Effects of a diet rich in resistant starch on hepatic lipid metabolism in the rat

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The aim of this study was to examine the effects of the replacement of a large part of absorbed glucose by volatile fatty acids on hepatic lipid metabolism. For this purpose, experiments were conducted in rats fed either a diet containing digestible wheat starch or amylase-resistant cornstarch.

Compared with the digestible wheat starch diet, plasma insulin was lower in rats fed the resistant cornstarch diet, and the fluctuations of insulinemia during the fed/postabsorptive period were smaller. The marked reduction of hepatic lipogenesis in rats fed the resistant cornstarch diet (−52% compared with the digestible wheat starch diet) resulted from the coordinated inhibition of all major enzymes implicated in the lipogenic pathway, except acetyl CoA synthetase activity. This suggests that volatile fatty acids, particularly acetate, constituted the major source of acetyl CoA for lipogenesis, rather than glucose. However, these modifications were not accompanied by a significant depressive effect on plasma triglycerides. In rats fed the resistant cornstarch diet, changes in lipogenesis activity were accompanied by a reduction of glycolysis as shown by the net inhibition of glucokinase and pyruvate kinase. In parallel to these modifications, with the resistant cornstarch phosphoenolpyruvate carboxykinase was markedly induced; with this diet, propionate should constitute the major gluconeogenic substrate removed by the liver.

HMG CoA reductase was markedly induced in rats adapted to the resistant cornstarch diet (1.6-fold higher than with the digestible wheat starch diet); this could be related to the increased fecal bile acids excretion. A significant hypocholesterolemic effect of the resistant cornstarch diet was only observed during the postabsorptive period.

In conclusion, hepatic fatty acid synthesis is tightly controlled by carbohydrate availability, but the possibility that volatile fatty acids exert specific effects on lipogenesis could not be ruled out. (J. Nutr. Biochem. 5: 138–144, 1994.)

Keywords: resistant starch; lipogenesis; volatile fatty acids; rats

Introduction

Various types of starches contain a high proportion of amylase-resistant starch, such as pea starch, crude potato starch, or amylose-rich maize starch (amylomaïze). In humans, amylase-resistant starch could, under some circumstances, represent a substantial part of polysaccharides entering the colon, besides fibers sensu stricto.[1–3] Even with digestible starch, a part of this starch escapes to the digestion in the small intestine and is fermented by colonic microflora.[4]

The chemical energy of these fermented starches may be recovered by the host in the form of organic acids, especially volatile fatty acids. The degradation of nonavailable polysaccharides and the absorption of volatile fatty acids is a relatively gradual process,[5] compared with the rapid absorption of glucose released from sucrose or readily digestible starch. Thus, during the 24-hour physiological cycle, it is conceivable that the processes of storage or catabolism of fuels are different in subjects fed a diet rich in digestible starch or containing carbohydrates fermented in the large intestine. In a previous report[6] we compared carbohydrates digestion and some parameters of liver metabolism of two groups of rats adapted either to a diet containing wheat starch (highly digestible starch) or a 60% cornstarch diet (rich in resistant starch). The relationship between lipid metabolism and dietary fiber has been extensively investigated,[7] particularly

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on lipid digestion and absorption and on circulating lipids. However, little is known about the adaptation of liver metabolism to a high availability of volatile fatty acids. Furthermore, studies have frequently been carried out during the postabsorptive period, yet absorption of volatile fatty acids is maximal during the late absorptive period. Thus, the present study was conducted during the fed and postabsorptive periods (just before food intake resumes during the normal light: dark cycle) in animals adapted to diets rich in digestible or resistant starches to investigate the effect of the replacement of a large part of absorbed glucose by volatile fatty acids on lipid metabolism.

Methods and materials

Animals and diets

Male Wistar rats (IFA-CREDO, l'Arbresle, France) were fed a commercial pellet diet (AO3 pellets, UAR, Villemoisson/Orge, France) until body weight reached about 170 g (6 weeks). The animals were then housed two per cage (wire-bottomed cages were used to limit coprophagy) and fed semipurified diet (distributed as a moistened powder) that contained the following (wt%): 18% casein (L. François, Paris, France), 5% corn oil (CIO, Genay, France), 6% mineral mix (UAR, Villemoisson/Orge, France), 1% vitamin mix (UAR) for 21 days. The carbohydrate supply was 70% wheat starch (digestible starch [DS] diet) or 60% cornstarch diet (Roquettes, Lestrem, France) plus 10% wheat starch (resistant starch [RS] diet). The rats were maintained in temperature-controlled rooms (22° C) with the dark period from 0900 to 2100 hours, and they had access to the food from 0900 to 1700.

