

Basic nutritional investigation

Sequential effects of a high-fiber diet with psyllium husks on the expression levels of hepatic genes and plasma lipids

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Abstract

Objective: We studied the sequential effects of a high-fiber diet using psyllium husks on hepatic gene expression and plasma lipid levels.

Methods: C57BL/6J mice were randomly assigned to a control diet or a high-fiber diet containing 10% psyllium husks for 3 weeks (PE-3wk) and 10 weeks (PE-10wk). Oligonucleotide microarrays were used to screen the transcriptional response at both time points. Genes encoding enzymes regulating key steps of lipid metabolism were then selected for further validation by quantitative real-time reverse transcription polymerase chain reaction and their protein expression by western blot assays.

Results: Plasma cholesterol and triacylglycerol levels were reduced in both high-fiber groups. Three weeks of high-fiber feeding downregulated genes involved in lipogenesis, whereas those involved in cholesterol and bile acid synthesis were upregulated. With prolonged high-fiber feeding, genes involved in lipogenesis such as fatty acid synthase (*Fasn*) were then upregulated. Additional genes in cholesterol synthesis such as 3-hydroxy-3-methylglutaryl coenzyme A reductase (*Hmgcr*) were also upregulated. At week 3, protein expression levels of *Fasn* were significantly lower in the high-fiber group but increased at week 10. Protein levels of *Hmgcr* were significantly increased in PE-10wk mice.

Conclusion: The high-fiber diet containing psyllium husks reduced plasma total cholesterol and triacylglycerol levels. Cholesterol lowering was most likely mediated by increased bile acid synthesis. The increased transcript levels of genes related to cholesterol synthesis throughout the entire feeding period and the subsequent increased lipogenic gene transcript levels could likely suggest a regulatory mechanism to restore the lowered plasma cholesterol and triacylglycerol levels. © 2008 Elsevier Inc. All rights reserved.

Keywords:

Hypercholesterolemia; Lipogenesis; Microarrays; Gene expression and C57BL/6J mice; High fiber; Psyllium

Introduction

It is estimated that by 2020, two-thirds of the global burden of disease will be attributable to chronic non-communicable diseases, with most strongly associated with diet [1]. A major diet-related risk factor contributing to the development of these chronic diseases in developed and developing countries is increased blood cholesterol levels. Increased dietary fiber may have beneficial effects in reducing blood cholesterol levels [2].

The completion of the human, mouse, and rat genome sequencing has allowed studies to examine the interplay between environment factors (e.g., diet) and the genome. With the recent advancements in expression array technology, several studies have examined the relation between diet and genetic responses. However, the literature on dietary fiber and gene expression remains scant. Studies are limited to the gastrointestinal tract [3–5] or only one to two hepatic genes [6–8]. No microarray studies have yet been carried out on the effect of dietary fiber on global hepatic gene expression.

The health benefits of increased dietary fiber intake were first suggested more than 30 y ago [9]. Since then, evidences of the link between dietary fiber and cardiovascular diseases have accumulated from epidemiologic observations [10–13] and clinical trials [14–16].

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Of the two main types of dietary fiber, non-viscous insoluble fiber has not been shown to have a consistent cholesterol-lowering effect. In contrast, consumption of a high-fiber diet containing viscous soluble fiber has been associated with significantly lower plasma total and low-density lipoprotein (LDL) cholesterol levels [17–19]. The cholesterol-lowering effects of viscous soluble dietary fiber could be due to 1) increased bile acid synthesis or 2) reduced cholesterol synthesis. Bile acid synthesis accounts for 40–50% of the daily elimination of cholesterol [20,21] and plasma cholesterol is quantitatively the most important substrate for bile acid synthesis [22]. Endogenous cholesterol biosynthesis accounts for approximately 75–80% of the total body cholesterol pool [22]. The liver is the main site of bile acid synthesis and endogenous cholesterol biosynthesis.

Of the viscous soluble fibers, psyllium husk appears to be the most effective in lowering cholesterol levels [23,24] and has the least adverse side effects [25]. Psyllium or ispaghula husk (the husk of the seeds of *Plantago ovata*) is a mixture of neutral and acid polysaccharides containing galacturonic acid. Some foods in the human diet could potentially be enriched with psyllium, such as breads, cereals, and pasta. The effect of psyllium husks on fasting plasma cholesterol has been evaluated in individuals with hypercholesterolemia, obesity, or diabetes [26,27]. In general, these studies showed that psyllium husk consumption could bring about a 5% reduction in total cholesterol and 7–8% reduction in LDL cholesterol, which were sustainable in the long term [28,29].

Thus far, there have been no studies on the sequential effects of a high-fiber diet on hepatic gene expression and changes in lipid metabolism. We therefore studied the effects of high-fiber feeding over a period of 10 wk using psyllium husks. Its effects on food intake, body weight, plasma lipids, and transcriptomic profile of hepatic genes, particularly those involved in lipid metabolism, were also determined. Two time-point measurements were used in the experimental design to elucidate the early responses to high-fiber feeding at week 3 and whether these responses were sustained over a longer period of 10 wk.

