Addition of inulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentrations in humans\(^1\)–\(^3\)

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**ABSTRACT**

**Background:** A high-carbohydrate, low-fat diet is recommended for the prevention of atherosclerosis, because it reduces plasma cholesterol concentrations. However, such a diet can increase plasma triacylglycerol concentrations—an undesirable side effect. The addition of nondigestible carbohydrate could reduce the risk of elevated triacylglycerol concentrations.

**Objective:** The objective was to determine whether the addition of a moderate dose of inulin to a moderately high-carbohydrate diet would decrease hepatic lipogenesis and plasma triacylglycerol concentrations and have a cholesterol-lowering action.

**Design:** Eight healthy subjects were studied twice in a double-blind, randomized, placebo-controlled crossover study after consuming for 3 wk a moderately high-carbohydrate, low-fat diet (55% of total energy) plus an oral placebo or 10 g high-performance inulin/d. Hepatic lipogenesis and cholesterol synthesis (deuterated water method), plasma lipid concentrations, fatty acid synthase, acetyl-CoA carboxylase 1, and sterol responsive element binding protein 1c messenger RNA concentrations were measured in adipose tissue at the end of the 2 diet periods.

**Results:** Plasma triacylglycerol concentrations and hepatic lipogenesis were lower after inulin than after placebo ingestion (\(P < 0.05\)), but cholesterol synthesis and plasma cholesterol concentrations were not significantly different between the 2 groups. None of the adipose tissue messenger RNA concentrations changed significantly after inulin ingestion.

**Conclusions:** The addition of high-performance inulin to a moderately high-carbohydrate, low-fat diet has a beneficial effect on plasma lipids by decreasing hepatic lipogenesis and plasma triacylglycerol concentrations. These results support the use of nondigestible carbohydrate for reducing risk factors for atherosclerosis. *Am J Clin Nutr* 2003;77:559–64.

**KEY WORDS** Inulin, stable isotopes, cholesterol, triacylglycerol, atherosclerosis, lipogenesis, adipose tissue, messenger RNA, fatty acid synthase, sterol responsive element binding protein 1c, SREBP-1c, acetyl-CoA carboxylase

**INTRODUCTION**

Atherosclerosis is one of the most common causes of death in industrialized societies. A reduction in risk factors for atherosclerosis, such as elevated concentrations of plasma total cholesterol and plasma LDL cholesterol (1), therefore, is a major goal in public health. Clinical trials concerning the prevention of coronary artery disease (CAD), especially with the use of inhibitors of \(\beta\)-hydroxy-\(\beta\)-methylglutaryl-CoA reductase (EC 1.1.1.88) activity, clearly showed the beneficial effect of the reduction of cholesterol concentrations (2). Such a reduction can be obtained, at least in part, through dietary advice, a relatively inexpensive approach that can be used for large cohorts of subjects (3). This role of diet is evidenced by the differences in mean cholesterol concentrations and in the incidence of CAD between populations with different dietary habits (4). A main dietary recommendation is to decrease the total intake of fat, especially of saturated fatty acids, and therefore to increase the intake of carbohydrate. A drawback is that low-fat, high-carbohydrate diets can result in an increase in plasma triacylglycerol concentrations (5), an undesirable side effect, because elevated triacylglycerol concentrations are an independent risk factor for CAD (6). However this increase in plasma triacylglycerol was observed with diets very high in carbohydrate (65–70% of energy intake) and, with some exceptions (7), with diets containing mainly simple carbohydrates such as glucose or fructose. These diets were thus different from the usual recommended diet. We compared previously the short-term effects of a moderately high-carbohydrate diet (55% of energy as carbohydrate, 30% of total energy intake as fat), which included mainly complex carbohydrates and met the usual dietary recommendations, with those of a high-fat diet (40% of energy as carbohydrate, 45% of total energy intake as fat) on cholesterol metabolism and plasma triacylglycerol concentrations (8). We observed that this moderately high-carbohydrate diet was effective in reducing plasma cholesterol concentration and had no adverse effect on plasma triacylglycerol concentrations.

