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ORIGINAL CONTRIBUTION

Effect of carrot intake on cholesterol metabolism and on antioxidant status in cholesterol-fed rat

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Summary *Background* Vegetables are major dietary sources of fibers and antioxidants such as carotenoids, polyphenols and vitamin C which contribute to explain their protective effects against cardiovascular diseases. *Aim of the study* We investigated in the rat the effects of a 3-week supplementation of the diet with carrot (15 % dry matter) on lipid metabolism and antioxidant status. *Results* A significant decrease of cholesterol level in liver (–44 %; $P = 0.0007$) was observed together with a reduction of the level of liver triglycerides (–40 %; $P = 0.0005$). Fecal total steroids excretion increased by 30 % upon feeding the carrot diet as compared to the control. The secretion of bile acids was maintained, whereas the cholesterol apparent absorption was reduced in rats fed carrot diet. Carrot consumption also improved the antioxidant status. It significantly decreased the urinary excretion of thiobarbituric acid reactive substances (TBARS), reduced the TBARS levels in heart, increased the vitamin E plasmatic level and tended to increase the ferric reducing ability of plasma (FRAP) as compared to the controls. The carrot diet provided carotenoid antioxidants: 5.1 mg β-carotene, 1.6 mg α-carotene and 0.25 mg lutein per 100 g diet. No carotenoids were found in plasma whereas the three carotenoids were detected in the plasma of the rats fed the carrot diet at 125, 41, 43 nmol/L respective concentrations. β-Carotene was also detected in liver and heart. *Conclusion* Carrot consumption modifies cholesterol absorption and bile acids excretion and increases antioxidant status and these effects could be interesting for cardiovascular protection.

Key words carrot – carotenoids – antioxidants – lipemia

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Introduction

Numerous studies support the view that diets rich in fruit and vegetable may protect against various pathologies, especially cardiovascular diseases (CVD) and cancers [1–4]. Epidemiological studies on vegetables, including one on smokers, underline the specific effect of carrot consumption on reduction of lung cancer [5]. Carrot is one of the major vegetables consumed in the world whatever the season. It represents about 10 % of the vegetables consumed in France. Its consumption has been increasing regularly in Western countries, particularly for cooked carrots [6] (Société d'étude de la consommation, distribution et publicité, Centre Technique Interprofessionnel des Fruits et Légumes investigation, 2000).

Carrot is one of the richest vegetables in fibers and carotenoids which are associated respectively to cholesterol metabolism and antioxidant protection. Fibers represent 3–4 % of carrot weight divided in soluble (48 %) and insoluble fiber (52 %). The effect of various fibers or related compounds on cholesterol metabolism has been extensively studied, and it is generally accepted that viscous water-soluble fractions are the most effective in lowering plasma cholesterol concentrations [7, 8].

EJN 419

Carrot is also a valuable source of carotenoids (5.6 mg/100 g). It also contains other antioxidants such as vitamin E (513 µg/100 g), vitamin C (7 mg/100 g) and phenolics such as *p*-coumaric, chlorogenic and caffeic acids [9]. This diversity of micronutrients may explain the positive effects on health associated with carrot consumption. Antioxidant micronutrients are associated with a reduction of the cardiovascular risk possibly explained by the protection of lipoproteins from peroxidation [10]. Antioxidants such as vitamin C, tocopherols, carotenoids and polyphenols are able to quench free radicals and together with endogenous systems of defense, they limit oxidative stress and reduce the risk of associated degenerative diseases [11–14].

We examined in the rat the effects of carrot consumption on lipid metabolism and antioxidant status. Rats were fed a semi-purified diet rich in carrot for three weeks. Lipids, bile acids and short chain fatty acids (SCFA) were estimated in different tissues and feces. Vitamin E and different markers of antioxidant status (FRAP, TBARS) were assayed in urine, plasma, and in heart and liver tissues. The respective contributions of carrot antioxidants and fibers to the different biological effects observed are discussed.