Materials and methods

Mice and diets

Twenty-four 8-wk-old female C57BL/6J mice were supplied by the Laboratory Animal Center, National University of Singapore (NUS). They were acclimatized in our animal holding unit for 2 wk, feeding on standard chow before commencing their respective diets. They were randomized into four groups of six and assigned to the control (C) or psyllium-enriched (PE) diet for both time points. The C diet was a standard AIN-93M diet (Specialty Foods, Perth, Australia), which consisted of 13.5% protein, 4% fat, and 82.5%

carbohydrate, with a total fiber content of 5%. The formulation of the PE diet was modified from the AIN-93M diet by supplementing it with 10% psyllium husks (SF03-034, Specialty Feeds, Perth, Australia). It had a total fiber content of 15%.

Food intake and body weight

The amount of food intake was measured by subtracting the residual food and food spillage from the total amount of food added weekly. Spilled food was collected on paper towels placed beneath the cages in which the individual mouse was kept. After removing feces and wood shavings from the paper towels, food spillage was collected and weighed.

Baseline body measurements of the mice were obtained when they first arrived. Subsequently, mice body weights were measured weekly at the same time of the day using the same machine.

Mice sacrifice and collection of tissue

After 3 and 10 wk of the respective dietary treatment, the mice were sacrificed by cardiac puncture. Blood was collected and livers were quickly removed. Liver samples comprising identical lobes were obtained from each mouse. These were immediately snap-frozen in liquid nitrogen and stored at -80°C . All animal experiments were performed with the approval of NUS Laboratory Animals Centre.

Lipid assays

Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and triacylglycerol levels were measured after an overnight fast using enzymatic assay kits on a Cobas Mira analyzer (Roche, Basel, Switzerland). LDL/very LDL cholesterol levels were determined as the difference between total and HDL cholesterol levels.

Analysis of gene expression profile by high-density oligonucleotide microarrays

To determine the gene expression profile of the hepatic genes in the different groups, the relative abundance of mRNAs in the PE groups was compared with the corresponding controls (e.g., PE-3wk versus C-3wk). The mouse genome 430A GeneChip (MOE430A, Affymetrix, Santa Clara, CA, USA) containing probes for about 14 400 known mouse genes were used. Total RNA was isolated from the individual frozen liver tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and purified using RNeasy Mini Kit (Qiagen, Hilden, Germany). A 2- μL sample was run on a 1% denaturing gel to ensure that RNA was not degraded after extraction. The purified total RNA was then pooled from each group. The preparation of biotinylated cRNA and hybridization onto the GeneChip were carried out according

to instructions provided by the Affymetrix protocol with 10–15 μg of total RNA.

GeneChip data analysis

Microarray Suite 5.0 (Affymetrix) was used to analyze the scanned image. Expression patterns for each group were analyzed according to the following pairwise comparisons. The mice fed on the high-fiber diet for 3 wk (PE-3wk) were compared with their corresponding controls (C-3wk). At week 10, the PE-10wk mice were compared with the mice fed on the standard control AIN-93M diet (C-10wk). The probe sets were sorted according to stringency criteria. To obtain the robust “increase” expression profile, the experimental probe sets designated “absent” were eliminated and only the probe sets flagged as “I” with a signal log ratio ≥ 0.5 were selected. For the robust “decrease” profile, the “absent” cells were eliminated from the C samples and only those flagged “D” with a signal log ratio ≤ -0.5 were selected. Data of “fold change” were calculated from the “signal log ratio.” The selected probe sets were then annotated and classified using the NetAffx Gene Ontology Mining Tool.

Quantitative real-time reverse-transcription polymerase chain reaction

Quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) was carried out to validate our microarray results. We selected five essential genes that showed differential expression at the two time points. The sequences of the primers used are listed in Table 1. The LightCycler SYBR Green I one-step RT-PCR (Roche Diagnostics, Basel, Switzerland) method was used to quantify the starting mRNA of the five target genes. For amplification, RNA template, primers, $\text{Mn}(\text{OAc})_2$ and LightCycler RNA Master SYBR Green I were mixed well and transferred to LightCycler capillaries (Roche Diagnostics) according to the Roche quantitative RT-PCR protocol. The signal of the housekeeping gene β -actin was used for normalization. Melting curve analysis was performed to assess the specificity of the amplified PCR products. The sizes of the amplified PCR products were verified with 2% agarose gel electrophoresis and visualized by ultraviolet fluorescence after staining with ethidium bromide.

Table 1
Sequences of primers used in quantitative real-time reverse transcription PCR

Gene name (gene symbol)	Forward (5'→3')	Reverse (5'→3')	PCR product
Actin, beta (β -actin)	taaagacctctatgccacacagt	cacgatggagggccggactcatc	241 bp
3-Hydroxy-3-methylglutaryl coenzyme A reductase (Hmger)	tgtgcccaggagtttggtgactga	taagattcaacaactctgctgacc	101 bp
Fatty acid synthase (Fasn)	tctgtgaagagtcagtgaggca	cagcagcctgtgtattgagctag	212 bp
Carnitine palmitoyltransferase 1A, liver (Cpt1L)	actgttaagtgtcaaggaggag	gctatattcgttcacacatgc	173 bp
Cytochrome P450, family 7, subfamily a, polypeptide 1 (Cyp7a1)	actgtgcttctgcttgccttg	tggatacattcagtcaggagtc	168 bp
Peroxisome proliferator-activated receptor- α (Ppar α)	tctacgcttggggatgaaga	tgaatcctattagcctccgatcac	126 bp

PCR, polymerase chain reaction.