Blood and tissue sampling

The rats were sampled either 8 hours (fed period) or 22 hours (postabsorptive period) after the onset of food intake. Animals were anesthetized with sodium pentobarbital, and blood was withdrawn from the hepatic vein, portal vein, and then the abdominal aorta. Portal and hepatic blood flows were determined by an indicator-dilution method, with p-aminohippurate as an indicator, using a procedure adapted from that of Katz and Bergman.[8] Then the cecum, complete with content, was removed and weighed (total cecal weight). A fraction of cecal contents was transferred to microfuge tubes that were immediately deep frozen in liquid nitrogen for 20 seconds and then stored at −20° C (duration of the procedure: about 90 seconds). In parallel, liver was excised, immediately freeze-clamped and stored at −80° C.

Analytical procedures

Volatile fatty acids were measured by gas-liquid chromatography, after ethanolic extraction from plasma samples,[9] and on aliquots of supernatants (8,000*g*, 5 minutes at 4° C) of cecal contents. Triglycerides (Biotrol, Paris, France) and cholesterol (BioMérieux, Charbonnières les Bains, France) were determined in plasma by enzymatic procedures. Bile acids were extracted from the feces samples by 6 volumes ethanol and quantified by an enzymatic method (Bile acid kit, Sigma, L'Isle d'Abeau, Chesnes, France).

Immuno reactive insulin was measured by a double antibody method using [^{125}I]insulin tracer (CEA, Gif/Yvette, France) and rat insulin standard (21 U/mg standard, Novo, Bagsvaerd, Denmark).

Fatty acid synthesis was measured in vivo using ^3H_2O.[10,11] On day 21 of the experiment, the animals were injected intraperitoneally with 100 μCi of ^3H_2O (108 mCi/ml, CEA, Gif sur Yvette, France)/100 g body wt. Injection was made at 0800 hours or at 1600 hours and animals were sacrificed 1 hour later. A sample was taken from the abdominal aorta for the determination of serum-specific radioactivity. Livers were perfused with 100 mL of cold saline, blotted on filter paper, weighed, and frozen in liquid nitrogen. A fraction of liver (1 to 2 g) was saponified in ethanolic KOH and acidified with HCl, and fatty acids were extracted with petroleum ether. The radioactivity was determined in a liquid scintillation analyzer 1600-TR (Packard). Results were corrected for recovery of internal standard ([1-^{14}C]palmitic acid, 45 to 60 mCi/mmol; CEA). The lipogenic activity was expressed in μmol of ^3H_2O incorporated into long chain fatty acids per hour per g liver.

The activities of glucose 6-P dehydrogenase[12] and malic enzyme[13] were measured spectrophotometrically at 37° C using a supernatant obtained by centrifugation at 50,000*g* of a liver homogenate in 0.25 M sucrose.

The activities of ATP-citrate lyase and fatty acid synthetase were measured using a supernatant obtained by centrifugation of a liver homogenate in 0.143 M phosphate buffer (pH 6.8). ATP citrate lyase was determined spectrophotometrically.[14] The enzyme activity was determined by an isotopic method, [2-^{14}C]malonyl CoA (CEA) was used as the tracer, adapted from that described by Maeda et al.[15] Fatty acid synthetase activity was expressed in μmol of [2-^{14}C]malonyl CoA incorporated into fatty acids per minute per g liver.

The activity of acetyl CoA carboxylase was assayed as described by Maeda et al.[15] using a liver homogenate in 0.06 M Tris buffer (pH 7.5). The 105,000*g* supernatant solution was subjected to gel filtration on sephadex G-25 in the homogenization medium prior to enzyme assay. Carboxylase assay involved activation of gel filtered cytosol at 37° C for 60 minutes in a medium containing in final concentration: 60 mM Tris-HCl buffer pH 7.0, 5 mM potassium citrate, 8 mM $MgCl_2$, 3 mM reduced glutathione, 0.1 mM EDTA and 0.06% serum albumin. Carboxylation was initiated at 37° C in the same medium containing in addition 0.2 mM acetyl CoA and 10 mM [^{14}C] $NaHCO_3$ (2.5 μCi/ml reaction mixture) (CEA). Acetyl CoA carboxylase activity was expressed as μmol of ^{14}Cmalonyl CoA formed per minute per g liver.

