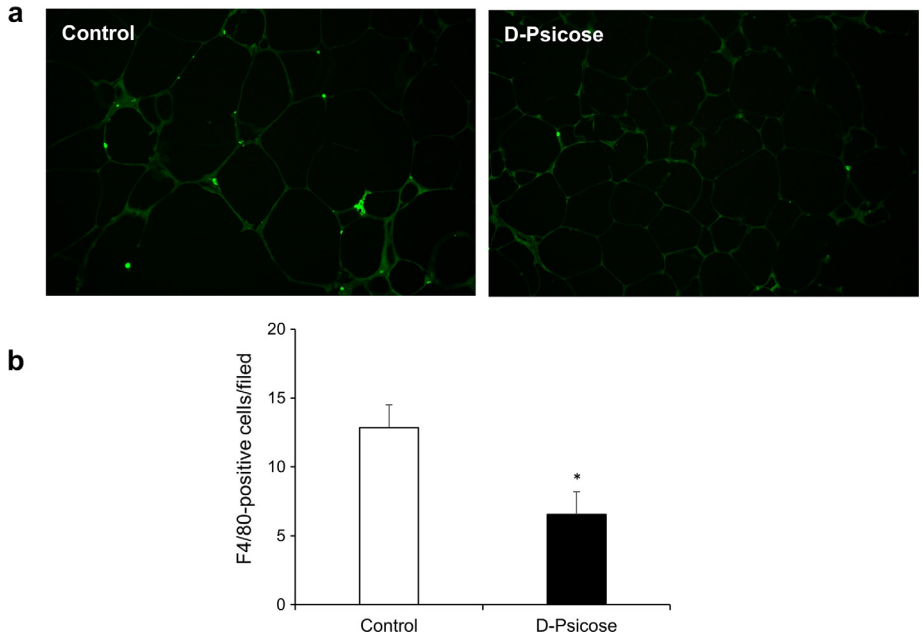

Fig. 3. Effects of D-psicose supplementation on adipose tissue macrophages in C57BL/6J-*ob*/*ob* mice. Representative F4/80 staining of white adipose tissue (a) and F4/80-positive cell numbers (b) were evaluated (magnification × 200). Each bar represents the mean ± SEM (n = 3 for the control diet group; n = 4 for the D-psicose diet group). $^{*}p < 0.05$ compared with the control diet.

homeostasis (Fry & Mackall, 2005). Notably, IL7R is up-regulated in the adipose tissue of obese men (Maury et al., 2007), whereas *Il7r* knockout mice show reduced body weight gain and visceral adiposity, decreased expression of genes associated with adipogenesis, and reduced pro-inflammatory cytokine production and macrophage infiltration (Lee, Song, Choi, Yu, & Park, 2015). It has been reported that toll-like receptors (TLRs) including TLR8 and pro-inflammatory signaling molecules are increased in the adipose tissue of mice with obesity induced by diet or leptin deficiency (Kim, Choi, Choi, & Park, 2012). The down-regulation of these genes involved in Network 1 agrees with the reduced final body weight, adipose tissue weight, and adipocyte size in obese mice fed the D-psicose diet observed in the present study.

In comparison, genes in Network 2 including *Abcd2* and *Mmp3* were associated with molecular transport, cellular development, and cellular growth and proliferation. ABCD2 (ATP-binding cassette, sub-family D, member 2) is a peroxisomal ABC transporter that facilitates the transport of very long-chain fatty acyl-CoA into peroxisomes for β–oxidation and is highly abundant in the adipose tissue of mice (Liu et al., 2010; Morita & Imanaka, 2012). A recent study demonstrated that ABCD2 is located in a subclass of peroxisomes associated with mitochondria and the endoplasmic reticulum in mouse adipose tissue (X. Liu, Liu, Lester, Pijut, & Graf, 2015). *Abcd2* knockout mice fed eruic acid (22:1 ω9) show rapid expansion of adipose tissue, increased adipocyte size, and insulin resistance (J. Liu et al., 2012). Thus, the up-regulation of *Abcd2* in response to D-psicose supplementation might be associated with positive regulation of β-oxidation. In addition, matrix metallopeptidases (MMPs) are known to play a role in adipose tissue remodeling and inflammatory responses in obesity (Chavey et al., 2003; Maquoi, Munaut, Colige, Collen, & Lijnen, 2002). An MMP inhibitor reduced adipogenesis *in vitro*, indicating that MMPs affect adipocyte differentiation (Chavey et al., 2003; Maquoi et al., 2002). Specifically, *Mmp3* has been reported to be up-regulated in the adipose tissue of obese mice (Chavey et al., 2003; Maquoi et al., 2002). It has been shown that FOS induces transcription of MMP3, which in turn activates MMP1, an interstitial collagenase (Vincenti, White, Schroen, Benbow, & Brinckerhoff, 1996). Recently, macrophage-secreted cytokines such as TNF-α and IL-1β have also been found to stimulate the expression of MMP1 and MMP3 in adipocytes, resulting in enhancement of adipose tissue remodeling (Gao & Bing, 2011; O'Hara, Lim, Mazzatti, & Trayhurn, 2009). Consistent with these findings, we identified decreased mRNA expression of *Fos* (Table 4), *Mmp3* (Table 4), *Mmp1* (Fig. S1), and TNF-α (Table 5) in the D-psicose-supplemented diet group, suggesting that D-psicose contributes to the decreased inflammation and adipogenesis observed in these animals.

