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Gene expression analysis of the liver and skeletal muscle of psyllium-treated mice

Naoyuki Togawa[1], Rumiko Takahashi[2], Shizuka Hirai[2], Tatsunobu Fukushima[1] and Yukari Egashira[2]*

[1]*Yokohama Corporate Research Laboratories, Mitsubishi Rayon Co., Ltd., Yokohama, Japan*
[2]*Laboratory of Food and Nutrition, Graduate School of Horticulture, Chiba University, Matsudo, Chiba, Japan*

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Abstract

Psyllium, a dietary fibre rich in soluble components, has both cholesterol- and TAG-lowering effects. Many studies have verified these actions using liver samples, whereas little information is available on the effects of psyllium treatment on other organs. The purpose of the present study was to evaluate the possible beneficial effects of psyllium. We investigated the gene expression profiles of both liver and skeletal muscle using DNA microarrays. C57BL/6J mice were fed a low-fat diet (LFD; 7% fat), a high-fat diet (HFD; 40% fat) or a HFD with psyllium (40% fat+5% psyllium; HFD+Psy) for 10 weeks. Body weights and food intake were measured weekly. After 10 weeks, the mice were killed and tissues were collected. Adipose tissues were weighed, and plasma total cholesterol and TAG blood glucose levels were measured. The expression levels of genes involved in glycolysis, gluconeogenesis, glucose transport and fatty acid metabolism were measured by DNA microarray in the liver and skeletal muscle. In the HFD+Psy group, plasma total cholesterol, TAG and blood glucose levels significantly decreased. There was a significant reduction in the relative weight of the epididymal and retroperitoneal fat tissue depots in mice fed the HFD+Psy. The expression levels of genes involved in fatty acid oxidation and lipid transport were significantly up-regulated in the skeletal muscle of the HFD+Psy group. This result suggests that psyllium stimulates lipid transport and fatty acid oxidation in the muscle. In conclusion, the present study demonstrates that psyllium can promote lipid consumption in the skeletal muscle; and this effect would create a slightly insufficient glucose state in the liver.

Key words: Psyllium: Gene expression: Skeletal muscle/metabolism

Psyllium is a dietary fibre rich in soluble components, and its cholesterol- and TAG-lowering effects have been reported in many studies[1–8]. Although it is still not clear whether psyllium adsorbs bile acids *in vivo*, the mechanism of the cholesterol-lowering effect of psyllium is due to an increase in the amount of bile acids in faeces[9–12]. When bile acid is excreted from the body, additional bile acids are needed to compensate for this loss. Cholesterol 7α hydroxylase (*Cyp7a1*) involved in the synthesis of bile acids from cholesterol and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*Hmgcr*) directly related to cholesterol synthesis increase expression in the liver, because bile acids are generated from cholesterol in the liver. More LDL-cholesterol is recovered to the liver from plasma. In consequence, plasma total cholesterol concentration is decreased. The administration of psyllium or the bile acid sequestrants such as cholestyramine activates enterohepatic circulation and a new synthesis of bile acid. Through the process of bile acid excretion and synthesis, it is also known that the composition of bile acids changes[12,13]. Psyllium selectively reduces taurine-conjugated bile acids, in particular, taurochenodeoxycholic acid. The ratio of glycocholic acids in the bile increases and the hydrophobicity of the bile acid is reduced, which inhibits the forming of gallstones[12]. Biliary bile acids usually consist of mixtures of individual bile acids. The primary bile acids are cholic acid and chenodeoxycholic acid; the two secondary bile acids are deoxycholic acid and lithocholic acid, resulting from bacterial action in the intestine, which are absorbed and resecreted by the liver. The primary and secondary bile acids are conjugated with either taurine or glycine, and changed as glycocholic acid, taurocholate acid, taurochenodeoxycholic acid and so on. Farnesoid X receptor (*Fxr*) related to feedback inhibition of bile acid synthesis is the nuclear receptor for bile acids. Although the potency of individual bile acids for activating *Fxr* is different, chenodeoxycholic acid activates *Fxr* with the highest potency[14]. Psyllium reduces the ratio of chenodeoxycholic acid in the bile, which then reduces to repress the expression of *Cyp7a1* through *Fxr*[12].

While *Fxr* involved in the feedback control mechanism in bile acid synthesis and enterohepatic circulation is well

Abbreviations: aRNA, antisense RNA; HFD, high-fat diet; HFD+Psy, high-fat diet with psyllium; LFD, low-fat diet; qPCR, quantitative PCR.

* **Corresponding author:** Y. Egashira, fax +81 47 308 8720, email egashira@faculty.chiba-u.jp

known as the nuclear receptor of bile acids, *Tgr5* is also considered as another membrane-type receptor of bile acids[15]. *Tgr5* is widely expressed in various tissues such as the heart, skeletal muscle, spleen, kidney, liver and small intestine and in leucocytes. *Tgr5* knockout mice fed a lithogenic diet are viable and develop normally. The mice do not develop gallstones and alter the feedback regulation of bile acid synthesis including the expression of *Cyp7a1* and *Cyp27a1*[16].

It has been found that *Tgr5* is involved in energy consumption more recently[17–19]. Bile acids bind to the G protein-coupled receptor, *Tgr5*. These signals increase cyclic AMP levels, thereby activating the expression of type 2 iodothyronine deiodinase (*D2*) in skeletal muscle and brown adipose tissue, which increases energy consumption. In the brown adipose tissue of mice fed a high-fat diet (HFD) with cholic acid, β-oxidation is increased and heat production is accelerated. In *D2*-knockout mice, the metabolic effect of bile acids was lost and thus, was dependent on the expression of *D2* through cyclic AMP production.

Incidentally, the potency of individual bile acids for activating *Tgr5*, as well as *Fxr*, is different. For example, lithocholic acid activates the receptor at nanomolar concentrations; and deoxycholic acid, chenodeoxycholic acid and cholic acid activate it at micromolar concentrations[15]. As previously stated, psyllium changes bile acid profiles during ingestion. Therefore, this means that the ligand molecule changes, which is expected to affect the signal activities of the membrane-type receptor of bile acids such as *Tgr5*, as well as the nuclear receptors of bile acids such as *Fxr*. The effectiveness of *Tgr5*-cyclic AMP-*D2* pathway has been reported particularly in mouse brown adipose tissue and human skeletal muscle and brown adipose tissue, but it is unclear in mouse skeletal muscle. Moreover, little information is available on activating the energy consumption of muscle with psyllium intake.

