

Effects of isoenergetic high-carbohydrate compared with high-fat diets on human cholesterol synthesis and expression of key regulatory genes of cholesterol metabolism¹⁻³

Caroline Vidon, Philippe Boucher, Ana Cachefo, Odile Peroni, Frédérique Diraison, and Michel Beylot

ABSTRACT

Background: High-carbohydrate diets improve plasma cholesterol concentrations but increase triacylglycerol concentrations; the latter effect increases the risk of cardiovascular disease (CVD). Triacylglycerol concentrations increase only during very-high-carbohydrate diets consisting mainly of simple sugars.

Objective: We compared the CVD risk profile, cholesterol metabolism, and glucose tolerance of 7 healthy subjects during 2 isoenergetic diets: a high-fat, low-carbohydrate diet (HF diet) and a moderately high-carbohydrate, low-fat diet (HC diet).

Design: In a randomized crossover study, we measured the effects of the HF diet [40% carbohydrate and 45% fat (15% saturated, 15% monounsaturated, and 15% polyunsaturated)] and HC diet [55% carbohydrate (mainly complex) and 30% fat (10% saturated, 10% monounsaturated, and 10% polyunsaturated)] (3 wk each) on plasma lipid concentrations, oral glucose tolerance, cholesterol synthesis rate, and the messenger RNA (mRNA) concentrations of β -hydroxy- β -methylglutaryl coenzyme A (HMG-CoA) reductase, the LDL receptor, and the LDL-receptor-related protein (LRP).

Results: Compared with the HF diet, the HC diet lowered total, LDL, and HDL cholesterol ($P < 0.05$ for all) without modifying the ratio of LDL to HDL cholesterol; triacylglycerol concentrations were unchanged. Lower cholesterol concentrations occurred despite a higher cholesterol synthesis rate ($P < 0.05$) and higher HMG-CoA reductase mRNA concentrations ($P < 0.05$). LDL receptor mRNA concentrations were unchanged, LRP mRNA concentrations were lower ($P < 0.01$), and oral glucose tolerance was better ($P < 0.05$) with the HC diet.

Conclusion: The beneficial effects of the HC diet on glucose tolerance and plasma cholesterol concentrations without increases in triacylglycerol show that this diet had favorable effects on both insulin sensitivity and the plasma lipid profile. *Am J Clin Nutr* 2001;73:878–84.

KEY WORDS Triacylglycerol, atherosclerosis, cardiovascular disease, cholesterol synthesis, HDL cholesterol, LDL cholesterol, LDL-receptor-related protein, LDL receptor, β -hydroxy- β -methylglutaryl coenzyme A reductase, messenger RNA, glucose tolerance, high-carbohydrate diet, stable isotopes

INTRODUCTION

Atherosclerosis is one of the most common causes of death in Western societies. Therefore, reducing risk factors, such as elevated concentrations of plasma total and LDL cholesterol (1), is a major public health goal. Clinical trials of prevention of coronary heart disease (CHD), particularly with β -hydroxy- β -methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, showed the beneficial effect of reducing blood cholesterol concentrations (2). Such reductions can also be obtained, at least in part, by dietary modification, which is a less expensive approach that is suitable for larger cohorts of subjects (3). Differences in mean cholesterol concentrations and incidence of CHD between populations with different dietary habits provide evidence of the importance of diet (4).

General guidelines for dietary advice have been established, but the mechanisms responsible for the decreases in cholesterol concentrations are not completely understood and probably differ between dietary constituents, eg, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) (3). A major goal of such dietary recommendations is to decrease intake of total fat, especially of saturated fatty acids (SFAs), and therefore to increase carbohydrate intake. In addition, higher carbohydrate consumption as a percentage of total energy could have beneficial effects on glucose tolerance, reducing the likelihood of diabetes, another risk factor for atherosclerosis. However, contradictory results on this point were reported (5). Low-fat, high-carbohydrate diets can also induce a rise in plasma triacylglycerol concentrations (6), which is an undesirable side effect because elevated triacylglycerol concentrations are also an independent risk factor for CHD (7). These increases in plasma triacylglycerol were observed when subjects consumed diets very high in

¹From INSERM U 499, Université RTH Laennec, Laboratoire de Biochimie and the Centre de Recherche en Nutrition Humaine, Hôpital E Herriot, Lyon, France.