We also identified up-regulation of *Ffar4* in Network 3 and down-regulation of *Cd68* in Network 4, both of which are involved in lipid metabolism and molecular transport. FFAR4 (free fatty acid receptor 4) functions as a receptor for long-chain fatty acids and regulates anti-inflammation and insulin sensitization (Oh et al., 2010). FFAR4 is also known to modulate body weight gain, adipocyte differentiation, and obesity (Ichimura et al., 2012). In turn, FFAR4 deficiency is associated with increases in body weight, obesity-related inflammation, adipogenesis, and fat accumulation (Ichimura et al., 2012; Li et al., 2015; Oh et al., 2010), whereas a FFAR4 agonist improves insulin resistance and chronic inflammation in obese mice by inhibiting the expression of proinflammatory cytokines such as TNF-α, IL-6, and MCP-1 (Oh da et al., 2014). Thus, the up-regulation of *Ffar4* might account for the deceased final body weight, adipogenesis, and inflammation in response to D-psicose supplementation observed in this study. Expression of CD68, a monocyte/macrophage marker, is positively correlated with adipocyte size, body mass, and fat accumulation (Michaud et al., 2014; Weisberg et al., 2003), which is also in line with our results. In addition, *Fgf21* (fibroblast growth factor 21) in Network 5 was down-regulated in the D-psicose-supplemented diet group. FGF21 functions as a hormone to regulate glucose and lipid metabolism. It has been recently reported that *Fgf21* knockout mice show reduced PPAR-γ activity and PPAR-γ-dependent gene expression in adipose tissue, resulting in a decrease in adipose tissue mass and adipocyte size (Dutchak et al., 2012), which is consistent with our observations. Overall, the primary involvement of the D-psicose-mediated gene expression profile in inflammation response and lipid metabolism supports our findings that