In the present study, we evaluate whether the intake of psyllium activates energy consumption. Mice fed excess lipid and the skeletal muscle were exposed to an excessive influx of fatty acids and TAG. The condition was easy to judge an activation of energy consumption in the skeletal muscle. The intake of psyllium is expected to change gene expression related to bile acid metabolism in the liver[20–22] and energy consumption in the skeletal muscle. We carried out microarray analysis of both the liver and skeletal muscle, and captured the changes in the expression of genes mainly associated with energy and bile acid metabolism.

Please refer to Appendix 1 for gene names and abbreviations.

Materials and methods

Experimental animals and diets

For the purpose of this study, twelve C57BL/6J mice (5 weeks old, male) were obtained from the Institute for Animal Reproduction (Charles River Laboratories Japan) and acclimatised for 1 week, and were fed on standard chow before starting experimental diets. Animals were assigned to three groups (n 4) alternately by order of body weight to minimise any differences between groups. The mice were housed individually under 12 h light–12 h dark photo-cycles, with food and water freely available. Mice were fed a low-fat diet (LFD), a HFD or a HFD with psyllium (HFD+Psy). The LFD was a standard chow diet with 20 % protein, 7 % fat and 63 % carbohydrate (15·9 % energy from fat diet). The HFD consisted of 20 % protein, 40 % fat and 30 % carbohydrate (64·2 % energy from fat diet). The formulation of the HFD+Psy was modified from the HFD by supplementing it with 5 % psyllium (PG200, MRC Polysaccharide Company, Limited) (Table 1). Mouse body weights and food intake were measured weekly at the same time of day. After 10 weeks of the respective dietary treatment, all mice were fasted for 16 h and anaesthetised by intraperitoneal injection of pentobarbital sodium at a dose of 50 mg/kg body weight and killed. Blood was withdrawn by cardiac puncture and the serum obtained was stored at −20°C until analysis. The liver, femur skeletal muscle and adipose tissues were dissected out immediately and were weighed. These tissues were quickly dipped into 1 ml of RNAlater (Ambion) and stored at −80°C. The care and treatment of the mice were in accordance with the Ethical Guidelines for the Care and Use of Laboratory Animals, Chiba University, and the present study was approved by the Ethics Committee for Animal Experiments of Chiba University.

Lipid and blood glucose assays

Plasma total cholesterol and TAG blood glucose levels were measured after 16 h of fasting using assay kits. Blood glucose concentrations were determined using glucose C II test kit (Wako Pure Chemicals). Total cholesterol and TAG concentrations in the serum were measured by enzymatic colorimetric methods using cholesterol E test and TAG E test kits (Wako Pure Chemicals).

DNA microarray

We designed 65-mer oligonucleotide DNA probes for one negative control and 205 mouse genes related to glycolysis, gluconeogenesis, GLUT, fatty acid metabolism, β-oxidation, energy sensor, nuclear receptor, cholesterol synthesis and

Table 1. Composition of the experimental diets (%)

	LFD	HFD	HFD+Psy
Casein	20	20	20
Maize starch	39·7486	6·7486	6·7486
Dextrin	13·2	13·2	13·2
Sucrose	10	10	10
Soyabean oil	7	7	7
Lard	–	33	33
Vitamin mixture*	1	1	1
Mineral mixture*	3·5	3·5	3·5
Cellulose	5	5	–
L-Cys	0·3	0·3	0·3
Choline bitartrate	0·25	0·25	0·25
t-Butylhydroquinone	0·0014	0·0014	0·0014
Psyllium	–	–	5

LFD, low-fat diet; HFD, 40 % high-fat diet; HFD+Psy, 40 % high-fat diet containing 5 % psyllium.
* Composition of the AIN-93G diet.

cholesterol transporter, using ProbeQuest software (Dynacom Company). The sequences of the probe for each gene were selected considering a melting temperature, specificity, secondary structure, and low complexity sequences, and were located to within 1000 bases from the 3′-end of the mRNA sequences. Melting temperatures of the designed probes were between 70 and 80°C. Synthesised probes were installed onto Genopal (Mitsubishi Rayon Company), which is composed of plastic hollow fibres. In this system, oligonucleotide DNA probes are immobilised to a hydrophilic gel within the three-dimensional space of each hollow fibre[23,24].

Total RNA isolation, antisense RNA synthesis and DNA microarray analysis

Total RNA was extracted from the liver and skeletal muscle samples using the RNeasy Mini Kit (Qiagen). All total RNA samples were run on the Agilent 2100 Bioanalyzer (Agilent Technologies) to check the quality of the samples. Here, three samples with a low degree of degradation in each group (n 4) were used for microarray analysis. Biotinylated antisense RNA (aRNA) were synthesised and amplified from 1 μg of total RNA using the MessageAmpII biotin enhanced amplification kit (Applied Biosystems), according to the manufacturer's instructions. After purification of the aRNA, 5 μg of the biotinylated aRNA were fragmented using 10× fragmentation reagents (Applied Biosystems) by heating at 94°C for 7·5 min. Hybridisation solutions (0·12 M-Tris–HCl, 0·12 M-NaCl, 0·05 % Tween-20 and 5 μg of fragmented biotinylated aRNA) were added to DNA microarrays, and hybridisation, washing and fluorescent labelling were performed by Genopal instrument systems (UE-104; Mitsubishi Rayon). Hybridisation signal acquisition was performed using a DNA microarray reader, adopting multibeam excitation technology (Yokogawa Electric Company)[24]. The median value of background spots was subtracted from the intensity value in each gene, and thereafter the value was normalised in relation to the expression of *Rplp0* (also known as *36B4*, *Arbp*).

Real-time quantitative PCR

Complementary DNA was synthesised from 1 μg of aRNA using the High Capacity complementary DNA Reverse Transcription Kit (Applied Biosystems)[25]. Gene expression was analysed by real-time quantitative PCR (qPCR) using the Applied Biosystems 7500 Fast Real-Time PCR system. Universal ProbeLibrary set, mouse and TaqMan probes (TaqMan Gene Expression Assays) were obtained from Applied Biosystems and Roche Applied Science. All primers are listed in Table 2. Gene expression levels of the target transcripts were normalised to the expression of an endogenous control, *Rp0p* (*36B4*) (NM_007475). Data were analysed using the comparative threshold cycle method.

