Effect of prebiotic fibre supplementation on hepatic gene expression and serum lipids: a dose–response study in JCR:LA-cp rats

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Prebiotic fibres have been proposed to promote weight loss and lower serum cholesterol; however, the mechanisms are not fully understood. The aim of the present research was to identify possible mechanisms through which prebiotic fibres improve serum lipids. Lean and obese JCR:La-cp rats aged 8 weeks consumed one of three diets supplemented with 0, 10 or 20 % prebiotic fibre for 10 weeks. Rats were anaesthetised and a fasting blood sample was taken for lipid analysis. Real-time PCR was used to determine gene expression for cholesterol and fatty acid regulatory genes in liver tissue. Liver and caecal digesta cholesterol and TAG content were quantified. Both doses of prebiotic fibre lowered serum cholesterol levels by 24 % in the obese hyperlipidaemic rats (P<0.05). This change was associated with an increase in caecal digesta as well as an up-regulation of genes involved in cholesterol synthesis and bile production. Additionally, there was a 42 % reduction in TAG accumulation in the liver of the obese rats with 10 % prebiotic diet (P<0.05); however, no change in liver fatty acid synthase (FAS). Prebiotic fibres appear to lower cholesterol levels through increased cholesterol excretion in the form of bile and inhibit the accumulation of TAG in the liver through a mechanism unrelated to FAS. These effects appear to be limited to the obese model and particularly the 10 % dose. The present work is significant as it provides insight into the mechanisms of action for prebiotic fibres on lipid metabolism and furthers the development of dietary treatments for hypercholesterolaemia.

Inulin and oligofructose: Lipid metabolism: Liver TAG: Cholesterol content: Gene expression

Individuals with obesity often display hyperlipidaemia, hypercholesterolaemia and insulin resistance(1). Therapies targeting both excessive body fat stores as well as lipid parameters may be beneficial, as they have the potential to simultaneously treat obesity and its comorbidities.

The effects of a variety of dietary fructans on lipid metabolism have been tested in rodent studies. Improvements in cholesterol, TAG, hepatic steatosis and plaque formation have been demonstrated when either regular chow or high-fat diets are supplemented with prebiotic fibre (2–7). Prebiotic supplementation has also been examined in pigs, where a decrease in plasma total cholesterol (TC), LDL cholesterol and TAG was reported(8). These studies generally support a conclusion that prebiotic fibres have beneficial effects on lipid metabolism in animal models(9). Studies in human subjects, however, tend to be conflicting and it has been suggested that the effectiveness of prebiotics is dependent on lipid status, with positive outcomes more commonly reported in individuals with hyperlipidaemia or hypercholesterolaemia(9).

Prebiotic fibres are considered to be both soluble as well as fermentable; however, they are unique with respect to their solubility as they do not gel in the gut nor modify viscosity(4). These characteristics are notable in that the traditionally proposed mechanism by which soluble fibre acts, namely complexing with dietary fat to form a stable gel-like emulsion that prevents pancreatic lipase from hydrolysing fat(10), does not apply. Consequently, it has been proposed that effects on lipid metabolism are due to inhibition of de novo fatty acid synthesis(11,12). One theory is that prebiotic fibres are able to downregulate hepatic lipogenic enzymes, specifically fatty acid synthase (FAS), through increased production of the SCFA propionate(9).

Prebiotic fibres likely have unique modes of action with respect to cholesterol metabolism specifically related to their fermentability and modulation of the microflora. Previously, SCFA have been implicated in cholesterol metabolism. In rodents, inulin supplementation results in an increase in SCFA in the caecum, as compared to a control diet(2). Concentrations measured in the portal vein suggest that the

Abbreviations: CYPT7A1, cholesterol 7-a-hydroxylase; FAS, fatty acid synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; OC, obese control; OF, obese fibre; OHF, obese high fibre; TC, total cholesterol.