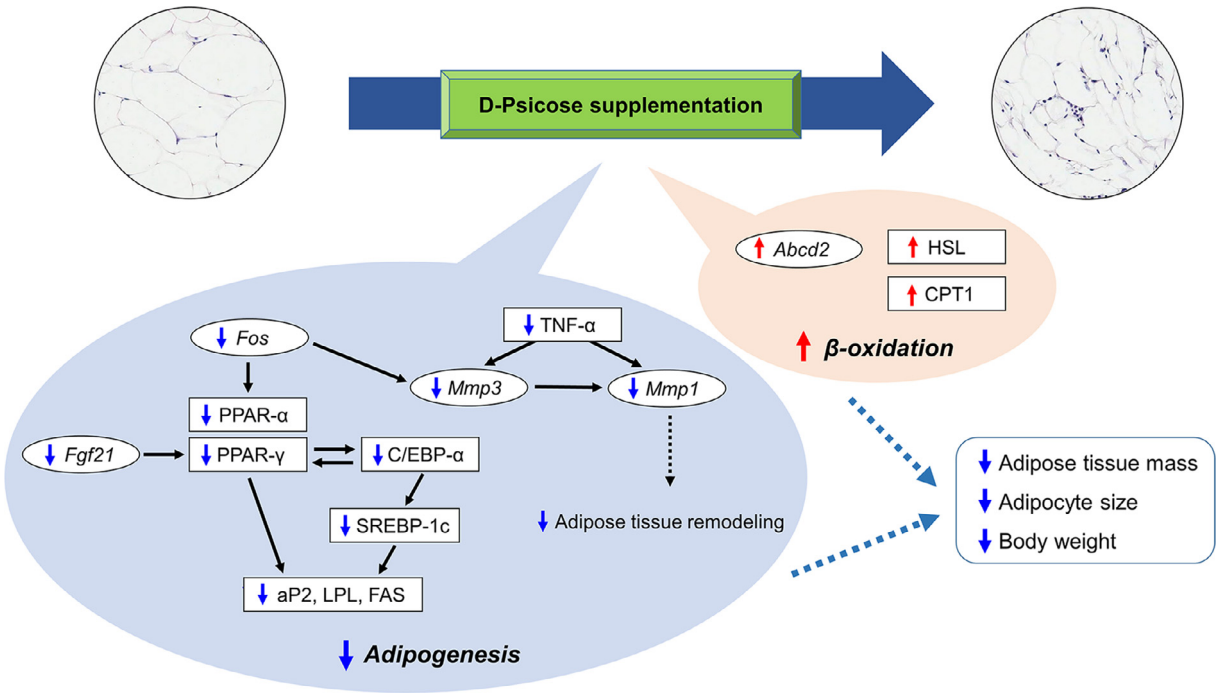

Fig. 4. Schematic diagram of the effect of D-psicose supplementation on adipose tissue metabolism. D-Psicose exerts anti-adipogenic activity in C57BL/6J-*ob*/*ob* mice by suppressing the expression of genes associated with inflammation and adipo/lipogenesis and increasing β-oxidation-related gene expression. Open circles represent genes detected by microarray analysis and confirmed by real-time qPCR. Open squares indicate genes determined by real-time qPCR.

D-psicose decreases body weight gain, adipocyte size, and adipogenesis and alleviates inflammation.

Given that obesity-associated chronic low-grade inflammation is associated with lipid accumulation (Liu, Mei, Yang, & Li, 2014), we further determined the expression of genes associated with lipid metabolism in our models. In particular, we identified decreased expression of adipo/lipogenesis-related genes encoding PPAR-α, PPAR-γ, C/EBP-α, SREBP-1c, aP2, LPL, and FAS in the adipose tissue of obese mice fed the D-psicose diet, whereas expression of β-oxidation-related genes including HSL and CPT-1 was increased. PPAR-α activation in adipose tissue stimulates adipocyte differentiation as well as β-oxidation, suggesting that both PPAR-α and PPAR-γ are involved in adipogenesis (Goto et al., 2011). It has been also reported that PPAR-α deficiency reduces epididymal adipose tissue mass (Islam, Knight, Frayn, Patel, & Gibbons, 2005). PPAR-γ and C/EBP-α, important transcription factors in adipogenesis, synergistically activate adipogenic gene expression (Madsen, Siersbaek, Boergesen, Nielsen, & Mandrup, 2014). In addition, SREBP-1c is a pro-adipogenic transcription factor that is regulated by C/EBP-α and stimulates the expression of lipogenic genes including FAS (Eberle, Hegarty, Bossard, Ferre, & Foufelle, 2004; Payne et al., 2010). aP2, also known as fatty acid binding protein 4 (FABP4), is abundant in adipose tissue and macrophages, and decreased levels of aP2 are associated with metabolic improvement (Erbay et al., 2009; Maeda et al., 2005). Similar to CD36, LPL is involved in fatty acid uptake and storage in adipose tissue (Goldberg, Eckel, & Abumrad, 2009). Accordingly, LPL deficiency results in reduced weight and fat mass in obese mice (Weinstock et al., 1997). Furthermore, HSL and CPT-1 are responsible for the hydrolysis of tri- and di-glycerides and mitochondrial β–oxidation, whereas adipose dysfunction leads to reduced lipolysis and increased fat mass. Together, these results highlight that the down-regulation of *Fos*, *Mmp3*, and *Fgf21* and up-regulation of *Abcd2* as identified by transcriptomic analysis have the potential to influence the expression of genes involved in adipo/lipogenesis and β-oxidation, which in turn are considered to likely be responsible for the anti-adipogenic activity of D-psicose (Fig. 4).

Recent studies have also suggested that D-psicose supplementation contributes to normalization of body weight and fat-pad mass in high-fat diet-fed obese mice by regulating lipid metabolism (Han et al., 2016). Expression of hepatic genes related to lipogenesis and β–oxidation is down-regulated, whereas that of white adipose tissue genes associated with β–oxidation is increased (Han et al., 2016), which is in agreement with our results. The same study also suggested that D-psicose inhibits intestinal lipid absorption, which might account for the increases in fecal lipid excretion and improvement in plasma lipid profile. In another study using Wistar rats fed an isocaloric high-sucrose diet, D-psicose decreases the activity of lipogenic enzymes including FAS and glucose-6-phosphate dehydrogenase in the liver and adipose tissue, thereby suppressing fat accumulation and body weight gain (Ochiai, Onishi, Yamada, Iida, & Matsuo, 2014). Levels of liver lipogenic enzymes are also found to be decreased in response to D-psicose supplementation in Sprague-Dawley rats (Nagata et al., 2015). In the present study, hepatic expression of genes involved in adipo/lipogenesis did not differ between dietary groups in C57BL/6J-*ob*/*ob* mice (data not shown), which might be associated with the lack of significant difference in serum triacylglycerol and non-esterified fatty acids concentrations. This is the first study, to our knowledge, to elucidate the molecular mechanisms underlying the anti-obesity and anti-adipogenic activities of D-psicose in *ob*/*ob* mice using transcriptomic techniques.