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³Address reprint requests to M Beylot, INSERM U 499, Université RTH Laennec, Rue Guillaume Paradin, 69008 Lyon, France. E-mail: beylot@laennec.univ-lyon1.fr.

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carbohydrates (65–70% of energy intake) and, with some exceptions (8), including mainly simple carbohydrates such as glucose or fructose. These diets were therefore quite different from the usual dietary recommendations.

Our aim was to determine in human subjects whether a moderate increase in the dietary carbohydrate-fat ratio, with a high percentage of complex carbohydrates and without any variations in the relative percentages of SFAs, MUFAs, and PUFAs, has beneficial effects on glucose tolerance and cholesterol concentrations without increasing plasma triacylglycerol concentrations. In addition, to gain some insight into the mechanisms responsible for the modification of cholesterol concentrations, we measured the fractional synthetic rate (FSR) of cholesterol and the expression of genes controlling key steps in its metabolism. We measured the messenger RNA (mRNA) concentrations of HMG-CoA reductase [the key regulatory enzyme of cholesterol synthesis (9)] and of the LDL receptor and the LDL-receptor-related protein (LRP), both of which are involved in the clearance of plasma cholesterol (10–13). These measurements were performed in blood mononuclear cells because it was shown that basic regulatory mechanisms are similar in these cells and in hepatocytes, at least for HMG-CoA reductase and the LDL receptor (14, 15).

SUBJECTS AND METHODS

Materials

Deuterated water (99% mole percent excess) was obtained from Cambridge Isotope Laboratory (Andover, MA). Chemicals and reactants were obtained from Sigma Chemical Co (St Louis), Boehringer Mannheim (Mannheim, Germany), or Pierce Chemical Co (Rockford, IL).

Subjects

We enrolled 7 healthy volunteers (4 women and 3 men) aged 22–48 y with a body mass index (in kg/m²) of 20–27. No subject had a personal or family history of diabetes or obesity or was taking any medication; all had normal results from physical examinations and normal plasma glucose and lipid concentrations. Subjects with unusual dietary habits were excluded.

The protocol of the study was approved by the Ethical Committee of Lyon and by the Institut National de la Santé et de la Recherche Médicale. The study was conducted according to the Hurriet law, and all subjects provided written, informed consent. All tests were performed in the Centre de Recherche en Nutrition Humaine of Lyon.

Methods

All subjects were studied twice, once after following a high-fat, low-carbohydrate diet (HF diet) for 3 wk and once after following a moderately high-carbohydrate, low-fat diet (HC diet) for 3 wk. The 2 diets were isoenergetic. The HF diet provided 45% of total energy as lipids (15% each of SFAs, MUFAs, and PUFAs) and 40% of energy as carbohydrates (same proportions of simple and complex carbohydrates as in the HC diet). The HC diet provided 55% of total energy as carbohydrates (20–25% simple and 30–35% complex carbohydrates) and 30% as fat (10% each of SFAs, MUFAs, and PUFAs). Cholesterol intake was the same during both diets. The sources of SFA were mainly butter, cheese, and meat. Intakes of *trans* fatty acids were <500 mg during both diets. Subjects received 3 meals/d.