Western blots

Four mice from each group were randomly selected for western blot assays, which were carried out on individual liver samples. The liver tissues were homogenized in RIPA buffer containing a mixture of protease inhibitors (Complete Mini EDTA-free, Roche Applied Science, Basel, Switzerland). Denatured proteins (30 μg) were loaded onto polyacrylamide gels. After electrophoresis, proteins were transferred to nitrocellulose membranes (Hybond ECL, Amersham, Buckinghamshire, United Kingdom) and stained with Ponceau S to confirm equal protein loading. Membranes were then blocked with phosphate buffered saline containing 0.1% Tween and 5% skimmed milk powder for 1 h at room temperature and later incubated overnight at 4°C with a rabbit antibody against 3-hydroxy-3-methylglutaryl coenzyme A reductase (Cell Signaling, Beverly, MA, USA) or antibody against fatty acid synthase (BD Biosciences, Franklin Lakes, NJ, USA). Subsequently, membranes were incubated with a horseradish peroxidase-linked goat anti-rabbit immunoglobulin G antibody (Cell Signaling) for 1 h at room temperature. After application of chemiluminescent detection reagents (Amersham), Clear-Blue X-ray films (Pierce, Rockford, IL, USA) were exposed to the blots and the band intensities were determined using Quantity One software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

All results are expressed as means $\pm$ SDs. Statistical significance of differences was determined by using Student's *t* test. SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The significance level was set at $P < 0.05$.

Results

Food intake

At week 3, food intake was significantly reduced in the high-fiber-fed mice (2.76 ± 0.06 g/d) compared with their controls (2.92 ± 0.08 g/d; Table 2), along with a significantly lower energy intake (38.1 ± 0.9 versus 44.3 ± 0.9

Table 2

Food intake, energy intake, and body weight*

	PE-3wk mice	C-3wk mice	PE-10wk mice	C-10wk mice
Food intake (g)	2.76 ± 0.06 [†]	2.92 ± 0.08	3.24 ± 0.14 [†]	3.01 ± 0.04
Energy intake (kJ)	38.1 ± 0.9 [†]	44.3 ± 0.9	44.8 ± 2.0	45.7 ± 0.6
Final body weight (g)	17.5 ± 1.3	18.4 ± 0.7	19.6 ± 0.5	19.8 ± 0.3
Gain in body weight (g)	0.5 ± 0.4	0.6 ± 0.4	2.8 ± 1.3	2.3 ± 1.2

C-3wk, 3-wk control diet; C-10wk, 10-wk control diet; PE-3wk, 3-wk high-fiber diet containing psyllium husk; PE-10wk, 10-wk high-fiber diet containing psyllium husk.

* Values are means ± SDs ($n = 6$ /group).

[†] $P < 0.01$ versus corresponding C group.

kJ/d). At week 10, we observed a significant increase in food intake but not in energy intake in the high-fiber fed mice compared with the controls.

Mouse body weights

At baseline, there were no significant differences in body weights between PE-3wk and C-3wk mice (17.0 ± 0.9 versus 17.8 ± 0.5 g, respectively) and between PE-10wk and C-10wk mice (16.9 ± 1.6 versus 17.5 ± 1.4 g, respectively). Fiber feeding did not seem to affect the body weights because there were no significant differences in the final body weight between the PE groups and their respective controls at weeks 3 and 10 (Table 2). Although PE-3wk mice gained slightly less weight from their initial body weights (0.5 ± 0.4 g) compared with the C-3wk mice (0.6 ± 0.4 g), this was not significantly different. At week 10, there was also no significant difference in weight gained between PE-10wk and C-10wk mice (2.8 ± 1.3 versus 2.3 ± 1.2 g, respectively).

Plasma lipid levels

At the start of the feeding study, there were no differences in plasma total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerol levels in the PE groups versus their respective C groups (Fig. 1A). Total cholesterol levels were significantly lower in the PE-3wk group compared with the C-3wk group. The levels were reduced by 27%, from 2.02 ± 0.08 mmol/L (C-3wk mice) to 1.49 ± 0.18 mmol/L (PE-3wk mice). At week 10, total cholesterol levels remained significantly lower in the PE-fed mice compared with their controls (1.39 ± 0.06 versus 2.14 ± 0.22 mmol/L, respectively). There was no significant reduction in total cholesterol levels between the PE-fed mice at weeks 3 and 10. There were no significant changes in LDL cholesterol levels, although there was a trend of reduction with psyllium feeding (Fig. 1B). Significant reductions in HDL cholesterol were observed in the PE-fed groups compared with their controls (Fig. 1C). Because the benefit of lowering total cholesterol levels (which confer lower cardiovascular risks) are usually accompanied by a reduction in protective HDL chole-

sterol levels, we calculated the total/HDL cholesterol ratio to determine the net effect (Fig. 1D). There was a marginally lower ratio (not significant) in the PE-3wk mice compared with the C-3wk mice. In contrast, the ratio appeared to have increased in the PE-10wk mice versus C-10wk mice, although not significantly. There was no significant difference in the ratio between the two PE groups. We observed a slight and non-significant reduction in plasma triacylglycerol levels in PE-3wk compared with C-3wk mice (Fig. 1E). At week 10, the levels were significantly reduced by 32% in the PE groups compared with the controls. There was further significant reduction of plasma triacylglycerol levels in the PE-10wk mice versus the PE-3wk mice as the high-fiber feeding continued.