Statistical analysis

Relative expression values of each gene were calculated using those of the median values of the LFD group. Differences in the variables and mRNA levels among mice fed the LFD, HFD and HFD+Psy were evaluated using Tukey's test. A $P<0{\cdot}05$ was considered significant and the values of bar graphs are presented as the means with their standard error of the mean. All calculations were performed using Excel Statistics 2008 for Windows (Social Survey Research Information Company).

Results

Changes in body weight

There was no statistically significant difference in body weight between groups during the experiment, although the gain in body weight of the mice in the HFD+Psy group was less than that in the HFD group (Table 3). There was no statistically significant difference in total food intake between the HFD and HFD+Psy groups (Table 4).

White adipose tissue weight, plasma lipids and glucose levels

There was a significant reduction in the relative weight of the epididymal fat tissue and retroperitoneal fat tissue depot in mice fed the HFD+Psy. As reported previously[11,26], the HFD+Psy group exhibited a significant decrease in plasma total cholesterol and TAG (Table 4). Blood glucose levels were also decreased in the HFD+Psy group.

Gene expression profiles of the liver and skeletal muscle from microarrays

Approximately 12 % of 205 genes mounted in the microarray were significantly changed in expression as a result of dietary treatment. In the liver, twenty-five genes were significantly changed; and twenty-six genes in the skeletal muscle were likewise changed (Fig. 1(a) and (b)). Under *in vivo* conditions,

Table 2. Sequences of primers and universal probe libraries (UPL) number used for quantitative PCR*

Gene name and gene symbol	Forward 5′–3′	Reverse 5′–3′	UPL no.
Cytochrome P450, family 7, subfamily a, polypeptide 1 (*Cyp7a1*)	tcaataccatgcttttgtctgc	gacctgcacagcatccact	19
3-Hydroxy-3-methylglutaryl-coenzyme A reductase (*Hmgcr*)	gcttacagagccaatgatgga	aactcctggccacaggaac	1
ATP-binding cassette, sub-family G (WHITE), member 5 (*Abcg5*)	caacagtatagtggccctgct	ggaatgggcatctcttgtatg	26
ATP-binding cassette, sub-family G (WHITE), member 8 (*Abcg8*)	tgtgttcagaccccagtgtg	gctgtgtggactccctgag	89
ATP-binding cassette, sub-family B (MDR/TAP), member 11 (*Abcb11*)	tttacctctgacacccgtga	ccctgagcctgggagatt	55

*The relative amount of each transcript was normalised to the amount of used *Rplp0* (also known as *36B4*, *Arbp*) transcript in the same complementary DNA. TaqMan assay ID no. of *Rplp0* is Mm99999223_gH.

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Table 3. Body weight (g) of mice fed different types of diets for 10 weeks*
(Mean values with their standard errors, *n* 4)

Diets…	LFD		HFD		HFD+Psy	
	Mean	SE	Mean	SE	Mean	SE
Week 0	19·38	0·27	19·38	0·61	19·38	0·67
Week 1	22·05	0·48	21·65	0·85	21·85	0·49
Week 2	23·20	0·44	22·95	1·07	23·05	0·69
Week 3	24·13	0·50	24·45	1·17	24·10	0·95
Week 4	25·20	0·41	26·30	1·31	25·33	1·05
Week 5	26·33	0·40	27·73	1·43	26·55	1·17
Week 6	27·75	0·45	30·05	1·57	28·15	1·34
Week 7	28·45	0·42	31·30	1·53	29·25	1·47
Week 8	29·75	0·85	33·30	1·71	30·65	1·61
Week 9	30·75	0·84	34·80	1·67	31·90	1·57
Week 10	31·43	0·74	36·30	1·79	33·18	1·68

LFD, low-fat diet; HFD, 40 % high-fat diet; HFD+Psy, 40 % high-fat diet containing 5 % psyllium.
*Mean values were not significantly different between the groups ($P>0·05$; Tukey's test).

changes in differential gene expression because of dietary treatment are expected to be small and often a small number of genes are significantly changed[27]. Tukey's test was performed 205 times at the 5 % significance level, and the false discovery rate was estimated at approximately 41 % in the liver and skeletal muscle.

Genes involved in energy metabolism in the liver

A gene encoding enzymes involved in lipogenesis, stearoyl-CoA desaturase 1 (*Scd1*), was down-regulated in the HFD and HFD+Psy groups (Fig. 2(a1)). Genes involved in fatty acid oxidation and lipid transport (pyruvate dehydrogenase E1 alpha 1 (*Pdha1*), *Ucp2*, *Ucp3*, *Octn1*, *Slc27a1* and *Cd36*) were up-regulated in the HFD group. One of the genes encoding enzymes involved in glycogenolysis, *G6pc*, was up-regulated in the HFD+Psy group. One of the enzymes catalysing the initial step of the mitochondrial fatty acid β-oxidation pathway, *Acads*, was up-regulated in the HFD and HFD+Psy groups.

Genes involved in cholesterol and bile acid metabolism in the liver

The expression levels of key genes involved in the cholesterol synthetic pathway (*Sqle*, *Hmgcs1* and *Hmgcr*) were enhanced in the HFD and HFD+Psy groups (Fig. 2(a2)). Genes involved in cholesterol and bile acid transport (*Abcb11*, *Abcg1 and Abcg4*) were significantly down-regulated in the HFD+Psy group. One of the genes involved in phosphatidylcholine biosynthesis, *Pcyt1a*, changed slightly.

Genes involved in transcription factors and miscellaneous genes in the liver

Here, two genes involved in transcription factors (*Nr5a2* and *Ppara*) were significantly down-regulated in the HFD+Psy group (Fig. 2(a3)). In addition to the genes previously mentioned, seven genes (C-reactive protein (*Crp*), *Nos1*, *Ifng*, *Ppia*, *Retn*, phosphoinositide-3-kinase regulatory subunit 1 (*Pik3r1*) and *Sgk1*) were significantly changed among the different diet groups (Fig. 2(a4)).

Genes involved in fatty acid oxidation and lipid transport in the skeletal muscle

Genes encoding key enzymes and transporters involved in fatty acid oxidation and lipid transport (*Acadm*, *Acadl*, *Hadh*, *Hadha*, *Hadhb*, *Acsl1*, *Lpl*, *Cd36*, *Cpt1b* and *Cact*) greatly increased in the HFD+Psy group (Fig. 2(b1)). Almost all of these genes were up-regulated 2- to 4-fold relative to the LFD group.