For each subject, ≥ 4 mo elapsed between the times when the 2 diet periods began. This interval was necessary because of the long half-lives of body water and of plasma cholesterol. The order of the 2 diets was randomized. For women, the tests were performed during the first 10 d of the menstrual cycle to take into account the known variations in lipogenesis during the menstrual cycle; there are no variations in cholesterol synthesis during the menstrual cycle (16). All diet instruction was provided by a dietitian, who met with each subject before the first diet period to obtain a report of the usual diet and to establish the subject's energy requirement. The dietitian also met with each subject during and at the end of each diet period to teach subjects how to choose the correct foods. A detailed report of each subject's dietary intake during the last week of each diet period was obtained. All subjects abstained from alcohol and heavy physical activity during the week before the blood tests.

The night before the tests, each subject consumed his or her last meal between 1900 and 2000 and drank a loading dose of deuterated water (3 g/kg body water), consuming one-half after the evening meal and one-half at 2200. Thereafter until the end of the study, subjects drank only water enriched with ²H₂O (4.5 g ²H₂O/L drinking water). All tests were initiated in the postabsorptive state after an overnight fast. At 0730, an indwelling catheter was placed in a forearm vein for blood sampling. Three blood samples were drawn at 15-min intervals. For glucose, insulin, glucagon, and triacylglycerol, we obtained and averaged 3 measurements for each subject. For the other metabolites and hormones, only one measurement was made for each subject. Blood was also analyzed to determine enrichment measurements, and circulating monocytes were separated. At 0800, subjects underwent an oral-glucose-tolerance test in which they ingested 75 g glucose. Blood samples were then collected during the subsequent 5 h to follow changes in glucose and insulin concentrations.

Analytic procedures

Metabolites were assayed with enzymatic methods by using neutralized perchloric extracts of plasma for glucose analysis (17) and by using plasma for fatty acid and triacylglycerol analyses (18). Plasma insulin and glucagon concentrations were determined by radioimmunoassay (19, 20). Total and free cholesterol were measured with enzymatic assays; the HDL fraction was measured as described previously (21). Plasma lipids were extracted by using the method of Folch et al (22). Free cholesterol was separated by thin-layer chromatography, scraped off the silica plates, and eluted from silica with ether before preparing its trimethylsilyl derivative (23, 24). Deuterium enrichment was determined with 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 helium. Ions 368 to 370 were selectively monitored. Special care was taken to obtain comparable ion peak areas between standard and biological samples; we adjusted the volume injected or diluted the sample when necessary. Deuterium enrichment in plasma water was measured by using the method of Yang et al (25).

Measurement of LDL receptor and HMG-CoA reductase mRNA concentrations

Mononuclear cells were immediately isolated by centrifugation of whole venous blood on a Ficoll gradient at 250 × g for

15 min at 4°C as described previously (26). The isolated cells were then stored at -80°C. Total RNA was prepared from frozen samples as described previously (21). Total RNA was then quantified by electrophoresis on 1% agarose gel; serial dilutions were compared with known amounts of standard RNA (Boehringer Mannheim). LDL receptor and HMG-CoA reductase mRNA copy numbers were determined by competitive reverse transcriptase-polymerase chain reaction (RT-PCR). Total cellular RNA was reverse transcribed into cDNA in the presence of a dilution series of AW 109 cRNA (Perkin Elmer Cetus Instruments, Courtaboeuf, France), an internal standard that contains primer sites for the LDL receptor and HMG-CoA reductase. Reactions occurred in a final volume of 100 µL containing 10 mmol tris-HCl/L (pH 8.3), 50 mmol KCl/L, 2.5 mmol MgCl₂/L, 150 µmol of each of the 4 deoxyribonucleoside triphosphates/L (Pharmacia LKB Biotechnology Inc, Saint-Quentin sur Yvelines, France), and 0.65 µmol LDL receptor or HMG-CoA reductase downstream primers/L. Primer sequences were identical to those described by Powell and Kroon (15).