Material and methods

Animals and diets

Male Wistar rats (about 150 g each) were obtained from the colony of laboratory animals of the National Institute of Agronomic Research (INRA, Clermont-Ferrand/Theix, France). Rats were housed two per cage in a room maintained at 22 °C with a 12 hours light-dark cycle (light from 8:00 to 20:00 h) with access to food from 16:00 to 8:00 h. They were all fed a semi-purified diet from UPAE (INRA, Jouy-en-Josas, France) for 6 days before the beginning of the experiment. After this period, rats were randomized in two groups and fed *ad libitum* for 3 weeks either a control diet or a carrot enriched diet (Table 1). Carrots were purchased from the local supermarket. The freeze-dried carrot accounted for 15 % (dry matter) of the total diet and provided 0.25 ± 0.02, 1.6 ± 0.12 and 5.1 ± 0.44 mg/100 g dry matter of lutein, α-carotene and β-carotene, respectively. During the last week of the experimental period rats were housed in metabolic cages for urine and feces collection. Daily food consumption and body weight were recorded twice a week. Animals were maintained and handled according to the recommendations described in the "Principles of laboratory animal care" (NIH publication N° 86–23, revised 1985). The experiment was also approved by the Institutional Ethic Committee (INRA).

Table 1 Composition of diets

Components (g/kg diet)	Minus carrot	15 % Carrot
Casein	160	160
Wheat starch	657.5	586.5
Sucrose	30	/
Glucose	20	/
Fructose	20	/
Mineral mixture[1]	50	42.5
Vitamin mixture[2]	10	8.5
Corn oil	50	50
Cholesterol	2.5	2.5
Carrot[3]	/	150

[1] Mineral mixture AIN-93M (per kg of diet): Ca HPO_4 15 g; K_2HPO_4 2.5 g; KCl 5 g; NaCl 5 g; $MgCl_2$ 2.5 g; Fe_2O_3 2.5 mg; $MnSO_4$ 125 mg; $CuSO_4$ 125 mg; $ZnSO_4 \cdot 7H_2O$ 100 mg; KI 0.4 mg.

[2] Vitamin mixture (mg/kg of diet): thiamin (15), riboflavin (20), pyridoxine (10), nicotinamide (100), pantothenate (70), folic acid (5), biotin (0.3), cyanocobalamin (0.05), retinyl palmitate (1.5), dl-a-tocopheryl acetate (42), cholecalciferol (0.15), menadion (1.5), ascorbic acid (50), myo-inositol (100), choline (1360). The mixture was prepared by mixing two AIN-76A preparations purchased from UAR, one with α-tocopherol and one without, which was further supplemented with choline.

[3] Carrot composition (% of dry weight): sucrose 20 %; glucose 13.5 %, fructose 13.5 %, fiber 37 %, protein 7 %, mineral 3 %, lipid 2 %

Sampling procedures

Rats were anesthetized during the post absorptive period [8:00–10:00], when the cecal fermentation is still active, by sodium pentobarbital intraperitoneal injection (40 mg/kg of body weight). Blood (6 mL) was drawn from the abdominal aorta into heparinized tubes and centrifuged at 12,000 g for 2 min. Plasma samples were either stored at 4 °C for lipid and lipoprotein analysis or immediately frozen at –80 °C for antioxidant assay.

After blood sampling, the cecum (wall with contents) was removed and weighed. The cecal wall was flush cleaned with water, dried and weighed (cecal wall weight). Samples of cecal contents were collected, immediately frozen at –20 °C. Supernatants were obtained by centrifuging the microtubes at 20,000 g for 10 min at 4 °C for short-chain fatty acid (SCFA) analysis.

The liver was freezed-clamped and stored at –80 °C for the measurement of lipid and carotenoid contents and for peroxidation assay. Heart was rapidly washed in physiological serum and immediately stored at –80 °C for lipid peroxidation assay.

Analytical procedures

SCFA were measured on aliquots of cecal supernatants by gas-liquid chromatography as previously described [15].

