Global Liver Gene Expression Analysis on a Murine Metabolic Syndrome Model Treated by Low-molecular-weight Lychee Fruit Polyphenol (Oligonol®)

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Abstract. *Background/Aim: Oligonol® (OLG) is a low-molecular-weight lychee fruit polyphenol mainly containing catechin-type monomers and oligomers of proanthocyanidins. Dietary OLG supplementation reportedly improves lipid metabolism disorder and lowers the visceral fat level in animal and human studies. Thus, we investigated the mechanism behind the protective and beneficial effects of OLG on a Western diet (WD)-induced metabolic syndrome (MetS) of a murine model. Materials and Methods: Using the C57BL/6J mouse for the MetS model, mice were divided into three groups: control (normal diet: ND), Western diet (WD) and WD + 0.5% OLG (OLG) groups. The WD group was fed a high-calorie (high fructose plus high fat) diet for 12 weeks to develop MetS. At week 12, all mice were sacrificed and the blood and liver were obtained for histological and biological examinations and RNA sequencing (RNA-Seq). Results: Body weight, liver weight, plasma triglycerides (TG), total cholesterol (T-Cho) and alanine aminotransferase (ATS) levels of both OLG groups were significantly lower than those of the WD group. On histological examination of the liver, the area of fatty deposits was shown to be suppressed by OLG administration. Expression gene analysis in the liver of WD- versus OLG-fed mice by RNA-Seq showed that 464/45,706 genes exhibited a significant change of expression (corrected p-value <0.05, absolute value of fold change (FC) ≥2). Gene network analysis showed that genes related to hepatic steatosis, liver inflammation and tumor invasion were inactivated in the OLG group. In particular, the lipid metabolism-related genes Lpin1, Adig and Cidea were regulated by OLG administration. Conclusion: OLG may function to suppress MetS and the progression of geriatric diseases in WD-fed mice by regulating the expression of lipid metabolism, inflammation and tumor-related genes in the liver.*

Polyphenols are widespread in the plant kingdom and comprise various types, including proanthocyanidin, a known dye, and flavonoids. Proanthocyanidin in particular consists of polymerized catechin, with its oral absorption being inefficient. In contrast, low-molecular-lychee polyphenol ((OLG) Oligonol®) is a health food ingredient, industrially prepared by decomposing lychee-derived polyphenols into oligomers, that, in contrast to high-molecular-polyphenols, its superior absorption has been reported (1).

OLG has various physiological functions, such as an antioxidative effect common in polyphenols. In particular, a study regarding OLG's involvement in lipid metabolism reported that when mice with high-fat diet were administered OLG, it prevented the decrease of adiponetcin (*ADIPOQ*) mRNA in adipose tissues and decreased mRNA expressions of *TNF-α, PAI-1* and *MCP-1*. Furthermore, a human intervention study targeting 18 males and females, with abdominal girth >85 cm, revealed that 10-week administration of OLG decreased the area of visceral fat (2-4). As described, OLG has superior absorption and its function has been elucidated; however, most its mechanisms are yet unknown.

Metabolic syndrome (MetS) is a serious issue in Japan. MetS is a pathological condition where risk factors for

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arteriosclerotic disease and type II diabetes accumulate in an individual; these risk factors include visceral obesity, insulin resistance and hyperglycemia, abnormal lipid metabolism and hypertension. The increase of visceral fat accompanies high risk of hepatic steatosis, hepatic cirrhosis and hepatocarcinoma (5).

MetS treatment mainly aims to improve lifestyle, such as dietetic treatment, therapeutic exercise and abstaining from smoking, while other preventive measures with functional foods are used. This may be because, for instance, 5%-10% decrease in body weight is considered to effectively suppress the onset of diabetes and cardiovascular events; therefore, prevention and treatment of MetS is regarded to be necessary to prolong the healthy life expectancy in a nation (6, 7).

Therefore, we prepared a Western diet (WD) containing high load of sucrose and fat to mimic Western style diet and given to mice to construct a MetS model. Furthermore, MetS animals were simultaneously administered WD and OLG and the genetic change in their liver was systematically analyzed by RNA sequencing (RNA-Seq) to study OLG's preventive effect on MetS model.

Materials and Methods

Animals. Six-week-old C57BL/6 male mice were purchased from Charles River Japan, Inc. (Kanagawa, Japan). They were housed in individual cages under conventional conditions with a 12-h light–dark cycle, 23˚C±1˚C temperature and 55%±15% humidity. At the end of the experiment, the animals were sacrificed using ether anesthesia.

After 1 week of acclimation, the mice were divided into three groups (n=8 per group): (i) normal diet ((ND) distilled water), (ii) Western diet (WD) and (iii) WD + 0.5% OLG (500 mg of OLG in 100 ml of distilled water, *ad libitum*). The food for the WD to produce the MetS mouse model was F2WTD (sucrose 34%, salt-free butter 20%, 417 kcal), while the ND food was MF (359 kcal) purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Low-molecular-weight lychee fruit polyphenol (OLG-F; Lot OLF1405S) was obtained from Amino Up Chemical Co., Ltd. (Sapporo, Japan). This study conforms to the Guiding Principles for the Care and Use of Experimental Animals of Hokkaido Pharmaceutical University (published 1998, revised in 2001 and 2007). The protocol approval number is H27-008.

