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RNA-seq analysis of diet-driven obesity and anti-obesity effects of quercetin glucoside or epigallocatechin gallate in *Drosophila* adults

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Abstract. – OBJECTIVE: High-fat diet (HFD) feeding stimulates fat accumulation in mammals and *Drosophila*. In the present study, we examined whether simultaneous feeding of familiar anti-obesity drugs, quercetin glycosides (QG) and epigallocatechin gallate (EGCG), to *Drosophila* has the same suppressive effect on fat accumulation as previously reported in rats and mice. To understand the underlying molecular mechanisms of HFD diet-induced obesity and the suppression effect of the drugs, we performed transcriptome analyses.

MATERIALS AND METHODS: We induced extra fat accumulation by feeding *Drosophila* fly food containing 20% coconut oil and quantified the triglyceride accumulated in flies. The effects of anti-obesity drugs were also evaluated. We isolated total RNA from each sample and performed RNA-seq analyses and quantitive Real Time-Polymerase Chain Reaction (qRT-PCR) to investigate altered gene expression.

RESULTS: The mRNA levels of several genes involved in lipid metabolism, glycolysis/gluconeogenesis, and anti-oxidative stress changed in HFD-fed adults. Moreover, the levels altered in those fed an HFD with QG or EGCG. The qRT-PCR further confirmed the RNA-seq data, suggesting that the expression of five essential genes for lipid metabolism changed in HFD-fed flies and altered in the flies treated with anti-obesity drugs. The most remarkable alteration was observed in the dHSL gene encoding a lipase involved in lipid-storage after HFD feeding and HFD with QG or EGCG. These alterations are consistent with HFD-induced fat accumulation as well as the anti-obesity effects of the drugs in mammals, suggesting that the genes play an important role in anti-obesity effects.

CONCLUSIONS: These are the first reports to date of entire profiles of altered gene expression under the conditions of diet-induced obesity and its suppression by anti-obesity drugs in *Drosophila*.

Key Words
RNA sequence, Drosophila, High-fat diet, Fat accumulation, Catechin, Quercetin.

Introduction

Overweight and obesity are defined as an abnormally excessive fat accumulation that presents a risk to health. This condition is currently considered to be an important clinical and public health burden worldwide. Epidemiologic studies suggest that overweight and obesity are important risk factors for a number of chronic diseases including diabetes, cardiovascular disease, cancer, and premature death[1,2]. The prevention and treatment of overweight, obesity and its related co-morbidities and early mortalities have been among the most important issues in public health[2,3]. To this end, several chemical compounds have recently been discovered and applied as medicines that may mitigate or prevent obesity, including orlistat, lorcaserin, rimonabant and metformin[4-7]. In addition, naturally occurring compounds have also drawn attention as appealing anti-obesity agents, because of their anticipated multimodal action and limited toxicity[8]. For example, catechin, which is contained in many dietary products, plants, fruits, green tea and red wine is known for its multiple health beneficial effects[8-10]. Clinical studies[11] have shown that the beneficial effects of catechin are related to its antioxidant action. Catechin is also involved in the regulation of cell death, angiogenesis and multidrug resistance in cancers and related disorders. Major catechin found in green tea is (-)-epigallocatechin-3-gal-

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late (EGCG)[8]. This polyphenolic compound and several related compounds are believed to be responsible for the health benefits associated with the consumption of green tea. The potential health benefits ascribed to green tea and EGCG include antioxidant effects, cancer chemoprevention, improvement of cardiovascular health, weight loss, protection of the skin from damage caused by ionizing radiation, and so on[11-15]. Recently, investigations of the anti-obesity effect of EGCG and its underlying molecular mechanism have become attractive topics of research. It has been reported that EGCG suppresses the onset of obesity in mice[16-18] and ameliorates excess fat storage induced by high-fat diet (HFD) in *Drosophila*[19]. However, the mechanism underlying the effects of EGCG on obesity are not described to date. Another group of naturally occurring compounds considered as an attractive candidate for the prevention of obesity is quercetin glycosides (QGs), which have one to seven glucose molecules. The QG belongs to a member of the flavonoid family and is one of the most prominent dietary antioxidants[20]. The compound is commonly present in many foods including vegetables, fruit, tea and wine, as well as many food supplements. A variety of beneficial health effects have been attributed to QG. It was illustrated that a diet including QG exhibits anti-diabetic effects in mammals[22-25]. Furthermore, its beneficial effects are considered to prevent various diseases such as osteoporosis, certain forms of cancer, respiratory and cardiovascular diseases[26,27]. In particular, the ability of QG to scavenge highly reactive species such as peroxynitrite and the hydroxyl radical is suggested to play a role in these beneficial health effects. Numerous studies[26] have been performed to gather scientific evidence for these beneficial health claims, as well as data regarding the exact mechanism of action and possible toxicological aspects of this flavonoid. However, the anti-obesity effect of quercetin and the corresponding molecular mechanism is not entirely understood.

To evaluate the anti-obesity effects of these candidates for use in potential medicine, it is essential to perform biological assays on an individual level using experimental animals. Mouse and rat have been used as experimental animals in the majority of such assays. However, bioassays using rats and mice are time-consuming and cost-intensive. In addition, the use of these model organisms has recently become more difficult due to ethical issues. On the other hand, *Drosophila melanogaster* serves as another useful genetic model due to its high fecundity and short life cycle[28,29]. Advances in genetic techniques for *Drosophila* have facilitated the experimental investigation, providing numerous advantages in drug discovery particularly as an experimental model for studies on several human diseases[29-32]. Furthermore, not only lipid metabolism pathways, but also enzymes and genes involved in lipid uptake, transport, storage, and mobilization are highly conserved between human and *Drosophila*[33]. Therefore, *Drosophila* is a more practical model for studies to identify and characterize the mechanisms causing obesity and related diseases. It can also provide an excellent experimental tool to evaluate anti-obesity drugs in a shorter period. In this work, we established a conventional method for diet-induced extra fat accumulation, which allowed us to evaluate the influence of both QG and EGCG with respect to obesity using the *Drosophila* model. Thus, we performed RNA-seq analysis to identify the genes involved in HFD-induced obesity and those involved in the effects of anti-obesity drugs.

Materials and Methods

Feeding Assay

Canton S was used as a standard control stock *Drosophila* strain. Parent flies were reared on the normal diet (ND) described below. The offspring were used for HFD feeding experiments. Five- to ten-day-old flies were collected, keeping males and females apart. Twenty flies of both sexes were reared in a single plastic vial containing normal food. ND: for every 150 ml of the food, 2.1 g of agarose, 6 g of cornmeal, 3 g of dry yeast, and 6 g of sucrose was added to 150 ml of water, mixed and boiled. Food was cooled, after which 1.5 mL of 10% parahydroxybenzonate and 1.5 mL of propionic acid were added as anti-skeptics. HFD: The ND was supplemented with 20% of coconut oil (Nacalai Tesque, Kyoto, Japan). (-)-Epigallocatechin-3-gallate (EGCG, Taiyo Kagaku, Mie, Japan) was administered to flies at a concentration of 10, 20, 40 μg/ml. Quercetin glycosides (QG; Saneigenffi, Osaka, Japan) were administered at 10, 40, 80 μg/ml. Treatments with EGCG and QG: 0.5 ml of 10 times concentrated solution of EGCG and QG were prepared, respectively. Each drug was added to a single plastic vial containing 4.5 ml HFD, and stirred immediately. The foods were kept at room temperature overnight to allow them to solidify and to dry their surface. Flies

were reared on ND, or HFD with or without each for 7 days at 25ºC. After the treatments, the flies were returned to the standard diet for 2 hours to let them excrete undigested foods from their guts. Subsequently, the flies were collected and rinsed with Phosphate-Buffered Saline (PBS) containing 1% Triton X-100 (Nacalai Tesque, Kyoto, Japan). Wings and legs were removed using a pair of fine tweezers.

Triglyceride (TG) Measurement

Treated adult flies were collected and dried by SpeedVac for 20 min at 65°C. Six to twelve flies were measured together to calculate dry weights of individual flies. After that, the dry flies were crushed in 1 ml of chloroform and methanol (1:1) by using tissue lyser (Qiagen, Hilden, Germany). After incubating for 10 min at room temperature, they were centrifuged at 4 000 x g for 5 min at room temperature, and the supernatant was collected. Residual chloroform and methanol were removed by precipitation with isopropanol. The quantification of TG was performed with the triglyceride E-test kit (Wako, Osaka, Japan). A comparison of TG quantity between adults reared on ND and those on HFD was performed using Student's *t*-test. One-way ANOVA with Dunnet's multiple comparison was applied to assess the differences in more than two groups. Statistical analyses were performed using Prism Software (GraphPad Software, La Jolla, CA, USA). Data were considered significant at p-values <0.05.

RNA-Seq Analysis

Total RNA was extracted from 15-day-old adults using the Isogen kit (Nippon Gene, Tokyo, Japan). The isolated RNAs were used for the construction of single-end mRNA-seq libraries, using a Sureselect strand specific RNA Library Prep Kit (Agilent, Santa Clara, CA, USA) according to the manufacturer's recommendations. The mRNA-seq was performed on an Illumina NextSeq 500 instrument (Illumina, San Diego, CA, USA) using 75-bp single-end reads. Read quality was checked for each sample using FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) (version 0.11.5). We aligned more than 40 M reads onto the reference *D. melanogaster* genome sequence (Dmel-all-chromosome-r6.12.fasta) using the TopHat programme version 2.0.13, with default parameters[34]. Cufflinks (version 2.2.1) was employed with default parameters for transcript assembly[35]. The expression level of each gene was quantified as FPKMs (fragments per kilo base of exon per million mapped fragments). We carried out gene ontology (GO) analysis of genes expressed in adults and aligned the genes whose expression was significantly changed, with p-values of <0.1, using DESeq2[36]. The GO classification system was applied by employing the database for annotation, visualization and integrated discovery using MeV. The obtained RNA-seq data have been submitted to the Gene Expression Omnibus database (accession number: GSE121531).

Quantitative Real Time-Polymerase Chain Reaction Analysis

Total RNA was extracted from adult flies from each genotype using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA synthesis from total RNA, including miRNAs with artificial poly (A) tails, was carried out using the PrimeScript II Fidelity Real Time-Polymerase Chain Reaction Kit (qRT-PCR; TaKaRa, Otsu, Shiga, Japan) with an oligo dT primer. Real Time-PCR was performed using the FastStart Essential DNA Green Master (Roche, Basel, Switzerland) and a Light Cycler Nano instrument (Roche, Basel, Switzerland). The qPCR primers were constructed as follows: Mag Fw: 5'CCGGAGTACAATGCCCTCATT3', Mag Rv: 5'GACCCAGGGCATTGAAGATGA3', FASN1 Fw: 5'AAAGCTTCAGTGGGTGGACAA3', FASN1 Rv: 5'CAGGTACAGCTCACGAAGGTT3', Lsd-1 Fw: 5'CCGCTGCTAATCGGGTCATAT3', Lsd-1 Rv: 5'GCACTAGTGTGGGACTCCAAA3', dHSL Fw: 5'CTGAGACCCCGACAATGAACA3', dHSL Rv: 5'GAGAAATGGCCAATGGGGGTA3', Bmm Fw: 5'ACTCTTCCAGGACGCTTTGAG3', Bmm Rv: 5'AGTTGGCCAGACCCTTATTGG3', RP49 FW: 5'TTCCTGGTGCACAACGTG3', RP49 RV: 5'TCTCCTTGCGCTTCTTGG3'.