Transcription profiling of hepatic genes involved in fatty acid oxidation and lipogenesis

Under in vivo conditions, changes in differential gene expression due to dietary treatment are expected to be small and often below a two-fold change [30,31]. As such, any gene expression changes from the microarray screening greater than a threshold of signal-log (base 2) ratio ≥ 0.5 (i.e., 1.4-fold difference in levels) were considered to be potentially relevant.

Gene expression profiles at week 3

Genes involved in β -oxidation of fatty acids, such as Acox1, Acaa2, Acsl1, Acsl4, Crat, Cpt1a, Ehhadh, Hadhb, and Pte2b, were upregulated in the PE-3wk mice (Fig. 2). Cpt1a, the gene encoding the rate-limiting enzyme for fatty acid oxidation, was upregulated by 1.6-fold. Concomitant with the upregulation of genes involved in fat breakdown, genes encoding enzymes involved in lipid biosynthesis were downregulated. These included Srebf1, Bucs1, Dgat2, and Fasn. Srebf1, which was highly suppressed by 3.7-fold, is a membrane-bound transcription factor that enhances transcription of genes required for fatty acid synthesis. Fasn, a key lipogenic gene, was highly suppressed by four-fold (Fig. 2).

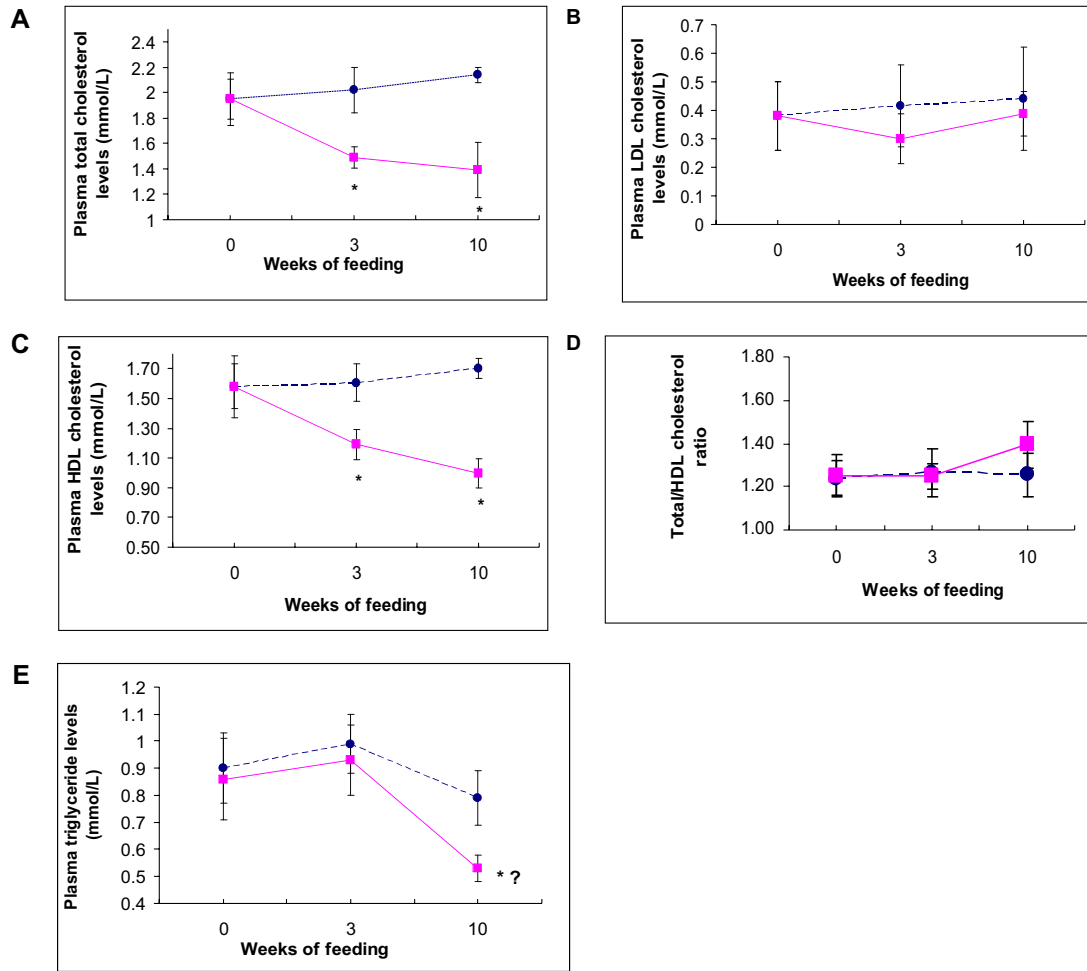


Fig. 1. Comparison of plasma levels of (A) total cholesterol, (B) LDL cholesterol, and (C) HDL cholesterol, (D) total/HDL cholesterol ratio, and (E) triacylglycerol levels between psyllium-treated (squares/solid lines) and control (circles/dotted lines) mice. Values are means $\pm$ SDs ($n = 6$ /group); * $P < 0.05$ and $^{\phi}P < 0.01$ versus corresponding control group. Data at week 0 (i.e., start of the feeding period) were calculated from data from all mice in the four groups. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Gene expression profiles at week 10

Genes involved in fatty acid β -oxidation were no longer upregulated in week 10. In fact some were actually downregulated (e.g., *Cpt1a*, *Cpt2*, and *Dci*; Fig. 2). *Ppar α* , which controls the peroxisomal β -oxidation pathway of fatty acids, was downregulated by 2.3-fold. Some of the downregulated genes involved in fatty acid biosynthesis (e.g., *Fasn* and *Acly*) at week 3 were found to be upregulated after 10 wk of the high-fiber diet. *Fasn* was upregulated by 1.7-fold. Other genes such as *Scd1*, *Ptgs1*, and *Alox5ap* that were involved in fatty acid biosynthesis were also upregulated.