The activity of the cytosolic form of acetyl CoA synthetase was determined according to Liedvogel[16] on supernatant obtained after centrifugation at 50,000*g* for 30 minutes of a 10% liver homogenate in 10 mM Tris-HCl, pH 7.5, 0.3 mM mannitol and 1 mM EGTA. Acetyl CoA present in the supernatant was measured by high performance liquid chromatography (at 254 nm) in isocratic conditions using a Spherisorb ODS2 5μ column (150 × 4.6 mm). Elution was performed using a phosphate buffer 25 mM, pH 5.5 and methanol 15% (flow rate: 1.2 ml/min). Acetyl CoA synthase activity was expressed in μmol of acetyl CoA formed per minute per g liver.

The activity of hydroxymethyl glutaryl CoA reductase (HMG CoA reductase) was measured on liver microsomal fractions as described by Wilce and Kroone.[17] Labeled mevalonolactone was separated from unreacted HMG CoA by column chromatography, using AG1-X8 resin (200 to 400 mesh; formate form) (Biorad, Paris, France). The activity of HMG CoA reductase was given in pmol of ^{14}C HMG CoA transformed in mevalonolactone per minute per mg protein.

The activities of glucokinase[18] and pyruvate kinase[19] were measured spectrophotometrically using a 100,000*g* supernatant obtained after homogenization of a portion of liver in a buffer containing: 50 mM potassium phosphate, pH 7.2, 100 mM KCl, 1 mM EDTA, 0.5 mM DTT, and 20% glycerol.

The activity of phosphoenolpyruvate carboxykinase (PEPCK) was measured by an isotopic method[20] using a supernatant obtained by centrifugation at 50,000*g* of a liver homogenate in 0.25 M sucrose. The activity was expressed in μmol of [^{14}C]$NaHCO^{3-}$ incorporated into malate per minute per g liver.

Calculations and data analysis

The cecal pool was calculated as: cecal concentration (mmol/L) × cecal water (mL). The portal balance was [(portal vein) − (artery)] × (portal blood flow); the hepatic balance was [(hepatic vein) − (afferent)] × (hepatic blood flow); the afferent concentration was calculated from portal vein and artery, considering their respective blood flow (hepatic arterial blood flow was calculated by differences between hepatic and portal blood flows).

Values are given as means ± SEM and statistical differences were determined by the Student's t test. Values of $P < 0.05$ were considered significant.

Results

Cecal digestion

In rats fed the two experimental diets, the daily food intake (DS: 25.0 ± 1.1 g/d; RS: 23.8 ± 1.2 g/d) and weight gain (DS: 4.7 ± 0.3 g/d; RS: 4.5 ± 0.3 g/d) were not significantly different. During the postabsorptive period there was a 10% decrease of the body weight with the two diets. Whatever the diet, the cecum weight did not vary significantly between the fed and postabsorptive periods *(Table 1)*. However, in rats fed the RS diet, the cecum (≈11 g) was more developed than in rats fed the DS diet (≈3 g). In parallel, there was a considerably higher cecal pool of volatile fatty acids in rats fed the RS diet (butyrate eightfold, acetate 10 fold, and propionate 16 fold) than in rats fed the DS diet. During the postabsorptive period, before food intake resumed, there were still large amounts of volatile fatty acids in the cecum of rats adapted to the RS diet, nevertheless the cecal pool declined from 1237 to 861 μmol.

Changes in the portal and hepatic balances of glucose and volatile fatty acids

As shown in *Figure 1*, there was a net positive balance of glucose across the portal-drained viscera in rats fed the DS or RS diets, but the flux was 2.7 fold higher in rats adapted to the DS diet. In these rats, 32% of absorbed glucose was removed by the liver, whereas there was a small but detectable release of glucose in rats fed the RS diet. The resulting splanchnic balance of glucose (which represents the difference between the hepatic and portal balances) was 30% higher in rats fed the DS diet (43.5 μmol/min) than in those fed the RS diet (33.6 μmol/min). During the postabsorptive period, in both groups of rats, the digestive tract was a site of net glucose utilization (about 9 μmol/min), and the liver released substantial amounts of glucose (20.7 or 15.6 μmol/min with the DS or RS diet, respectively).