Results

Establishment of the Assay System to Quantify Fat Accumulation in Drosophila by Feeding With a Higher Fat Diet and a Diet Supplemented With Anti-Obesity Drugs

We used a standard wild-type strain of *Drosophila*, called Canton S, to examine fat accumulation. We measured the amount of TG accumulated in the entire adult body, separately for male and female flies. After feeding of HFD containing 20% coconut oil for 7 days, the amount of TG in adult flies was found to

be higher than that of control flies reared on ND. Adult males contained TG corresponding to 5.0% of body weight on average (three repeated measurements in 40 flies). This value increased to up to two times that of control males (10.0% of body weight) after HFD treatment. The average TG amount accumulated in the whole body increased significantly (p<0.001) (Figure 1A). Similarly, adult females contained 6.5% of body weight on average (n=40) (Figure 1B). This value increased to up to 10.0% of total body weight after HFD feeding for 7 days. The average TG amount accumulated in the female bodies also increased by 1.5 times compared to the controls (p<0.01). Thus, the amount of TG contained in both males and females sharply increased in this feeding condition. These results allowed us to conclude that this simple feeding method readily reproduces diet-induced obesity in both males and females of *Drosophila*. This success encouraged us to further examine whether two chemical compounds, EGCG and QG, both of which have an anti-obesity effect in mammals, would influence TG accumulation of wild-type flies reared on the HFD for 7 days. We fed the flies the HFD supplemented with QG or EGCG at three different concentrations. First, we measured and calculated the amount of TG accumulated in fly bodies reared on ND (control), HFD without the drug (HFD), HFD containing QG at 10 μg/ml concentration (Q10), HFD with 40 μg/ml QG (Q40), or HFD with 80 μg/ml QG (Q80) (Figure 2A, B). We found a significant decrease in the TG accumulated in wild-type males after 7-day-feeding of HFD supplemented with QG at every concentration, although dose-dependency of the anti-obesity drug was not observed in these conditions. The QG treatment (i.e. continuous feeding of HFD supplemented with QG) noticeably decreased the amount of TG accumulated in adult male flies to 7.2%, 7.0%, and 7.0% for each above-mentioned concentration, respectively, compared to the 10.0% of the whole body on average for HFD without the drug (n=30 for each treatment, p<0.001, one-way ANOVA with Dunnet's multiple comparison) (Figure 2A). Likewise, in adult females, the TG amount decreased to 8.7% and 8.5% of the body weight by Q10 and Q40 treatment, respectively, compared with HFD without the drug (n=30 for each treatment). The difference in decrease of the TG amount was less noticeable, but still statistically significant for the two concentrations (p<0.05). Conversely, the TG increased in amount when QG was administered to adult females at the highest concentration (Q80) (Figure 2B). By the same methods, we examined whether another known anti-obesity drug, EGCG, demonstrates a similar effect in *Drosophila*. We measured the quantity of TG in males and females reared on ND (control), HFD without the drug (HFD), HFD containing EGCG at 10 μg/ml concentration (E10), HFD with 20 μg/ml EGCG (E20), or HFD with 40 μg/ml EGCG (E40) for 7 days. We found that the EGCG treatment had a significant effect of suppressing excessive TG accumulation in both males (Figure 3A) and females (Figure 3B). We observed a more remarkable decrease of TG in males fed HFD with EGCG, compared with the decrease found in males fed HFD with QG (p<0.001) (Figure 2A, 3A).

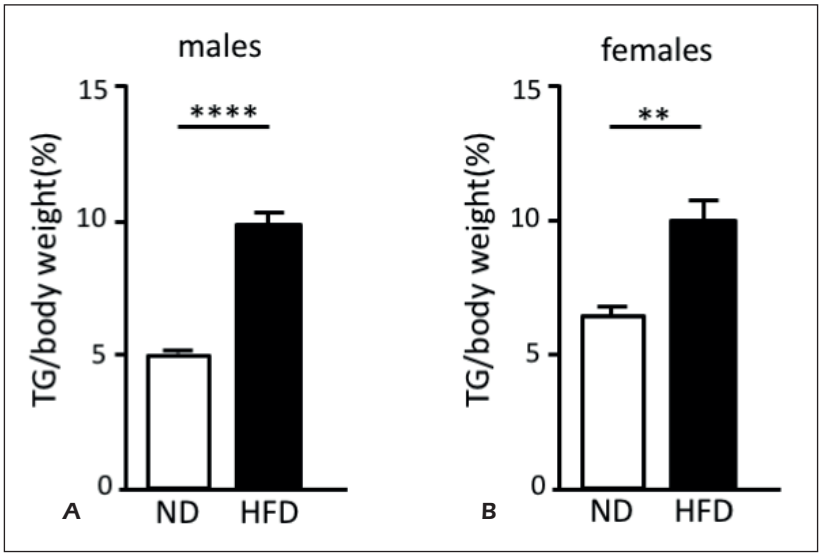

Figure 1. Accumulation of triacylglycerol (TG) in male (**A**), and female (**B**), flies. Normal diet (ND), n=40 for adult males. n = 40 for adult females; high-fat diet (HFD), n=35 for adult males, n=38 for adult females. The statistical analyses were carried out with the Student's t-test. **p<0.01, ****p<0.0001.

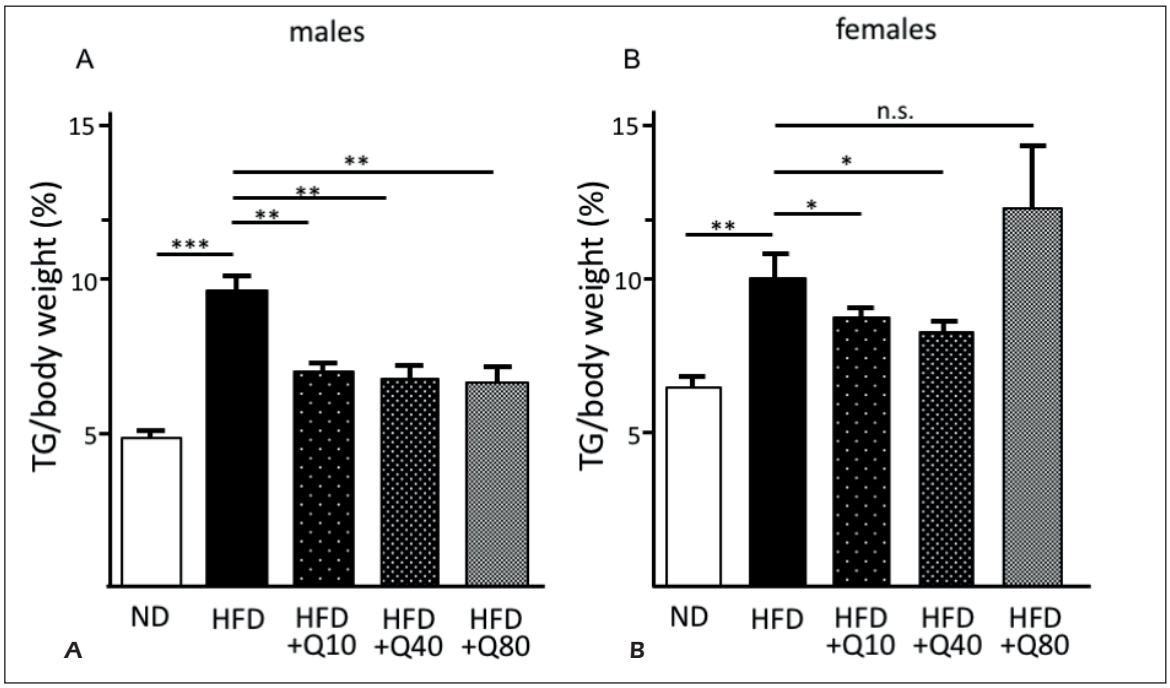

Figure 2. QG suppressed the accumulation of TG in the wild-type male **(A)** and female flies **(B)** reared on high-fat diet (HFD). Ten flies were weighed for each concentration. Normal diet (ND), high-fat diet (HFD), HFD and 10 μg/ml QG (Q10), HFD and 40 μg/ml QG (Q40), HFD and 80 μg/ml QG (Q80), n=30 for each treatment. Statistical analyses were performed using one-way ANOVA with the Dunnet's multiple comparison. ns, not significant; **p*<0.05; ***p*<0.01; ***p*<0.01; ****p*<0.001.

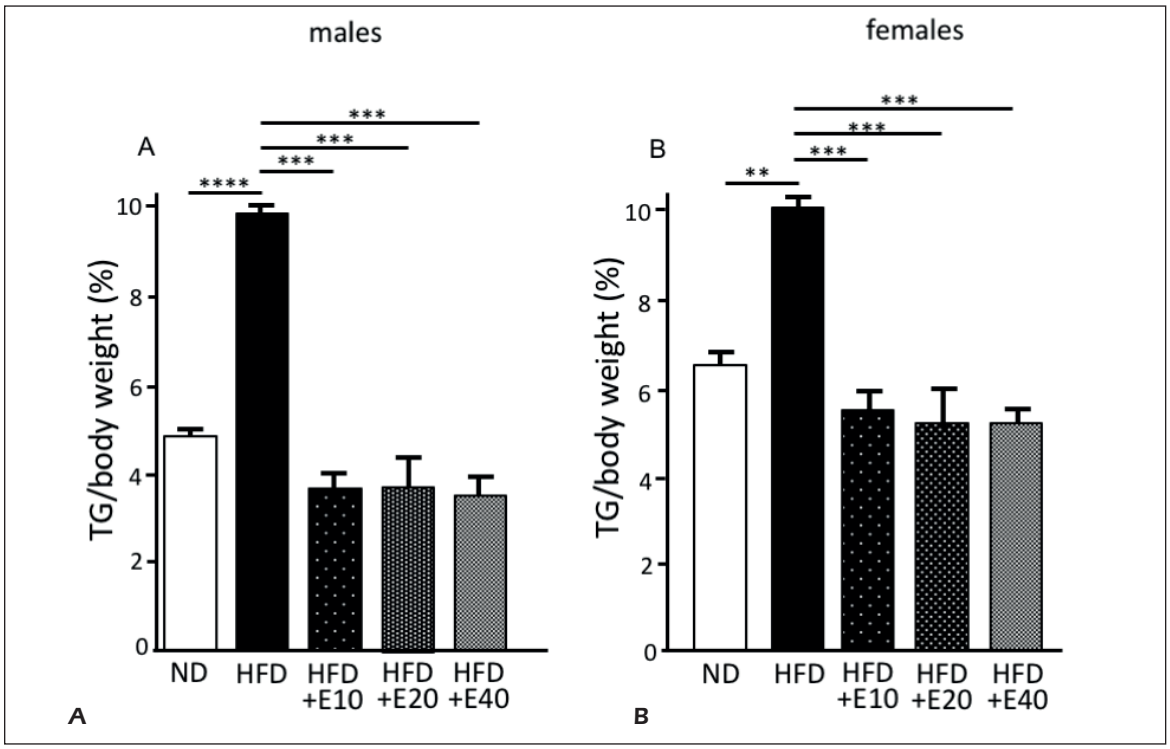

Figure 3. Effects of EGCG on TG accumulation induced by high-fat diet in wild-type male and female flies. Normal diet (ND), high-fat diet (HFD), HFD and 10 μg/ml EGCG (E10), HFD and 20 μg/ml EGCG (E20), HFD and 40 μg/ml EGCG (E40), n=20 for ND males and n=30 for HFD, E10, E20, E40 males, n=60 for all treatments of females. The Statistical analyses were performed using one-way ANOVA with the Dunnet's multiple comparison. ***p*<0.01, ****p*<0.001, *****p*<0.001.