Genes involved in energy metabolism in the skeletal muscle

Genes involved in the citrate cycle and glucose and energy metabolism (*G6pc*, *Idh2*, *Mdh1*, *Ldhb* and *Ndufab1*) were significantly up-regulated in the HFD+Psy group (Fig. 2(b2)).

Genes involved in signal transduction and miscellaneous genes in the skeletal muscle

Genes involved in signal transduction (*Cckar*, *Camkk2* and *Fgf15*) were slightly lowered in the HDF+Psy group relative to the HFD group (Fig. 2(b3)). Here, two genes involved in the stress response and the regulation of lipid metabolic processes (*Sgk1* and *Angptl4*) were up-regulated in the HFD+Psy group (Fig. 2(b3)). In addition to the genes previously mentioned, six genes (*Il17a*, *Il18*, *Il12b*, *Nos3*, *Ppara* and

Table 4. Total food intake, white adipose tissue weight, plasma lipids and glucose levels
(Mean values with their standard errors)

Diets…	LFD		HFD		HFD+Psy	
	Mean	SE	Mean	SE	Mean	SE
Total food intake (g)	223·71^{a}	9·73	157·39^{b}	3·84	147·76^{b}	5·40
Epididymal fat tissue weight (g/100 g body weight)	4·33^{a,b}	0·19	5·95^{a}	0·53	3·96^{b}	0·49
Retroperitoneal fat tissue weight (g/100 g body weight)	1·69^{a,b}	0·16	2·21^{a}	0·15	1·53^{b}	0·16
Glucose (mg/l)	2319·2^{a}	241·0	1988·9^{a,b}	81·7	1499·0^{b}	187·9
Total cholesterol (mg/l)	1186·1^{a,b}	99·2	1407·6^{a}	40·3	1030·1^{b}	88·2
TAG (mg/l)	622·6^{a}	54·5	594·3^{a}	29·3	386·8^{b}	25·0

LFD, low-fat diet; HFD, 40 % high-fat diet; HFD+Psy, 40 % high-fat diet containing 5 % psyllium.
^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$, Tukey's test).

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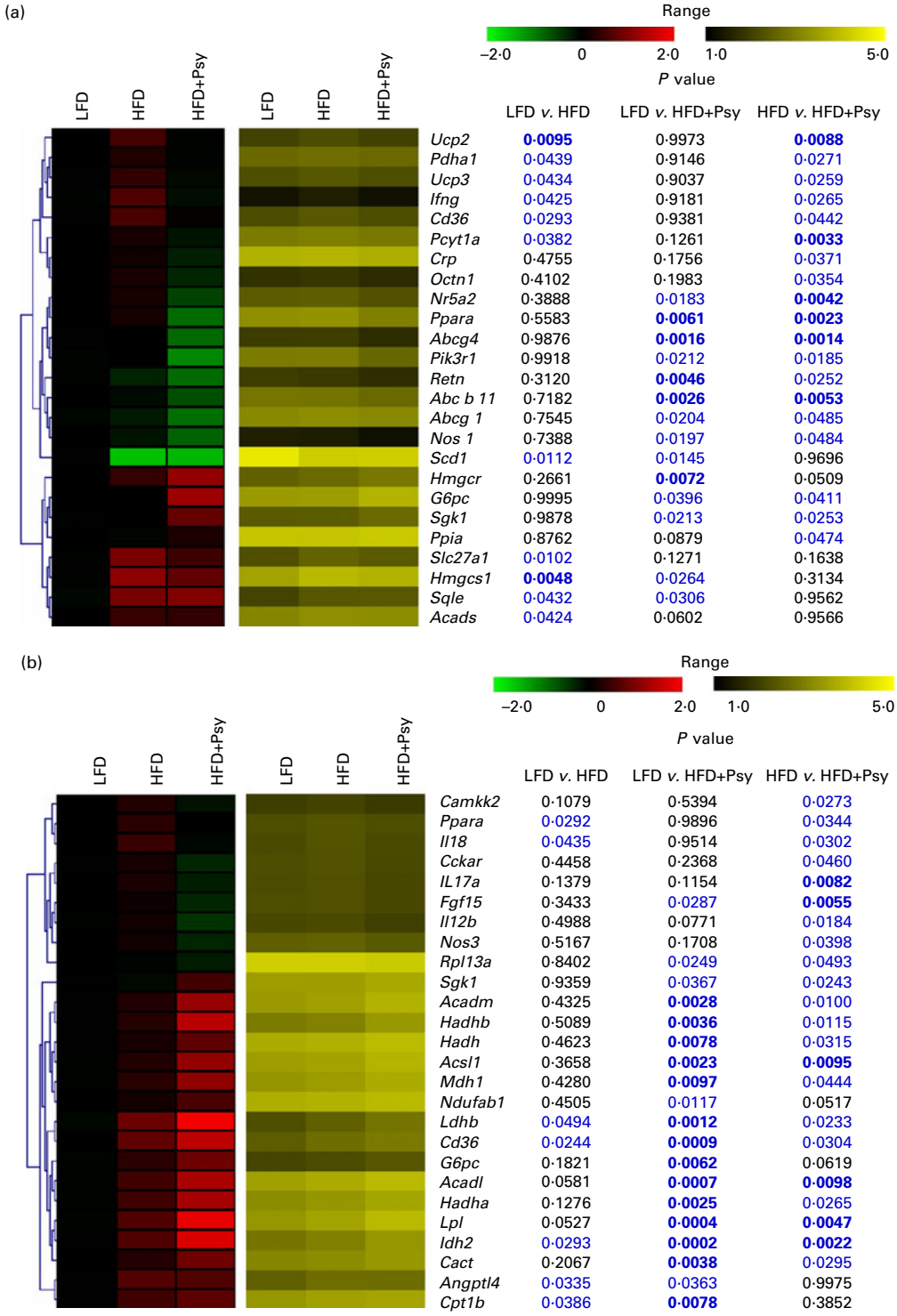


Fig. 1. Genes that changed significantly in (a) the liver and (b) the skeletal muscle between three different dietary groups. The left box is expressed as relative expression values to the low-fat diet (LFD) group for each gene (log2-transformed values). The right box is a heat map that represents intensity values after background subtraction and normalisation (log10-transformed values). The right upper panel is a colour intensity key representing relative expression values from low (green) to high (red) and intensity values from low (black) to high (yellow). Of the 205 genes that were mounted on the microarray, twenty-five genes were significantly changed in the liver and twenty-six genes were significantly changed in the skeletal muscle between three different dietary groups (Tukey's test; $P<0{\cdot}05$). Hierarchical clustering was carried out using statistical TIGR Multiple Experiment Viewer software (Dana-Farber Cancer Institute; http://www.tm4.org/mev/). The correlation coefficient was used as a distance metric and complete linkage was used as the clustering algorithm. *P* values of the three-group comparisons are listed on the right side of the heat map. *P* values$<0{\cdot}05$ are shown in blue text and *P* values$<0{\cdot}01$ are shown in bold blue text. The average estimated number of falsely significant genes was 10·3 genes ($205\times0{\cdot}05$). HFD, high-fat diet; HFD + Phy, 40 % HFD containing 5 % psyllium. Please refer to Appendix 1 for gene names and abbreviations. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn)

Rpl13a) were significantly changed among the different diet groups (Fig. 2(b4)).