Plasma biochemical examinations and histological analysis. At week 12, all mice were sacrificed and blood and liver samples were obtained. Blood samples were collected in a heparin-containing plasma separator tube (PST) (BD Japan Co., Ltd., Tokyo, Japan) from the caudal vein of the mice. Each plasma sample was obtained from whole blood, separated by centrifugation at 12,000 rpm/min for 10 min and stored at −80˚C. The levels of triglyceride (TG), total cholesterol (T-Cho), glucose (GLU), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma were determined using a DRI-CHEM 4000 device (Fujifilm Medical Co., Ltd., Tokyo, Japan).

Each liver sample was fixed in 10% neutral-buffered formalin, embedded in paraffin and cut in thin sections (5 μm). These sections were stained with hematoxylin & eosin (HE) solution. HE-stained liver tissues were histologically examined using an Olympus AX70 light microscope, (Olympus Co., Ltd., Tokyo, Japan), equipped with 40× and 100× objective lenses.

Global analysis of gene expression of the liver using RNA-Seq. Livers were dissected and stored in RNAlater® Solution (Thermo Fisher Scientific Inc., Waltham, MA, USA) and, then, total RNA was extracted using an RNeasy Lipid Tissue Mini Kit (Qiagen Co., Ltd., Venlo, Netherlands). The RNA quality and quantity were assessed on a 2100 Bioanalyzer using the RNA 6000 Nano kit (Agilent Technologies Inc., Santa Clara, CA, USA).

One microgram of total RNA from the livers of the ND (n=3), WD (n=3) and OLG groups (n=3) was applied to library preparation. Sequencing libraries were generated using TruSeq RNA Library Preparation Kit v2 (Illumina Inc., San Diego, CA, USA). Library construction procedures were as follows: Total RNA was purified to mRNA and then mRNA was fragmented and converted to double-stranded cDNA. Sequencing adapters containing index sequences were ligated to the end of the cDNA. Libraries were sequenced on Illumina HiSeq 2500 (Illumina Inc.,) to generate 100-bp reads.

Statistical analysis. The results are expressed as means±S.E. One-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test, was used for comparing differences among multiple groups. Differences were considered significant at $**p<0.01$ and $*p<0.05$.

Sequence reads were trimmed and filtered by quality and these reads were mapped to GRCm38 (mm10) with the RNA-Seq mapping algorithm in CLC Genomics Workbench v8.0.3 (CLC Bio Japan Inc., Tokyo, Japan). We conducted an analysis of differentially expressed genes (DEGs) using the empirical analysis of digital gene expression (DGE) test. DEGs were generated based on a false discovery rate (FDR)-corrected p-value <0.05 using the empirical analysis of DGE tool in the CLC Genomics Workbench. Functional analyses of DEGs were performed using the ingenuity pathway analysis (IPA) software Ingenuity Systems (Qiagen Co., Ltd.). Activation z-scores of gene networks were predicted using IPA. The activation z-scores represent activation status (activated or inactivated state) of biofunction and diseases related to gene networks.

Results

Change in body weight and plasma biochemical examinations. Significant increases in weight were observed in the WD group compared to the ND group on the final day of the experiment. In contrast, OLG suppressed the increase in body weight caused by WD. The amount of feed intake was in a decreasing trend in the WD group; however, no significant difference was found (data not shown).

Biochemical indicators in the plasma samples, including TG, T-Cho, AST and ALT, showed significant increases in the WD group compared to the ND group. The blood glucose level showed an increasing trend in the WD group; however it was not significant. In contrast, no significant changes in TG, T-Cho and ATL were found in the OLG group compared to the ND group (Table I).

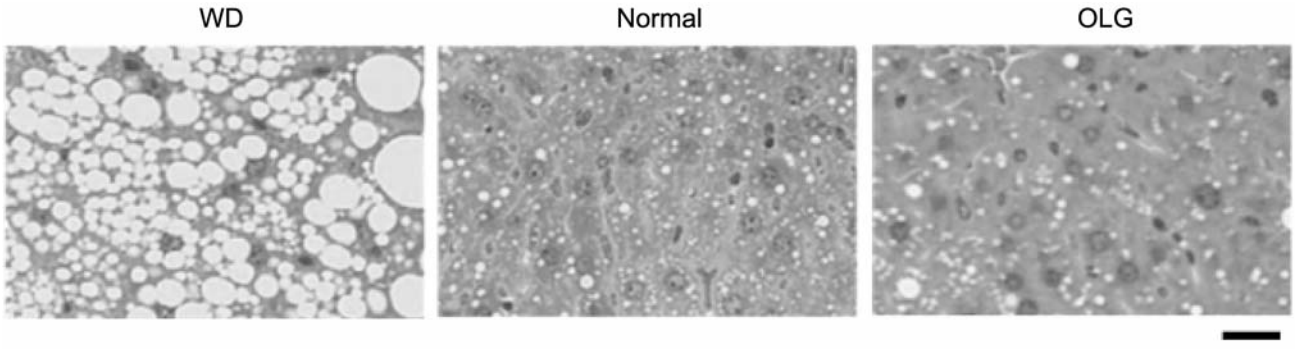

Figure 1. *Effect of Oligonol® (OLG) on histological features of Western diet (WD)-induced metabolic syndrome (MetS) in a murine model. Livers were fixed with formaldehyde and examined after HE staining. Numerous large lipid droplets were observed in WD, not in Normal group. In contrast to WD, the size of lipid droplets in OLG group decreased. Scale bars=100 μm.*