Transcription profiling of hepatic genes involved in cholesterol metabolism

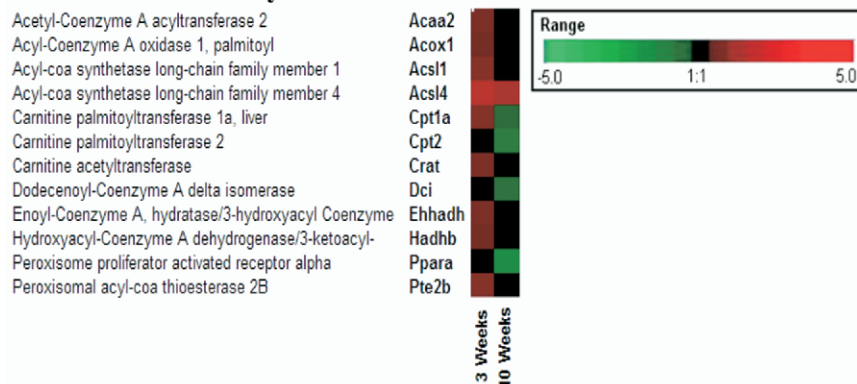
After 3 wk of high-fiber feeding, three genes involved in the cholesterol synthesis pathway (*Hmgcs1*, *Hsd17b7*, and

Cyp51) were upregulated by 1.6-, 1.5-, and 1.9-fold, respectively (Fig. 2). The mice fed on 10 wk of the high-fiber diet continued to show an upregulation for these three genes, with a greater increase in *Hmgcs1* (2-fold) and *Cyp51* (2.2-fold) compared with their controls. In addition, more genes involved in the cholesterol biosynthesis pathway, such as *Nsdh1*, *Hmgcr*, *Idil*, *Sc4mol*, *Stard4*, and *Dhcr7*, were upregulated in the PE-10wk group (Fig. 2). *Hmgcr*, which encodes the rate-limiting enzyme for cholesterol synthesis, was upregulated by two-fold at week 10. None of the genes involved in the cholesterol synthesis pathway were downregulated at both time points.

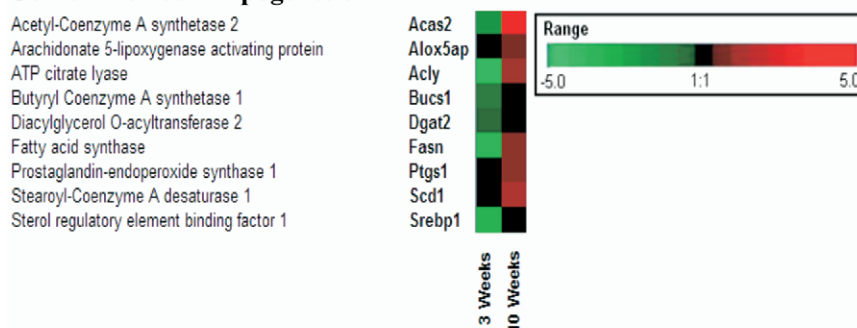
Transcription profiling of hepatic genes involved in bile acid synthesis

The gene encoding the rate-limiting enzyme involved in bile acid synthesis, *Cyp7a1*, was highly upregulated by 3.3-fold in the PE-3wk mice (Fig. 2). However, *Cyp7a1*

Genes involved in fatty acid oxidation



Genes involved in lipogenesis



Genes involved in cholesterol metabolism and bile acid synthesis

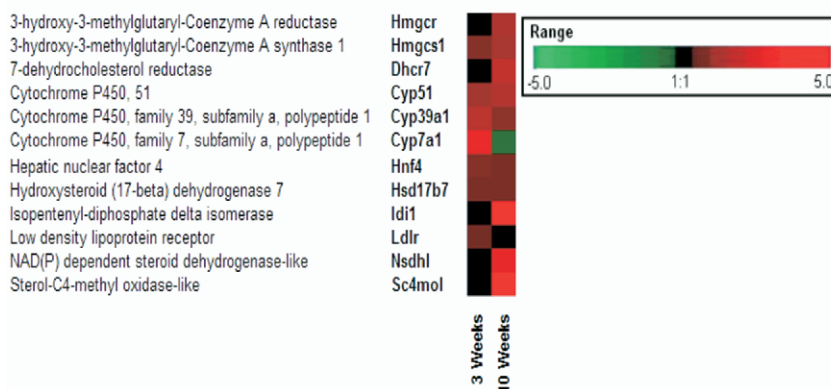


Fig. 2. Microarray profiling of hepatic genes involved in lipid metabolism regulated by high-fiber feeding (green = downregulation, red = upregulation). Data of “fold change” were calculated from the “signal log ratio.” The scale is presented as fold change.

expression was downregulated by 1.4-fold after 10 wk of high-fiber feeding enriched with psyllium husks compared with the control group. HNF4 α , a key hepatic transcription factor that can regulate Cyp7a1, showed increased expression in PE-3wk and PE-10wk mice by 1.6- and 1.5-fold, respectively. Cyp39a1 is also involved in the conversion of cholesterol to bile acids. Its substrates include oxysterols such as 25-hydroxycholesterol, 27-hydroxycholesterol, and 24-hydroxycholesterol. It has been shown that Cyp39a1 has a preference for the substrate 24-hydroxycholesterol. Its expression was elevated

in the livers of PE-3wk and PE-10wk mice by 2.3- and 1.7-fold, respectively.