TG quantities in adult males fed HFD with EGCG at each concentration ranging from 10 to 40 μg/ml decreased to less than 40% of the TG in males fed HFD (Figure 3A). A markedly higher amount of TG in females fed an HFD is observed compared to females fed an ND (p<0.001). A feeding on HFD with EGCG at 10 to 40 μg/ml for 7 days remarkably decreased the TG amount in females, compared with the amount in females on HFD (p<0.001).

RNA-Seq Analyses for Overview of Alteration of Gene Expression in Adults on HFD

We performed a comprehensive RNA-seq analysis to identify genes whose expression altered in flies harboring extra fat accumulation. Initially, DNA sequences of cDNA clones derived from adult males fed ND were determined to identify each clone. Similarly, DNA sequences of cDNA clones derived from adult males fed HFD were determined to identify each clone. We determined the DNA sequences of 47,392,413 cDNA reads (35-76 nucleotides in length) prepared from mRNAs expressed in ND-fed adult males and 54,695,739 cDNA reads from HFD-fed males. The RNA-seq reads were mapped to a total of 43,922,631 and 51,027,431 mRNAs, respectively. The mapping frequencies of the reads were 92.7% for the controls and 93.3% for the HFD-fed adults. Similarly, we determined the DNA sequences of 47,296,852 cDNA reads (35-76 nucleotide in length) prepared from mRNAs expressed in ND-fed adult females and 59,643,082 cDNA reads from HFD-fed females. The RNA-seq reads were mapped to a total of 44,477,960 and 55,848,066 mRNAs, respectively. The mapping frequencies of the reads were 94.0% for the controls and 93.6% for the HFD-fed adults. Subsequently, we carried out analysis of expression using Cuffdiff v2.2.1 software. For males and females, we separately determined the FPRK value of ND-fed adults and that of HFD-fed adults and performed their logarithmic transformation. We found 203 genes (112 up-regulated and 91 down-regulated genes) whose mRNA levels significantly altered (p<0.1, Wald test) due to the difference in diet. Among the down-regulated genes (47 genes) or up-regulated genes (16 genes) in the HFD-fed adult males, we compiled lists of genes showing significant alterations (top 20 genes or all genes if fewer than 20 were present) in mRNA levels, with down-regulated and up-regulated genes in Tables I and III, respectively. GO analysis revealed that some of the genes differentially expressed in ND-fed and HFD-fed adults are involved in carbohydrate metabolism pathways, lipid metabolism pathways and immune responses, such as response to bacteria. Subsequently, we carefully examined the RNA-seq analysis data to discern whether the expression of genes related to obesity changed in adults fed HFD, compared to adults fed ND. First, we inspected the following 21 genes related to lipid metabolism as follows: mag, FASN1, FASN2, AkhR, Lsd-1, bmm, Hsl, srl, Desat1, CG3394, CG3961, whd, CPT-2, SCP-X, CG12262, Acox57D-d, ILK, PDK1, Ubi-p63E, CG10924, and Gk2. The mRNA of the FASN1 gene (FBtr0077659) encoding Fatty acid synthetase 1 markedly decreased in amount in the HFD-fed males, compared to that in the ND-fed adults. In contrast, mRNA of the *FASN2* gene (FBtr0077657) encoding Fatty acid synthetase 2 significantly increased in the HFD-fed adults. The mRNA level of the *Desat1*gene (FBtr0082627) encoding stearoyl-CoA desaturase remarkably decreased in HFD-fed males but increased in HFD-fed females. The expression of the other listed genes was not significantly altered in HFD-fed adults of both sexes. Secondarily, we examined whether the expression of oxidative stress-related genes, which are known to be closely associated with obesity, is altered in adults fed HFD. With respect to six anti-oxidative stress-related genes (Sod1, Sod2, Sod3, catalase, hsp83, and CG9510), mRNAs of both Sod1 and Sod2 genes increased in amount in both HSD-fed males and females, although these changes were not statistically significant.

Furthermore, we focused on other lipid metabolism-related genes that are likewise involved in glucose metabolism, gluconeogenesis, and cell cycle regulation to examine whether the expression of these genes has altered in adults fed HFD. Previous studies reported that the expression levels of these types of genes were altered after feeding of HFD or simultaneous feeding of HFD with Quercetin or EGCG in *Drosophila*[37,38] and mice[39]. Therefore, we first examined the expression of three subsequent lipid metabolism-related genes, *Acsl, FABP,* and *CCt1* in *Drosophila* adults fed HFD and those fed ND. The mRNA level of *Acsl* and *FABP* did not remarkably change. The mR-

Table I. A list of genes whose mRNA levels remarkably decreased in HFD-fed male flies, compared to ND-fed male flies[*1].

Gene short name	Gene name	ND[*2]	HFD[*3]	log2 (Ratio)[*4]	Function[*5]
CG34040	CG34040	62	2	- 4.954	Unknown
CG4847	CG4847	83	6	- 3.79	Proteolysis involved in cellular protein catabolic process
Dro	Drosocin	223	17	- 3.713	Antibacterial peptide with activity against Gram-negative and Gram-positive bacteria
CG43897	CG43897	73	6	- 3.605	Mesoderm development
Tobi	Target of brain insulin	273	23	- 3.569	Glycoside hydrolase superfamily
CG18180	CG18180	165	23	- 2.843	Serine proteases
Jon99Ci	Jon99Ci	70	12	- 2.544	Serine-type endopeptidase
CG42807	CG42807	167	29	- 2.526	Known
Mal-A6	Maltase A6	359	63	- 2.511	Carbohydrate metabolic process
CR45045	CR45045	74	13	-2.509	ncRNA
Mal-A4	Maltase A 4	73	13	- 2.489	Carbohydrate metabolic process
Mal-A8	Maltase A 8	213	39	- 2.449	Carbohydrate metabolic process
Mal-A7	Maltase A 7	140	26	- 2.429	Carbohydrate metabolic process
DptA	Diptericin A	99	19	- 2.381	Antibacterial peptide against Gram-negative bacteria
CG34166	CG34166	418	81	- 2.368	Known
CG4363	CG4363	488	98	-2.316	Known
Mtk	Metchnikowin	350	79	-2.147	Antifungal peptide that is secreted from the fat body
Obp99b	Odorant-binding protein 99b	87	20	-2.121	Response to pheromone; olfactory behaviour; sensory perception of smell
Amy-p	Amylase proximal	752	182	-2.047	Alpha amylase
Jon25Bi	Jon25Bi	132	33	-2.000	Peptidase S1, chymotrypsin family

[*1]Among genes whose mRNA levels decreased in male flies fed HFD, compared with those in males fed ND, top 20 genes (p<0.1) are listed in ascending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in control flies fed ND.
[*3]The FPKM of mRNA reads expressed in flies fed HFD.
[*4]The log2 (ratio of the FPKM of mRNA reads) in HFD-fed flies to that in ND-fed flies.
[*5]http://flybase.org/

NA level of *Cct1* gene encoding CTP: phosphocholine cytidylyltransferase 1 increased in HFD-fed adult males, while amounts of mRNAs of *Asc1* and *FABP* genes did not change. Secondarily, among eight glucose metabolism and gluconeogenesis-related genes (*srl, Pepck, Ilp5, Amylase-p, Amylase-d, sug, tobi,* and *upd2*), the mRNA levels of both *Amylase-p* and *Amylase-d* genes significantly decreased in HFD-fed females. The mRNA level of the *sug* gene encoding a transcription factor for DILP genes markedly increased in HFD-fed females. The mRNA level of the *tobi* gene encoding a target gene for the DILP signaling pathway significantly decreased in HFD-fed adults in both males and females.

Overview of Alteration in Gene Expression Identified by RNA-Seq Analysis in Adults Fed HFD with QG or Those Fed HFD With EGCG

After we identified genes whose mRNA levels were changed in adults with extra fat accumulation, we examined whether the altered expression of these genes further changed in adults fed HFD supplemented with either of QG or EGCG at 40 mg/ml. We initially determined DNA sequences of cDNA clones derived from adult males fed HFD with QG or EGCG, and then identified the genes corresponding to the cDNA clones. Similarly, DNA sequences of cDNA clones derived from adult males fed HFD with QG or EGCG were determined to

Table II. A list of genes whose mRNA levels remarkably decreased in HFD-fed female flies, compared to ND-fed female flies[*1].