Table I. *Effects of Oligonol® (OLG) on a murine metabolic syndrome (MetS) model.*

	Normal	Western diet	OLG
Body weight (g)	31.4±0.9	38.9±1.3**	34.6±0.5
Liver/Body (weight %)	3.4±0.1	4.9±0.3**	3.5±0.4
TG (mg/dl)	86.4±3.2	118.7±5.2**	98.4±6.0
T-Cho (mg/dl)	102.9±4.3	238.9±9.9**	164.7±25.5*
GLU (mg/dl)	102.8±16.4	141.3±5.7	105±9.7
ALT (IU/l)	24.9±1.66	130±14.8**	50.7±12.8
AST (IU/l)	75.7±5.6	191.9±12.2**	155.8±16.8**

Body weight, liver weight/body weight, blood plasma triglycerides (TG), total cholesterol (T-Cho), glucose (GLU), aspartate aminotransferase (ALT) and alanine aminotransferase (AST) were measured at 12 weeks (mean±S.E.). Asterisks indicate significant differences between the untreated Western diet and (Normal) group (*$p<0.05$, **$p<0.01$).

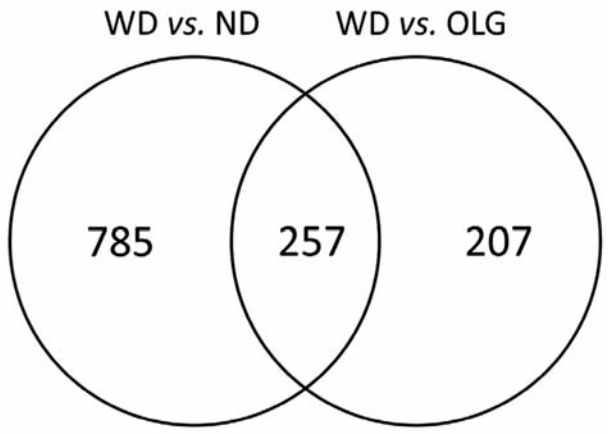

Figure 2. *Venn diagram of differentially expressed genes (DEGs). The number in each area showed the number of DEGs. This Venn diagram summarizes the overlap of DEGs from the left circle (WD vs. ND) and the right circle (WD vs. OLG). More than half of DEGs in WD vs. OLG are shared with WD vs. ND. WD, Western diet; ND, normal diet; OLG, Oligonol®.*

Histological analysis of the liver. Figure 1 shows the image of hepatic tissue. A lipid droplet is observed as white sections in HE staining. In the WD group, numerous depositions of lipid droplets were visible; in contrast, the size of lipid droplets was smaller in the ND and OLG groups than in the WD group. The state of hepatic tissue in the OLG group resembled that of the ND group (Figure 1).

Analysis of gene expression of the liver by RNA-Seq. Each sequence read in the ND, WD and OLG groups was subjected to gene mapping and statistical tests for differential gene expression were conducted to compare the levels of gene expression based on WD's level. DEGs with statistical significance were listed up when the absolute value of fold change (FC) was 2 or higher (FC ≥2), while adjusted *p*-value using FDR is <0.05.

The difference in number of DEGs between the WD group and other groups was larger in the ND group than in the OLG group. DEGs between the WD group and Normal group were 1,042 (767 were ≤–2, 275 were ≥2), whereas between the WD group and OLG group, DEGs were 464 (272 were ≤–2, 192 were ≥2). Comparing each DEG, 257 DEGs were found to be common in both conditions, where 166 were ≤–2 in the ND group (FC ≤–2) and 91 were ≥2 (FC > 2); in the OLG group, 168 were ≤–2 (FC ≤–2) and 89 were ≥2 (FC ≥2) (Figure 2).