Bile acids and neutral steroids were extracted twice

from feces at 70 °C for 2 hours with 40 volumes of alkaline ethanol (KOH 4 mmol/L). Bile acids were quantified using the reaction catalyzed by 3 α-hydroxysteroid dehydrogenase (EC 1.1.1.50; Sigma Chemical Co., L'Isle d'Abeau Chesnes, France) [16]. Neutral steroids (100 µL) were extracted three times with hexane (500 µL) after addition of 5 α-cholestane (internal standard, Sigma St Louis). The hexane extract was concentrated to 200 µL and 2 µL were injected into the gas chromatograph (Danieducational, Paris, France) fitted with a 12 m x 0.25 mm fused silica capillary column (BP 10) and a flame-ionization detector. Helium was used as a carrier gas, and an isocratic temperature (260 °C) was used for the steroid separation. Sterol concentrations were calculated from the peak area relative to the area of the internal standard.

Plasma total cholesterol concentration was enzymatically determined using a kit purchased from BioMerieux (Charbonnières-les-bains, France) and plasma triglyceride concentrations were determined using a kit from Biotrol (Paris, France). Liver lipids were extracted with chloroform/methanol (2:1, v/v) according to the method previously described [8].

Plasma lipoproteins were separated by ultracentrifugation (100,000 g for 24 hours at 15 °C) of 1 ml plasma samples on a density gradient of potassium bromide. The gradient was divided into 24 fractions of 500 µL and cholesterol and triglyceride content of each fraction were determined as described above for plasma samples. Results were expressed for pools with $d < 1.040$ kg/L (chiefly triglyceride-rich lipoprotein: TGRLP, with a minor contribution of LDL) and $d > 1.040$ kg/L fraction (essentially HDL).

Carotenoids and vitamin E were analyzed by HPLC-UV [17]. Briefly, carotenoids were extracted twice from plasma after addition of echinenone (internal standard) by 2x2 volumes of hexane. Tissues were previously homogenized with potter in 3 volumes of PBS 1X before hexane extraction. The separation was carried out on a Vydac TP54 (250 x 4.6 mm; Hesperia, CA) and a Nucleosil column (150 x 4.6 mm; Interchim, Montluçon, France) in series. Elution was performed with an isocratic mobile phase: acetonitrile/methanol containing 50 mM ammonium-acetate/dichloromethane/water (70/15/10/5: v/v/v/v), at a constant flow of 2 mL/min. Carotenoids were analyzed in the diets and in feces as follows: aliquots (100 mg) were treated with a mixture of 7 mL methanol containing 0.04 g of magnesium carbonate, 7 mL trichloromethane and 5 mL water and the internal standard. The organic phase was removed and the remaining phase extracted again by a mixture of dichloromethane (5 mL), tetrahydrofuran (5 mL) and water (3 mL). The two organic phases were pooled, evaporated and resuspended in acetonitrile/dichloromethane for HPLC analysis.

Ferric reducing ability of plasma (FRAP) was determined on 100 µL plasma samples diluted 1:2 and the tripyridyltriazine complex formed with the reduced ferrous ions were measured by spectrofluorimetry (LS 5, Perkin Elmer, Norwalk, CT) [18].

The levels of TBARS in urine samples were measured by the modified procedure of Lee et al. [19] by reading absorbance at 535 nm. The amounts of TBARS in urine were expressed as equivalents of malondialdehydes (MDA) and corrected by creatinine values. Results were determined as nmol/mg creatinine excreted with creatinine being measured with the kit purchased from BioMerieux (Charbonnières-les-bains, France).

MDA was also determined in heart homogenates by measuring the formation of TBARS upon induction of oxidation by a mixture with 2 mmol/L $FeSO_4$ and 50 mmol/L of ascorbic acid for 30 min at 37 °C in an oxygen-free medium [20].