A complementary approach for reducing the risk of elevated triacylglycerol concentrations during consumption of a high-carbohydrate diet, or to obtain—in addition to the cholesterol-lowering effect of this diet—a decrease in plasma triacylglycerol, is to include a nondigestible carbohydrate such as inulin or one of its fractions in the high-carbohydrate diet (9). Indeed, such nondigestible oligosaccharides can lower plasma triacylglycerol and

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cholesterol concentrations (10). Such effects were observed in experimental models (11–15), normolipidemic volunteers (16, 17), and hyperlipidemic subjects (18, 19). No-effect studies in humans have been reported (20, 21). The decrease in plasma triacylglycerol has been ascribed in animals to a reduction in VLDL-triaclyglycerol secretion and to the inhibition of hepatic lipogenesis (11, 22) through a reduction of the activities of lipogenic enzymes and of the messenger RNA (mRNA) concentration of fatty acid synthase (FAS; EC 2.3.1.85) (10, 11, 22). In addition, reesterification of fatty acids by the liver is decreased (15). This inhibition of hepatic lipid synthesis could be due to the colonic production of short-chain fatty acids from the nondigestible oligosaccharides and, more particularly, of propionate (14), which has been shown to inhibit hepatic lipogenesis (23).

On the basis of these previous studies, we tested in humans the effects on lipid metabolism of a preparation of inulin. This preparation, high-performance inulin (HP-inulin), is obtained by physical fractionation of inulin extracted from chicory and has an average degree of polymerization of 25 (range: 12–65). More specifically, our aim was to determine whether the addition of HP-inulin to a moderately high-carbohydrate diet would decrease hepatic lipogenesis and triacylglycerol concentrations in healthy subjects and whether it could have beneficial effects on cholesterol metabolism. In addition, we tested whether the dietary intake of HP-inulin could decrease the expression, as determined on the basis of mRNA concentrations, of lipogenic genes in adipose tissue.

SUBJECTS AND METHODS

Materials

Deuterated water (99% mole percent excess) was from Cambridge Isotope Laboratory (Andover, MA). Chemicals and reagents were from Sigma Chemical Co (St Louis), Boehringer (Mannheim, Germany), or Pierce Chemical Co (Rockford, IL). HP-inulin was obtained from Orafti (Raftiline HP; Tienen, Belgium). The placebo was maltodextrin (Avebe, Amsterdam).

Subjects

Written informed consent was obtained from 8 healthy volunteers after they received a full explanation of the nature, purpose, and possible risks of the study. This group consisted of 4 women and 4 men aged 23–32 y with a body mass index (in kg/m²) of 19–25. No subject had a personal or familial history of diabetes or obesity or was taking any medication. For all subjects, the results of a physical examination were normal as were plasma glucose and lipid concentrations. Subjects with unusual dietary habits were excluded. The protocol was approved by INSERM and the Ethical Committee of Lyon (France), and the study was conducted according to the Hurriet law.

Protocol

All tests were performed in the Human Nutrition Research Center in Lyon. All subjects were studied twice according to a randomized, double-blind, placebo-controlled, crossover design. There were ≥4 mo between the 2 studies. This interval was necessary because of the long half-lives of body water and of plasma cholesterol. During the 6 wk preceding the study, the subjects consumed either 10 g HP-inulin/d (5 g before breakfast and 5 g before the evening meal) or 10 g placebo/d (5 g maltodextrin twice daily). During the 3 wk preceding the study, the subjects consumed a controlled diet. This diet provided 55% of total energy as carbohydrate (20–25% simple, mainly as fruit; 30–35% complex, mainly as bread, potatoes, rice, and noodles) and 30% as fat (10% each of saturated, monounsaturated, and polyunsaturated fatty acids). Cholesterol intake was the same as during the 2 periods of the controlled diet. Intakes of trans fatty acids were <500 mg/d. The sources of saturated fatty acids were mainly butter, cheese, and meat. Food plants rich in inulin (24) were excluded. The subjects consumed 3 meals/d. All diet instruction was provided by a dietitian, who met with each subject before the first diet period to obtain a report of the subject’s usual diet and to establish the subject’s energy requirement. The dietitian met with each subject during and at the end of each diet period. A detailed report of each subject’s dietary intake during the last week of each diet period was obtained, and the actual intakes were calculated by using the CIQUAL [Center Informatique pour la Qualité des Aliments (Computer Center for Food Quality)] tables. All subjects abstained from alcohol or heavy physical activity during the week before the study. For women, the test was performed during the first 10 d of the menstrual cycle to take into account the known variations of lipogenesis during the menstrual cycle (there is no menstrual variation for cholesterol synthesis) (25).