5. Conclusions

In conclusion, we provide evidence that D-psicose supplementation ameliorated body weight gain and improved adipose tissue weight, adipocyte size, and serum lipid profile in C57BL/6J-*ob*/*ob* mice. The beneficial metabolic effects appear to be associated with the reduced inflammation and alterations in lipid metabolism observed in adipose tissue in response to D-psicose supplementation. Therefore, our data suggest that D-psicose represents a promising sugar substitute possessing a direct gene-regulatory function related to the suppression of body fat deposition.

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Author contributions

S.-E.K., H.-J.K., and M.-K.S. contributed to the conceptualization and design of the research; S.J.K. conducted the research and analyzed the data; H.-J.K. provided the material; S.-E.K. contributed to the analysis and interpretation of the data and drafted the manuscript; S.-E.K. and M.-K.S. participated in the critical revision of the manuscript; M.-K.S. had primary responsibility for final content. All authors read and approved the final manuscript.

Disclosure

The authors declare no commercial or financial conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2016.11.029.

References

Arner, E., Westermark, P. O., Spalding, K. L., Britton, T., Ryden, M., Frisen, J., ... Arner, P. (2010). Adipocyte turnover: Relevance to human adipose tissue morphology. *Diabetes, 59*(1), 105–109. http://dx.doi.org/10.2337/db09-0942.

Bluher, M. (2009). Adipose tissue dysfunction in obesity. *Experimental and Clinical Endocrinology & Diabetes, 117*(6), 241–250. http://dx.doi.org/10.1055/s-0029-1192044.

Chavey, C., Mari, B., Monthouel, M. N., Bonnafous, S., Anglard, P., Van Obberghen, E., & Tartare-Deckert, S. (2003). Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation. *Journal of Biological Chemistry, 278*(14), 11888–11896. http://dx.doi.org/10.1074/jbc.M209196200.

Chung, Y. M., Hyun Lee, J., Youl Kim, D., Hwang, S. H., Hong, Y. H., Kim, S. B., ... Hye Park, C. (2012). Dietary D-psicose reduced visceral fat mass in high-fat diet-induced obese rats. *Journal of Food Science, 77*(2), H53–58. http://dx.doi.org/10.1111/j.1750-3841.2011.02571.x.

Chung, Y. M., Lee, J. H., Kim, D. Y., Hwang, S. H., Hong, Y. H., Kim, S. B., ... Park, C. H. (2012). Dietary D-psicose reduced visceral fat mass in high-fat diet-induced obese rats. *Journal of Food Science, 77*(2), H53–H58. http://dx.doi.org/10.1111/j.1750-3841.2011.02571.x.

Chung, M. Y., Oh, D. K., & Lee, K. W. (2012). Hypoglycemic health benefits of D-psicose. *Journal of Agriculture and Food Chemistry, 60*(4), 863–869. http://dx.doi.org/10.1021/jf204050w.

Coburn, C. T., Knapp, F. F., Jr., Febbraio, M., Beets, A. L., Silverstein, R. L., & Abumrad, N. A. (2000). Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *Journal of Biological Chemistry, 275*(42), 32523–32529. http://dx.doi.org/10.1074/jbc.M003826200.

Dutchak, P. A., Katafuchi, T., Bookout, A. L., Choi, J. H., Yu, R. T., Mangelsdorf, D. J., & Kliewer, S. A. (2012). Fibroblast growth factor-21 regulates PPARgamma activity and the antidiabetic actions of thiazolidinediones. *Cell, 148*(3), 556–567. http://dx.doi.org/10.1016/j.cell.2011.11.062.

Eberle, D., Hegarty, B., Bossard, P., Ferre, P., & Foufelle, F. (2004). SREBP transcription factors: Master regulators of lipid homeostasis. *Biochimie, 86*(11), 839–848. http://dx.doi.org/10.1016/j.biochi.2004.09.018.

Erbay, E., Babaev, V. R., Mayers, J. R., Makowski, L., Charles, K. N., Snitow, M. E., ... Hotamisligil, G. S. (2009). Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. *Nature Medicine, 15*(12), 1383–1391. http://dx.doi.org/10.1038/nm.2067.

Farnier, C., Krief, S., Blache, M., Diot-Dupuy, F., Mory, G., Ferre, P., & Bazin, R. (2003). Adipocyte functions are modulated by cell size change: Potential involvement of an integrin/ERK signalling pathway. *International Journal of Obesity and Related Metabolic Disorders, 27*(10), 1178–1186. http://dx.doi.org/10.1038/sj.ijo.0802399.

Forshee, R. A., Anderson, P. A., & Storey, M. L. (2008). Sugar-sweetened beverages and body mass index in children and adolescents: A meta-analysis. *American Journal of Clinical Nutrition, 87*(6), 1662–1671.