Statistical analysis

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

Results

Effects of carrot diet on food intake and on symbiotic fermentation in the rat cecum

The incorporation of 15 % (w/w) freeze-dried carrot did not alter either growth, the body weight gain was similar in both groups (7.4 ± 0.4 g/day, *i.e.*, from 184.4 ± 3.7 to 340.3 ± 12.2 g in controls rats *vs* 7.1 ± 0.4 g/day, *i.e.*, from 182.5 ± 1.6 to 331.0 ± 8.4 g in rats fed carrot diet), or daily food intake (21.2 ± 0.7 *vs* 20.7 ± 0.8 g of dry matter/day). The fecal excretion was significantly higher in rats fed the carrot diet as compared to those fed the control diet (0.85 ± 0.11 *vs* 0.45 ± 0.05 g of dry matter/day; $P < 0.01$). The carrot diet led to a 20 % increase (1.00 ± 0.03 *vs* 0.84 ± 0.04 $P = 0.008$) of the cecal wall weight (total cecal weight – cecal content) and to a significant acidification of the cecal content (pH of cecal content were 6.5 ± 0.1 in rats fed carrot *vs* 6.9 ± 0.1 in control rats; $P = 0.003$). Cecal total short chain fatty acids (SCFA) in rats fed carrot diet increased significantly (+ 40 %; $P = 0.013$). This increase was mainly due to the 123 % increase of butyrate and to the 43 % increase of acetate whereas propionic acid concentrations were similar to controls (Fig. 1).

Influence of carrot diet on lipid metabolism

As shown in Table 2, the daily cholesterol intake was similar and the cholesterol balance was positive in both

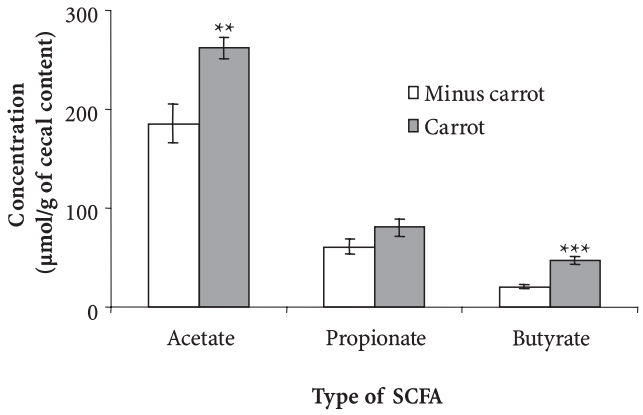

Fig. 1 Cecal short chain fatty acids in rats of a 21-day period of feeding experimental diet

groups. Carrot diet led to an increase of fecal excretion of coprostanol ($P < 0.05$) and total neutral sterol. Therefore the efficiency of cholesterol apparent absorption (calculated by the ratio of cholesterol intake minus total neutral sterols excretion divided by total cholesterol intake) was lower in the rats fed the carrot diet (34 % *vs* 45 % for the control group; $P < 0.05$) (Fig. 2). The fecal bile acid losses were apparently not affected by carrot consumption. Thus, for different levels of cholesterol absorption the same level of bile acids excretion (29 µmol/day) was observed. We can calculate that the percentage of cholesterol absorbed, eliminated as bile acids was higher in rats fed carrot diet (63 %) than in control rats (45 %).

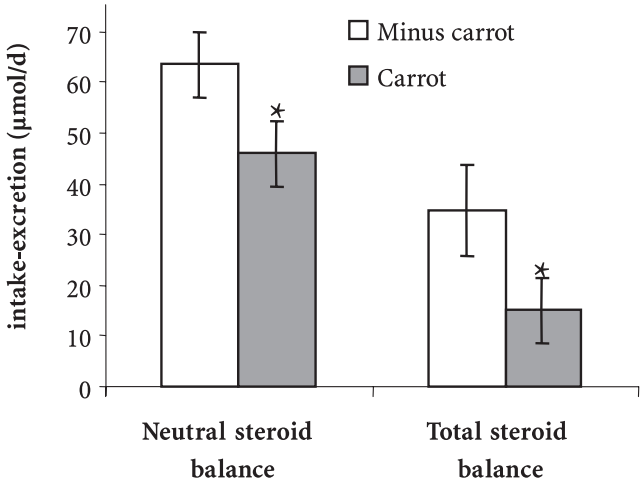

Fig. 2 Changes in fecal excretion of neutral steroids and in the total steroid balance. The total steroid balance was calculated as: [daily cholesterol intake – (fecal excretion of bile acids + fecal excretion of neutral steroids)]. Each value is a mean ± SEM for eight rats in each diet