Functional analysis of DEGs showed many diseases and functions, such as lipid metabolism, tumor, inflammation and inactivated immune system (Figure 3). Upon comparison between the WD and OLG groups, inactivation of genetic network involved in lipid metabolism was predicted in the OLG group, among the genetic networks involved in disease

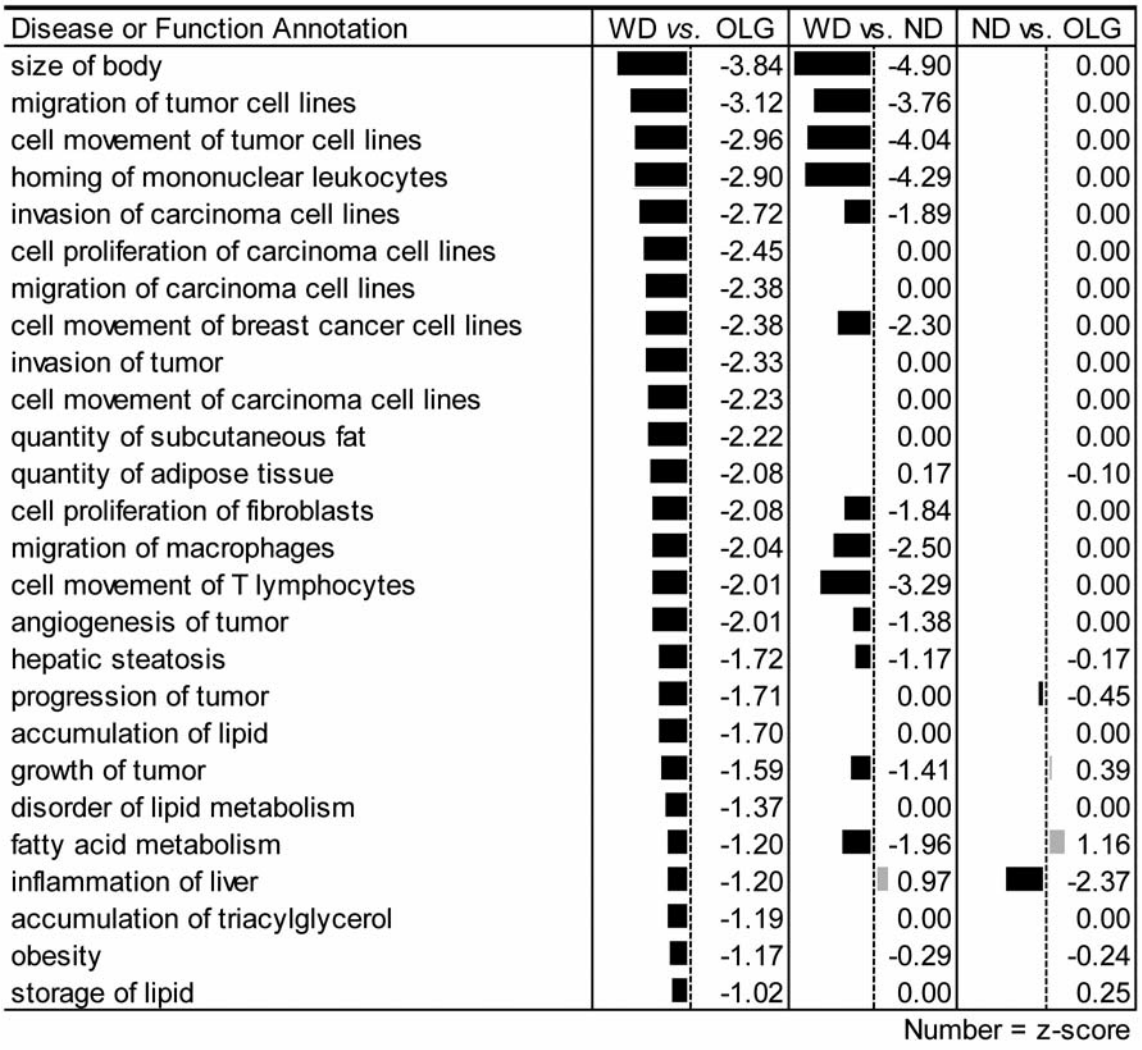

Figure 3. *Comparison of inactivated diseases or function annotation. Diseases or functions were annotated from DEGs and the activation z-score was predicted in Ingenuity Pathway Analysis (IPA). Black bars indicate the level of z-score, whereas the corresponding number shows value of z-score. Most of inactivated diseases or functions were shared with WD vs. OLG and WD vs. ND. DEG, differentially expressed gene; WD, Western diet; ND, normal diet; OLG, Oligonol®.*

and biological function. In particular, inflammation of liver (z=–1.200) and hepatic steatosis (z=–1.723) was predicted to be inactive. In addition, invasion of tumor (z=–2.333) was predicted to be inactive in the OLG group. When three conditions (WD *vs.* OLG, WD *vs.* ND and ND *vs.* OLG) were compared, the inflammation of liver was found to be slightly active in the WD *vs.* ND group; however, it was inactive under both conditions in the OLG group. Therefore, inflammation of liver is considered to be affected by OLG. Hepatic steatosis was predicted to be most inactive in the OLG group when the WD and OLG groups were compared (Figure 4). Table II shows the expression networks of genes involved in each disease.

With regard to FC of expression value of genes between WD and ND groups, 30 genes were extracted from top and bottom, respectively. All these genes showed a similar expression pattern within the ND and OLG groups. We further selected those genes with regards to difference of normalized amount of gene expression and reads per killobases per million (RPKM) between WD and OLG. Then, 20 genes were listed (Table III). In particular, expression of *Eif4ebp3, Serpina4-ps1, Lpin1, Selenbp2* and *Serpina1e* were repressed by WD, whereas their expression were facilitated by OLG. Furthermore, although *Sprr1a, Adig, Ly6d, Cidea* and *Apoa4* were expressed by WD, they were repressed in the OLG group (Figure 5).