The night before the test, the subjects consumed their last meal between 1900 and 2000. For each subject, the total energy and composition of this meal were the same at the end of the 2 periods of the controlled diet. The subjects drank a loading dose of deuterated water (3 g/kg body water; one-half after the evening meal and one-half at 2200 to reduce the risk of vertigo; 2 subjects experienced vertigo). From then until the end of the study, the subjects drank only water enriched with 2H2O (4.5 g 2H2O/L). All tests were initiated in the postabsorptive state, after an overnight fast. At 0730, an indwelling catheter was placed in a forearm vein, and blood was sampled for the various concentrations and enrichment measurements. A sample (150–250 mg) of abdominal subcutaneous adipose tissue was obtained by needle biopsy under local anesthesia and stored in liquid nitrogen.

Analytic procedures

Plasma glucose, nonesterified fatty acids, and triacylglycerol were assayed with enzymatic methods (26, 27). Plasma insulin and glucagon concentrations were measured by radioimmunoassay. Total cholesterol was measured by enzymatic assay; the HDL fraction was measured as described previously (28), and the LDL fraction was calculated by using the equation of Friedewald. Detailed methods for the measurement of deuterium enrichment in plasma cholesterol and in the palmitate of plasma triacylglycerol were published previously (29, 30). In brief, plasma lipids were first extracted by using the method of Folch et al (31). Free cholesterol and triacylglycerols were separated from other lipid fractions by thin-layer chromatography. Free cholesterol was scraped off the silica plates and eluted from silica with ether before trimethylsilyl derivative was prepared (29, 30). The transmethylated derivatives of the palmitate of triacylglycerols were prepared as described previously (32). Deuterium enrichment was determined with the use of a gas chromatograph (HP5890; Hewlett-Packard, Palo Alto, CA) equipped with a 25-m fused silica capillary column (OV1701; Chrompack, Bridgewater, NJ) and interfaced with a mass spectrometer (HP5971A; Hewlett-Packard) operating in the electronic impact ionization mode (70 eV). The carrier gas
was diluted when necessary. Deuterium enrichment in plasma water was measured as described previously (33).

**mRNA concentrations**

Total RNA was extracted from adipose tissue samples by using the RNeasy total RNA kit (Qiagen, Couteauboeuf, France). Concentrations and purity were verified by measuring absorbance at 260 and 280 nm. Their integrity was checked by agarose gel electrophoresis. Total RNA was suspended in water and kept at −80°C until quantification of the target mRNAs. FAS, acetyl-CoA carboxylase 1 (ACC1; EC 6.4.1.2), and sterol responsive element binding protein 1c (SREBP-1c) mRNA concentrations were published previously (34, 35). For ACC1, a 504–base pair cDNA fragment was synthesized by in vitro transcription (Riboprobe System; Promega). The antisense primer of the ACC1 mRNA, including the target sequences, were synthesized by RT-PCR assays, RNAs corresponding to part of the ACC1 mRNA, including the target sequences, were synthesized by in vitro transcription (Riboprobe System; Promega). Known amounts of synthesized mRNA were quantified to create a dose-response curve, as recommended previously (36). For the mRNA assays in the tissue samples, serial dilutions of the competitor plasmids were performed (10−1 to 10−5 mmol/μL) in 10 mmol tris-HCl/L (pH 8.3) 1 mmol/L EDTA buffer.