The RT-PCR protocol consisted of 1 cycle at 42°C for 40 min followed by 33 cycles at 94°C for 2 min, 58°C for 1 min, and 72°C for 3 min. All PCRs included a negative control. The absence of genomic DNA contamination in the RNA samples was confirmed by the use of RT-negative RNA samples. PCR products were analyzed on 2% agarose gel stained with ethidium bromide. For quantitation, densitometric scanning of photographic images was performed and the relative amounts of target and competitor products in each sample were compared. The initial amounts of target and competitor were assumed to be equal when the molar ratio of target to competitor was 1. The results were expressed as copy number/µg total cellular RNA.

Measurement of LRP mRNA concentrations

LRP mRNA concentrations were measured by using semi-quantitative RT-PCR. LRP and the ubiquitous glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were simultaneously amplified in each assay tube as described previously (21). Reactions were performed by using the following LRP primer sequences: 5'-CTCAGGTACGTGGCCAAGAT-3', located at position 1311-1330, and 5'-CGAGTTGGTGGACTA GAGAT-3', located at position 1730-1750 according to the LRP gene sequence published previously (27). GAPDH primers were those used by Dante et al (28). Regarding the specificity of the RT-PCR assay, we confirmed the authenticity of PCR products by DNA sequence analysis. RT-PCR was also performed by using a serial dilution of total RNA in sterile water; this indicated that LRP mRNA expression was linear for total RNA amounts ranging from 10 to 200 ng. PCR products were analyzed on agarose gel stained with ethidium bromide. For quantitation of relative band intensities, densitometric scanning was performed and the ratio of LRP to GAPDH was determined for each sample. All PCRs included a negative control; the absence of genomic DNA contamination in the RNA samples was confirmed by using RT-negative RNA samples.

Calculations

The fractional contribution of cholesterol synthesis to plasma free cholesterol was calculated from the deuterium enrichments in free cholesterol and in plasma water as described previously (23, 24). In short, the deuterium enrichments that would have been obtained if endogenous synthesis

were the only source of plasma cholesterol were calculated from plasma water enrichment. The comparison of the actual enrichments observed with these theoretical values gives the contribution, expressed as the FSR, of endogenous synthesis to the pool of rapidly exchangeable free cholesterol during the 12-h interval between ingestion of the loading dose of deuterated water and blood sampling. An important assumption (3), and possible limitation, in these calculations of cholesterol synthesis is that the number of incorporation sites of deuterium in the molecule synthesized is not modified significantly by diet. This value was then transformed as the absolute synthetic rate (ASR), expressed in mg/12 h. For this calculation, we first calculated the total pool of rapidly exchangeable cholesterol (M_1) according to the equation of Goodman et al (29). ASR was then calculated as $ASR = FSR \times M_1$. Because M_1 comprises both esterified and free cholesterol, and we found deuterium enrichment only in free cholesterol, we calculated the ASR in the rapidly exchangeable free cholesterol pool, estimating that the ratio in plasma of free to total cholesterol concentrations represents this ratio in the whole pool.

Statistical analyses

The results are shown as means ± SEMs. We compared values measured at the ends of the 2 diet periods with two-tailed Student's *t* tests for paired values, except for glucose and insulin values during the oral-glucose-tolerance test, which were analyzed with two-way analysis of variance followed by *t* tests with Bonferroni correction. Results were analyzed with EXCEL (version 4.0; Microsoft, Redmond, WA).

RESULTS

Dietary intake

The actual dietary intakes as calculated from the diet survey performed during the last week of each diet period are shown in **Table 1**. Total energy intake was slightly but not significantly ($P > 0.30$) higher during the HF diet, and body weight increased slightly during the HF diet but was unchanged during the HC diet. Subjects consumed the expected amounts of fat and carbohydrates and the intended proportions of SFAs, MUFAs, and PUFAs. Dietary intakes of cholesterol were comparable during the 2 diets. During the HC and HF diets, fiber intake was 27.7 ± 1.5 and 23.1 ± 1.4 g/d, respectively ($P < 0.05$), and fructose intake was 20.2 ± 4.2 and 17.9 ± 1.8 g/d, respectively.