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Liver is exposed to high concentrations of propionic acid. The authors conclude that the availability of propionic acid to the liver depresses cholesterolaemic responses \(^{(2)}\). In vitro, in rat hepatocytes, propionate competitively inhibits acetate thereby attenuating its effects on the cholesterogenic pathway; however, the effects were much less pronounced in human hepatocytes, suggesting the mechanism may be species specific \(^{(8,13)}\). Alterations in gut microflora may also influence cholesterol absorption. Addition of oligofructose and inulin to the diet is associated with increased bifidobacteria and lactobacilli \(^{(14,15)}\). In vitro, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* strains demonstrate enhanced bile acid deconjugation, thereby enhancing bile acid and cholesterol precipitation. The physiological effectiveness of this process is contentious however, as a low pH has been proposed as a requirement for the co-precipitation of cholesterol \(^{(16)}\). A similar mechanism has also been proposed for the hypocholesterolaemic effect of *L. acidophilus* on serum cholesterol in pigs \(^{(17)}\). Generally, acidification of the caecal contents renders bile acids and cholesterol insoluble \(^{(2,18)}\), and, in vitro, oligofructose supplementation has been shown to bring the pH of faecal cultures down to 3.7 \(^{(15)}\). Theoretically, if these effects translated in vivo, less bile would be reabsorbed in the gut, serum concentrations would decrease and less bile acid would reach the liver through enterohepatic circulation. The liver would compensate by increasing bile production from cholesterol further lowering serum cholesterol levels \(^{(17,19)}\). Recent microarray profiling of hepatic genes in rodents fed psyllium fibre supports this theory as the authors report increased expression of several genes involved in cholesterol synthesis \(^{(20)}\).

Given the unique characteristics of prebiotic fibres, a detailed examination of genes regulating hepatic cholesterol and fatty acid metabolism, cholesterol excretion and liver lipid content is warranted to explain their lipid-modulating effects. Furthermore, a dose response to the prebiotic fibres, oligofructose and inulin, has not previously been performed and is examined here.

**Experimental methods**

**Animal model**

Male lean (+/?) and obese (cp/cp) JCR:La-cp rats 8 weeks of age were obtained from the colony of Dr Spencer Proctor (University of Alberta, Edmonton, AB, Canada). The obese cp/cp rat lacks a functional leptin receptor and exhibits hepatic (University of Alberta, Edmonton, AB, Canada). The obese control (*n* = 8 rats). Experimental diets were formulated based on the AIN-93M diet (Dyets, Inc., Bethlehem, PA, USA) with the addition of a 1:1 mix of inulin and oligofructose (the mixture resembles the commercially available Orafti Synergy 1 \(^{(1)}\) and was prepared by mixing equal amounts of Raftiline HP and Raftilose P95 supplied by Quadra Chemicals Ltd, Burlington, ON, Canada) to create diets that were 10 or 20 % prebiotic fibre by weight (Table 1). The standard AIN-93M diet was used as the control. The diets were formulated to match protein, fat and micronutrient content as closely as possible. The prebiotic fibre diets have lower energy contents due to the energy-diluting effect of the prebiotic fibre; however, a change in simple sugars was avoided by adjusting maize starch rather than sucrose to make up the difference. Food intake was recorded daily.

**Dietary intervention**

At 8 weeks of age, the animals were randomly allocated to a control (0 % prebiotic fibre), fibre (10 % prebiotic fibre) or high-fibre (20 % prebiotic fibre) w/w diet for 10 weeks. Groups included: lean control; lean fibre; lean high fibre; OC, obese control; OF, obese fibre; OHF, obese high fibre (n = 8 rats). Experimental diets were formulated based on the AIN-93M diet (Dyets, Inc., Bethlehem, PA, USA) with the addition of a 1:1 mix of inulin and oligofructose (the mixture resembles the commercially available Orafti Synergy 1 \(^{(1)}\) and was prepared by mixing equal amounts of Raftiline HP and Raftilose P95 supplied by Quadra Chemicals Ltd, Burlington, ON, Canada) to create diets that were 10 or 20 % prebiotic fibre by weight (Table 1). The standard AIN-93M diet was used as the control. The diets were formulated to match protein, fat and micronutrient content as closely as possible. The prebiotic fibre diets have lower energy contents due to the energy-diluting effect of the prebiotic fibre; however, a change in simple sugars was avoided by adjusting maize starch rather than sucrose to make up the difference. Food intake was recorded daily.

**Body composition**

Body weight was recorded weekly using an electronic scale. Body composition was measured 1 d before sacrifice by a dual energy X-ray absorptiometry scan in conjunction with the use of Hologic QDR software for small animals (QDR 4500; Hologic, Inc., Bedford, MA, USA).