Figure 1 also shows that the portal balance of volatile fatty acids was 10 fold higher in rats fed the RS diet than in rats fed the DS diet. The portal balance of volatile fatty acids and that of glucose were of comparable magnitude (about 28 μmol/min) in rats fed the RS diet. In these animals, acetate absorption was particularly high (15 μmol/min), and 54% of absorbed acetate was removed by the liver. Nevertheless, the splanchnic balance was positive, thus allowing substantial amounts of acetate to be available for extrasplanchnic tissues. In rats fed the DS diet, there was no net hepatic uptake of acetate. Under all conditions, propionate and butyrate were almost completely taken up by the liver, even when absorption was very high and the splanchnic balance was close to zero. In rats fed the RS diet, propionate absorption was eightfold higher than that of butyrate. Because propionate is extensively taken up by the liver, it represented 55% of the total volatile fatty acid uptake. During the postabsorptive period, the portal supply of volatile fatty acids was markedly depressed: it remained noticeable with the RS diet (13.0 μmol/min) but negligible with the DS diet (1.5 μmol/min) (data not presented).

These results show that feeding a resistant starch diet depresses the intestinal absorption of glucose and spreads the absorption of volatile fatty acids all along the light-dark cycle. In these conditions the main source of C units for the liver was no longer glucose but volatile fatty acids.

Table 1 Changes in cecal digestion in rats adapted to the digestible starch (DS) or resistant starch (RS) diet, sampled during the fed or postabsorptive period

	Cecal weight	Cecal pool		
		Acetate	Propionate	Butyrate
	g	μmol		
Fed period				
DS	2.7 ± 0.2	66 ± 7	30 ± 4	11 ± 2
RS	12.3 ± 0.3†	670 ± 51†	480 ± 42†	87 ± 10†
Postabsorptive period				
DS	3.1 ± 0.3	70 ± 7	28 ± 4	13 ± 2
RS	11.1 ± 0.9†	518 ± 45*†	281 ± 27*†	62 ± 9†

Results are the means ± SEM for 12 rats.
*$P < 0.05$: fed period versus postabsorptive period.
†$P < 0.05$: DS versus RS.

Effect of a diet rich in resistant starch on insulinemia and lipogenesis

As shown in *Table 2*, in rats fed the DS diet insulinemia was higher (155 mU/L plasma) than in those fed the RS diet (86 mU/L plasma). During the postabsorptive period, the plasma insulin concentration was depressed with both diets, however, the diurnal fluctuations were greater in rats fed the DS diet (−60 mU/L) than in those adapted to the RS diet (−26 mU/L). These wider variations of insulinemia with the DS diet could be related to the high uptake of glucose by the liver during the absorptive period and with the net release of this substrate in the postabsorptive state. Nevertheless, insulinemia was still significantly higher during the postabsorptive period in rats adapted to the DS diet.

Table 2 also shows that, in parallel to insulinemia, hepatic lipogenesis was halved in animals fed the RS diet. On the other hand, with both diets lipogenesis was markedly reduced during the fed/postabsorptive transition (DS: −80%; RS: −50%), and the remaining lipogenic activity was similar in the two groups of animals (about 10 μmol ^3H_2O incorporated in fatty acids/hour g liver). Thus, the difference in insulin level between the two diet groups during the postabsorptive period did not result in noticeable difference in hepatic basal lipogenesis.