Gene short name	Gene name	ND[*2]	HFD[*3]	log2 (Ratio)[*4]	Function[*5]
Fcp3C	Follicle cell protein 3C	47	1	- 5.555	Unknown
CG6422	CG6422	27	1	- 4.755	YTH N6-methyladenosine RNA binding protein
Vm26Ac	Vitelline membrane	52	3	- 4.115	Chorion-containing eggshell formation
Jon99Ci	Jonah 99Ci	71	5	- 3.828	Serine proteases
Psd	Palisade	111	9	- 3.624	Chorion-containing eggshell formation
Vm26Aa	Vitelline membrane	37	3	- 3.624	Chorion-containing eggshell formation
Obp19c	Odorant-binding protein 19c	60	5	- 3.585	Sensory perception of chemical stimulus
CG10407	CG10407	35	3	-3.544	Haemolymph juvenile hormone binding;
Sam-S	S-adenosylmethionine Synthetase	50	5	-3.322	Determination of adult lifespan; S-adenosylmethionine biosynthetic process
CG14834	CG14834	55	6	-3.196	Unknown
tobi	Target of brain insulin	59	7	-3.075	Carbohydrate metabolic process
Fas1	Fasciclin 1	41	5	-3.036	Calcium-independent cell-cell adhesion
yellow-g2	yellow-g2	69	9	-2.939	Melanin biosynthetic process
ctrip	circadian trip	37	5	-2.888	Ubiquitin-dependent protein catabolic process
CG11381	CG11381	117	18	-2.700	Unknown
Drsl4	Drosomycin-like 4	101	16	-2.658	Defence response to fungus
CG13114	CG13114	88	14	-2.652	Unknown
CG43965	CG43965	182	34	-2.420	Unknown
bif	Bifocal	60	12	-2.322	Axon guidance; negative regulation of axon extension
RpS10b	Ribosomal protein S10b	96	20	-2.263	cytoplasmic translation

[*1]Among genes whose mRNA levels decreased in male flies fed HFD, compared with those in males fed ND, top 20 genes (p<0.1) are listed in ascending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in control flies fed ND.
[*3]The FPKM of mRNA reads expressed in flies fed HFD.
[*4]The log2 (ratio of the FPKM of mRNA reads) in HFD-fed flies to that in ND-fed flies.
[*5]http://flybase.org/

identify each clone. We determined the DNA sequences of 56,025,970 cDNA clones prepared from mRNAs expressed in HFD- and QG-fed adult males and 56,005,963 cDNA reads from HFD- and EGCG-fed adult males. The RNA-seq reads were mapped to a total of 52,197,941 mRNAs and 52,093,948 mRNAs, for the QG and EGCG supplement, respectively (Tables V-XII). The respective mapping frequencies of the reads were 93.2% and 93.0%. Similarly, we examined whether the mRNA levels of some genes have changed in adult females fed HFD supplemented with QG or EGCG. We determined DNA sequences of 58,051,399 cDNA clones prepared from mRNAs expressed in HFD- and QG-fed adult females and 44,457,855 cDNA reads from HFD- and EGCG-fed adult females. The RNA-seq reads were mapped to a total of 54,492,862 mRNAs and 41,033,386 mRNAs for QG and EGCG, respectively. The respective mapping frequencies of the reads were 93.9% and 92.3%.

We compiled lists of top 20 genes showing the most remarkable alterations in mRNA levels between males or females fed ND and those fed HFD (Tables I-IV). We found that several genes involved in glucose metabolism, protein synthesis, energy production and some genes for immune response were significantly up-regulated in HFD-fed adults compared to

Table III. A list of genes whose mRNA levels remarkably increased in HFD-fed male flies, compared to ND-fed male flies[*1].

Gene short name	Gene name	ND[*2]	HFD[*3]	log2 (Ratio)[*4]	Function[*5]
ND-AGGG	NADH dehydrogenase (ubiquinone) AGGG subunit	3	121	5.334	Unknown
Lmpt	Limpet	6	56	3.222	Defence response to fungus
Msr-110	Msr-110	13	96	2.885	Unknown
MtnB	Metallothionein B	98	446	2.186	Metal ion homeostasis
CG11911	CG11911	67	293	2.129	Serine proteases
RpL12	Ribosomal protein L12	42	174	2.051	Ribosomal large subunit assembly
CG13947	CG13947	56	202	1.851	Unknown
CG43680	CG13947	40	126	1.655	Unknown
RpL17	Ribosomal protein L17	273	764	1.485	Ribosomal large subunit assembly
CR34685	snoRNA:Me18S-A425	82	202	1.301	Unknown
RpS10b	Ribosomal protein L10b	156	340	1.124	Ribosomal large subunit assembly
mt:ND5	mitochondrial NADH-ubiquinone oxidoreductase chain 5	1059	2018	0.930	NADH dehydrogenase (ubiquinone) activity
msopa	male-specific opa containing gene	1753	3295	0.910	Multicellular organism reproduction; defence response
CG42481	CG42481	431	791	0.876	Unknown
RpL7A	Ribosomal protein L7A	389	694	0.835	Ribosomal large subunit assembly
LysD	Lysozyme D	1402	2322	0.728	Defence response to Gram-negative bacterium

[*1]Among genes whose mRNA levels decreased in male flies fed HFD, compared with those in males fed ND, top 16 genes ($p<0.1$) are listed in ascending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in control flies fed ND.
[*3]The FPKM of mRNA reads expressed in flies fed HFD.
[*4]The log2 (ratio of the FPKM of mRNA reads) in HFD-fed flies to that in ND-fed flies.
[*5]http://flybase.org/

those fed ND. Conversely, genes involved in the carbohydrate metabolic process were markedly down-regulated in HFD-fed adults, compared to that in those fed ND. The mRNA levels of several genes involved in chorion-containing eggshell formation commonly decreased in females fed HFD. However, a significant increase of many lipid metabolism genes in mRNA levels was not observed. Moreover, we drew up lists of top 20 genes showing the most remarkable alterations in mRNA levels between males or females fed HFD and those fed HFD supplemented with EGCG (Tables V-VIII). We observed a down-regulation of genes required for either ribosomal function or mitochondrial functions in both males and females after feeding of HFD with EGCG. Some genes involved in ATP synthesis-coupled electron transport and genes involved in innate immune response were conversely up-regulated in adults fed HFD with EGCG, compared to those in males or females fed HFD. We also made up lists of top 20 genes showing the most remarkable alterations in mRNA levels between males or females fed HFD and those fed HFD supplemented with QG (Tables IX-XII). Although distinctive alterations of gene expression in genes involved in mitochondrial function and immune response were also observed in adults fed HFD with QG, we failed to identify significant alterations of gene expression in genes involved in fat metabolism.

Table IV. A list of genes whose mRNA levels remarkably increased in HFD-fed female flies, compared to ND-fed female flies[*1].

Gene short name	Gene name	ND[*2]	HFD[*3]	log2 (Ratio)[*4]	Function[*5]
Gyg	Glycogenin	67	65	3.907	Glycogen biosynthetic process
Cpr	Cytochrome P450 reductase	183	177	3.833	Oxidation-reduction process
CG2765	CG2765	207	233	3.652	Myosin binding
CG14963	CG14963	77	95	3.544	Unknown
CG2765	CG2765	66	51	3.392	Unknown
Mgstl	Microsomal glutathione S-transferase-like	61	58	3.285	Unknown
Cpr72Ec	Cuticular protein 72Ec	168	178	3.015	Chitin-based cuticle development
Cct1	Chaperonin containing TCP1 subunit 1	32	25	2.888	De novo protein folding
TotM	Turandot M	39	10	2.791	Response to bacterium
CG18815	CG18815	91	87	2.558	Unknown
DptB	Diptericin B	101	17	2.487	Defence response to Gram-positive bacterium
CG14022	CG14022	61	60	2.445	Unknown
CG3604	CG3604	64	84	2.437	Unknown
plh	Pasang lhamu	12	59	2.298	Response to hypoxia
pirk	Poor Imd response upon knock-in	16	77	2.267	A negative feedback activity to the severity of infection
Mur29B	Mucin related 29B	39	185	2.246	Extracellular matrix structural constituent
CG8299	CG8299	43	199	2.210	Chymotrypsin family; Serine proteases
Mlc1	Myosin alkali light chain 1	26	115	2.145	Mesoderm development; muscle contraction
FASN2	Fatty acid synthase 2	20	87	2.121	Methyl-branched fatty acid biosynthetic process
Npl4	Nuclear protein localisation 4	26	111	2.094	Ubiquitin-dependent protein catabolic process

[*1]Among genes whose mRNA levels increased in female flies fed HFD, compared with those in females fed ND, top 20 genes (p<0.1) are listed in descending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in control flies fed ND.
[*3]The FPKM of mRNA reads expressed in flies fed HFD.
[*4]The log2 (ratio of the FPKM of mRNA reads) in HFD-fed flies to that in ND-fed flies.
[*5]http://flybase.org/

qRT-PCR Analysis for Investigating Alteration of mRNA Levels of Genes Involved in Fat Metabolism

We carefully examined by RNA-seq analysis whether the expression of genes involved in fat metabolism altered in male flies fed HFD, and HFD with either of the anti-obesity drugs, QG and EGCG. We focused on the following 21 genes: mag, FASN1, FASN2, AkhR, Lsd-1, bmm, Hsl, srl, Desat1, CG3394, CG3961, whd, CPT-2, SCP-X, CG12262, Acox57D-d, ILK, PDK1, Ubi-p63E, CG10924, and Gk2. Among them, we found a notable alteration in mRNA levels of three genes: mag, FASN1, and FASN2. We further examined mRNA levels of these genes in addition to Lsd-1, Hsl and bmm genes by RT-PCR. The mag/CG5932 gene encodes an enzyme required for fat absorption from intestinal epithelia. The RNA-seq analyses indicate that the mRNA level of the *mag/CG5932* gene was reduced in adult males and females fed HFD, compared with those fed ND. Feeding of HFD with QG resulted in a 35% and 22% decrease in gene mRNA levels in HFD-fed adult males and females, respectively. The RT-PCR confirmed that the mRNA level of *mag/CG5932* in adults fed HFD decreased by 40% of the level of adults fed ND (p<0.01 [p=0.00023]). Moreover, the level in adults fed HFD with EGCG

Table V. A list of genes whose mRNA levels decreased in male flies fed HFD supplemented with anti-obesity drug EGCG, compared to those in male flies fed HFD only[*1].

Gene short name	Gene name	HFD[*2]	HFD + EGCG[*3]	log2 (Ratio)[*4]	Function[*5]
CG31644	CG31644	190	40	-2.248	Mitochondrial cytochrome c oxidase subunit
Msr-110	Msr-110	202	55	-1.159	Unknown
cype	cyclope	96	43	-0.977	Mitochondrial electron transport
ocn	ocnus	311	158	-0.884	Peptidyl-histidine dephosphorylation
Mst84Da	Male-specific RNA 84Da	419	227	-0.847	Sperm axoneme assembly
CG31788	CG31788	20	11	-0.828	Unknown
CG42688	CG42688	457	254	-0.801	Unknown
CG31740	CG31740	718	412	-0.722	Unknown
CG12699	CG12699	944	625	-0.595	Unknown
MtnC	Metallothionein C	4656	3222	-0.531	Metal ion homeostasis; response to metal ion
Acp98AB	Accessory gland protein 98AB	2815	2027	-0.474	Negative regulation of female receptivity, post-mating
CG42852	CG42852	6518	4705	-0.470	Unknown
Sfp60F	Seminal fluid protein 60F	4826	3753	-0.363	Multicellular organism reproduction
Mst57Db	Male-specific RNA 57Db	34231	27656	-0.308	Oviposition; post-mating behaviour

[*1]Among genes whose mRNA levels decreased in male flies fed HFD with EGCG, compared with those in males fed HFD, top 14 genes ($p<0.1$) are listed in ascending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in flies fed HFD.
[*3]The FPKM of mRNA reads expressed in flies fed HFD supplemented with EGCG.
[*4]The log2 (ratio of the number of mRNA reads) in flies fed HFD supplemented with EGCG to that in HFD-fed flies.
[*5]http://flybase.org/

and that in adults fed HFD with QG resulted in a 20% decrease from the level of adults fed HFD ($p=0.043<0.05$) (Figure 4A). As down-regulation of the enzyme required for fat absorption is expected for suppression of obesity, the alterations observed in the flies administered these two anti-obesity drugs are consistent with their anti-obesity effects. *FASN1* genes encoding FA synthase: RNA-seq analyses revealed that the mRNA level of the gene in adult males fed HFD decreased compared with that in adult males fed ND. In males fed HFD supplemented with QG, as well as

Table VI. A list of genes whose mRNA levels decreased in female flies fed HFD supplemented with anti-obesity drug EGCG, compared to those in female flies fed HFD only[*1].