Quantitative real-time RT-PCR

We validated our microarray findings by conducting real-time PCR assays on selected genes, such as Cpt1, Fasn, Hmgcr, Cyp7a1, and Ppara. These were selected because they encode enzymes regulating the key steps of lipid metabolism. For instance, Cpt1a is the gene encoding the rate-limiting enzyme for mitochondrial fatty acid oxidation.

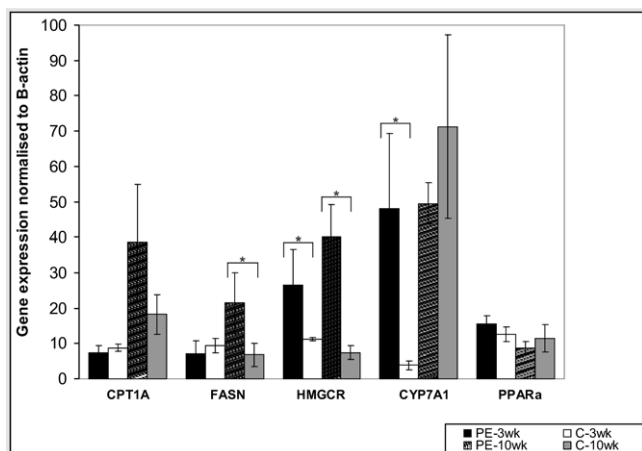


Fig. 3. Gene expression levels measured by quantitative real-time reverse-transcription polymerase chain reaction. Results are presented as means $\pm$ SD ($n = 6$ /group). * $P < 0.05$ versus corresponding C group. C-3wk, 3-wk control diet; C-10wk, 10-wk control diet; PE-3wk, 3-wk high-fiber diet containing psyllium husk; PE-10wk, 10-wk high-fiber diet containing psyllium husk.

Fasn encodes a key lipogenic enzyme, and Hmgcr encodes the rate-limiting enzyme for cholesterol synthesis. Cyp7a1 encodes the enzyme regulating the rate-limiting step in the neutral pathway of bile acid synthesis.

Figure 3 presents the gene expression levels of Cpt1, Fasn, Hmgcr, Cyp7a1, and Ppar α measured by quantitative RT-PCR. There was a slight but non-significant reduction in the expression of Cpt1a in the PE-3wk mice but a non-significant increase was observed at week 10. PE-3wk mice had a lower expression of Fasn but this trend was reversed and was statistically significant from the controls at week 10. There were no significant differences in Ppar α expression levels between the PE mice and their corresponding controls at weeks 3 and 10.

Both PE groups had significantly higher expression of Hmgcr than their respective C groups ($P < 0.05$). The PE-3wk mice had significantly higher mRNA levels of Cyp7A1 than the C-3wk mice. At week 10, expression levels of Cyp7A1 were lower in PE-10wk than in C-10wk mice (not significant).

Western blot

We verified the differential expression of Hmgcr and Fasn at the protein levels by western blot analysis. We demonstrated that the PE-10wk group had significantly higher protein expression levels of Hmgcr compared with the C-10wk group (Fig. 4). There was no observable difference in protein expression in the PE-3wk group versus the C-3 wk group (data not shown). We also found that the PE-3wk group had a significantly lower expression of Fasn protein levels than the C-3wk group. At week 10, we observed increased Fasn protein levels in the PE mice (Fig. 5).

Discussion

Our findings confirmed the beneficial effects of consuming a high-fiber diet containing psyllium husks in lowering cholesterol levels. Within 3 wk of starting the high-fiber diet, total cholesterol levels were significantly reduced by 27% compared with the control mice. Total cholesterol levels remained suppressed with prolonged high-fiber feeding. However, there was no further significant reduction as the feeding period continued. We observed a significant reduction in HDL cholesterol but not in LDL cholesterol levels in the high-fiber groups. This could be due to the fact that the major fraction of plasma cholesterol in mice is HDL and not LDL. This could be because mice do not have plasma cholesterol ester transfer protein activity. There are two major suggested mechanisms through which soluble fiber can reduce cholesterol. These are through the inhibition of hepatic cholesterol synthesis or through higher rate of clearance with increased bile acid synthesis. With regard to cholesterol synthesis, we found that none of the genes involved in hepatic cholesterol biosynthesis were down-regulated after high-fiber feeding at both time points. Instead, they were found to be upregulated at week 3. Additional genes were upregulated at week 10. Some even showed further increases in their gene expression levels. These gene expression levels were further verified by western blot assay of Hmgcr. Hmgcr encodes the rate-limiting enzyme for cholesterol synthesis. Its protein levels were significantly higher in the PE-wk10 mice compared with the controls. This might be a response by the mice to adapt to the reduced plasma cholesterol levels. Miettinen and Tarpila [32] suggested that cholesterol synthesis could increase to offset fecal sterol losses when hyperlipidemic subjects were fed soluble viscous fiber. Our observations of the hepatic genes involved in cholesterol synthesis at the mRNA level provided further molecular evidence for their work.