For each target mRNA, an RT reaction was performed from 0.1 μg total RNA with 2.5 U thermostable reverse transcriptase, or Tth DNA polymerase (EC 2.7.7.49; Promega Corp) in 10 mmol tris-HCl buffer (pH 8.3) with 1 mmol EDTA/L, 90 mmol KCl/L, 1 mmol MnCl2/L, 0.2 mmol deoxyxynoside triphosphates/L, and 15 pmol of the specific antisense primer in a final volume of 20 μL. The reaction was allowed to proceed for 3 min at 60°C followed by 15 min at 72°C and then 5 min at 99°C. After the RT medium was chilled, 4 μL water was added, from which 20 μL was used for the competitive PCR reaction. Sense primers labeled in the 5’ position with Cy-5 fluorescent dye (Eurogentec, Seraing, Belgium) were used during the PCR. For this reaction, 20 μL RT medium was added to 80 μL PCR mix (10 μL of 10 mmol tris-HCl/L (pH 8.3), 100 mmol KCl/L, 0.75 mmol ETG/L, and 50 g glycerol/L) containing 0.2 mmol deoxyxynoside triphosphates/L, 5 U Taq polymerase (Life Technologies, Cergy Pontoise, France), 45 pmol of the corresponding sense primer, and 30 pmol of the antisense primer. Four 20-μL aliquots were then transferred into PCR tubes containing 5 μL of defined working solutions of the competitor cDNA. The PCR conditions were as follows: 2 min at 94°C followed by 40 cycles (40 s at 94°C, 60 s at 55°C, and 40 s at 72°C) and finally 10 min at 72°C. The PCR products were analyzed with an automated laser fluorescence DNA sequencer (ALFexpress; Pharmacia, Uppsala, Sweden) in denatured 40-g/L polyacrylamide gels. The amounts of PCR products (competitor and target) were calculated by integrating peak areas with the use of FRAGMENT MANAGER software from Pharmacia. The initial concentration of target mRNA was determined at the competition equivalence point as described previously (36). To compare the results of RT-competitive PCR accurately, the target mRNAs of different samples were measured in the same run of RT and PCR by using the same mix of medium.

**Calculations**

The fractional contributions of cholesterol synthesis and hepatic lipogenesis to plasma free cholesterol and to plasma triacylglycerol pools, respectively, were calculated from the deuterium enrichments in free cholesterol, palmitate triacylglycerol, and in plasma water, as described previously (30, 37). In short, the deuterium enrichments that would have been obtained if endogenous synthesis was the only source of plasma cholesterol or of palmitate triacylglycerol were calculated from plasma water enrichment. The comparison of the enrichments observed with these theoretical values gives the contributions, expressed as the fractional synthetic rate, of endogenous synthesis to the pool of hepatic lipogenesis to plasma free cholesterol and to plasma triacylglycerol (30, 32). The average enrichment of different samples were measured in the same run of RT and PCR by using the same mix of medium.

**RESULTS**

**Dietary intake**

The dietary intakes, as calculated from the diet surveys conducted during the last week of each controlled-diet period, are shown in Table 1. The total energy intake and the daily intakes of fiber (excluding inulin intake during the inulin period), fructose, and cholesterol during the 2 periods of the controlled diet were not significantly different. The expected intakes of fat and carbohydrate were obtained, as was the relative repartition between