TABLE 1
Dietary intakes during the 2 diet periods¹

Intake	High-carbohydrate diet	High-fat diet
Energy (kJ · kg ⁻¹ · d ⁻¹)	128.0 ± 9.2	141.0 ± 5.0
Protein (% of energy)	14.0 ± 0.4	15.1 ± 0.3
Carbohydrate (% of energy)	55.0 ± 0.3	40.9 ± 0.9
Fat (% of energy)	30.9 ± 0.3	44.0 ± 0.9
Fatty acids		
Saturated (g/d)	20.8 ± 1.5	30.9 ± 1.3
Monounsaturated (g/d)	21.2 ± 1.8	32.8 ± 1.4
Polyunsaturated (g/d)	21.9 ± 1.4	30.7 ± 1.2
Cholesterol (mg/d)	157 ± 17	178 ± 14

¹ $\bar{x} \pm SEM$; $n = 7$.

TABLE 2

Plasma hormone and metabolite concentrations measured in the basal postabsorptive state¹

	High-carbohydrate diet	High-fat diet
Glucose (mmol/L)	4.29 ± 0.15	4.84 ± 0.11 ²
Insulin (pmol/L)	47 ± 7	65 ± 7 ²
Glucagon (ng/L)	170 ± 27	146 ± 29
Triacylglycerol (mmol/L)	0.67 ± 0.08	0.68 ± 0.15
Total cholesterol (mmol/L)	4.78 ± 0.19	5.41 ± 0.21 ²
Free cholesterol (mmol/L)	1.18 ± 0.19	1.16 ± 0.14
LDL cholesterol (mmol/L)	3.00 ± 0.13	3.42 ± 0.17 ²
HDL cholesterol (mmol/L)	1.37 ± 0.10	1.59 ± 0.09 ²

¹ $\bar{x} \pm \text{SEM}$; $n = 7$.

²Significantly different from high-carbohydrate diet, $P < 0.05$ (Student's t test).

Metabolites and hormones

Concentrations in the basal, postabsorptive state are shown in **Table 2**. Glucose and insulin concentrations were significantly higher after the HF diet than after the HC diet. Concentrations of total cholesterol, but not of free cholesterol, were significantly lower after the HC diet than after the HF diet. Both the HDL and LDL fractions were significantly lower after the HC diet than after the HF diet, but the ratio of HDL to LDL cholesterol did not differ significantly between the 2 diets. Plasma triacylglycerol concentrations did not differ significantly between the HF and HC diets. During the oral-glucose-tolerance test, both glucose and insulin rose to higher concentrations after the HF diet than

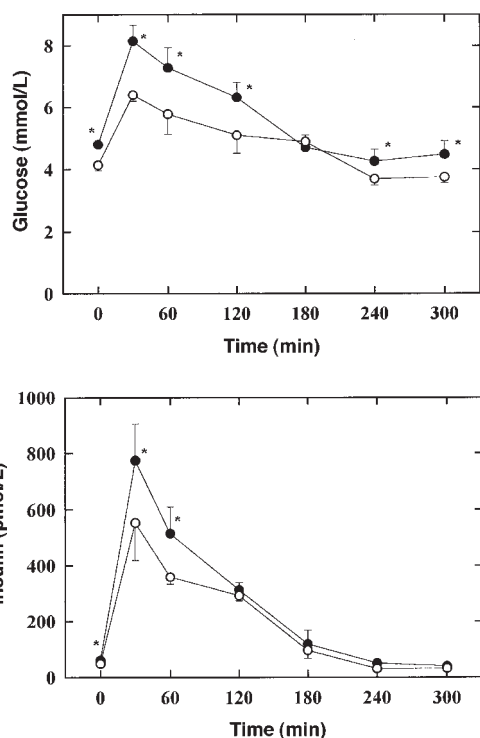


FIGURE 1. Evolution of plasma glucose and insulin concentrations during the oral-glucose-tolerance tests performed after 3 wk of the high-fat (●) and high-carbohydrate (○) diets. *Significantly different from the high-carbohydrate diet, $P < 0.05$. $n = 7$.