Validation of gene expression results from the microarrays and measurement of key enzyme gene expression using quantitative PCR

To validate the values of the expression patterns from the microarrays, we selected key genes of bile acid and cholesterol metabolism (*Hmgcr*, *Abcg5*, *Abcg8*, *Abcb11*, *Cyp7a1*), and conducted further qPCR tests (Fig. 3(a) and (b)). Gene expression levels of *Hmgcr* were significantly elevated in the HFD+Psy group. Gene expression levels of *Abcb11* gene were significantly reduced in the HFD+Psy group. Although the gene expression levels of *Cyp7a1* were up-regulated in the HFD and HFD+Psy groups, there was no statistically significant change in the gene expression of *Abcg5*, *Abcg8* or *Cyp7a1* (Fig. 3(b)). The qPCR results showed that the levels of mRNA for the selected genes followed the same pattern of expression as those observed within the microarray experiment, thus confirming the values obtained from the microarrays.

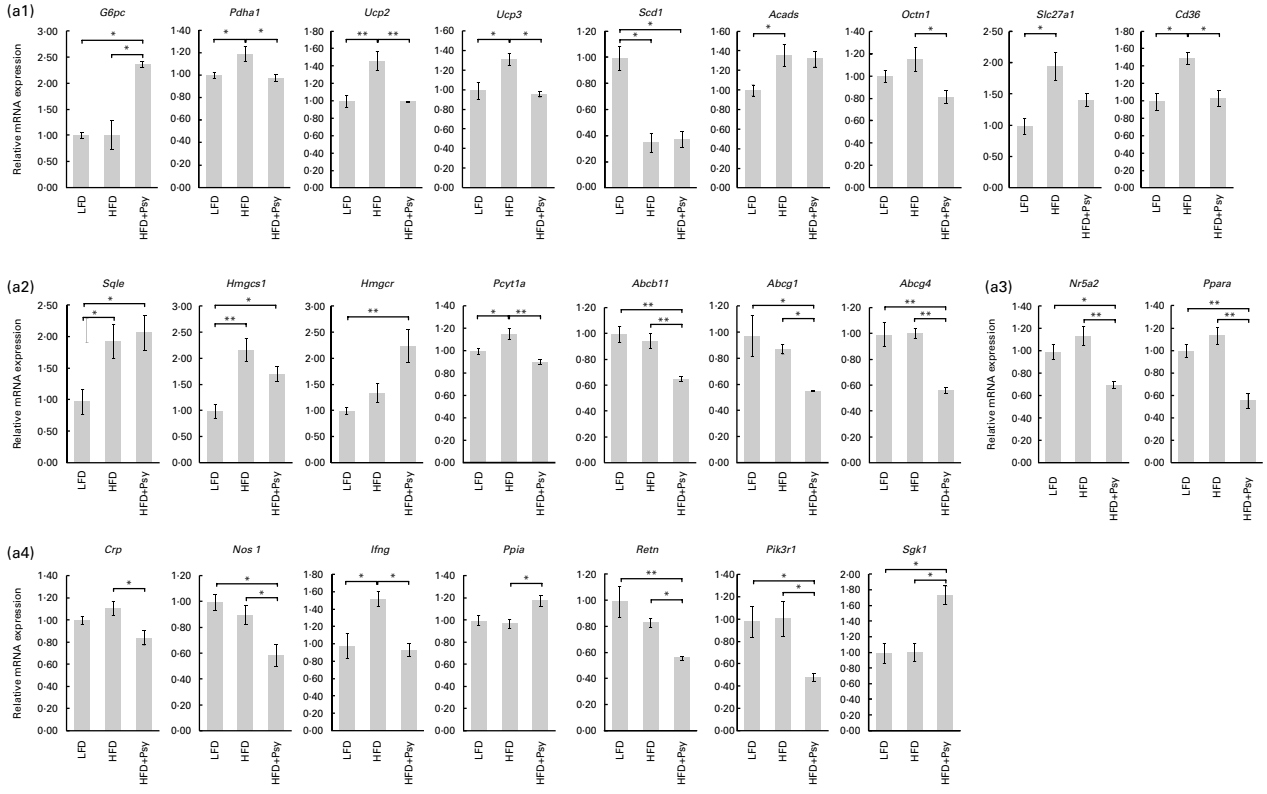
Discussion

The aim of the present study was to evaluate the novel function of psyllium using DNA microarray analysis. The results of this study suggest that psyllium promotes lipid consumption in the skeletal muscle.

Although a significant difference in dietary intake was not found between the HFD and HFD+Psy groups, body weights tended to be lower in mice fed the HFD+Psy; and adipose tissue weights of the HFD+Psy group were significantly lower relative to the HFD group (Tables 3 and 4). These results are incongruent with those reported by Galisteo *et al.*[27]. Chan *et al.*[21] reported that psyllium treatment did not seem to affect the body weight of mice. A possible reason for these discrepancies could be due to differences in dietary fat content because the diets used in the previous studies contained 4 % fat.

de Wilde *et al.*[28] measured the gene expression levels in mouse skeletal muscle tissue. They reported that on the gene expression levels of mouse skeletal muscle, a HFD effected little change relative to a LFD. In contrast, we observed that genes involved in fatty acid oxidation and lipid transport in the skeletal muscle were significantly up-regulated in the HFD+Psy group (Fig. 1(b)). This result suggests that feeding psyllium stimulates lipid transport and fatty acid oxidation in the muscle.