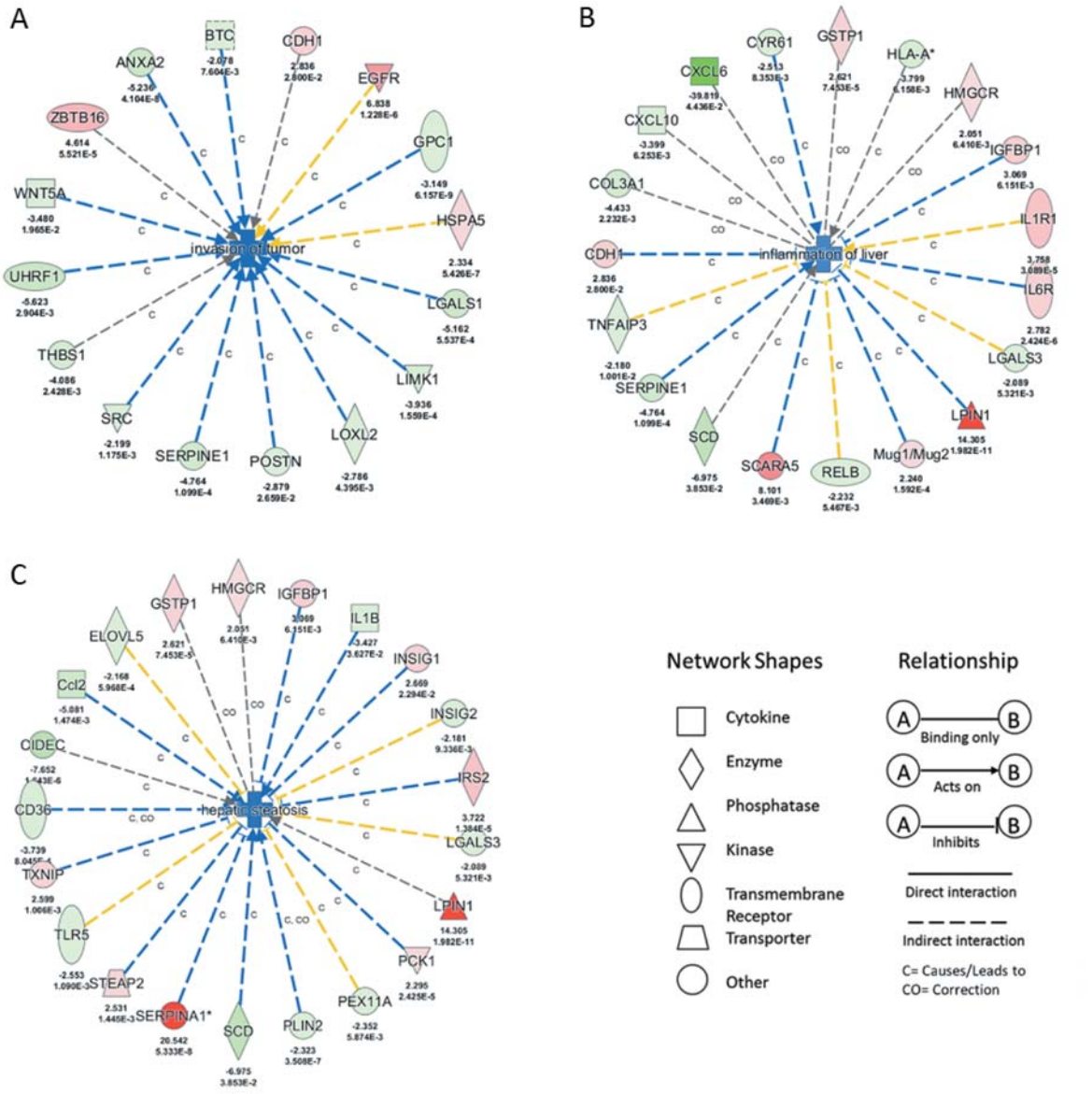

Figure 4. *Gene networks of inactivated diseases or functions in WD versus OLG groups. Attributed to OLG feed, A) hepatic steatosis, B) inflammation of liver and C) invasion of tumor were inactivated. WD, Western diet; OLG, Oligonol®.*

Discussion

The Western diet used in the present study is a special feed designed for the onset of metabolic syndrome and contains high load of fat and sucrose. This study essentially demonstrated the onset of a MetS-like syndrome based on the increase in body weight, hepatic steatosis and change of biochemical indicators. Recently, the dietary habits of Japanese have been changing to contain high load of fat and sucrose, as observed in the Western countries; therefore, Western diet for an animal model was deigned to study the change of dietary habits. In rats, fed with WD for 6 weeks, symptoms of hepatic steatosis appeared and the expression level of inflammation-related genes increased, thus leading to onset of non-alcoholic fatty liver disease (8).