During the absorptive period, there was no significant difference in triglyceridemia and cholesterolemia between the DS and RS diet *(Table 2)*. With both diets, the plasma concen-

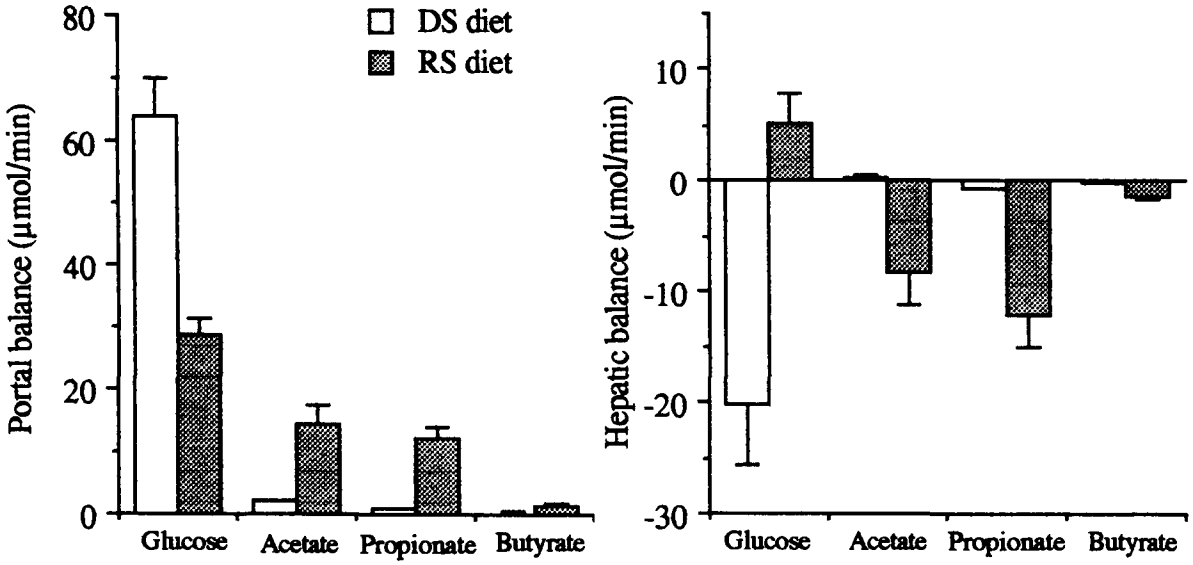

Figure 1 Changes in the balance of glucose and volatile fatty acids across the digestive tract (portal balance) and liver (hepatic balance) in rats adapted to the digestible starch (DS) or resistant starch (RS) diet, sampled during the fed period. Results are means ± SEM for 12 rats in each diet. All data obtained with the RS diet were significantly different from that obtained with the DS diet ($P < 0.01$).

Table 2 Changes in insulinemia, lipogenesis, and lipid metabolites in rats adapted to the digestible starch (DS) or resistant starch (RS) diet, sampled during the fed or postabsorptive period

	Fed period		Postabsorptive period	
	DS	RS	DS	RS
		mU/L plasma		
Insulin	148 ± 16	80 ± 11†	91 ± 9*	56 ± 6†
		µmol ^3H_2O incorporated in fatty acids/h.g liver		
Lipogenesis	47.3 ± 4.4	22.6 ± 1.5†	8.5 ± 1.2*	11.0 ± 0.8*
		mmol/L plasma		
Triglycerides	0.85 ± 0.05	0.79 ± 0.06	0.49 ± 0.04*	0.33 ± 0.01*†
Cholesterol	1.59 ± 0.03	1.40 ± 0.04	1.65 ± 0.05	1.25 ± 0.03*†

Results are means ± SEM for 12 rats for each period of sampling.
*$P < 0.05$: fed period versus postabsorptive period.
†$P < 0.05$: DS versus RS.

tration of triglyceride decreased during the fed/postabsorptive transition (−30% and −40% with the DS and RS diets, respectively). Whereas there was no noticeable change in plasma concentration of cholesterol during the light-dark cycle with the DS diet, this parameter was significantly depressed during the postabsorptive period in animals adapted to the RS diet. The daily fecal excretion of bile acids was highly enhanced in rats adapted to the RS (15.2 µmol) compared to those adapted to the DS diet (6.5 µmol) (data not presented).

Effect of a diet rich in resistant starch on lipogenic enzyme activities

Table 3 shows the activities of the main enzymes involved in hepatic lipogenesis in rats fed a DS or an RS diet. Because of the low level of hepatic lipogenesis in the postabsorptive period, the activities of lipogenic enzymes during this period are not presented.

The activities of the glucose 6-P dehydrogenase and malic enzyme, known for their role in the supply of NADPH for hepatic lipogenesis, have been measured. It appears that both enzyme activities were markedly lower in the liver of rats fed the RS diet (−35% for glucose 6-P dehydrogenase and −43% for malic enzyme).