Hannan *et al.*[29] proposed that psyllium inhibits glucose absorption in the intestinal tract and Matsumoto *et al.*[22] reported that blood glucose was significantly reduced in mice fed a diet with 1 % cholestyramine for 8 weeks. In the present study, blood glucose levels were 24 % lower in mice fed psyllium (Table 4). This result suggests that psyllium has a serum glucose-decreasing effect. The fact that glucose levels tend to be lower in the HFD+Psy group and the expression level of hepatic *G6pc* was significantly higher



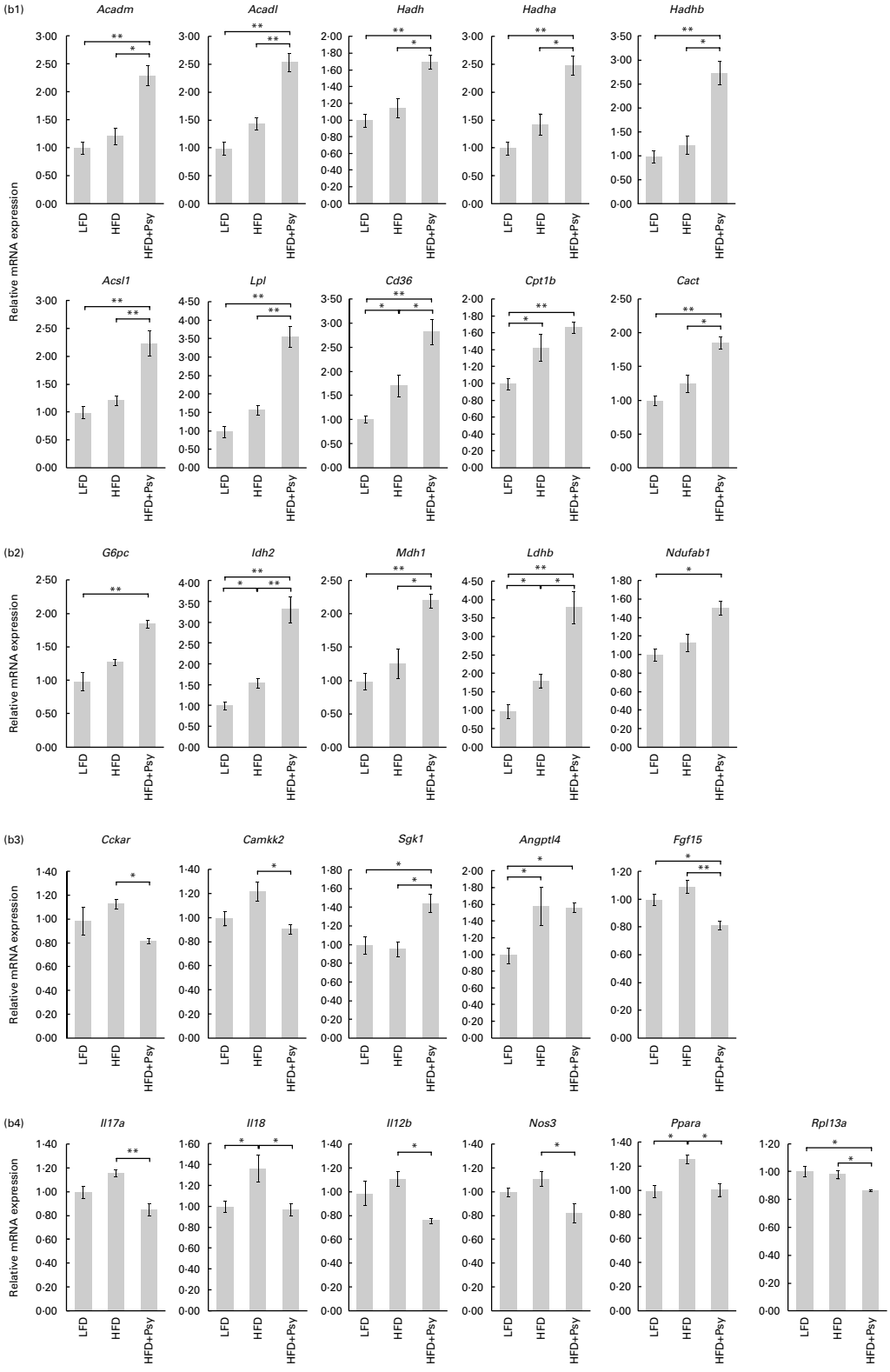


Fig. 2. Expression profiles that changed significantly in the (a) liver and (b) skeletal muscle between three different dietary groups. Genes were categorised by function: (a1) energy metabolism; (a2) cholesterol and bile acid metabolism; (a3) nuclear receptors; (a4) miscellaneous; (b1) fatty acid oxidation and lipid transport; (b2) energy metabolism; (b3) signal transduction; (b4) miscellaneous. Relative mRNA expression data were means, with their standard errors of three mice at each dietary group and are expressed as relative values to the low-fat diet (LFD) group for each gene. Mean values were significantly different: $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$; Tukey's pair-wise comparisons. HFD, high-fat diet; HFD+Phy, 40 % HFD containing 5 % psyllium. Please refer to Appendix 1 for gene names and abbreviations.