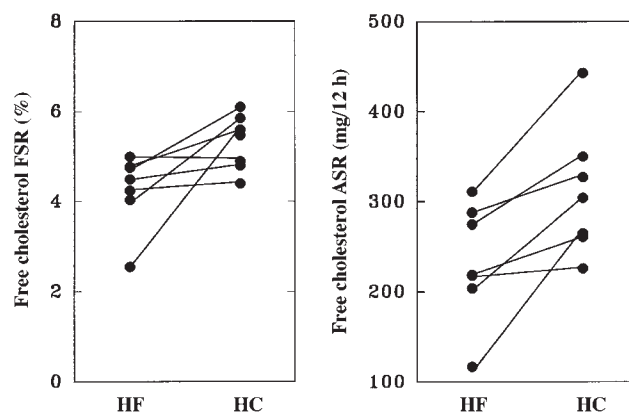


FIGURE 2. Individual values ($n = 7$) for plasma free cholesterol fractional synthetic rate (FSR) and absolute synthetic rate (ASR) measured in the postabsorptive state at the end of the high-fat (HF) and high-carbohydrate (HC) diets.

after the HC diet, showing lower glucose tolerance and insulin sensitivity with the HF diet (**Figure 1**).

Cholesterol metabolism

Deuterium enrichments in plasma water were $0.32 \pm 0.02\%$ and $0.33 \pm 0.01\%$ during the HC and HF diets, respectively. The corresponding enrichments in plasma free cholesterol were $0.45 \pm 0.03\%$ and $0.38 \pm 0.03\%$, respectively. These enrichments were higher than those in plasma water because there are multiple possible incorporation sites (27 sites) of deuterium in the molecule of cholesterol during its synthesis. During the interval between the 2 diets, the abundance of deuterium in plasma water returned to basal values, whereas some enrichment persisted in cholesterol; the values observed in cholesterol after the second dose of deuterated water were corrected accordingly.

The individual FSRs and ASRs of plasma free cholesterol in the postabsorptive state measured at the ends of the 2 diet periods are shown in **Figure 2**. The FSR was moderately higher after the HC diet than after the HF diet ($5.29 \pm 0.24\%$ compared with $4.26 \pm 0.31\%$, $P < 0.05$). When calculated with the total pool of rapidly exchangeable cholesterol, the ASRs during the period of the study (12 h) were 1091 ± 115 and 1286 ± 112 mg during the HF and HC diets, respectively ($P = 0.06$). This difference was highly significant when the ASR was calculated for the fraction of free cholesterol in this pool (233 ± 25 and 311 ± 27 mg after the HF and HC diets, respectively, $P < 0.01$).

The concentrations of the different types of mRNA measured in circulating mononuclear cells are shown in **Figures 3** and **4**. These results are shown as absolute values for HMG-CoA reductase and LDL receptors and as relative values for LRP. We measured higher HMG-CoA reductase mRNA concentrations ($P < 0.05$) and lower LRP mRNA concentrations ($P < 0.01$) during the HC diet than during the HF diet. There were no significant differences in LDL receptor mRNA concentrations between the 2 diets.