Fried, S. K., Bunkin, D. A., & Greenberg, A. S. (1998). Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: Depot difference and regulation by glucocorticoid. *Journal of Clinical Endocrinology and Metabolism, 83*(3), 847–850. http://dx.doi.org/10.1210/jcem.83.3.4660.

Fry, T. J., & Mackall, C. L. (2005). The many faces of IL-7: From lymphopoiesis to peripheral T cell maintenance. *The Journal of Immunology, 174*(11), 6571–6576.

Gao, D., & Bing, C. (2011). Macrophage-induced expression and release of matrix metalloproteinase 1 and 3 by human preadipocytes is mediated by IL-1beta via activation of MAPK signaling. *Journal of Cellular Physiology, 226*(11), 2869–2880. http://dx.doi.org/10.1002/jcp.22630.

Goldberg, I. J., Eckel, R. H., & Abumrad, N. A. (2009). Regulation of fatty acid uptake into tissues: Lipoprotein lipase- and CD36-mediated pathways. *Journal of Lipid Research, 50*(Suppl), S86–90. http://dx.doi.org/10.1194/jlr.R800085-JLR200.

Goto, T., Lee, J. Y., Teraminami, A., Kim, Y. I., Hirai, S., Uemura, T., ... Kawada, T. (2011). Activation of peroxisome proliferator-activated receptor-alpha stimulates both differentiation and fatty acid oxidation in adipocytes. *Journal of Lipid Research, 52*(5), 873–884. http://dx.doi.org/10.1194/jlr.M011320.

Han, Y., Han, H. J., Kim, A. H., Choi, J. Y., Cho, S. J., Park, Y. B., ... Choi, M. S. (2016). D-Allulose supplementation normalized the body weight and fat-pad mass in diet-induced obese mice via the regulation of lipid metabolism under isocaloric fed condition. *Molecular Nutrition & Food Research, 60*(7), 1695–1706. http://dx.doi.org/10.1002/mnfr.201500771.

Hayashi, N., Yamada, T., Takamine, S., Iida, T., Okuma, K., & Tokuda, M. (2014). Weight reducing effect and safety evaluation of rare sugar syrup by a randomized double-blind, parallel-group study in human. *Journal of Functional Foods, 11*, 152–159. http://dx.doi.org/10.1016/j.jff.2014.09.020.

Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science, 259*(5091), 87–91.

Ichimura, A., Hirasawa, A., Poulain-Godefroy, O., Bonnefond, A., Hara, T., Yengo, L., ... Froguel, P. (2012). Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature, 483*(7389), 350–354. http://dx.doi.org/10.1038/nature10798.

Iida, T., Hayashi, N., Yamada, T., Yoshikawa, Y., Miyazato, S., Kishimoto, Y., ... Izumori, K. (2010). Failure of D-psicose absorbed in the small intestine to metabolize into energy and its low large intestinal fermentability in humans. *Metabolism, 59*(2), 206–214. http://dx.doi.org/10.1016/j.metabol.2009.07.018.

Iida, T., Yamada, T., Hayashi, N., Okuma, K., Izumori, K., Ishii, R., ... Matsuo, T. (2013). Reduction of abdominal fat accumulation in rats by 8-week ingestion of a newly developed sweetener made from high fructose corn syrup. *Food Chemistry, 138*(2–3), 781–785. http://dx.doi.org/10.1016/j.foodchem.2012.11.017.

Islam, K. K., Knight, B. L., Frayn, K. N., Patel, D. D., & Gibbons, G. F. (2005). Deficiency of PPARalpha disturbs the response of lipogenic flux and of lipogenic and cholesterogenic gene expression to dietary cholesterol in mouse white adipose tissue. *Biochimica et Biophysica Acta, 1734*(3), 259–268. http://dx.doi.org/10.1016/j.bbalip.2005.03.009.

Jernas, M., Palming, J., Sjoholm, K., Jennische, E., Svensson, P. A., Gabrielsson, B. G., ... Lonn, M. (2006). Separation of human adipocytes by size: Hypertrophic fat cells display distinct gene expression. *The FASEB Journal, 20*(9), 1540–1542. http://dx.doi.org/10.1096/fj.05-5678fje.

Kamei, N., Tobe, K., Suzuki, R., Ohsugi, M., Watanabe, T., Kubota, N., ... Kadowaki, T. (2006). Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. *Journal of Biological Chemistry, 281*(36), 26602–26614. http://dx.doi.org/10.1074/jbc.M601284200.

Kennedy, D. J., Kuchibhotla, S., Westfall, K. M., Silverstein, R. L., Morton, R. E., & Febbraio, M. (2011). A CD36-dependent pathway enhances macrophage and adipose tissue inflammation and impairs insulin signalling. *Cardiovascular Research, 89*(3), 604–613. http://dx.doi.org/10.1093/cvr/cvq360.