Plasma cholesterol concentrations tend to be lower in rats fed carrot diet ($P = 0.06$) but plasma triglyceride (TG) concentrations were not affected (Table 3). Analysis of plasma lipoprotein profile showed a marginal reduction of cholesterol in the TGRLP fractions (d < 1.040 kg/L) in rats fed carrot diet (Fig. 3). There was no change in the higher density fractions (mainly HDL). Triglyceride concentrations were 16 % lower in TGRLP of rats fed the carrot diet as compared to the control. The hepatic cholesterol and triglyceride concentrations were both significantly depressed in rats fed the carrot diet as compared to controls ($P = 0.0007$ and $P = 0.0005$, respectively).

Influence of carrot diet on carotenoid status

The carotenoids recovered in diet and tissues were lutein, α-carotene and β-carotene, whereas no carotenoids had been detected either in the control diet (Table 4) or in the plasma of rats fed control diet. In the rats fed carrot diet, β-carotene was the major carotenoid in plasma (60 %) followed by lutein and α-carotene (21 % each). Fecal carotenoid concentrations were also measured and we observed a particularly low level of xanthophylls (2 % of total carotenoids) as compared to α- and β-carotene (respectively 33 % and 64 %). For lutein, α-carotene and β-carotene, the percent recovery

Table 3 Plasma and hepatic concentration of cholesterol and triglycerides in rats fed control or carrot diet for 21 days[1]

	Plasma		Liver	
	Cholesterol	Triglycerides	Cholesterol	Triglycerides
	(mmol/L)		(mmol/g tissue)	
Minus Carrot	2.26±0.11	1.90±0.22	12.56±0.88	18.15±1.24
Carrot 15 %	1.99±0.08	1.76±0.16	6.97±0.77*	11.06±0.70*

[1] Values are means ± SEM, n = 8 in each group. * $P < 0.001$

Table 2 Fecal excretion of cholesterol and its absorption in rats after a 21-day period of feeding experimental diet[1]

	Cholesterol intake (µmol/d)	Fecal bile acids (µmol/d)	Fecal cholesterol (µmol/d)	Fecal coprostanol (µmol/d)	Total steroid balance (intake–excretion)
Minus carrot	140±5.6	28.7±2.6	42.2±3.3	34.3±4.6	34.8±7.0
Carrot 15 %	137±5.6	29.1±1.9	40.6±1.2	50.5±5.9*	16.8±6.6