The known functions of OLG have been reported as follows: suppression of oxidative stress-induced inflammation against C6 glial cells by suppressing NF-κB, COX-2 and iNOS; suppression of inflammatory cytokines and STAT3-SOCS3 and AMPK-mTOR pathways in human

Table II. *Differentially expressed genes (DEGs) involved in gene networks of Western diet (WD) versus Oligonol® (OLG) from analysis of disease and function annotation in Ingenuity Pathway Analysis.*

Categories	Disease or Function annotation	Genes	Number of genes z-score	Activation	*p*-Value
Organismal development	Size of body	*ACTG1, ANXA2, AQP7, BTC, Ccl2, CCNB2, CD36, COL3A1, COL5A2, DACT1, E2F1, E2F8, EGFR, FANCB, FMOD, FOSB, HELLS, HEYL, IGFBP1, IL1R1, INSIG1, IRS2, LAMA3, LEPR, MESP2, Mug1/Mug, NQO1, NTRK2, PCK1,PIM1, PIM3, PLIN2, POSTN, PTCH1, RCAN1, RDH16, RHBDF1, SERPINE1, SMPD3, SOCS2, SPRY4, SRC, STAT5A, STX1B, SULF2, TNFAIP3, VLDLR, WNT5A, ZNF423*	49	–3.84	9.40E-08
Cancer, organismal injury and abnormalities, tumor morphology	Invasion of tumor	*ANXA2, BTC, CDH1, EGFR, GPC1, HSPA5, LGALS1, LIMK1,LOXL2, POSTN, SERPINE1, SRC, THBS1, UHRF1, WNT5A, ZBTB16*	16	–2.33	1.64E-04
Gastrointestinal disease, hepatic system disease, metabolic disease, organismal injury and abnormalities	Hepatic steatosis	*ANXA2, ASNS, CCNA2, CDCP1, CDH1, COL1A1, CYR61, E2F1, E2F8, EGFR, IL1B, IRF6, JUN, NEK2, Nrg1, POSTN, S100A10, SCD, SRC, TLR5, TUBB2A, TXNIP, UHRF1, WNT5A, ZBTB16*	21	–1.72	4.39E-06
Gastrointestinal disease, hepatic system disease, inflammatory response, organismal injury	Inflammation of liver	*ANXA2, CXCL6, CYR61, EGFR, GPC1, IL1BLGALS1, SERPINE1, SMPD3, THBS1*	19	–1.2	1.10E-04

Table III. *Differentially expressed genes (DEGs) with high absolute values of fold change.*

	WD *vs*. OLG		WD *vs*. ND		ND *vs*. OLG	
Feature ID	Fold change	FDR corrected *p*-Value	Fold change	FDR corrected *p*-Value	Fold change	FDR corrected *p*-Value
Sprr1a	–262.26	1.46E-06	–262.26	4.65E-07	1.00	1.00E+00
Cidea	–23.30	6.60E-07	–67.20	3.10E-11	2.88	5.87E-01
Ly6d	–29.04	1.63E-12	–56.48	2.63E-16	1.95	1.00E+00
Ubd	–6.20	1.50E-05	–27.17	1.09E-13	4.39	3.20E-02
Smpd3	–2.42	3.45E-03	–26.52	5.78E-30	10.93	2.60E-14
Sdcbp2	–13.77	3.54E-02	–23.93	3.63E-03	1.74	1.00E+00
Col1a1	–4.99	2.13E-04	–21.91	1.63E-13	4.39	7.30E-03
Adig	–37.23	3.48E-08	–19.95	3.72E-07	–1.87	1.00E+00
Apoa4	–4.16	5.74E-04	–19.94	8.21E-16	4.79	1.54E-04
Phospho1	–8.07	1.03E-05	–14.64	2.47E-09	1.81	1.00E+00
Il1r1	3.76	3.09E-05	4.78	1.04E-07	–1.27	1.00E+00
Gm3839	5.50	1.27E-10	4.82	1.61E-09	1.14	1.00E+00
Sds	2.88	2.82E-03	5.21	3.16E-08	–1.81	3.48E-01
Zbtb16	4.61	5.52E-05	5.91	4.31E-07	–1.28	1.00E+00
Serpina1e	20.54	5.33E-08	10.54	1.19E-05	1.95	1.00E+00
Fkbp5	4.94	3.62E-09	13.38	0.00E+00	–2.71	1.40E-03
Selenbp2	20.02	2.34E-04	14.78	5.67E-04	1.35	1.00E+00
Serpina4-ps1	9.41	2.99E-16	25.46	3.36E-32	–2.70	2.11E-03
Eif4ebp3	31.59	3.90E-73	36.78	1.73E-78	–1.16	1.00E+00
Lpin1	14.30	1.98E-11	50.81	0.00E+00	–3.55	8.15E-03

Highly changed differentially expressed genes (DEGs) showed similar expression pattern between WD *vs*. ND and WD *vs*. OLG. Fold change cut-offs of >2 and corrected p-values (<0.05) by calculating the FDR indicate significant differences in gene expression. WD, Western diet; ND, normal diet; OLG, Oligonol®; FDR, false discovery rate.