In the liver, acetyl CoA for lipogenesis is mainly provided by ATP citrate lyase and to a lesser extent, particularly in conditions of high acetate uptake, by the cytosolic form of acetyl CoA synthetase. *Table 3* shows that in rats fed with the RS diet the activity of ATP citrate lyase was markedly lower (−50%) than that measured with the DS diet. On the other hand, in the liver of rats adapted to the RS diet, the activity of acetyl CoA synthetase was significantly higher than that measured with the DS diet (+20%); however, this was probably not sufficient to compensate for the low activity of ATP citrate-lyase.

Acetyl CoA carboxylase, the key enzyme of lipogenesis,

Table 3 Changes in enzyme activities implicated in long chain fatty acids synthesis in rats adapted to the digestible starch (DS) or resistant starch (RS) diet, sampled during the fed period

	Fed period	
	DS	RS
	μmol/min.g liver	
Glucose 6-P dehydrogenase	4.25 ± 0.29	2.75 ± 0.18†
Malic enzyme	7.24 ± 0.57	4.14 ± 0.37†
ATP-citrate lyase	2.79 ± 0.25	1.42 ± 0.11†
Acetyl CoA synthetase	0.59 ± 0.04	0.73 ± 0.07†
Acetyl CoA carboxylase	0.66 ± 0.03	0.50 ± 0.04†
Fatty acid synthetase	0.140 ± 0.006	0.086 ± 0.004†

Values are means ± SEM for 12 rats.
†$P < 0.05$: DS versus RS.

was also affected by the dietary conditions because its activity significantly decreased (−25%) when the rats were fed the resistant starch (see *Table 3*). Moreover, with this diet the activity of fatty acid synthetase was also markedly diminished (−39%) compared with that measured with the DS diet.

These data show that, when volatile fatty acids availability was increased at the expense of glucose, namely with the RS diet, all the lipogenic enzymes activities are depressed, leading to the marked reduction of lipogenesis (see *Table 2*).

Effect of the diet on the activity of HMG CoA reductase

Table 4 shows that the activity of HMG CoA reductase, the regulatory enzyme of cholesterol synthesis, was markedly induced during the feeding period in rats adapted to the RS diet (1.6 fold higher than that measured with the DS diet). The same difference was observed between the two diets during the postabsorptive period. Moreover, with both diets the diurnal fluctuations of HMG CoA reductase were marked: during the postabsorptive period this activity was 70% lower than during the fed period.

Effect of a diet rich in resistant starch on glycolytic and gluconeogenic enzymes

The activities of glucokinase and pyruvate kinase were maximal during the fed period in both groups of rats *(Table 5)*. In rats fed the RS diet, there was a significant inhibition of glucokinase: −23% in fed rats and −32% in postabsorptive rats compared with the DS diet. Pyruvate kinase activity was also markedly reduced in rats adapted to the RS diet and the magnitude of the inhibition (about −30%) was similar in fed and postabsorptive rats.

In the two diet conditions, the activity of PEPCK, a rate-controlling enzyme of gluconeogenesis, was highly induced during the postabsorptive period and declined in fed rats. Moreover, this enzyme activity was higher in rats adapted to the RS diet than in those receiving a digestible starch. This difference was particularly strong during the postabsorptive period (+52% with RS), but it was still significant in fed rats. This high activity of phosphoenolpyruvate carboxykinase with the RS diet during the fed period could be connected to the metabolism of propionate because this volatile fatty acid was particularly abundant in the liver during this period.

These data suggest that the replacement of a large part of glucose absorbed by volatile fatty acids led in the liver to an inhibition of the glycolytic process with a parallel stimulation of some steps of gluconeogenesis.