DISCUSSION

The present study compared the effects of isoenergetic HC and HF diets on glucose tolerance and some aspects of lipid

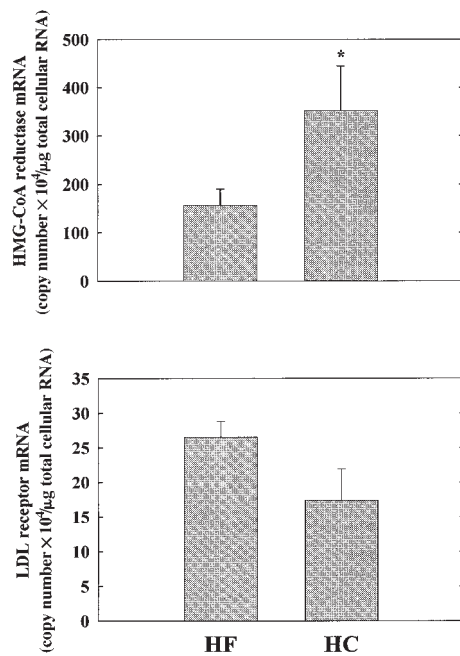


FIGURE 3. Mean (\pm SEM) messenger RNA (mRNA) concentrations of β -hydroxy- β -methylglutaryl coenzyme A (HMG-CoA) reductase and LDL receptor in circulating mononuclear cells after the high-fat (HF) and high-carbohydrate (HC) diets. *Significantly different from the HF diet, $P < 0.05$. $n = 7$.

metabolism. The HC diet was low in fat, whereas the HF diet was low in carbohydrates. Contrary to most previous studies (6, 8, 30, 31), the fat-carbohydrate ratios of both the HC and HF diets in the present study were moderate and were within the range usually observed in the general population; in addition, the HC diet was low in simple sugars. We were also careful to control the percentages of total fat intake as SFAs, MUFAs, and PUFAs, because variations in these percentages can alter lipid metabolism (32–36).

We observed beneficial effects of the HC diet compared with the HF diet with respect to plasma total and LDL-cholesterol concentrations and glucose tolerance. These beneficial effects occurred without any increases in plasma triacylglycerol concentrations or in the LDL-HDL cholesterol ratio. Thus, these results provide additional support for recommending a low-fat, high-carbohydrate diet rich in complex carbohydrates for the prevention of risk factors for CHD.

Previous studies found increases in plasma triacylglycerol concentrations during high-carbohydrate diets (6, 8, 30, 31, 37). These increases in triacylglycerol were ascribed to increased secretion (8, 30, 31, 37) and decreased clearance rates (8) (for a review, *see* 38). However, these studies evaluated the effects of diets that had very high ratios of carbohydrate to fat and, with some exceptions (8, 37), that were rich in simple sugars. We found no rise in plasma triacylglycerol concentrations when healthy subjects changed from the HF diet to the HC diet, which was rich in complex carbohydrates and had a moderately higher ratio of carbohydrates to fat. Although previous studies with rather unphysiologic diets provided important information on some aspects of metabolic regulation, their relevance to more physiologic and usual variations in fat and carbohydrate intakes

is limited. One could argue that the moderately higher fiber intake observed in our study during the HC diet than during the HF diet also may have played a role in the lack of increase in plasma triacylglycerol concentrations. However, in a study by Parks et al (8), a much more substantial increase in fiber intake did not prevent a large increase in plasma triacylglycerol concentrations, making this hypothesis unlikely.

The reductions in total and LDL-cholesterol concentrations that we observed during the HC diet are consistent with the findings of previous studies (3, 34). An exception is the study by Parks et al (8), who did observe a reduction in LDL cholesterol but found no reduction in total cholesterol after their subjects consumed a high-carbohydrate diet. The lack of decrease in total cholesterol was explained by a rise in VLDL cholesterol, which was probably related to increases in total and VLDL triacylglycerol. Taken together, these results highlight the importance of preventing any increase in triacylglycerol concentrations as a goal of dietary advice to reduce the risk factors for CHD.