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Dietary intakes during the 2 diet periods 1</th>
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<tbody>
<tr>
<td>Placebo</td>
<td>Inulin</td>
</tr>
<tr>
<td>Energy intake (kJ·kg⁻¹·d⁻¹)</td>
<td>151 ± 5</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>15.1 ± 0.5</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>56.6 ± 0.6</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>27.9 ± 0.7</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>26.1 ± 1.4</td>
</tr>
<tr>
<td>Fructose (g/d)</td>
<td>14.4 ± 3.3</td>
</tr>
<tr>
<td>Saturated fatty acid (g/d)</td>
<td>22.1 ± 1.2</td>
</tr>
<tr>
<td>Monounsaturated fatty acid (g/d)</td>
<td>23.6 ± 1.4</td>
</tr>
<tr>
<td>Polyunsaturated fatty acid (g/d)</td>
<td>22.9 ± 1.3</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>171 ± 15</td>
</tr>
</tbody>
</table>

1 x ± SEM; n = 8. There were no significant differences between the 2 periods.
saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. No subject experienced any adverse effect during the ingestion of inulin or placebo or felt any sensory difference between inulin and placebo. Body weights were stable during the 2 periods of the controlled diet.

Hormonal and metabolites values

Addition of HP-inulin to the high-carbohydrate diet induced no significant changes in glucose, insulin, or glucagon concentrations (Table 2). Total cholesterol, HDL-cholesterol (placebo 1.20 ± 0.11, inulin 1.31 ± 0.10 mmol/L), and LDL-cholesterol (placebo 2.77 ± 0.21, inulin 2.90 ± 0.22 mmol/L) concentrations also did not change significantly. Plasma triacylglycerol concentrations were significantly lower after consumption of HP-inulin (P < 0.05).

Rates of lipid synthesis

Deuterium enrichment values obtained in plasma water, plasma free cholesterol, and palmitate plasma triacylglycerol at the end of the 2 diet periods are shown in Table 3. The values observed for palmitate and cholesterol can be higher than in plasma water because there are multiple sites of deuterium incorporation in the molecules during their synthesis.) The calculated fractional synthetic rates of cholesterol were not changed significantly by inulin. The fractional contribution of hepatic lipogenesis to plasma triacylglycerol, on the contrary, decreased (P < 0.05) after HP-inulin consumption.

mRNA concentrations

Despite a trend for lower values at the end of the HP-inulin period, no significant difference in any of the mRNA concentrations measured in adipose tissue was observed at the end of the 2 diet periods (Table 4).

**TABLE 2**

<table>
<thead>
<tr>
<th>Hormonal and metabolite values at the end of the 2 diet periods¹</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Glucose (mmol/L)</td>
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<tr>
<td>Nonesterified fatty acids (μmol/L)</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
</tr>
<tr>
<td>Glucagon (ng/L)</td>
</tr>
</tbody>
</table>

¹T ± SEM; n = 8.
²Significantly different from placebo, P < 0.05.

**TABLE 4**

<table>
<thead>
<tr>
<th>Adipose messenger RNA concentrations at the end of the 2 diet periods¹</th>
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<tr>
<td></td>
</tr>
<tr>
<td>FAS</td>
</tr>
<tr>
<td>ACCl</td>
</tr>
<tr>
<td>SREBP-lc</td>
</tr>
</tbody>
</table>

¹T ± SEM; n = 8. FAS, fatty acid synthase; ACCl, acetyl-CoA carboxylase l; SREBP-lc, sterol responsive element binding protein l.

**DISCUSSION**

In the current study we tested whether the addition of a nondigestible oligosaccharide, HP-inulin, to a moderately high-carbohydrate diet could prevent the potential adverse effects on hepatic lipogenesis and plasma triacylglycerol concentrations of this diet and could in addition decrease plasma cholesterol concentrations. We found that the oral ingestion of a well-tolerated, moderate amount of HP-inulin reduced hepatic lipogenesis and plasma triacylglycerol concentrations. However, no significant effect on either plasma cholesterol concentrations or the fractional synthetic rate was observed. The expression of the lipogenic pathway in adipose tissue, as appreciated by the measurement of mRNA concentrations, did not decrease significantly.