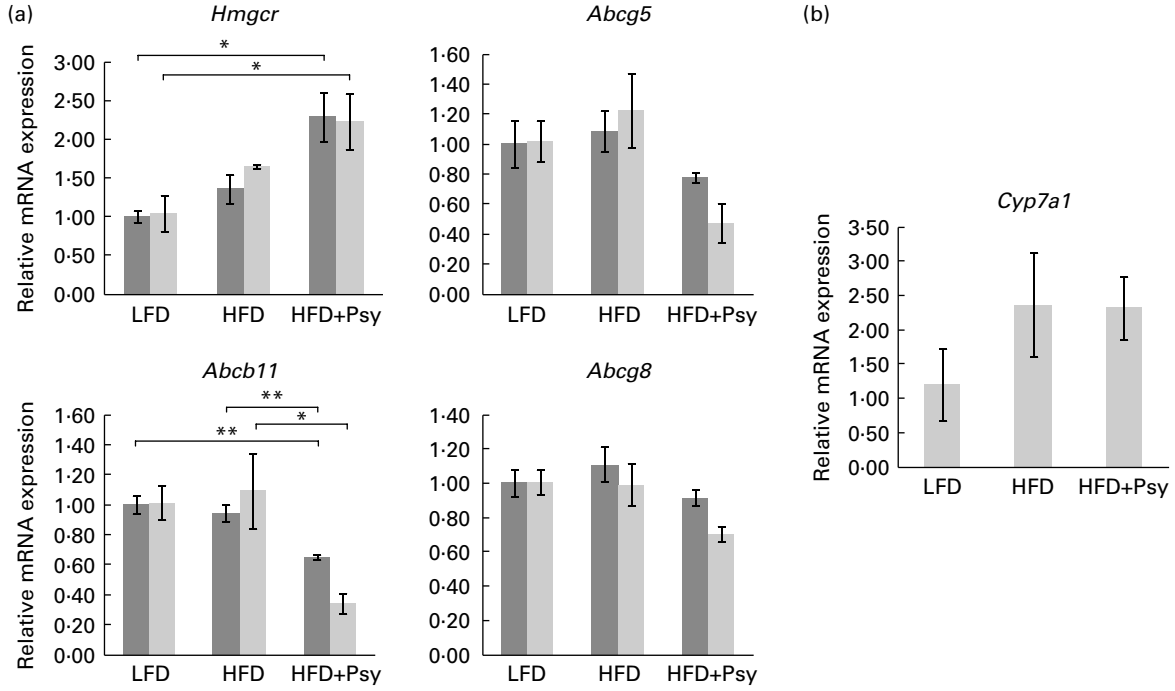


Fig. 3. Quantitative PCR (qPCR; ■) gene expression analysis. (a) Validation of gene expression patterns from the microarray analysis (■) by relative quantification through qPCR. Real-time PCR units indicate relative expression to the internal standard *Arbp* (*36B4*). Mean values were significantly different: **P*<0·05, ***P*<0·01; Tukey's pair-wise comparisons. (b) Relative mRNA expression data of cholesterol 7α hydroxylase (*Cyp7a1*) at each dietary group. Validation of gene expression patterns of *Cyp7a1* were carried out by qPCR. Relative mRNA expression data were means, with their standard errors of three mice at each dietary group and were expressed as relative values to the low-fat diet (LFD) group for each gene. Mean values were not significantly different between the groups. HFD, high-fat diet; HFD+Phy, 40 % HFD containing 5 % psyllium. Please refer to Appendix 1 for gene names and abbreviations.

than in the HFD group (Fig. 2(a1)) may indicate that glucose supply in the liver was insufficient in the HFD+Psy group. *Pik3r1* is also known as p85alpha and is involved in insulin signalling and energy metabolism. *Pdha1* is a component of pyruvate dehydrogenase complex catalysing the oxidative decarboxylation of pyruvate and producing acetyl-CoA and CO_2. The complex is a key enzyme in controlling the balance between lipid and glucose oxidation. The expression levels of *Pik3r1* and *Pdha1* were significantly suppressed in the HFD+Psy group (Fig. 2(a1) and (a4)), which may indicate the feedback regulation of low blood glucose levels. Because of the low glucose levels, fatty acid oxidation may be stimulated in the skeletal muscle and glycerol and ketone bodies may be produced as alternative energy substrates.

It was also reported that psyllium reduced cholesterol and TAG. Trautwein *et al.*[12] made a comparison between hamsters fed a control diet and those fed a diet containing 6 % psyllium, reporting that plasma total cholesterol was reduced to 61 % and TAG reduced to 75 %. Chan *et al.*[21] also compared mice fed a control diet and mice fed a diet containing 10 % psyllium for 10 weeks, reporting that plasma total cholesterol was lowered 35 % and TAG lowered 32 %. Our results agree with these reports on the effect, given that plasma total cholesterol concentrations were significantly 26 % lower and TAG concentrations significantly 35 % lower in mice fed the HFD+Psy relative to mice fed the HFD (Table 4).

Microarray analysis in the liver showed that *Sqle* and *Hmgcr*, cholesterol metabolism-related genes, were significantly up-regulated in the HFD+Psy group compared with other groups (Fig. 2(a2)), which indicates that cholesterol was insufficient in the liver for the increased cholesterol excretion. While the expression levels of *Cyp7a1* measured by qPCR were higher in the HFD and HFD+Psy groups than those in the LFD group, the expression levels of *Cyp7a1* between the HFD group and the HFD+Psy group were not different (Fig. 3(b)). This result indicated that a HFD by itself stimulates the expression levels of *Cyp7a1* to some extent. We also measured the expression levels of *Abcb11* (*Besp*), *Abcg5* and *Abcg8*, genes; encoding a bile acid and cholesterol transporter molecule. The expression levels of these molecules in the HFD+Psy group were greatly decreased (Fig. 3(a)). Matsumoto *et al.*[22] observed that the gene expression levels of *Abcg5* and *Abcg8* were decreased in a cholestyramine-containing diet, consistent with the results of our present study.

Both psyllium and cholestyramine change the expression levels of cholesterol transporter molecules. Down-regulation of *Abcb11*, *Abcg5* and *Abcg8* would be the result of a feedback effect of bile acid excretion.

By general consensus, unsaturated fatty acids suppress the expression of *Scd1* and *Acads* is required to catalyse the β-oxidation of SCFA. In the HFD and HFD+Psy groups, the *Scd1* expression was greatly down-regulated and the *Acads* expression was up-regulated relative to those in the LFD group (Fig. 2(a1)), which may indicate that the liver was exposed to excess fatty acids and produced excess SCFA. Moreover, it is known that *Ppara* directly up-regulates *Ucp3*

and *Cd36*. The expression levels of genes involved in fatty acid oxidation and lipid transport in the liver (*Ppara*, *Octn1*, *Ucp2*, *Ucp3* and *Cd36*) were significantly up-regulated in the HFD group relative to the HFD+Psy group (Fig. 2(a1) and (a3)). These results indicated that in the HFD group, fatty acid oxidation is activated in the liver, but in the HFD+Psy group, the fatty acid oxidation level is not so stimulated as that in the HFD group, which suggests that psyllium has the ability to resist excess influx of fatty acids into the liver and protect it.

North *et al.*[30] reported that psyllium might possess the quality of reducing *Crp* levels. In the present study, the expression levels of *Crp*, *Nos1*, *Ifng* and *Retn* were significantly down-regulated in the HFD+Psy group (Fig. 2(a4)). Thus, psyllium might possess the ability to suppress stress-induced inflammation.