Kim, S. J., Choi, Y., Choi, Y. H., & Park, T. (2012). Obesity activates toll-like receptor-mediated proinflammatory signaling cascades in the adipose tissue of mice. *Journal of Nutritional Biochemistry, 23*(2), 113–122. http://dx.doi.org/10.1016/j.jnutbio.2010.10.012.

Knebel, B., Kotzka, J., Lehr, S., Hartwig, S., Avci, H., Jacob, S., ... Muller-Wieland, D. (2013). A mutation in the c-fos gene associated with congenital generalized lipodystrophy. *Orphanet Journal of Rare Diseases, 8*, 119. http://dx.doi.org/10.1186/1750-1172-8-119.

Kodama, K., Horikoshi, M., Toda, K., Yamada, S., Hara, K., Irie, J., ... Butte, A. J. (2012). Expression-based genome-wide association study links the receptor CD44 in adipose tissue with type 2 diabetes. *Proceedings of the National Academy of Sciences USA, 109*(18), 7049–7054. http://dx.doi.org/10.1073/pnas.1114513109.

Kuda, O., Jenkins, C. M., Skinner, J. R., Moon, S. H., Su, X., Gross, R. W., & Abumrad, N. A. (2011). CD36 protein is involved in store-operated calcium flux, phospholipase A2 activation, and production of prostaglandin E2. *Journal of Biological Chemistry, 286*(20), 17785–17795. http://dx.doi.org/10.1074/jbc.M111.232975.

Lee, M., Song, S. J., Choi, M. S., Yu, R., & Park, T. (2015). IL-7 receptor deletion ameliorates diet-induced obesity and insulin resistance in mice. *Diabetologia, 58*(10), 2361–2370. http://dx.doi.org/10.1007/s00125-015-3684-7.

Li, X., Ballantyne, L. L., Che, X., Mewburn, J. D., Kang, J. X., Barkley, R. M., ... Funk, C. D. (2015). Endogenously generated omega-3 fatty acids attenuate vascular inflammation and neointimal hyperplasia by interaction with free fatty acid receptor 4 in mice. *Journal of the American Heart Association, 4*(4). http://dx.doi.org/10.1161/jaha.115.001856.

Liu, L. F., Kodama, K., Wei, K., Tolentino, L. L., Choi, O., Engleman, E. G., ... McLaughlin, T. (2015). The receptor CD44 is associated with systemic insulin resistance and proinflammatory macrophages in human adipose tissue. *Diabetologia, 58*(7), 1579–1586. http://dx.doi.org/10.1007/s00125-015-3603-y.

Liu, J., Liang, S., Liu, X., Brown, J. A., Newman, K. E., Sunkara, M., ... Graf, G. A. (2012). The absence of ABCD2 sensitizes mice to disruptions in lipid metabolism by dietary erucic acid. *Journal of Lipid Research, 53*(6), 1071–1079. http://dx.doi.org/10.1194/jlr.M022160.

Liu, X., Liu, J., Lester, J. D., Pijut, S. S., & Graf, G. A. (2015). ABCD2 identifies a subclass of peroxisomes in mouse adipose tissue. *Biochemical and Biophysical Research Communications, 456*(1), 129–134. http://dx.doi.org/10.1016/j.bbrc.2014.11.046.

Liu, L., Mei, M., Yang, S., & Li, Q. (2014). Roles of chronic low-grade inflammation in the development of ectopic fat deposition. *Mediators of Inflammation, 2014*, 418185. http://dx.doi.org/10.1155/2014/418185.

Liu, J., Sabeva, N. S., Bhatnagar, S., Li, X. A., Pujol, A., & Graf, G. A. (2010). ABCD2 is abundant in adipose tissue and opposes the accumulation of dietary erucic acid (C22:1) in fat. *Journal of Lipid Research, 51*(1), 162–168. http://dx.doi.org/10.1194/jlr.M900237-JLR200.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods, 25*(4), 402–408. http://dx.doi.org/10.1006/meth.2001.1262.

Madsen, M. S., Siersbaek, R., Boergesen, M., Nielsen, R., & Mandrup, S. (2014). Peroxisome proliferator-activated receptor gamma and C/EBPalpha synergistically activate key metabolic adipocyte genes by assisted loading. *Molecular and Cellular Biology, 34*(6), 939–954. http://dx.doi.org/10.1128/mcb.01344-13.

Maeda, K., Cao, H., Kono, K., Gorgun, C. Z., Furuhashi, M., Uysal, K. T., ... Hotamisligil, G. S. (2005). Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metabolism, 1*(2), 107–119. http://dx.doi.org/10.1016/j.cmet.2004.12.008.

Malik, V. S., Popkin, B. M., Bray, G. A., Despres, J. P., Willett, W. C., & Hu, F. B. (2010). Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: A meta-analysis. *Diabetes Care, 33*(11), 2477–2483. http://dx.doi.org/10.2337/dc10-1079.