**Liver cholesterol and TAG**

An electronic scale accurate to three decimal places was used to measure mass. For cholesterol quantification, approximately 10 mg of wet liver tissue was used for the extraction of liver cholesterol and was sonicated in 200 μl of chloroform–Triton X-100 solution. The extract was spun at top speed in a microcentrifuge for 10 min. The organic phase was collected and dried at 50°C followed by a second 30 min vacuum dry. The dried lipids were dissolved in Triton X-100 and 200 μl of cholesterol reaction buffer. For determination of cholesterol reaction buffer. For determination of cholesterol, a colorimetric assay (BioVision Research Products, Mountain View, CA, USA) was used. Briefly, the cholesterol standard was diluted to create a standard curve. Reaction mix was added to the standard and test samples. The reaction was incubated for 1 h at 37°C. Optical densities of the samples were measured at 570 nm. For calculations, the background subtraction was performed.

**Table 1. Composition of the experimental diets**

<table>
<thead>
<tr>
<th></th>
<th>Control* (g/kg)</th>
<th>10% prebiotic fibre (g/kg)</th>
<th>20% prebiotic fibre (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize starch/glucose</td>
<td>46.69</td>
<td>39.45</td>
<td>33.51</td>
</tr>
<tr>
<td>Casein</td>
<td>14.04</td>
<td>13.11</td>
<td>12.03</td>
</tr>
<tr>
<td>Dextrinised maize starch</td>
<td>15.54</td>
<td>14.51</td>
<td>13.32</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.03</td>
<td>9.36</td>
<td>8.59</td>
</tr>
<tr>
<td>Soyabean oil</td>
<td>4.01</td>
<td>3.75</td>
<td>3.44</td>
</tr>
<tr>
<td>AIN-93M-MX</td>
<td>3.51</td>
<td>3.48</td>
<td>3.39</td>
</tr>
<tr>
<td>AIN-93-VX</td>
<td>1.00</td>
<td>0.99</td>
<td>0.96</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.18</td>
<td>0.18</td>
<td>0.17</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.25</td>
<td>0.25</td>
<td>0.24</td>
</tr>
<tr>
<td>Alphacel</td>
<td>5.01</td>
<td>4.97</td>
<td>4.83</td>
</tr>
<tr>
<td>Fibre source†</td>
<td>0.00</td>
<td>9.94</td>
<td>19.53</td>
</tr>
<tr>
<td>Digestible energy</td>
<td>15.90</td>
<td>14.81</td>
<td>13.81</td>
</tr>
</tbody>
</table>

*Based on AIN-93 (Dyets, Inc.) diet for the maintenance of adult rats.
†1:1 Blend of inulin (supplied as Orafline HP by Quadra Chemicals Ltd) and oligofructose (supplied as Oraflilose by Quadra Chemicals Ltd).
was first subtracted and then cholesterol concentration was generated based on the standard curve. For TAG, approximately 25 mg of wet liver tissue were weighed and the TAG extracted with a KOH–EtOH solution. Samples were placed at 70°C for 1 h and then allowed to rest overnight. The volume was brought up to 500 μl with 2x Tris–HCl. Samples were diluted 1:5 with the Tris–HCl. Quantification of the TAG was done colorimetrically with a TAG (glycerol-3-phosphate oxidase) liquid reagent set (Point Scientific, Inc., Lincoln Park, MI, USA). One millilitre of glycerol-3-phosphate oxidase was added to a tube for each standard or sample and heated to 37°C for 5 min. Standard or sample was added to the glycerol-3-phosphate oxidase and heated for another 5 min; 200 μl of each were added to a plate and read at 500 nm. TAG content in mmol/l was determined based on the linear curve (Protocol provided by DH Wasserman’s Lab, Vanderbuilt University School of Medicine, Nashville, TN, USA)\(^{(22)}\).

**Caecal TAG and cholesterol**

Caecal digesta samples were dried overnight in a freeze drier. Extraction of cholesterol and TAG from the caecal samples and calculations were performed as described earlier.

**Serum lipid analysis**

To determine serum lipid profiles, 0·6 ml of blood was collected from each rat into a serum tube. The blood was centrifuged at 1600 g for 15 min at 4°C and the serum was collected for analysis. Samples were sent to Calgary Laboratory Services (Calgary, AB, Canada) for quantification of TC, HDL cholesterol and TAG. LDL cholesterol levels were calculated by subtracting HDL cholesterol and (TAG/5) from TC.

**RNA isolation and real-time PCR**

RNA isolation and real-time PCR procedures were completed as previously described\(^{(23)}\). Briefly, total RNA was extracted with TRIzol reagent and reverse transcription was performed to generate complementary DNA. The resultant complementary DNA was amplified using primers synthesised by University of Calgary Core DNA Services (Calgary, AB, Canada).