Discussion

Diets containing resistant starch are useful models to study the replacement of glucose absorption by volatile fatty acids. Our study has shown that the replacement of 50% of wheat starch by amylomaïze induced a 55% decrease of glucose absorption while the volatile fatty acid absorption was enhanced ninefold, and in contrast to glucose, the absorption of volatile fatty acids is relatively continuous during the 24 hour light:dark cycle, but with a significant decrease in postabsorptive rats.[6] The question arises as to the recovery of resistant starch as volatile fatty acids. Because only 30% of amylomaize starch is actually amylase-resistant,[6] the rats consumed about 4.3 g of resistant starch daily. During fermentation in the large intestine, about 40% of carbohydrate carbon is transformed into non-metabolizable compounds (CO_2, CH_4, bacterial molecules, etc.). From the data previously published,[6] it may be calculated that about 2 g of volatile fatty acids are absorbed daily. Thus, insofar as the daily food intake was not significantly affected by the type of starch, the two experimental diets were not strictly isocaloric, but the difference was quite limited (10 to 15%).

The replacement of a part of dietary glucose by volatile fatty acids may considerably alter liver and peripheral metabolism, as well as the hormonal status.[6,21] When present in physiological concentrations in the portal vein, propionate and butyrate are almost completely taken up by the liver.[22,23] However, butyrate availability for liver metabolism is very low because it constitutes an energetic fuel for colonocytes.[24] He-

Table 4 Changes in HMG CoA reductase activity in the liver of rats adapted to the digestible starch (DS) or resistant starch (RS) diet, sampled during the fed or postabsorptive period

	Fed period		Postabsorptive period	
	DS	RS	DS	RS
		pmol/min.mg protein		
HMG CoA reductase	233.2 ± 31.3	374.2 ± 53.3†	63.7 ± 5.9*	112.4 ± 12.7†*

Values represent means ± SEM of 12 rats.
*$P < 0.05$: fed period versus postabsorptive period.
†$P < 0.05$: DS versus RS.

Table 5 Changes in enzyme activities implicated in glycolysis and gluconeogenesis in the liver of rats adapted to the digestible starch (DS) or resistant starch (RS) diet, sampled during the fed or postabsorptive period.

	Fed period		Postabsorptive period	
	DS	RS	DS	RS
Glucokinase	3.65 ± 0.15	2.80 ± 0.20†	2.98 ± 0.16*	2.03 ± 0.15
Pyruvate kinase	85.32 ± 3.66	59.90 ± 1.84†	72.50 ± 3.93*	52.90 ± 2.59†*
PEPCK	1.84 ± 0.26	2.72 ± 0.30†	2.70 ± 0.20	4.70 ± 0.37†*

Results are means ± SEM of 12 rats.
*$P < 0.05$: fed period versus postabsorptive period.
†$P < 0.05$: DS versus RS.

patic acetate uptake is dependent on the portal concentrations (in the physiological range, the higher the portal acetate the higher hepatic uptake) and on the physiological conditions.[25,26]

In the liver, the general orientation of metabolism depends on substrate availability and on hormonal status. In rats fed the digestible starch diet, the high availability of C units (chiefly as glucose) should favor storing processes, such as glycogen synthesis and lipogenesis, all the more because the insulinemia was elevated. With the resistant starch diet, the increased utilization of volatile fatty acids by the liver was not sufficient to compensate for the deficit in C units supply due to the absence of glucose uptake. In spite of the various factors unfavorable for lipogenic activity (no net glucose uptake, depressed activity of lipogenic enzymes), lipogenesis was not negligible (about 50% of DS diet), suggesting that volatile fatty acids, particularly acetate, are effective precursors of acetyl CoA. Even if glucose did not constitute a major substrate for lipid synthesis in rats fed the RS diet, it is still absorbed in significant amounts to maintain insulinemia at 86 mU/L plasma. In rats fed the RS diet all the major enzymes of lipogenesis were inhibited (from 25% for acetyl CoA carboxylase to 40% for fatty acid synthetase), which indicates that the substantial inhibition of lipogenesis may result from a coordinated depression of the activity of the various enzymes of the pathway. The changes in hepatic fatty acid synthesis also depend on the catalytic efficiency modulated by a number of metabolites or by phosphorylation/dephosphorylation of key enzymes such as acetyl CoA carboxylase. There are considerable diurnal changes in liver lipogenesis with both diets, whereas it has been shown with a high carbohydrate diet that lipogenic enzymes activities are not subjected to noticeable variations.[27] In this view, it has been shown that mRNA quantities of lipogenic enzymes vary in 1 day, but the diurnal variations have no measurable effects on the enzymes levels.[28] It may be because the half-lives of the enzymes are too long to be affected by the mRNAs, which were high only for a few hours.