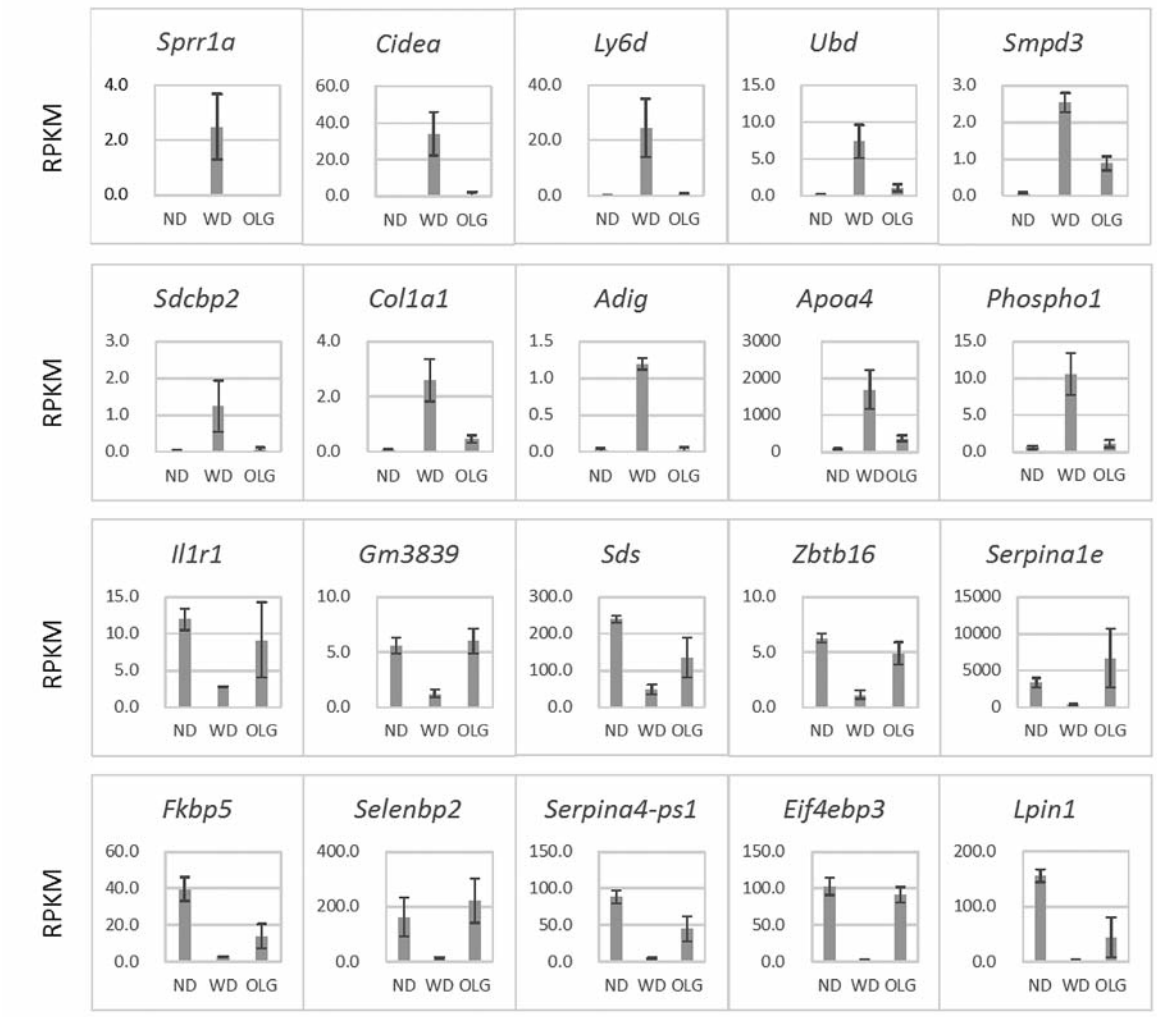

Figure 5. *Expression value (RPKM: estimation of gene expression) of genes in each condition. DEGs that showed similar expression pattern between ND and OLG groups were selected. Bars indicate value of RPKM means with ±S.E. in each condition. These genes' full names are Adig (adipogenin), APOA4 (apolipoprotein A-IV), CIDEA (cell death-inducing DFFA-like effector a), COL1A1 (collagen, type I, alpha 1), EIF4EBP3 (eukaryotic translation initiation factor 4E binding protein 3), FKBP5 (FK506 binding protein 5), Gm3839 (glyceraldehyde-3-phosphate dehydrogenase pseudogene), IL1R1 (interleukin 1 receptor, type I), LPIN1 (lipin 1), LY6D (lymphocyte antigen 6 complex, locus D), PHOSPHO1 (phosphatase, orphan 1), SDCBP2 (syndecan binding protein 2), SDS (serine dehydratase), SELENBP1 (selenium binding protein 1), SERPINA1 (serpin peptidase inhibitor, clade A, alpha-1 antiproteinase, antitrypsin, member 1), Serpina4-ps1 (serine or cysteine peptidase inhibitor, clade A, member 4, pseudogene 1), SMPD3 (sphingomyelin phosphodiesterase 3), SPRR1A (small proline-rich protein 1A) and UBD (ubiquitin D), ZBTB16 (zinc finger and BTB domain containing 16). RPKM, Reads per killobases per million; DEG, differentially expressed gene; WD, Western diet; ND, normal diet; OLG, Oligonol®.*