Gene short name	Gene name	HFD[*2]	HFD + EGCG[*3]	log2 (Ratio)[*4]	Function[*5]
mt:ND4L	Mitochondrial NADH-ubiquinone oxidoreductase chain 4L	99	42	-2.222	ATP synthesis-coupled electron transport
CG32368	CG32368	196	935	-0.974	Unknown
MtnB	Metallothionein B	446	226	-0.964	Metal ion homeostasis
CG10911	CG10911	1478	1060	-0.480	Unknown

[*1]Among genes whose mRNA levels decreased in female flies fed HFD with EGCG, compared with those in females fed HFD, top 4 genes ($p < 0.1$) are listed in ascending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in flies fed HFD.
[*3]The FPKM of mRNA reads expressed in flies fed HFD supplemented with EGCG.
[*4]The log2 (ratio of the number of mRNA reads) in flies fed HFD supplemented with EGCG to that in HFD-fed flies.
[*5]http://flybase.org/

Table VII. A list of genes whose mRNA levels increased in male flies fed HFD supplemented with anti-obesity drug EGCG, compared to those in male flies fed HFD only[*1].

Gene short name	Gene name	HFD[*2]	HFD + EGCG[*3]	log2 (Ratio)[*4]	Function[*5]
CR44138	CR44138	8	62	2.954	Unknown
CG44250	CG44250	9	65	2.852	Unknown
CG32198	CG32198	16	82	2.358	Unknown
CG15043	CG15043	71	218	1.618	Unknown
mt:ND4L	Mitochondrial NADH-ubiquinone oxidoreductase chain 4L	99	303	1.614	ATP synthesis coupled electron transport
CG13905	CG13905	67	166	1.309	Unknown
CG4363	CG4363	98	235	1.262	Unknown
CG14125	CG14125	118	273	1.21	Chitin metabolic process
FASN1	Fatty acid synthase 1	105	226	1.106	Triglyceride biosynthetic process
CG13947	CG13947	202	403	0.996	Unknown
IM2	Immune induced molecule 2	133	259	0.962	Defence response; response to bacterium
CG32368	CG32368	196	370	0.917	Unknown
CG13323	CG13323	575	1043	0.859	Unknown
IM3	Immune induced molecule 3	1225	1971	0.686	Defence response; response to bacterium
CG10911	CG10911	511	767	0.586	Unknown
GstD1	Glutathione S transferase D1	806	1178	0.547	Glutathione metabolic process
RpL7A	Ribosomal protein L7A	1018	1436	0.496	Ribosomal large subunit assembly
RpL29	Ribosomal protein L29	1386	1856	0.421	Ribosomal large subunit assembly

[*1]Among genes whose mRNA levels increased in male flies fed HFD with EGCG, compared with those in males fed HFD, top 18 genes (p<0.1) including a ncRNA gene are listed in descending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in flies fed HFD.
[*3]The FPKM of mRNA reads expressed in flies fed HFD supplemented with EGCG.
[*4]The log2 (ratio of the FPKM of mRNA reads) in flies fed HFD supplemented with EGCG to that in HFD-fed flies.
[*5]http://flybase.org/

with EGCG, the mRNA level of the gene significantly increased in comparison to HFD-fed male flies. We further confirmed these results by qRT-PCR. The mRNA level in adult males fed HFD markedly reduced to 50% of the level in males fed ND (p=0.0047<0.01). A feeding on HFD with QG

Table VIII. A list of genes whose mRNA levels increased in female flies fed HFD supplemented with anti-obesity drug EGCG, compared to those in female flies fed HFD only[*1].

Gene short name	Gene name	HFD[*2]	HFD + EGCG[*3]	log2 (Ratio)[*4]	Function[*5]
Cyp6w1	Cyp6w1	29	155	2.418	Cytochrome P450 superfamily
CG13947	CG13947	203	484	1.254	Unknown
lcs	la costa	1411	2562	0.861	Unknown
Jon99Ciii	Jonah 99Cii	401	689	0.781	Serine proteases
Yp3	Yolk protein 3	1886	2510	0.412	Embryonic development; response to bacterium
Yp1	Yolk protein 1	2353	3028	0.364	Lipase/vitellogenin, sex differentiation
LysD	Lysozyme D	3503	4384	0.324	Defence response to Gram-negative bacterium

[*1]Among genes whose mRNA levels increased in female flies fed HFD with EGCG, compared with those in females fed HFD, top 8 genes (p<0.1) are listed in descending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in flies fed HFD.
[*3]The FPKM of mRNA reads expressed in flies fed HFD supplemented with EGCG.
[*4]The log2 (ratio of the number of mRNA reads) in flies fed HFD supplemented with EGCG to that in HFD-fed flies.
[*5]http://flybase.org/

Table IX. A list of genes whose mRNA levels decreased in male flies fed HFD supplemented with anti-obesity drug QG, compared to those in male flies fed HFD only[*1].

Gene short name	Gene name	HFD[*2]	HFD + QG*3	log2 (Ratio)[*4]	Function[*5]
ND-AGGG	NADH dehydrogenase (ubiquinone) AGGG subunit	121	3	-4.334	snoRNA with unknown function
CR34685,	CR34685,	202	48	-3.073	Unknown
CG31644	CG31644	190	0	-1.639	Mitochondrial cytochrome c oxidase subunit
RpL17	Ribosomal protein L17	764	847	-1.311	Ribosomal large subunit assembly
Jon99Cii	Jonah 99Cii	373	358	-1.092	Serine proteases
CG12374	CG12374	578	830	-1.046	Metallocarboxypeptidase-like
Acp54A1	Accessory gland protein 54A1	1376	0	-0.738	Multicellular organism reproduction
yip7	yippee interacting protein 7	2254	2596	-0.635	Serine proteases
betaTry	βTrypsin	3811	2590	-0.557	Proteolysis
Jon65Aiv	Jonah 65Aiv	3025	2134	-0.503	Proteolysis, Serine proteases, trypsin domain
Acp98AB	Accessory gland protein 98AB	2815	2013	-0.484	Negative regulation of female receptivity
alphaTry	aTrypsin	2373	1707	-0.475	Proteolysis
MtnC	Metallothionein C	4656	3363	-0.469	Metal ion homeostasis; response to metal ion
Sfp60F	Seminal fluid protein 60F	4826	3731	-0.371	Multicellular organism reproduction receptivity
Mst57Db	Male-specific RNA 57Db	34231	27482	-0.317	Oviposition; post-mating behaviour

[*1]Among genes whose mRNA levels decreased in male flies fed HFD with QG, compared with those in males fed HFD, top 16 genes ($p < 0.1$) including a ncRNA gene are listed in ascending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in flies fed HFD.
[*3]The FPKM of mRNA reads expressed in flies fed HFD supplemented with QG.
[*4]The log2 (ratio of the number of mRNA reads) in flies fed HFD supplemented with QG to that in HFD-fed flies.
[*5]http://flybase.org/

Table X. A list of genes whose mRNA levels decreased in female flies fed HFD supplemented with anti-obesity drug QG, compared to those in female flies fed HFD only[*1].

Gene short name	Gene name	HFD[*2]	HFD + QG*3	log2 (Ratio)[*4]	Function[*5]
Cp36	Chorion protein 36	149	16	-3.219	Eggshell chorion assembly
Cp38	Chorion protein 38	236	27	-3.128	Unknown
DptB	Diptericin B	213	34	-2.647	Defence response to Gram-positive bacterium
lectin-37Da	lectin-37Da	522	136	-1.94	C-type lectin-like domain superfamily
CG42825	CG13324	553	238	-1.216	Transcription activator unknown
Mtk	Metchnikowin	783	343	-1.191	Defence response to fungus, Gram-negative bacterium, Gram-positive bacterium
CG33926	CG33926	338	152	-1.153	Unknown
MtnB	Metallothionein B	441	203	-1.119	Metal ion homeostasis
CG10912	CG10912	633	358	-0.822	Cold acclimation

[*1]Among genes whose mRNA levels decreased in female flies fed HFD with QG, compared with those in females fed HFD, top 9 genes ($p < 0.1$) are listed in ascending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in flies fed HFD.
[*3]The FPKM of mRNA reads expressed in flies fed HFD supplemented with QG.
[*4]The log2 (ratio of the number of mRNA reads) in flies fed HFD supplemented with QG to that in HFD-fed flies.
[*5]http://flybase.org/

Table XI. A list of genes whose mRNA levels increased in male flies fed HFD supplemented with anti-obesity drug QG, compared to those in male flies fed HFD only[*1].

Gene short name	Gene name	HFD[*2]	HFD + QG*3	log2 (Ratio)[*4]	Function[*5]
cype	cyclope	103	65	6.375	Mitochondrial electron transport
Dro	Drosocin	134	138	2.654	Antibacterial peptide with activity against Gram-negative and Gram-positive bacteria
DptB	Diptericin B	213	34	2.336	Defence response to Gram-positive bacterium
IM18	Immune induced molecule 18	53	43	1.278	Defence response to other organisms
mt:ND4L	mitochondrial NADH-ubiquinone oxido-reductase chain 4L	196	97	1.278	ATP synthesis coupled electron transport
Cyp6w1	Cyp6w1	29	76	1.221	Cytochrome P450 superfamily
FASN1	Fatty acid synthase 1	138	155	1.144	Triglyceride biosynthetic process
IM3	Immune induced molecule 3				Defence response to bacterium
RpL17	Ribosomal protein L17	569	922	0.696	Ribosomal large subunit assembly
IM4	Immune induced molecule 4	944	1400	0.569	Defence response; cell morphogenesis; humoral immune response; response to bacterium

[*1]Among genes whose mRNA levels increased in male flies fed HFD with QG, compared with those in males fed HFD, top 10 genes (p<0.1) are listed in descending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in flies fed HFD.
[*3]The FPKM of mRNA reads expressed in flies fed HFD supplemented with QG.
[*4]The log2 (ratio of the number of mRNA reads) in flies fed HFD supplemented with QG to that in HFD-fed flies.
[*5]http://flybase.org/

resulted in a 20% increase of the mRNA level in adult males, compared with that in HFD-fed males (p=0.05). In contrast, the mRNA level in males fed HFD with EGCG resulted in a 5% increase of the mRNA level on average, compared with that in HFD-fed males (p=0.47). This increase was not statistically significant (Figure 4B). These qRT-PCR results are generally consistent with the results obtained from the RNA-seq analyses. However, these alterations in gene expression in flies fed with anti-obesity drugs are inconsistent with their corresponding anti-obesity effects. *Lsd-1* gene: The RNA-seq analyses revealed that the mRNA level of the gene (FBtr0335002) in adult males fed HFD decreased by 10% compared with that in ND-feeding adults. By contrast, the mRNA level of the gene remarkably increased in males fed HFD supplemented with QG, as well

Table XII. A list of genes whose mRNA levels increased in female flies fed HFD supplemented with anti-obesity drug QG, compared to those in female flies fed HFD only[*1].