Primer sequences are available upon request from the authors.

A DNA iCycler was used for the PCR, and the resulting melt curve showed the melting point of the PCR product of interest. Actin primers were included as an internal control. In both the lean and obese groups, the control diet was used as the control condition. For comparisons between the lean and obese groups, the lean group was used as the control. Fold change from control was calculated using the comparative threshold cycle time method\(^{(24)}\).

**Statistical analysis**

All data are presented as mean (±SEM). Changes in physiology, serum lipids and caecal digesta were determined using two-factor ANOVA (with diet and genetic group (lean v. obese) as variables) with a Holm–Sidak test for multiple comparisons. Changes in gene expression between the diets within the lean and obese groups were analysed using one-way ANOVA with a Bonferroni adjustment for multiple comparisons. Data were analysed using SigmaStat 3.5 (Systat Software, Inc., San Jose, CA, USA) software and STATA 8 (STATA Corporation, College Station, TX, USA). A result was considered significant when \(P \leq 0.05\).

**Results**

**Liver characteristics**

Energy intake was reduced \((P < 0.05)\) with prebiotic supplementation compared to control across all groups except lean fibre group (Table 2). The lower body weight and fat mass observed with increasing doses of fibre, however, were not significantly different \((P > 0.05)\). Caecal weight was dose dependently increased with increasing doses of fibre \((P < 0.05)\). Total liver weight decreased in the OF \((P < 0.01)\) and OHF \((P < 0.01)\) groups compared to OC group (Fig. 1(a)). Liver TAG expressed per gram of tissue was unchanged with prebiotic supplementation. Total TAG, however, which takes into account liver weight and TAG content, showed a significant group by diet interaction effect \((P < 0.05);\) Fig. 1(b)). Separate analysis of the lean and obese groups indicated that the combination of decreased liver mass and decreased TAG/g tissue resulted in a 42% decrease

**Table 2.** Physical characteristics of lean and obese JCR:LA-cp rats fed control (0 %), fibre (10 %) and high-fibre (20 %) prebiotic diets

(Mean values with their standard errors for \(n = 8\) except obese high fibre (OHF) group \(n = 7\))

<table>
<thead>
<tr>
<th></th>
<th>LC</th>
<th>LF</th>
<th>LHF</th>
<th>OC</th>
<th>OF</th>
<th>OHF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>Energy intake (MJ)</td>
<td>16·39±0·2</td>
<td>15·4±0·2</td>
<td>14·7±0·3</td>
<td>27·0±0·6</td>
<td>25·4±0·5</td>
<td>24·9±0·6</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>345·6±5·0</td>
<td>340·6±8·4</td>
<td>334·4±3·7</td>
<td>567·2±9·5</td>
<td>563·2±8·5</td>
<td>561·5±10·9</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>7·6±0·2</td>
<td>5·9±1·0</td>
<td>5·6±0·6</td>
<td>55·1±1·1</td>
<td>54·3±1·5</td>
<td>52·6±1·2</td>
</tr>
<tr>
<td>Caecal TAG (mg/g)</td>
<td>2·7±0·4</td>
<td>2·6±0·5</td>
<td>2·6±0·5</td>
<td>2·7±0·5</td>
<td>2·6±0·4</td>
<td>2·7±0·9</td>
</tr>
<tr>
<td>Caecal cholesterol (mg/g)</td>
<td>13·0±2·7</td>
<td>15·1±2·7</td>
<td>14·7±2·7</td>
<td>19·0±2·7</td>
<td>26·7±2·7</td>
<td>14·9±2·7</td>
</tr>
</tbody>
</table>

\(^{†}\) Mean values were significantly different between the 10 and 20 % fibre diets within lean and obese groups as determined by a two-way ANOVA with a Holm–Sidak adjustment for multiple comparisons \((P < 0.05)\).
Figure 1. Liver characteristics for the lean and obese rats fed control (0 %), fibre (10 %) and high-fibre (20 %) prebiotic diets. Panel (a) depicts total liver weight. Panel (b) depicts liver TAG content. The series on the left shows TAG content per g of wet liver tissue and the series on the right shows total TAG content of the liver. Total TAG content was calculated by multiplying TAG/g of tissue by total liver weight. For ease of presentation, total liver TAG content was reduced by a factor of 10. Actual values from left to right are 1393.7, 1276.7, 4626.5, 2701.2 and 3719.2 mg. Panel (c) depicts liver cholesterol content. The series on the left shows cholesterol content per gram of wet liver tissue and the series on the right shows total cholesterol (TC) content of the liver. TC content was calculated by multiplying cholesterol per gram of tissue by total liver weight. Data are mean (SE); n 8 except obese high fibre (OHF) group n 7. * Mean values were significantly different from control diet within lean or obese groups. † Mean values were significantly different between the 10 % and 20 % fibre diets within lean or obese groups as determined by ANOVA with Holm–Sidak adjustment for multiple comparisons or a one-way ANOVA with Bonferroni adjustment where there was a significant diet and group interaction (P<0.05). LC (■), lean control; LF (▲), lean fibre; LHF (□), lean high fibre; OC (■), obese control; OF (▲), obese fibre; OHF (□), obese high fibre.