hepatic HepG2 cells; suppression of renal disorder and CCL4-induced rat hepatic disorder in type-2 diabetes murine model by suppressing NF-κB; accelerating fat degradation in rat adipose cell culture system by activating ERK1/2; and suppression of chemically-induced skin cancer caused by phorbol ester or decreased COX-2 expression level after UV-B irradiation (4, 9-12). These studies and our results suggest that OLG has suppressive effects on lipid metabolism and inflammatory action on a metabolic syndrome model.

The present study revealed that OLG suppresses the raise of biochemical indicators of lipid metabolism in serum and liver. In addition, the suppressive effect of OLG on lipid droplet accumulation was visually confirmed in the liver. Thus, OLG was shown to have suppressive effects on the onset of metabolic syndrome caused by high calorie diet.

To elucidate the mechanism of OLG on metabolic syndrome, we conducted a systematic genetic analysis by RNA-Seq and compared DEGs in the WD and OLG groups

with that in the WD and ND groups. The results showed that more than half of DEGs in the WD and OLG groups (257) were the genes commonly observed in DEGs in the WD and ND groups (Figure 2). This suggests, when OLG is ingested with high calorie diet, then the state of gene expression resembles that in the normal state; in contrast, the WD group demonstrated that high calorie intake drastically changed gene expression in the liver.

When we further scrutinized the genes commonly expressed in the Normal and OLG groups, the following genetic networks were predicted to be suppressed: inflammation of liver, hepatic steatosis, invasion of tumor, obesity, disorder of lipid metabolism (cell proliferation of carcinoma cell lines, invasion of tumor, angiogenesis of tumor, concentration of fatty acid, accumulation of lipid). These results enabled us to estimate the types of pathology that are affected by OLG regarding the onset and prevention of metabolic syndrome (Figure 3). We prepared a schematic of genetic networks involved in inflammation of liver, hepatic steatosis and invasion of tumor (Figure 4) that enable us to obtain insights regarding genetic dynamics and disease on OLG administration. However, since the results revealed some genes with unknown function, further analysis is required.

The present study focused particularly on the amount of gene expression (RPKM) in ND, WD and OLG groups and picked 20 genes that showed correlative behavior in the WD and OLG groups. Among these 20 genes, OLG administration facilitated the expression of *Serpina1e, Selenbp2, Eif4ebp3, Serepina4-ps1* and *Lipin1* and suppressed the expression of *Cidea, Ly6d, Apoa4, Phospho1, Sprr1a, Col1a1* and *Adig*, those that are reportedly involved in lipid metabolism. Expression of Serpina1a was decreased in WD, while highly augmented by OLG feed. Deficiency of Serpina1a protein increases fatty liver in the organism (13) and hepatocellular cancer in humans (14).

Lipin1 was discovered in 2001 from a lipoatrophy murine model and is reportedly involved in two functions: fatty acid synthesis and degradation depending on intracellular localization (15, 16). In experimental alcohol-induced hepatic steatosis, liver-specific *Lipin1* deficiency in mice exacerbates the development and progression of steatohepatitis (17). It has been suggested that Lipin1 plays a protective role likely *via* transcriptional regulation of genes involved in fatty acid catabolism (17). In our study, we confirmed Lipin1 deficiency in WD. However, Lipin1 showed up-regulated expression in the OLG group. IPA predicted that Lipin1 occurs in the anti-inflammatory network (Figure 4).

Adig (adipogenin) has been reported to be produced by subcutaneous and visceral adipose tissue in mice fed by high-fat diet and known to control adipocyte differentiation. More recently, the decreases of subcutaneous adipose tissue and liver fat have been reported in *Adig* knock-out (KO) mice (17). Furthermore, single nucleotide polymorphism (SNP) of *CIDEA* and *V114* is reportedly associated with the risk of metabolic syndrome and three types of cell death-inducing DNA fragmentation factor alpha-like effector (CIDE) protein, Cdiea, Cideb and CIdec (FSP27), are known (18, 19). They are found in adipose droplets or endoplasmic reticulum in adipose cells or liver cells. KO mice for Cdiea, Cideb and CIdec are less prone to be obese and consume a large amount of energy. Cide protein influences lipid metabolism (lipid and lipid droplet synthesis) by regulating the stability of AMP kinase (20).

Therefore, OLG is suggested to prevent metabolic syndrome *via* gene regulation involved in lipid metabolism. Furthermore, since OLG represses genetic networks associated with inflammation and tumor, it is expected to suppress -at the gene level- the risk of lipid accumulation, hepatic steatosis, hepatitis and carcinogenesis in the liver.

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