Gene short name	Gene name	HFD[*2]	HFD + QG*3	log2 (Ratio)[*4]	Function[*5]
Vm32E	Vitelline membrane 32E	24	226	24	Vitelline membrane formation involved in chorion-containing eggshell formation
dec-1	defective chorion 1	19	143	19	Eggshell chorion assembly
Jon65Aii	Jonah 65Aii	161	403	161	Serine proteases
LysD	Lysozyme D	3503	5327	0.605	Defence response to Gram-negative bacterium
CR9111	Lysozyme C	4591	6868	0.581	Lysozyme-like, unknown

[*1]Among genes whose mRNA levels increased in female flies fed HFD with QG, compared with those in females fed HFD, top 5 genes (p<0.1) including a RNA gene are listed in descending order of log2 (Ratio).
[*2]The FPKM of mRNA reads expressed in flies fed HFD.
[*3]The FPKM of mRNA reads expressed in flies fed HFD supplemented with QG.
[*4]The log2 (ratio of the number of mRNA reads) in flies fed HFD supplemented with QG to that in HFD-fed flies.
[*5]http://flybase.org/

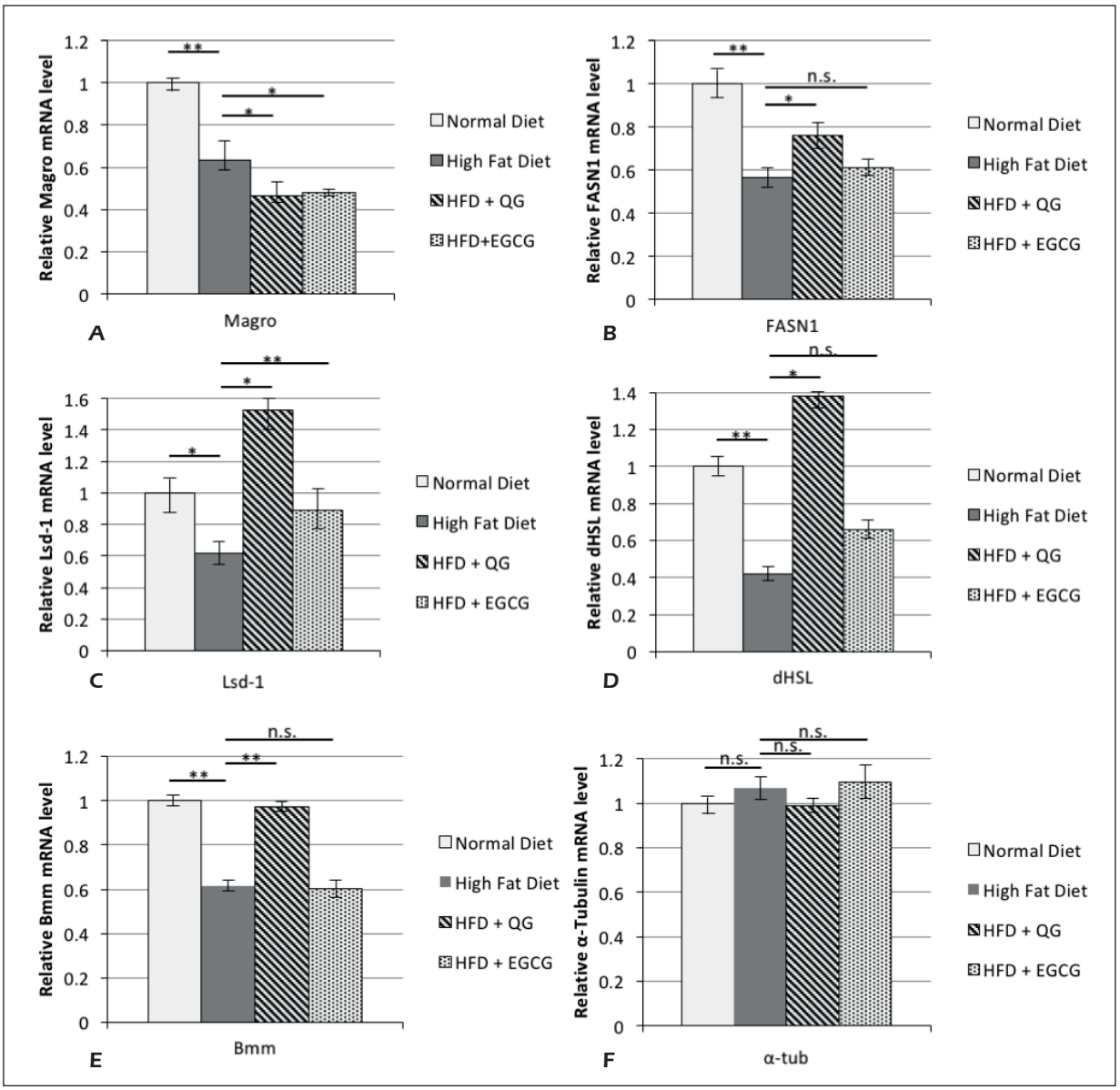

Figure 4. Quantification of mRNAs for six fat metabolism-related genes in the Drosophila adults fed HFD, HFD supplemented with QG, and HFD supplemented with EGCG. Relative mRNA levels for each gene in adult males fed HFD, HFD supplemented with 80 μg/ml QG, and HFD with 30 μg/ml EGCG with respect to adults fed ND were obtained using the results from qRT-PCR analysis. Total RNA was isolated from adult males fed HFD, HFD supplemented with QG and HFD supplemented with EGCG or from adults fed with normal diet (ND) for a control sample. A-E, Genes for fat metabolism enzymes. **A**, Mag; **B**, FASN1; **C**, Lsd-1; **D**, dHSL; **E**, Bmm; **F**, A housekeeping gene encoding a-tubulin. qRT-PCR analysis to quantitate the amount of mRNA of each gene was repeated three times. Statistical analysis was performed using one-way ANOVA with Dunnet's multiple comparison. ns, not significant; $p<0.05$; $^{**}p<0.01$.

as with EGCG, compared with ND-fed male flies. We further confirmed these results by qRT-PCR (Figure 4C). The mRNA level in adult males fed HFD markedly was reduced to 60% of the level in males fed ND (p=0.05). A feeding on HFD with QG resulted in the two-fold increase of the mRNA level in adult males, compared with that in HFD-fed males (p=0.0039<0.01). In contrast, the mRNA level in males fed HFD with EGCG resulted in a 25% increase of the mRNA level on average, compared with that in HFD-fed males, although the increase was not statistically significant (p=0.47>0.05). These qRT-PCR results are generally consistent with the results obtained from

the RNA-seq analyses. However, these alterations of gene expression in flies fed with the anti-obesity drugs are inconsistent with the anti-obesity effects of the two drugs. dHSL gene: The RNA-seq analyses showed that the mRNA level of the gene (FBtr0086270) in adult males fed HFD was reduced compared with that in adults fed ND. This change is not statistically significant. In adult males fed HFD with QG as well as with EGCG, the mRNA level of the gene increased, compared with that in HFD-fed adults. We further confirmed these results by qRT-PCR (Figure 4D). The mRNA level in adult males fed HFD was reduced by 40% of the level in males fed ND (p=0.0009<0.01). The level in males fed HFD with QG also resulted in a 3.5-fold increase, compared with that of HFD-fed males (p=0.0002<0.01). The mRNA level in males fed HFD with EGCG also resulted in a 20% increase on average, compared with that in ND-fed males (p=0.018<0.05). These qRT-PCR results confirmed the results derived from the RNA-seq analyses. The up-regulation of the gene by administration of QG was detected more distinctively by qRT-PCR rather than with RNA-seq. These alterations of gene expression in flies fed HFD with QG, as well as with EGCG, are consistent with the anti-obesity effects of the drugs.

Bmm gene: The RNA-seq analyses revealed that mRNA level of the gene in adult males fed HFD decreased, compared with that in adults fed ND. In adult males fed HFD supplemented with QG as well as with EGCG, mRNA level of the gene increased slightly. We further confirmed these results by qRT-PCR (Figure 4E). The mRNA level in adult males fed HFD was reduced to 50% of the mRNA level in males fed ND (p=0.0083<0.01). The mRNA level in males fed HFD with QG significantly increased up to the level of ND-feeding adults, compared with that in HFD-fed males (p=0.004<0.01). The mRNA level in males fed HFD with EGCG did not show any increase compared with that in HFD-fed males (Figure 4E). These qRT-PCR results, except for the case of adults fed HFD with EGCG, confirmed the results obtained from the RNA-seq analyses. The up-regulation of the gene by QG feeding was detected more distinctively by qRT-PCR rather than with RNA-seq. In summary, the altered expression of five out of six genes in flies administered with QG and EGCG are consistent with the anti-obesity effects of the drugs, with QG exhibiting more distinctive effects in alterations of gene expression than EGCG.

Discussion

Animal models have made an important contribution to describing the molecular mechanism that underlying obesity. To this end, convenient experiment animals are essential for discovery and testing of anti-obesity drugs. In lipid metabolism, enzymes and regulatory proteins involved in lipid uptake, transport, storage, mobilization, fat storage, and lipolysis are highly conserved between mammals and *Drosophila*[33,40]. Therefore, we established a simple fly model of HFD-induced obesity. Like mammals, flies store and mobilize energy in the form of carbohydrates and lipids, and utilize highly conserved insulin- and glucagon-like signaling pathways to regulate their glucose homeostasis[41]. Thus, a high sugar diet also results in extra accumulation of fat in fly bodies. Because of easier manipulation in feeding, the high sugar diet has often been used to produce obese flies[42]. In turn, we reproduced progressive obesity in *Drosophila* adults having excess TAG accumulation by feeding of a fly food supplemented with coconut oil. Our results are consistent with previous results of HFD-induced obesity in the fly model[43,44,19]. Using this fly obesity model, we further demonstrated that known chemical compounds, EGCG and QG, which have been believed to have anti-obesity effects in mammals, have similar effects in *Drosophila* adults. These preliminary data encourage us to conduct new molecular studies to investigate genes involved in HFD-induced obesity and describe the alteration of gene expression in the suppression of obesity by QG and EGCG using this obesity model. RNA-seq experiments conducted in this work investigated whether this diet-induced obesity and simultaneous feeding of QG or EGCG with HFD modify gene expression in *Drosophila* adults. The results demonstrated that multiple genes involved in *Drosophila* egg development and a TGA lipase gene were significantly up-regulated in adult females, suggesting excess TAG induces *Drosophila* oogenesis. Some metabolic genes and genes possibly involved in defence were up-regulated in both males and females. However, a remarkable increase in the mRNA levels of many lipid metabolism genes was not observed. A previous microarray analysis also indicated that annotation groups showing more significant alterations in their mRNA levels in *Drosophila* fed HFD included genes for proteins for immune response, carbohydrate binding, ion transport. The analyses also suggested that

the expression of genes involved in pathways for amino acid metabolism, glycerophospholipid metabolism and breakdown of sugars and waste products were markedly altered[44]. Some of the genes involved in metabolism and immune response were commonly altered in their gene expression in flies reared on HFD. In contrast, the transcriptome analysis of diet-induced mice model indicated that the genes involved in metabolic processes, such as lipid metabolism, carbohydrate metabolism and energy production were more remarkably altered[45].