These results agree with those of animal studies, which showed a net effect of nondigestible oligosaccharides on triacylglycerol, whereas the reduction in cholesterol concentration was moderate and observed only during long-term administration (14, 15). Data on the effects of nondigestible oligosaccharide consumption on lipid metabolism in humans are inconsistent (38). Some studies in control subjects showed that the administration of inulin for 4–8 wk lowered triacylglycerol concentrations but had no effect on plasma cholesterol (19), whereas other studies failed to show any effect on plasma lipid concentrations (20, 21). In these latter 2 studies, however, the subjects consumed rather large amounts of fat. As discussed by Williams (16), this is an unfavorable situation in which to observe any effect of inulin on endogenous triacylglycerol metabolism because a high-fat diet alone results in decreased lipogenesis and triacylglycerol secretion compared with the consumption of a high-carbohydrate diet (39, 40).

Our data clearly show beneficial effects (ie, lowered plasma triacylglycerol concentrations) of adding HP-inulin to a moderately high-carbohydrate, low-fat diet, which is recommended for the prevention of CAD. However, it should be stressed that we studied only control subjects and for only a short period. Further studies are needed to determine whether this effect persists during the long-term administration of inulin. Such studies would allow us to determine whether long-term inulin administration might, as in animals (14, 15), have a cholesterol-lowering effect. Last, it remains to be established whether these effects would occur in persons with elevated lipid concentrations or an elevated hepatic lipogenic rate, such as obese people. The previous finding in type 2 diabetic patients and in subjects with modest hyperlipidemia (18, 41, 42) that nondigestible oligosaccharides lower plasma cholesterol but not triacylglycerol concentrations and have no effect on lipid concentrations (43) is intriguing and suggests that some of the metabolic effects of nondigestible oligosaccharide ingestion on lipid metabolism could be different under different pathologic situations. This possibility is strengthened by the finding that oligofructose did not decrease serum triacylglycerol
concentrations in obese Zucker rats and, among the key enzymes of fatty acids synthesis and esterification, decreased the activity of only the malic enzyme (44).

Nondigestible oligosaccharides have been shown to decrease lipogenesis and the triacylglycerol secretion rate in animals (11); however, no data on their effect on lipids synthesis in humans are available. Clearly, HP-inulin decreased hepatic lipogenesis but not cholesterol synthesis. This reduced lipogenic rate probably played a role in the triacylglycerol-lowering action of inulin. However, because hepatic lipogenesis is only a minor contributor to the amount of triacylglycerol secreted by the liver (37), other mechanisms—such as the decreasing hepatic reesterification of plasma free fatty acids (15) or the increased clearance rate of triacylglycerol—probably contributed to the decrease in triacylglycerol concentrations. In the absence of liver biopsy specimens, which were not obtained for obvious ethical reasons, we could not show that HP-inulin administration decreased liver FAS activity and mRNA concentration, as was shown in animals (22). We looked for such an effect in adipose tissue and found no changes in FAS, ACC1, or SREBP-1c mRNA concentrations. However, this negative result does not preclude an effect on the expression of the lipogenic pathway in liver. The effects of nondigestible oligosaccharides are considered to be mediated, at least in part, through the colonic production of propionate. Actually, most of the short-chain fatty acids produced by the colonic fermentation of nondigestible oligosaccharides and released into the portal circulation are extracted by the liver, thus limiting the effects of propionate mainly to hepatic metabolism.

In conclusion, the addition of nondigestible oligosaccharides such as HP-inulin to a moderately high-carbohydrate, low-fat diet strengthens their beneficial effect on the plasma lipid profile by lowering hepatic lipogenesis and by resulting in a triacylglycerol-lowering effect in addition to the previously observed cholesteryl-lowering action of such diets (8). These results support the use of nondigestible oligosaccharides for reducing risk factors for atherosclerosis.

We thank all the subjects who participated in this study and CP, IP, CM, and MS for their help during the performance of the tests. DL and FD performed the mRNA measurements and helped perform the deuterium enrichment measurements. MB was responsible for the study design, the selection and follow-up of the subjects, and for most of the mass spectrometry analysis. All authors participated in the data analysis and in writing the manuscript. No author had any financial or personal interest in the organization supporting the research.

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