The mechanisms leading to decreases in plasma cholesterol concentrations during high-carbohydrate diets remain unclear. In our study, the small decrease in cholesterol intake could have played a role. However, previous reports showed that such small variations in cholesterol intake are not likely to have significant effects on plasma cholesterol (3). The decrease in plasma cholesterol concentrations that we observed was not explained by decreased synthesis and secretion in the plasma pool; to the contrary, we found a moderate increase in cholesterol synthesis. This increased synthesis was associated with higher concentrations of HMG-CoA reductase mRNA, suggesting that it resulted from not only an increased supply of acetyl coenzyme A available for synthesis but also from an increase in the expression of key regulatory genes of cholesterol synthesis during the HC diet. Because synthesis and secretion in the plasma pool increased, it appears that the lower plasma cholesterol concentrations were related to increased removal from plasma. However, the mRNA concentrations of 2 main lipoprotein receptors that control the clearance of cholesterol from plasma were unchanged (LDL receptors) or decreased (LRP). In the absence of direct measurement, increased activity of these receptors despite lower mRNA concentrations cannot be excluded.

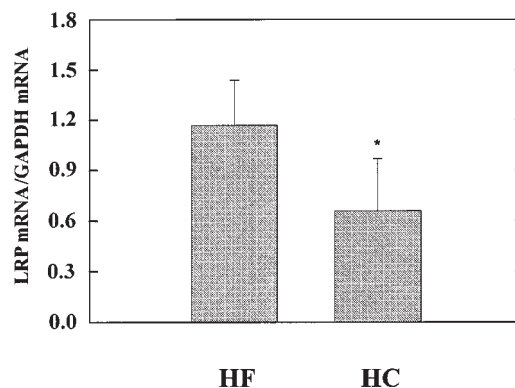



FIGURE 4. Mean (\pm SEM) messenger RNA (mRNA) concentrations of LDL-receptor-related protein (LRP), expressed relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, in circulating mononuclear cells after the high-fat (HF) and high-carbohydrate (HC) diets. *Significantly different from the HF diet, $P < 0.01$. $n = 7$.

Another possibility would be an increase in the expression, activity, or both of other receptors such as those responsible for the uptake of HDL cholesterol (eg, SR-B1) (39). The difference in variations of mRNA concentrations between the LDL receptor and HMG-CoA reductase is surprising because variations in these mRNA concentrations are usually parallel and the expressions of both genes are thought to be regulated in a comparable way, ie, mainly through the transcription factor SREBP-2 (40). However, other transcription factors intervene in these regulatory processes, at least for the LDL receptor (41), and therefore might contribute to such differences in the variations of mRNA concentrations. The regulation of LRP gene expression is still poorly understood. Stimulatory effects of insulin (42) and glucocorticoids (43) were shown. It is thus conceivable that the observed difference in insulinemia between the 2 diet periods in this study, although small, may have played a role in the variations of LRP mRNA. The LRP promoter contains no sterol regulatory element (44); however, there are data showing upregulation of LRP by dietary cholesterol (21). Therefore, we cannot exclude the possibility that the moderate decrease in cholesterol intake during the HC diet could have contributed to the decrease in LRP mRNA concentration.

Lastly, glucose tolerance improved during the HC diet compared with the HF diet, despite a lower response of insulin to the oral glucose load. This finding, together with the results for basal glucose and insulin concentrations, suggests enhanced sensitivity to insulin during the HC diet compared with the HF diet. There are conflicting data on the effects of high-carbohydrate diets on insulin sensitivity in humans (5), with the overall result depending on the subjects studied (control or insulin-resistant subjects) and the amount and type (simple or complex) of carbohydrate used. An important factor in controlling the modifications of glucose tolerance and insulin sensitivity during high-carbohydrate diets could be whether hypertriglyceridemia is induced, because there is evidence that elevated plasma triacylglycerol concentrations can contribute to the development of insulin resistance (5, 45). Our observation of improved glucose tolerance when plasma triacylglycerol concentrations did not increase during the HC diet support this idea. Again, this indicates that controlling triacylglycerol concentrations should be an important goal, along with the goal of lowering total and LDL cholesterol concentrations, when giving dietary recommendations to reduce CHD risk. 

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