To examine whether the expression of the genes involved in lipid metabolism was modified, we focused on five genes, *Mag*, *Fatty acid synthase* (*FAS*), *Plin1*, *dHSL* and *Bmm* among 22 lipid-metabolism-related genes. The four genes, exempting *FAS*, showed that the mRNA levels significantly changed, according to the feeding of HFD as well as a simultaneous feeding of QG or EGCG with HFD. One of the genes, *Mag*, encodes a *Drosophila* ortholog of the mammalian lipase A that plays a role in fat absorption from intestinal epithelia[46]. It is possible that the reduced level of the lipase involved in fat absorption is correlated with the suppression of HFD-induced obesity by QG and EGCG. Unexpectedly, a decreased mRNA level of the lipase gene was observed in adults fed HFD, compared with that of ND-feeding adults. In diet-induced obesity mice, marked alteration of the lipase A family protein expression has not been reported[47-49]. Conversely, other two lipid-metabolism-related genes, *dHSL* and *Bmm*, encode lipases required for lipolysis of TAG stored in adipose tissues called fat bodies[49,50]. The mRNA levels of both genes are down-regulated in flies fed HFD, and the levels commonly increased by administering QG and EGCG in both cases. Particularly, QG feeding provided a more significant alteration of mRNA levels of both genes, compared to EGCG feeding. We further confirmed a reproducibility of the results by repeated qRT-PCR experiments. The results allow us to interpret that the increased level of this type of lipases is correlated with suppression of HFD-induced obesity by QG and EGCG. Interestingly, it was reported that the activation of the mammalian ortholog, Hormone-sensitive lipase (HSL) occurred after QG treatment in mammalian mature adipocytes, although the alteration of the mRNA level was not investigated[51]. Another lipid-metabolism-related gene, *Lsd-1* encodes a protein that protects lipid droplets from lipase-mediated remobilization and facilitates lipolysis by serving as an anchoring point for HSL[52]. Thus, it is reasonable to consider that the up-regulation of the gene in adults fed QG and EGCG eventually results in suppression of diet-induced obesity. It is still uncertain whether the expression of mammalian HSL alters as obesity progresses in mouse models and whether the expression changes by feeding of anti-obesity drugs such as EGCG[53-55]. Our *Drosophila* study provides new insight for investigation of these lipase genes as targets of those anti-obesity drugs in mammals. *FASN1*, encoding a fatty acid synthase, was contrary to our expectations down-regulated in *Drosophila* adults fed HFD, as observed in our qRT-PCR experiments. Furthermore, the mRNA level decreased by administration of QG. If the mRNA level of the gene increases in HFD-induced adults, synthesis of TAG is enhanced, which eventually results in the production of obese flies. Our current result regarding the FASN1 mRNA level is not consistent with the expected alteration of the gene expression associated with anti-obesity effect of QG and EGCG. In contrast, the mRNA level of the gene in diet-induced obese mice remarkably increased[47,56,57]. Although these reported results are consistent with an interpretation that altered *FASN* gene expression plays a critical role in the mouse obesity, another recent study[58] failed to detect a significant up-regulation of the gene in a similar obese mouse model. Further detailed studies remain to be performed. In addition, our RNA-seq analysis suggests that mRNA levels of several other genes involved in anti-oxidative stress have also changed in adults fed the HFD. An up-regulation of the *SOD* genes was commonly observed in mouse models of obesity[59]. Therefore, it is of interest to further examine the relationship between up-regulation of anti-oxidative stress genes and HFD-induced obesity in *Drosophila*. Continuous feeding of QG resulted in reduced body weight gain, and significant reduction of visceral fat weight in diet-induced obese mice[60]. In cultured adipocytes, quercetin also accelerated the glycerol release and phosphorylation of HSL in a dose-dependent manner[61]. We speculated that genes for the lipase A family, HSL, perilipin, and adipose triglyceride lipase (ATGL) play more important roles in the anti-obesity effects of the drugs in this diet-induced *Drosophila* obesity model. There is preliminary evidence showing entire profiles of altered gene expression under the condition of a diet-induced obesity and suppression of the

accumulation by anti-obesity drugs in these organisms. It has been commonly observed that the effects of QG to inhibit obesity in several animal models seemed to exhibit more effectively than those of other compounds[60,62,63]. In previous studies[64], diets supplemented with QC demonstrated reduction of excess weight gain in obese Zucker rats. In addition, the dietary supplements protected against obesity-associated insulin resistance and diabetes in mice[65]. Furthermore, the QG supplement showed clearer effects on males rather than females. This may explain that the fat can accumulate at the ovary of female flies reported by the previous studies[66]. Our data indicating that female flies contained more TG than males are consistent with this information. As it seems more difficult to release TG accumulated in ovaries, this might explain this sexual difference that the anti-obesity effects of QG supplement appeared more sharply in males than in females.

EGCG also demonstrated its positive effects to reduce the TG accumulation, although the effects of EGCG supplements were less effective than those of QG. A previous study using *Drosophila* as obesity model demonstrated a similar result that tea polyphenols ameliorate fat storage induced by HFD in *Drosophila* adults[19]. This group and others also reported that a novel tea polyphenol, epitheaflagallin strongly suppresses lipid accumulation in *Drosophila* adults[11,19]. However, the current study is the first report describing genome-wide alterations of the gene expression by EGCG treatment in *Drosophila* adults. It could be considered that the effect of EGCG might influence the expression of genes related to lipid metabolism in *Drosophila*. In diet-induced mouse models, it has been reported that the regulation of multiple gene expression has changed[54,56,18]. Our *Drosophila* results are partially consistent with these previous studies. We observed some unique alteration of the gene expression in both anti-obesity drugs. These possible target genes speculated from our *Drosophila* study should be carefully investigated in mouse models of obesity.

Conclusions

In this work, we have established a convenient monitoring system to study diet-induced fat accumulation and to evaluate the effects of anti-obesity drugs using *Drosophila* adults. Using this system, we showed that known anti-obesity drugs, EGCG and QG have the same effect that inhibits TG accumulation. Our RNA-seq analysis suggested that the mRNA levels of several genes involved in lipid metabolism, glycolysis/gluconeogenesis and anti-oxidative stress have changed in adults fed the HFD. Moreover, the levels altered in *Drosophila* fed HFD supplemented with QG or EGCG, suggesting that these genes play important roles in the anti-obesity effects of the drugs.

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Declaration of funding interests

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Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Abelson P, Kennedy D. The obesity epidemic. Science 2004; 304: 1413.
2) WHO. Epidemic of obesity and overweight linked to increased food energy supply. The Bulletin of the World Health Organization 2015; http://www.who.int/bulletin/releases/NFM0715/en/
3) Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes (Lond) 2008; 32: 1431-1437.
4) Hvizdos KM, Markham A. Orlistat: a review of its use in the management of obesity. Drugs 1999; 58: 743-760.
5) Fong TM, Heymsfield SB. Cannabinoid-1 receptor inverse agonists: current understanding of mechanism of action and unanswered questions. Int J Obes (Lond) 2009; 33: 947-955.
6) Aronne LJ, Powell AG, Apovian CM. Emerging pharmacotherapy for obesity. Expert Opin Emerg Drugs 2011; 16: 587-596.
7) Shukla AP, Kumar RB, Aronne LJ. Lorcaserin Hcl for the treatment of obesity. Expert Opin Pharmacother 2015; 16: 2531-2538.
8) Yung JW. Possible anti-obesity therapeutics from nature – a review. Phytochemist 2010; 71: 1625-164