Because of their high rate of hepatic uptake with a diet rich in resistant starch, volatile fatty acids could constitute a source of C2 units for lipogenesis. In contrast to propionate and butyrate, for which the activation takes place almost exclusively in the mitochondria, there are two forms of acetyl CoA synthetase: a mitochondrial one (Km ≈ 10 mM) and a cytosolic one sensitive to insulin (Km ≈ 0.1 mM). The relative importance of these two enzymes in acetate activation is still being discussed.[29,30] When acetate is activated in the cytosol, acetate should constitute an effective precursor for lipogenesis[31]; when activated in the mitochondria, acetyl CoA produced is preferentially used for citrate synthesis.[32] In contrast to ATP citrate lyase, the activity of the cytosolic form of acetyl CoA synthetase was slightly induced in rats fed the RS diet; this could correspond to the increased hepatic acetate uptake. Consequently, with this type of diet the acetyl CoA supply for lipogenesis could be mainly provided by acetate rather than by the glycolytic pathway.

Despite the marked reduction of hepatic lipogenesis, we have failed to put forward a hypotriglyceridemic effect of the resistant starch diet. This could be due to a lesser peripheral uptake of triglycerides with this diet, in accordance with the low insulinemia. Furthermore, it has been shown that the triglyceride secretion rate is less affected by a fiber diet than hepatic lipogenesis.[22]

Diets rich in resistant starch favor gluconeogenesis, which in turn affects hepatic lipid metabolism because lipogenesis and gluconeogenesis are strongly antagonistic. This antagonism concerns the utilization of substrates, the production of reduced equivalents (NADH, NADPH), the utilization of energy, and the insulin to glucagon ratio. The orientation of hepatic metabolism toward gluconeogenesis with a diet rich in resistant starch is confirmed by the inhibition of key glycolytic enzymes (glucokinase and pyruvate kinase) and by the marked stimulation of PEPCK during the postabsorptive period. Propionate is an effective gluconeogenic substrate in ruminants as well as in monogastric species[23] because: it is readily taken up and activated by the liver; it does not depend on the rate-controlling step of pyruvate carboxylase; and the net ATP requirement for the conversion of propionate into glucose is particularly low. In our nutritional conditions (RS diet), propionate was the major volatile fatty acid for liver metabolism because its hepatic uptake was almost quantitative, so it is probable that propionate may be the major glucogenic substrate removed by the liver, as previously shown in rats fed a high fiber diet.[24] Moreover, in the liver of rats adapted to a diet rich in resistant starch, the activity of PEPCK remained at a relatively high value during the absorptive period, suggesting a significant production of glucose from propionate, even in the fed state.

It has been frequently reported that nonavailable carbohydrates may depress plasma cholesterol, this effect being ascribed to an enhanced secretion of bile acids and/or to more general metabolic effects.[33,34] The replacement of a part of absorbed glucose by volatile fatty acids appeared insufficient to depress cholesterol in fed rats; nevertheless, a significant

hypocholesterolemic effect was observed during the postoperative period. Moreover, the present work shows an induction of HMG CoA reductase, the rate-limiting enzyme of cholesterol synthesis, in the liver of rats fed the diet rich in resistant starch. In these animals, the simultaneous high rate of propionate utilization and HMG CoA reductase activation does not favor a potent negative effect of propionate on this enzyme activity, as proposed by Chen et al.,[35] to explain the hypocholesterolemic effect of propionate. The stimulation of HMG CoA reductase with a diet rich in resistant starch probably corresponds to the net rise of fecal bile acid excretion observed with this diet. This proposal could explain why HMG CoA reductase activity is enhanced with a diet rich in resistant starch during the fed period, whereas cholesterolemia is poorly affected. This is in keeping with the observation that some soluble fibers are hypocholesterolemic but enhance hepatic cholesterol biosynthesis.[36]

In conclusion, the substitution of glucose by volatile fatty acids appears to favor hepatic gluconeogenesis at the expense of lipogenesis. Generally it has been assumed that hepatic fatty acid synthesis is tightly controlled by carbohydrate availability (which is generally depressed by high fiber diets). However, the possibility that volatile fatty acids have specific effects on lipogenesis could not be ruled out and deserves further investigations.

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