Maquoi, E., Munaut, C., Colige, A., Collen, D., & Lijnen, H. R. (2002). Modulation of adipose tissue expression of murine matrix metalloproteinases and their tissue inhibitors with obesity. *Diabetes, 51*(4), 1093–1101.

Masaki, M., Kurisaki, T., Shirakawa, K., & Sehara-Fujisawa, A. (2005). Role of meltrin alpha (ADAM12) in obesity induced by high- fat diet. *Endocrinology, 146*(4), 1752–1763. http://dx.doi.org/10.1210/en.2004-1082.

Masoodi, M., Kuda, O., Rossmeisl, M., Flachs, P., & Kopecky, J. (2015). Lipid signaling in adipose tissue: Connecting inflammation & metabolism. *Biochimica et Biophysica Acta, 1851*(4), 503–518. http://dx.doi.org/10.1016/j.bbalip.2014.09.023.

Matsuo, T., Baba, Y., Hashiguchi, M., Takeshita, K., Izumori, K., & Suzuki, H. (2001a). Dietary D-psicose, a C-3 epimer of D-fructose, suppresses the activity of hepatic lipogenic enzymes in rats. *Asia Pacific Journal of Clinical Nutrition, 10*(3), 233–237.

Matsuo, T., Baba, Y., Hashiguchi, M., Takeshita, K., Izumori, K., & Suzuki, H. (2001b). Less body fat accumulation with D-Psicose diet versus D-fructose diet. *Journal of Clinical Biochemistry and Nutrition, 30*, 55–65.

Maury, E., Ehala-Aleksejev, K., Guiot, Y., Detry, R., Vandenhooft, A., & Brichard, S. M. (2007). Adipokines oversecreted by omental adipose tissue in human obesity. *American Journal of Physiology-Endocrinology and Metabolism, 293*(3), E656–665. http://dx.doi.org/10.1152/ajpendo.00127.2007.

McKee, C. M., Penno, M. B., Cowman, M., Burdick, M. D., Strieter, R. M., Bao, C., & Noble, P. W. (1996). Hyaluronan (HA) fragments induce chemokine gene expression in alveolar macrophages. The role of HA size and CD44. *Journal of Clinical Investigation, 98*(10), 2403–2413. http://dx.doi.org/10.1172/jci119054.

Michaud, A., Boulet, M. M., Veilleux, A., Noel, S., Paris, G., & Tchernof, A. (2014). Abdominal subcutaneous and omental adipocyte morphology and its relation to gene expression, lipolysis and adipocytokine levels in women. *Metabolism, 63*(3), 372–381. http://dx.doi.org/10.1016/j.metabol.2013.11.007.

Montague, C. T., Farooqi, I. S., Whitehead, J. P., Soos, M. A., Rau, H., Wareham, N. J., & O'Rahilly, S. (1997). Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature, 387*(6636), 903–908. http://dx.doi.org/10.1038/43185.

Morita, M., & Imanaka, T. (2012). Peroxisomal ABC transporters: Structure, function and role in disease. *Biochimica et Biophysica Acta, 1822*(9), 1387–1396. http://dx.doi.org/10.1016/j.bbadis.2012.02.009.

Nagata, Y., Kanasaki, A., Tamaru, S., & Tanaka, K. (2015). D-psicose, an epimer of D-fructose, favorably alters lipid metabolism in Sprague-Dawley rats. *Journal of Agriculture and Food Chemistry, 63*(12), 3168–3176. http://dx.doi.org/10.1021/jf502535p.

Ochiai, M., Nakanishi, Y., Yamada, T., Iida, T., & Matsuo, T. (2013). Inhibition by dietary D-psicose of body fat accumulation in adult rats fed a high-sucrose diet. *Bioscience, Biotechnology, and Biochemistry, 77*(5), 1123–1126. http://dx.doi.org/10.1271/bbb.130019.

Ochiai, M., Onishi, K., Yamada, T., Iida, T., & Matsuo, T. (2014). D-psicose increases energy expenditure and decreases body fat accumulation in rats fed a high-sucrose diet. *International Journal of Food Sciences and Nutrition, 65*(2), 245–250. http://dx.doi.org/10.3109/09637486.2013.845653.

Ogden, C. L., Carroll, M. D., Fryar, C. D., & Flegal, K. M. (2015). Prevalence of obesity among adults and youth: United States, 2011-2014. *NCHS Data Brief, 219*, 1–8.

Oh da, Y., Walenta, E., Akiyama, T. E., Lagakos, W. S., Lackey, D., Pessentheiner, A. R., ... Olefsky, J. M. (2014). A Gpr120-selective agonist improves insulin resistance and chronic inflammation in obese mice. *Nature Medicine, 20*(8), 942–947. http://dx.doi.org/10.1038/nm.3614.