In contrast, our transcription profiling of the livers showed molecular evidence supporting the concept that there was increased bile acid synthesis in mice fed with high fiber enriched with psyllium husks, as evidenced by the changes in genes such as Cyp7A1, Cyp39A1, and Ldlr. Cyp7A1 governs the rate-limiting step in the neutral pathway of bile acid synthesis from cholesterol. In the liver, cholesterol is converted to bile acids. This removes it from the active cholesterol pool and results in an upregulation of hepatic LDL receptors (Ldlr) and, hence, decreased plasma cholesterol levels. Cyp7A1 was highly upregulated in the PE-3wk mice. This was confirmed by quantitative RT-PCR performed on individual mRNA samples from the PE-3wk mice. Our observation on the increased expression levels of Cyp7a1 in the PE-3wk mice are in agreement with findings reported by Yang et al. [6]. They found increased Cyp7a1 mRNA levels in rats fed on barley (which is also high in viscous soluble fiber) for 2 wk. However, it was notable that the prolonged high-fiber feeding induced a downward trend in Cyp7a1 mRNA levels. The suppressed Cyp7a1 mRNA levels

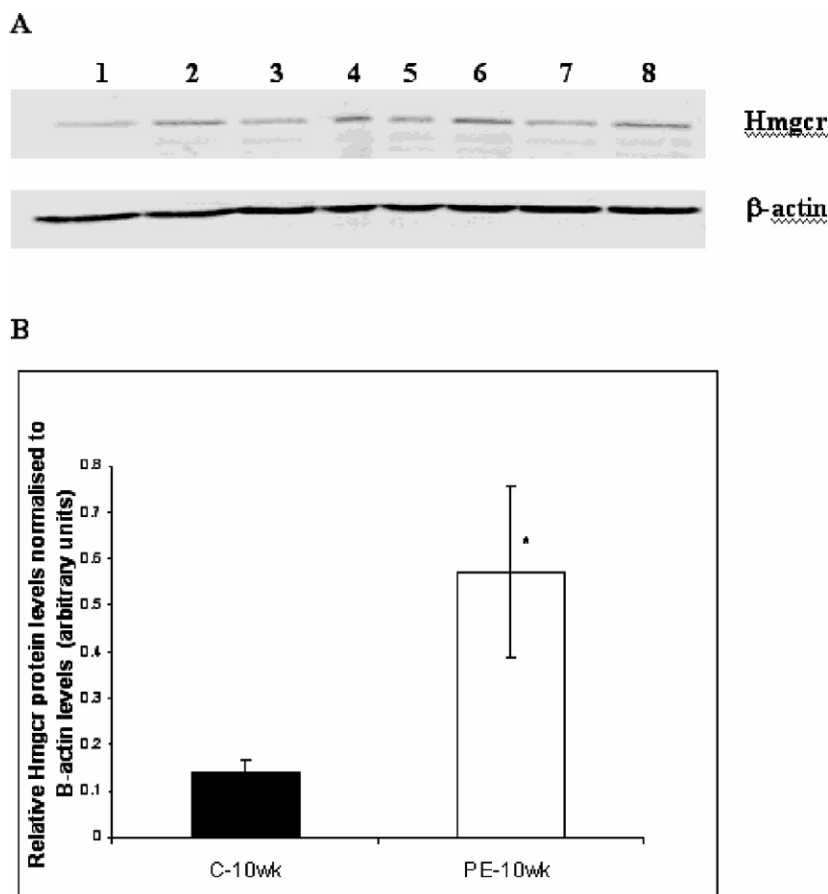


Fig. 4. Western blot analysis of 3-hydroxy-3-methylglutaryl coenzyme A reductase (Hmgcr). (A) Western blot analysis of Hmgcr in the livers from control mice (odd-numbered lanes) and mice fed on psyllium husks for 10 wk (even-numbered lanes). (B) Densitometry analysis of Hmgcr bands normalized to β -actin bands in C-10wk versus PE-10wk mice. Each bar represents mean $\pm$ SD (for each group of mice $n = 4$). * $P < 0.05$ versus control group. C-10wk, 10-wk control diet; PE-10wk, 10-wk high-fiber diet containing psyllium husk.

at week 10 could be due to feedback inhibition by the bile acids returning to the liver through the enterohepatic circulation.

In addition to Cyp7a1, we found that the PE mice had elevated mRNA levels of Cyp39a1. Cyp39a1 also preferentially converts 24-hydroxycholesterol into bile acids. Recently, there were numerous studies that suggested a pivotal role of 24-hydroxycholesterol in the link between increased cholesterol levels and the risk of developing Alzheimer's disease, as reviewed by Bjorkhem et al. [33]. There is evidence to suggest that plasma levels of 24-hydroxycholesterol are higher in patients with Alzheimer's disease than in healthy subjects [33]. Our findings with regard to the increased level of expression of Cyp39a1 could possibly highlight another potential benefit of consuming a high-fiber diet enriched with soluble viscous fiber. Our observations also suggested that the lowering of plasma cholesterol by psyllium husks could likely be exerted at a more upstream level of transcription, i.e., through the upregulation of HNF4 α . HNF4 α is a key hepatic transcription factor that can regulate the expression of Cyp7A1 [34].