The continual process of excretion and synthesis changes the composition of bile acids[12,13]. Bile acids function as signalling molecules through *Fxr* and *Tgr5* and the change in the composition of bile acids affects binding activity, influencing signal activity. Watanabe *et al.*[31] reported that a HFD containing cholic acid activated *D2* gene expression levels in mouse brown adipose tissue and increased energy production. In contrast, the *D2* gene was not detected in mouse skeletal muscle. In addition, Vassileva *et al.*[16] observed that the *Tgr5* gene was much expressed in the gall bladder but was little expressed in the skeletal muscle. These two reports suggest that the pathway via *Tgr5* gene and *D2* gene affect the metabolism of mouse skeletal muscle a little. Our study results showed that genes involved in fatty acid oxidation and lipid transport were significantly up-regulated, and thus, another signal may be involved in our present results.

Staiger *et al.*[32] reported that long-chain fatty acids enhance the expression level of *Angptl4* in C2C12 myocytes. *Angptl4* was up-regulated in the HFD and HFD+Psy groups (Fig. 2(b3)), which suggests that the skeletal muscle is exposed to excess long-chain fatty acids in the HFD and HFD+Psy groups.

Meanwhile, Campos *et al.*[33] reported that psyllium promotes the production of butyrate and acetate. Moreover, butyrate can change the properties of the skeletal muscle and activate AMP-activated protein kinase directly[34]. Yamauchi *et al.*[35] reported that thyroid hormone T_3 increases cellular oxygen consumption and *Camkk2* is involved in the effect of T_3 on AMP-activated kinase in C2C12 cells. In this study, the expression level of *Camkk2*, a gene involved in AMP-activated kinase phosphorylation[36], was slightly down-regulated in the HFD+Psy group (Fig. 2(b3)). This raises the possibility of a feedback effect enhancing genes involved in fatty acid oxidation and lipid transport in the skeletal muscle.

Sgk1 was up-regulated in the HFD+Psy group in both the liver and skeletal muscle (Fig. 2(a4) and (b3)). Sgk1 is a substrate for the mTORC2 complex and regulates a number of transcription factors like *Foxo3a* involved in the regulation of processes such as cell survival[37–40], which may indicate that the glucose-lowering effects of psyllium (Table 4) is involved in mTOR signals.

Although the mechanism of enhancing lipid consumption in the skeletal muscle remains controversial and a limitation of our study is mainly based on expression data, the present results are useful to evaluate the possible beneficial effects of psyllium.

In conclusion, the present study demonstrates that psyllium can enhance the transfer of excess fatty acid from the liver to the skeletal muscle and promote lipid consumption in the skeletal muscle; and this effect would create a slightly insufficient glucose state in the liver. To compensate for a low glucose level, fatty acid oxidation seems to be accelerated in the skeletal muscle and more glycerol and ketone bodies may be produced. Therefore, psyllium contributes to the consumption of lipids and ameliorates body weight gain.

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Appendix 1. Gene names and abbreviations

Abbreviation	Name
Abcb11	ATP-binding cassette, sub-family B (MDR/TAP), member 11
Abcb11 (Bsep)	ATP-binding cassette, sub-family B (MDR/TAP), member 11
Abcg1	ATP-binding cassette, sub-family G (WHITE), member 1
Abcg4	ATP-binding cassette, sub-family G (WHITE), member 4
Abcg5	ATP-binding cassette, sub-family G (WHITE), member 5
Abcg8	ATP-binding cassette, sub-family G (WHITE), member 8
Acadl	Acyl-coenzyme A dehydrogenase, long-chain
Acadm	Acyl-coenzyme A dehydrogenase, medium-chain
Acads	Acyl-coenzyme A dehydrogenase, short-chain
Acsl1	Acyl-CoA synthetase long-chain family member 1
Angptl4	Angiopoietin-like 4
Arbp (36B4)	Acidic ribosomal phosphoprotein P0
Cact	Carnitine/acylcarnitine translocase
Camkk2	Calcium/calmodulin-dependent protein kinase kinase 2, β
Cckar	Cholecystokinin A receptor
Cd36	CD36 antigen
Cpt1b	Carnitine palmitoyltransferase 1b
Crp	C-reactive protein
Cyp7a1	Cytochrome P450, family 7, subfamily a, polypeptide 1
Cyp27a1	Cytochrome P450, family 27, subfamily a, polypeptide 1
Fgf15	Fibroblast growth factor 15
Foxo3a	Forkhead box O3
Fxr	Farnesoid X receptor
G6pc	Glucose-6-phosphatase, catalytic
Hadh	Hydroxyacyl-coenzyme A dehydrogenase
Hadha	Hydroxyacyl-coenzyme A dehydrogenase/3-ketoacyl-coenzyme A thiolase/enoyl-coenzyme A hydratase (trifunctional protein), α subunit
Hadhb	Hydroxyacyl-coenzyme A dehydrogenase/3-ketoacyl-coenzyme A thiolase/enoyl-coenzyme A hydratase (trifunctional protein), β subunit
Hmgcr	3-Hydroxy-3-methylglutaryl-coenzyme A reductase
Hmgcs1	3-Hydroxy-3-methylglutaryl-coenzyme A synthase 1
Idh2	Isocitrate dehydrogenase 2
Ifng	Interferon γ
Il12b	Interleukin 12b
Il17a	Interleukin 17A
Il18	Interleukin 18
Ldhb	Lactate dehydrogenase B
Lpl	Lipoprotein lipase
Mdh1	Malate dehydrogenase 1
mTORC2 (Rictor)	RPTOR independent companion of MTOR, complex 2
Ndufab1	NADH dehydrogenase (ubiquinone) 1, α/β subcomplex, 1
Nos1	Nitric oxide synthase 1, neuronal
Nos3	Nitric oxide synthase 3, endothelial cell
Nr5a2	Nuclear receptor subfamily 5, group A, member 2
Octn1	Organic cation transporter 1
Pcyt1a	Phosphate cytidylyltransferase 1, choline, α isoform
Pdha1	Pyruvate dehydrogenase E1 α 1
Pik3r1	Phosphoinositide-3-kinase regulatory subunit 1
Ppara	Peroxisome proliferator activated receptor α
Ppia	Peptidylprolyl isomerase A
Retn	Resistin
Rpl13a	Ribosomal protein L13A
Scd1	Stearoyl-CoA desaturase 1
Sgk1	Serum/glucocorticoid regulated kinase 1
Slc27a1	Solute carrier family 27 (fatty acid transporter), member 1
Sqle	Squalene epoxidase
Tgr5	G protein-coupled bile acid receptor 1
Ucp2	Uncoupling protein 2
Ucp3	Uncoupling protein 3