Serum lipids

There was a significant group by diet interaction for serum TC (Fig. 2). When the groups were analysed separately, both doses of prebiotic fibre resulted in a 24 % decrease in TC (P=0.05 and 0.08 for OF and OHF groups, respectively). LDL cholesterol was significantly lower in OHF group compared to OC group (P<0.05). There was no effect on TAG in lean or obese animals, and HDL cholesterol was reduced in both the OF (P<0.01) and OHF (P=0.02) groups compared to OC group. There was a significant group by diet interaction for the ratio of total:HDL cholesterol; however, analysis in the individual groups did not indicate a diet effect.

Gene expression

With respect to fatty acid metabolism, there was a 3.5-fold up-regulation of FAS in the lean fibre and lean high fibre groups compared to lean control group (Fig. 3). This coincided with an up-regulation of sterol regulatory element-binding protein 1c, a known transcriptional regulator of FAS. Furthermore, acetyl-CoA carboxylase-α mRNA was increased approximately twofold in both the lean and obese groups on the 20 % fibre diet and in the 10 % obese group (Fig. 3). With respect to cholesterol metabolism, a fourfold increase in 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase was noted in the lean group with the 10 % and 20 % fibre doses compared to control (Fig. 4). The regulatory enzyme sterol regulatory element-binding proteins 2 also matched this up-regulation (P<0.05). A similar but non-significant pattern for HMG-CoA was seen in the OHF group. Conversely, lecithin:cholesterol acyltransferase was down regulated 19 % in the lean high fibre group compared to lean control group, while cholesterol 7-α-hydroxylase (CYP7A1), an enzyme involved in bile synthesis, was increased twofold with fibre supplementation in both the lean and obese 20 %
Prebiotic fibres alter lipid metabolism

Discussion

The goal of the present study was to determine the dose–response effect of prebiotic fibre supplementation on lipid metabolism. The major finding is that prebiotic fibres reduce serum cholesterol levels through lower cholesterol absorption and modulation of hepatic gene expression. Secondary findings suggest that prebiotic fibres are able to reduce liver TAG accumulation.

The present study provides evidence that prebiotic fibre supplementation lowers total serum cholesterol in a hypercholesterolaemic rat model. These results are in support of several other reports of lower cholesterol levels with prebiotic fibre intake. The present results suggest that the reduction in serum cholesterol is primarily due to a sequence of events consisting of (1) decreased cholesterol absorption from the gut; (2) reduced hepatic exposure to bile salts; (3) enhanced cholesterol production by the liver and/or cholesterol uptake from the plasma; (4) increased bile production; (5) greater cholesterol excretion from the body in the form of bile. Direct measurement of total dried caecal contents was not possible; however, we do report a significant increase in caecal size, including tissue and wet contents, and others report an increase of 330% in caecal digesta with prebiotic fibre, which would ultimately increase the amount of cholesterol excreted from the body.

This theory is further strengthened by our gene expression data. Firstly, an increase in the expression of HMG-CoA reductase, an enzyme that regulates the conversion of HMG-CoA to mevalonate, the rate-limiting step in cholesterol synthesis, was increased with prebiotic fibre. Furthermore, the sterol regulatory element-binding proteins, which are considered to be regulatory factors for HMG-CoA reductase, were upregulated. A possible explanation for this outcome is that genes involved in cholesterol synthesis are upregulated by reductions in bile absorption due to the prebiotic fibre. Support for bile salts as a regulatory product for HMG-CoA reductase has previously been demonstrated. Here, chronic biliary diversion stimulated HMG-CoA reductase, whereas taurocholate, a hydrophobic bile salt, inhibited HMG-CoA reductase suggesting bile salts can regulate HMG-CoA reductase through negative feedback.