9) Rayalam S, Della-Fera MA, Baile CA. Phytochemicals and regulation of the adipocyte life cycle. J Nutr Biochem 2008; 19: 717-726.
10) Thielecke F, Boschmann M. The potential role of green tea catechins in the prevention of the metabolic syndrome – a review. Phytochemist 2009; 70: 11-24.
11) Wolfram S, Wang Y, Thielecke F. Anti-obesity effects of green tea: from bedside to bench. Mol Nutr Food Res 2006; 50: 176-187.
12) Nagle DG, Ferreira D, Zhou YD. Epigallocatechin-3-gallate (EGCG): chemical and biomedical perspectives. Phytochemist 2006; 67: 1849-1855.
13) Boschmann M, Thielecke, F. The effects of epigallocatechin-3-gallate on thermogenesis and fat oxidation in obese men: a pilot study. J Am Coll Nutr 2007; 26: 389S-395S.
14) Moon HS, Lee HG, Choi YJ, Kim TG, Cho CS. Proposed mechanisms of (-)-epigallocatechin-3-gallate for anti-obesity. Chem Biol Interact 2007; 167: 85-98.
15) Sakurai N, Mochizuki K, Kameji H, Shimada M, Goda T, (-)-Epigallocatechin gallate enhances the expression of genes related to insulin sensitivity and adipocyte differentiation in 3T3-L1 adipocytes at an early stage of differentiation. Nutrition 2009; 25: 1047-1056.
16) Bose M, Lambert JD, Ju J, Reuhl KR, Shapses SA, Yang CS. The major green tea polyphenol, (-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice. J Nutr 2008; 138: 1677-1683.
17) Chen TC, Wang W, Golden EB, Thomas S, Sivakumar W, Hofman FM, Louie SG, Schonthal AH. Green tea epigallocatechin gallate enhances therapeutic efficacy of temozolomide in orthotopic mouse glioblastoma models. Cancer Lett 2011; 302: 100-108.
18) Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R, Bapat P, Kwun I, Shen CL. Novel insights of dietary polyphenols and obesity. J Nutr Biochem 2014; 25: 1-18.
19) Kayashima Y, Murata S, Sato M, Matsuura K, Asanuma T, Chimoto J, Ishii T, Mochizuki K, Kumazawa S, Nakayama T, Yamakawa-Kobayashi K. Tea polyphenols ameliorate fat storage induced by high-fat diet in Drosophila melanogaster. Biochemistry and Biophysics Reports 2015; 4: 417-424.
20) D'Andrea G. Quercetin: a flavonol with multifaceted therapeutic applications? Fitoterapia 2015; 106: 256-271.
21) Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comp Biochem Physiol C Toxicol Pharmacol 2003; 135: 357-364.
22) Katsube T, Yamasaki M, Shiwaku K, Ishijima T, Matsumoto I, Abe K, Yamasaki Y. Effect of flavonol glycoside in mulberry (Morus alba L.) leaf on glucose metabolism and oxidative stress in liver in diet-induced obese mice. J Sci Food Agric 2010; 90: 2386-2392.
23) Heyman L, Axling U, Blanco N, Sterner O, Holm C, Berger K. Evaluation of beneficial metabolic effects of berries in high-fat fed C57BL/6J mice. J Nutr Metab 2014; 2014: 403041.
24) Rodriguez-Rodriguez C, Torres N, Gutierrez-Uribe JA, Noriega LG, Torre-Villalvazo I, Leal-Diaz AM, Antunes-Ricardo M, Marquez-Mota C, Ordaz G, Chavez-Santoscoy RA, Serna-Saldivar SO, Tovar AR. The effect of isorhamnetin glycosides extracted from Opuntia ficus-indica in a mouse model of diet induced obesity. Food Funct 2015; 6: 805-815.
25) Sekhon-Loodu S, Ziaullah Z, Rupasinghe HP, Wang Y, Kulka M, Shahidi F. Novel quercetin-3-O-glucoside eicosapentaenoic acid ester ameliorates inflammation and hyperlipidemia. Inflammopharmacology 2015; 23: 173-185.
26) Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. Eur J Pharmacol 2008; 585: 325-337.
27) Boots AW, Drent M, Swennen EL, Moonen HJ, Bast A, Haenen GR. Antioxidant status associated with inflammation in sarcoidosis: a potential role for antioxidants. Respir Med 2009; 103: 364-372.
28) Jennings BH. Drosophila – a versatile model in biology & medicine. Mater Today 2011; 14: 190-195.
29) Inoue YH, Katsube H, Hinami Y. Drosophila models to investigate insulin action and mechanisms underlying human diabetes mellitus. Adv Exp Med Biol 2018; 1076: 235-256.
30) Giacomotto J, Segalat L. High-throughput screening and small animal models, where are we? Br J Pharmacol 2010; 160: 204-216.
31) Gladstone M, Su TT. Chemical genetics and drug screening in Drosophila cancer models. J Genet Genomics 2011; 38: 497-504.
32) Jafari M. Drosophila melanogaster as a model system for the evaluation of anti-aging compounds. Fly (Austin) 2010; 4: 253-257.
33) Trinh I, Boulianne GL. Modeling obesity and its associated disorders in Drosophila. Physiology (Bethesda) 2013; 28: 117-124.
34) Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol 2013; 14: R36.
35) Trapnell C, Hendrickson DG, Sauvageau M, Goff L, Rinn JL, Pachter L. Differential analysis of gene regulation at transcript resolution with RNA-seq. Nat Biotechnol 2013; 31: 46-53.
36) Anders S, Huber, W. Differential expression analysis for sequence count data. Genome Biol 2010; 11: R106.
37) Baranski TJ, Kraja AT, Fink JL, Feitosa M, Lenzini PA, Borecki IB, Liu CT, Cupples LA, North KE, Province MA. A high throughput, functional screen of human Body Mass Index GWAS loci using tissue-specific RNAi Drosophila melanogaster crosses. PLoS Genet 2018; 14: e1007222.
38) Kong Y, Li K, Fu T, Wan C, Zhang D, Song H, Zhang Y, Liu N, Gan Z, Yuan L. Quercetin ameliorates Abeta toxicity in Drosophila AD model by modulating cell cycle-related protein expression. Oncotarget 2016; 7: 67716-67731.
39) Cialdella-Kam L, Ghosh S, Meaney MP, Knab AM, Shanely RA, Nieman DC. Quercetin and green tea extract supplementation downregulates genes related to tissue inflammatory responses to a 12-week high fat-diet in mice. Nutrients 2017; 9: E773.

40) Padmanabha D, Baker KD. Drosophila gains traction as a repurposed tool to investigate metabolism. Trends Endocrinol Metab 2014; 25: 518-527.
41) Bharucha KN, Tarr P, Zipursky SL. A glucagon-like endocrine pathway in Drosophila modulates both lipid and carbohydrate homeostasis. J Exp Biol 2008; 211: 3103-3110.
42) Musselman LP, Fink, JL, Narzinski K, Ramachandran PV, Hathiramani SS, Cagan RL, Baranski TJ. A high-sugar diet produces obesity and insulin resistance in wild-type Drosophila. Dis Model Mech 2011; 4: 842-849.
43) Birse RT, Choi J, Reardon K, Rodriguez J, Graham S, Diop S, Ocorr K, Bodmer R, Oldham S. High-fat-diet-induced obesity and heart dysfunction are regulated by the TOR pathway in Drosophila. Cell Metab 2010; 12: 533-544.
44) Heinrichsen ET, Zhang H, Robinson JE, Ngo J, Diop S, Bodmer R, Joiner WJ, Metallo CM, Haddad GG. Metabolic and transcriptional response to a high-fat diet in Drosophila melanogaster. Mol Metab 2014; 3: 42-54.
45) Li R, Grimm SA, Mav D, Gu H, Djukovic D, Shah R, Merrick BA, Raftery D, Wade PA. Transcriptome and DNA methylome analysis in a mouse model of diet-induced obesity predicts increased risk of colorectal cancer. Cell Rep 2018; 22: 624-637.
46) Sieber MH, Thummel CS. Coordination of triacylglycerol and cholesterol homeostasis by DHR96 and the Drosophila LipA homolog magro. Cell Metab 2012; 15: 122-127.
47) Boots AW, Drent M, de Boer VC, Bast A, Haenen GR. Quercetin reduces markers of oxidative stress and inflammation in sarcoidosis. Clin Nutr 2011; 30: 506-512.
48) Zhang Y, Xu Q, Liu YH, Zhang XS, Wang J, Yu XM, Zhang RX, Xue C, Yang XY, Xue CY. Medium-chain triglyceride activated brown adipose tissue and induced reduction of fat mass in C57BL/6J mice fed high-fat diet. Biomed Environ Sci 2015; 28: 97-104.
49) Gronke S, Mildner A, Fellert S, Tennagels N, Petry S, Muller G, Jackle H, Kuhnlein RP. Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. Cell Metab 2005; 1: 323-330.
50) Bi J, Xiang Y, Chen H, Liu Z, Grönke S, Kühnlein RP, Huang X. Opposite and redundant roles of the two Drosophila perilipins in lipid mobilization. J Cell Sci 2012; 125: 3568-3577.
51) Tateishi N, Egawa K, Kanzaki N, Kitagawa Y, Shibata H, Kiso Y, Enomoto S, Fukada D, Nagai Y, Sada M. Effects of quercetin glucosides on diet-induced obesity in mice. Jpn Pharmacol Ther 2009; 37: 123-131.
52) Beller M, Bulankina AV, Hsiao HH, Urlaub H, Jäckle H, Kühnlein RP. PERILIPIN-dependent control of lipid droplet structure and fat storage in Drosophila. Cell Metab 2010; 12: 521-532.
53) Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu J. Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. Int J Obes Relat Metab Disord 2002; 26: 1459-1464.
54) Lee MS, Kim CT, Kim Y. Green tea (-)-epigallocatechin-3-gallate reduces body weight with regulation of multiple genes expression in adipose tissue of diet-induced obese mice. Ann Nutr Metab 2009; 54: 151-157.
55) Hasumura T, Hasumura T, Shimada Y, Kuroyanagi J, Nishimura Y, Meguro S, Takema Y, Tanaka T. Green tea extract suppresses adiposity and affects the expression of lipid metabolism genes in diet-induced obese zebrafish. Nutr Metab (Lond) 2012; 9: 73.
56) Rains TM, Agarwal, Sanjiv, Maki KC. Antiobesity effects of green tea catechins: a mechanistic review. J Nutr Biochem 2011; 22: 1-7.
57) Douglass JD, Zhou, Yin Xiu, Wu, A, Zadrogra JA, Gajda AM, Lackey AI, Lang W, Chevalier KM, Sutton SW, Zhang SP, Flores CM, Connelly MA, Storch J. Global deletion of MGL in mice delays lipid absorption and alters energy homeostasis and diet-induced obesity. J Lipid Res 2015; 56: 1153-1171.
58) Zheng G, Lin L, Zhong S, Zhang Q, Li D. Effects of puerarin on lipid accumulation and metabolism in high-fat diet-fed mice. PLoS One 2015; 10.
59) Kim HS, Quon, Michael J, Kim JA. New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. Redox Biol 2014; 2: 187-195.
60) Jung CH, Cho I, Ahn J, Jeon TI, Ha TY. Quercetin reduces high-fat diet-induced fat accumulation in the liver by regulating lipid metabolism genes. Phytother Res 2013; 27: 139-143.
61) Gaidhu MP, Gaidhu MP, Anthony NM, Patel P, Hawke TJ, Ceddia RB. Dysregulation of lipolysis and lipid metabolism in visceral and subcutaneous adipocytes by high-fat diet: role of ATGL, HSL, and AMPK. Am J Physiol Cell Physiol 2010; 298: C961-971.
62) Odbayar TO, Badamhand D, Kimura T, Takayashi Y, Thushida T, Ide T. Comparative studies of some phenolic compounds (Quercetin, Rutin, and Ferulic Acid) affecting hepatic fatty acid synthesis in mice. J Agric Food Chem 2016; 54: 8261-8265.
63) Hossain MS, Asano F, Fujiyama R, Miyoshi C, Sato M, Ikkyu A, Kanno S, Hotta N, Kakizaki M, Honda T, Staci J. Kim, H. Komiya, I. Miura, T. Suzuki, K. Kobayashi, H. Kaneda, V. Kumar, Takahashi JS, Wakana S, Funato H, Yanagisawa M. Identification of mutations through dominant screening for obesity using C57BL/6 substrains. Sci Rep 2016; 6: 32453.
64) Rivera L, Moron R, Sanchez M, Zarzuelo A, Galisteo M. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. Obesity (Silver Spring) 2008; 16: 2081-2087.
65) Dong J, Zhang X, Zhang L, Bian HX, Xu N, Bao B, Liu J. Quercetin reduces obesity-associated ATM infiltration and inflammation in mice: a mechanism including AMPKalpha1/SIRT1. J Lipid Res 2014; 55: 363-374.
66) Jiang Z, Wu XL, Michal JJ, McNamara JP. Pattern profiling and mapping of the fat body transcriptome in Drosophila melanogaster. Obesity Res 2005; 13: 1898-1904.