Oh, D. Y., Talukdar, S., Bae, E. J., Imamura, T., Morinaga, H., Fan, W., ... Olefsky, J. M. (2010). GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell, 142*(5), 687–698. http://dx.doi.org/10.1016/j.cell.2010.07.041.

O'Hara, A., Lim, F. L., Mazzatti, D. J., & Trayhurn, P. (2009). Microarray analysis identifies matrix metalloproteinases (MMPs) as key genes whose expression is up-regulated in human adipocytes by macrophage-conditioned medium. *Pflügers Archiv-European Journal of Physiology, 458*(6), 1103–1114. http://dx.doi.org/10.1007/s00424-009-0693-8.

Payne, V. A., Au, W. S., Lowe, C. E., Rahman, S. M., Friedman, J. E., O'Rahilly, S., & Rochford, J. J. (2010). C/EBP transcription factors regulate SREBP1c gene expression during adipogenesis. *Biochemical Journal, 425*(1), 215–223. http://dx.doi.org/10.1042/bj20091112.

Pietilainen, K. H., Rog, T., Seppanen-Laakso, T., Virtue, S., Gopalacharyulu, P., Tang, J., ... Oresic, M. (2011). Association of lipidome remodeling in the adipocyte membrane with acquired obesity in humans. *PLoS Biology, 9*(6), e1000623. http://dx.doi.org/10.1371/journal.pbio.1000623.

Prentice, A. M. (2006). The emerging epidemic of obesity in developing countries. *International Journal of Epidemiology, 35*(1), 93–99. http://dx.doi.org/10.1093/ije/dyi272.

Sartipy, P., & Loskutoff, D. J. (2003). Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proceedings of the National Academy of Sciences USA, 100*(12), 7265–7270. http://dx.doi.org/10.1073/pnas.1133870100.

Stanhope, K. L., Schwarz, J. M., Keim, N. L., Griffen, S. C., Bremer, A. A., Graham, J. L., ... Havel, P. J. (2009). Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *The Journal of Clinical Investigation, 119*(5), 1322–1334. http://dx.doi.org/10.1172/jci37385.

Takeshita, K., Suga, A., Takada, G., & Izumori, K. (2000). Mass production of D-psicose from D-fructose by a continuous bioreactor system using immobilized D-tagatose 3-epimerase. *Journal of Bioscience and Bioengineering, 90*(4), 453–455.

Tchernof, A., & Despres, J. P. (2013). Pathophysiology of human visceral obesity: An update. *Physiological Reviews, 93*(1), 359–404. http://dx.doi.org/10.1152/physrev.00033.2011.

Veilleux, A., Caron-Jobin, M., Noel, S., Laberge, P. Y., & Tchernof, A. (2011). Visceral adipocyte hypertrophy is associated with dyslipidemia independent of body composition and fat distribution in women. *Diabetes, 60*(5), 1504–1511. http://dx.doi.org/10.2337/db10-1039.

Vincenti, M. P., White, L. A., Schroen, D. J., Benbow, U., & Brinckerhoff, C. E. (1996). Regulating expression of the gene for matrix metalloproteinase-1 (collagenase): Mechanisms that control enzyme activity, transcription, and mRNA stability. *Critical Reviews in Eukaryotic Gene Expression, 6*(4), 391–411.

Weinstock, P. H., Levak-Frank, S., Hudgins, L. C., Radner, H., Friedman, J. M., Zechner, R., ... Breslow, J. L. (1997). Lipoprotein lipase controls fatty acid entry into adipose tissue, but fat mass is preserved by endogenous synthesis in mice deficient in adipose tissue lipoprotein lipase. *Proceedings of the National Academy of Sciences USA, 94*(19), 10261–10266.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. Jr., (2003). Obesity is associated with macrophage accumulation in adipose tissue. *The Journal of Clinical Investigation, 112*(12), 1796–1808. http://dx.doi.org/10.1172/jci19246.

Xiao, H., Leblanc, S. E., Wu, Q., Konda, S., Salma, N., Marfella, C. G., ... Imbalzano, A. N. (2011). Chromatin accessibility and transcription factor binding at the PPARgamma2 promoter during adipogenesis is protein kinase A-dependent. *Journal of Cellular Physiology, 226*(1), 86–93. http://dx.doi.org/10.1002/jcp.22308.

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., ... Chen, H. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *The Journal of Clinical Investigation, 112*(12), 1821–1830. http://dx.doi.org/10.1172/jci19451.

Zhang, H. H., Halbleib, M., Ahmad, F., Manganiello, V. C., & Greenberg, A. S. (2002). Tumor necrosis factor-alpha stimulates lipolysis in differentiated human adipocytes through activation of extracellular signal-related kinase and elevation of intracellular cAMP. *Diabetes, 51*(10), 2929–2935.