If these observations are extrapolated to this model, reduced uptake of bile from the gut would reduce hepatic exposure to bile salts thereby stimulating gene expression and consequently cholesterol production. Although increased cholesterol production would seem to contradict lower serum levels, it
appears to coincide with increased cholesterol excretion in the form of bile.

Cholesterol can be incorporated into bile salts by CYPT7A1, the initial and rate-determining enzyme for bile synthesis\(^{(29)}\). Newly synthesised cholesterol has been shown to be the preferred substrate for CYPT7A1 in rats\(^{(30)}\). Conversely, a study in hamsters reported increased incorporation of preformed cholesterol into bile acids under physiological conditions\(^{(31)}\). It is possible that the conflicting results are due to differences in lipid metabolism between the two species\(^{(32)}\). We did not test for increases in hepatic LDL receptor mRNA; however, increases we observed in sterol regulatory element-binding protein would likely predict increased cholesterol uptake from the plasma given their involvement in the activation of the LDL receptor gene\(^{(27,33)}\). Although the proportion of newly synthesised \(\nu\)-preformed cholesterol in bile salts remains unclear, both are incorporated in some manner\(^{(30,31)}\). As increased CYPT7A1 mRNA was noted in the present study, it is reasonable to assume that increased amounts of \(\textit{de novo}\) and preformed cholesterol are being incorporated into bile and subsequently excreted by the prebiotic fibre groups.

Further support for this model comes from the regulation of CYPT7A1. If the assumption of decreased bile absorption from the gut with prebiotic fibre intake is correct, reduced liver exposure to bile salts would occur. This in turn would affect bile production, as bile salts are reported to regulate CYPT7A1 through a negative feedback mechanism. Indeed, CYPT7A1 activity, mRNA and transcription rates have been reported to decrease with exposure to bile acids\(^{(34,35)}\). Specifically, high levels of bile salts reaching the liver through the portal vein suppress CYPT7A1 and consequently bile production, whereas biliary diversion and binding resins stimulate CYPT7A1\(^{(28,32)}\). Here, increased CYPT7A1 is reported suggesting increased bile production, an effect likely stimulated by reduced bile absorption initially. In summary, liver and caecal analyses support a chain of events whereby prebiotic fibres inhibit bile absorption, thereby leading to alterations in liver metabolism, of which the end product is increased cholesterol secretion in the form of bile.

A variety of other fibres have been proposed to exert their hypcholesterolaemic effects through inhibition of bile absorption\(^{(32)}\). Increased cholesterol metabolism and secretion in the form of bile acids have been noted with pectin and psyllium fibres. Matheson et al.\(^{(35)}\) report increased CYPT7A1 mRNA with both pectin and psyllium feeding and note that the magnitude correlates with the increased bile acid pool sizes reported in their previous experiments\(^{(36)}\). Psyllium has also been shown to increase CYPT7A1 mRNA and faecal bile acids in rats\(^{(37)}\). Finally, analysis of cholesterol metabolism in psyllium fibre-fed mice showed an up-regulation of HMG-CoA reductase and CYPT7A1\(^{(20)}\).

Prebiotic fibres likely inhibit bile salt absorption differently than other soluble type fibres due to their lack of viscosity. Possible mechanisms include enhanced bile acid deconjugation\(^{(40)}\), cholesterol assimilation into the bacterial cells\(^{(38,40)}\), and acidification of the caecal contents\(^{(2,18)}\).

Lecithin:cholesterol acyltransferase has a role in the esterification of free cholesterol, thus generating a cholesteryl ester, which can be transferred to the HDL particle resulting in an increase in HDL particle size\(^{(41,42)}\). A decrease in hepatic
The present research is significant as it helps to unravel the mechanism of action for prebiotic fibres. Several studies indicate that prebiotic supplementation may be beneficial for human subjects with dyslipidaemia; however, the mechanisms remain unclear and the results are inconsistent. A further understanding of the mechanisms of action and dose-dependent responses is crucial to optimise the benefits of prebiotic fibres. The present animal study provides insight into possible mechanisms of action in human subjects and suggests that different actions and outcomes may result depending on body weight status and underlying disease state. Additionally, it is the first experiment to assess the effects of increasing amounts of these fibres to determine optimal dosages. Future research should focus on the unique mechanisms of prebiotic fibre including microbial modifications. If it can be identified that prebiotic fibres function differently than other types of soluble fibres, the effects of fibre combinations should be explored.

Acknowledgements

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