Although both groups of PE mice did not show any reduced expression of hepatic genes involved in cholesterol

synthesis, genes related to lipogenesis were downregulated at week 3 in the high-fiber fed mice. Conversely, the genes involved in fatty acid oxidation were upregulated in PE-3wk compared with C-3wk mice. However, these trends reversed at week 10. The transcription profiling of the mice livers revealed that prolonged high-fiber feeding resulted in increased mRNA levels of lipogenic genes and decreased mRNA levels of genes involved in fatty acid oxidation. The western blot assays of Fasn, a key gene involved in lipogenesis, were also in agreement with its gene expression levels. Its protein levels were significantly lower in the livers of the PE-fed mice compared with controls at week 3. However, Fasn protein levels were significantly raised with prolonged high-fiber feeding. These differential mRNA levels of the genes involved in lipogenesis suggested that the mice might have been attempting to adapt to the lower plasma triacylglycerol levels. This could also possibly indicate that non-adipose tissue such as the liver was responding by regulating its triacylglycerol content.

The early responses to a high-fiber diet, aimed at reducing mRNA levels of lipogenic genes and increasing mRNA levels of lipolysis genes, changed after a longer high-fiber

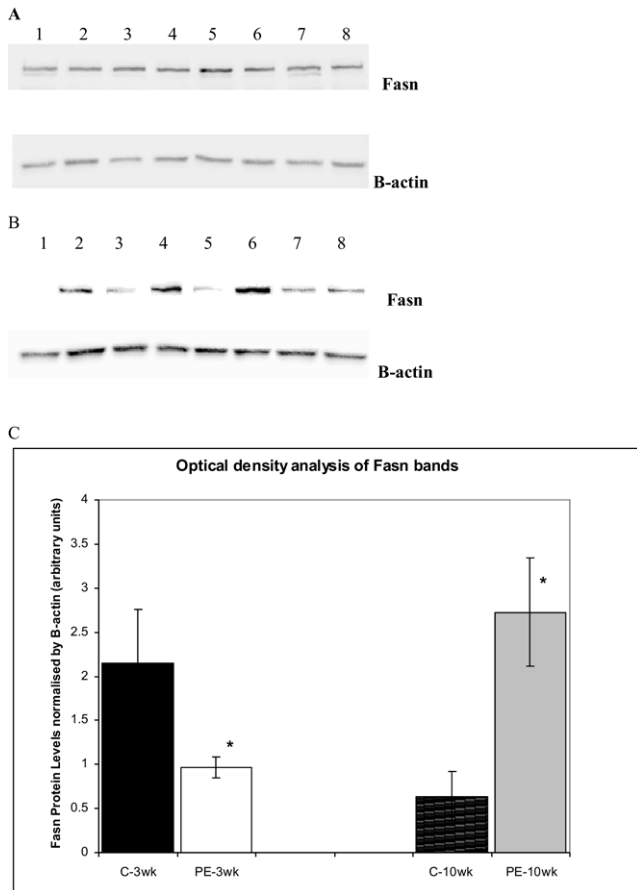


Fig. 5. Western blot analysis of fatty acid synthase (Fasn). (A) Western blot analysis of Fasn in the livers from the CE-3wk group (odd-numbered lanes) and PE-3wk group (even-numbered lanes). (B) Western blot analysis of Fasn in the livers from the C-10wk group (odd-numbered lanes) and PE-10wk group (even-numbered lanes). (C) Densitometric analysis of Fasn bands normalized by β -actin bands in the C versus corresponding PE groups. Each bar represents mean $\pm$ SD (for each group of mice $n = 4$). * $P < 0.05$ versus C group. C-3wk, 3-wk control diet; C-10wk, 10-wk control diet; PE-3wk, 3-wk high-fiber diet containing psyllium husk; PE-10wk, 10-wk high-fiber diet containing psyllium husk.

feeding period. This suggested that different mechanisms may be induced by prolonged high-fiber feeding to adapt to the decreased fat stores in the body. The increased mRNA levels of hepatic genes involved in cholesterol synthesis throughout the entire feeding period could likely point to a robust regulatory mechanism in place to regulate plasma cholesterol levels. In turn this could possibly explain why a more substantive reduction in plasma cholesterol levels was not observed in the PE-10wk mice.

Concerning weight, our findings revealed that a diet high in fiber enriched with psyllium husks did not promote weight loss because the weights of both PE groups were comparable to the C groups. It was notable that, although we observed a reduction in energy intake by the PE-3wk mice intake, they showed no significant weight loss. Because the average energy cost of weight loss is known to be about 30 kJ/g of body weight, the expected average weight

loss in the PE-3wk group should be about 4 g in the mice over the 3 wk of high-fiber feeding. Not only was there an absence of weight loss, but the PE-3wk mice had increased body weights by 0.5 ± 0.4 g. Therefore, our data over the entire fiber feeding period did not seem to support the theory suggested by others [35,36] that consumption of a high-fiber diet containing viscous soluble fiber would promote weight loss.

Conclusion

Weight was unaffected by the high-fiber diet containing psyllium husks. Conversely, plasma cholesterol and triacylglycerol levels were lowered. We observed that the high-fiber diet could differentially regulate the expression levels of hepatic genes involved in lipid metabolism and that these effects were dependent on the length of feeding.

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