[1] Values are means ± SEM, n = 8 in each group. * $P < 0.05$

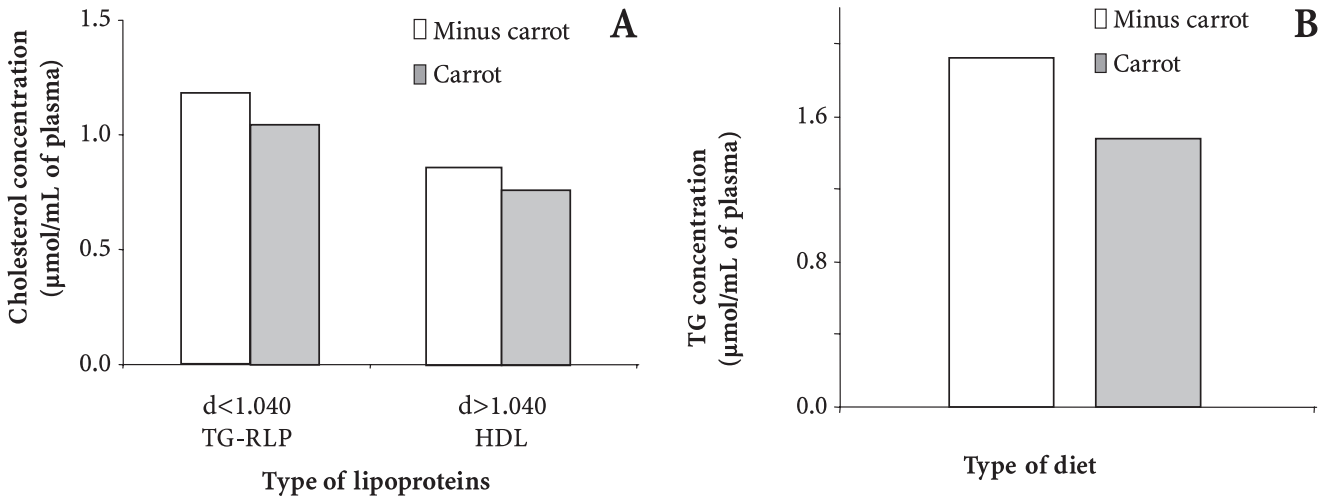

Fig. 3 Changes in the repartition of cholesterol in the various plasma lipoprotein fractions in rats fed control or carrot diets. Each value is a mean ± SEM of a triplicate analysis of a pool of eight plasma

in feces was, respectively, 15, 31 and 19 % of the ingested dose. Among the carotenoids, only β-carotene had been detected in liver and to a lesser extent in the heart (Table 4). Traces of β-carotene were also detected in lung and kidney.

Influence of carrot diet on antioxidant status

Carrot diet reduced significantly urinary TBARS elimination (30 %; $P = 0.007$) as compared to controls (Table 5). Concurrently, we observed a significant decrease by the carrot diet of the susceptibility to oxidation of heart lipids as assayed by an *ex vivo* induction of lipid oxidation by ferrous ions (–37 %; $P = 0.007$). Carrot diet tended to increase the FRAP values in plasma ($P = 0.06$).

The consequence of carrot intake on vitamin E status was evaluated. Plasma α-tocopherol concentrations were markedly increased in rats fed the carrot diet (20.2 µM *versus* 12.7 µM in controls; $P = 0.002$; Fig. 4). The vitamin E/TG ratio is also higher in rats fed the carrot diet ($P = 0.006$).

Table 5 FRAP level, urine-bladder TBARS excretion and heart tissue TBARS in rats fed a carrot or control diet

	FRAP (µmol/L)	Urine-TBARS (nmol/mg creatinin)	Heart tissue TBARS (nmol/g)
Minus carrot	186 ± 6.2	2.6 ± 0.1	143 ± 14.7
Carrot 15 %	240 ± 20.1	1.8 ± 0.3*	90 ± 8.4*

[1] Values are means ± SEM, n = 8 in each group. * P < 0.01

Table 4 Carotenoid content in the carrot diet, and in plasma and feces of rat fed the carrot[1]

	Lutein		α-carotene		β-carotene	
		% of total carotenoids		% of total carotenoids		% of total carotenoids
Carotenoids ingested (mg/d)	0.05 ± 0.06	3	0.34 ± 0.06	23	1.06 ± 0.06	73
Fecal excretion (µg/d)	7	2	106	33	205	64
% recovery ingested carotenoids in feces	15	/	31	/	19	/
Plasma carotenoids (nmol/L)	43 ± 0.6	21	41 ± 6.2	20	125 ± 23.9	60
Tissues carotenoids (µg/g)						
Liver	nd	/	nd	/	57 ± 9	
Heart	nd	/	nd	/	0.35 ± 0.08	
Lung	nd	/	nd	/	0.28 ± 0.04	
Kidney	nd	/	nd	/	0.29 ± 0.05	

[1] Values are means ± SEM, n = 8 in each group.
Detection limit is 2 ng for lutein and 5 ng for α- and β-carotene

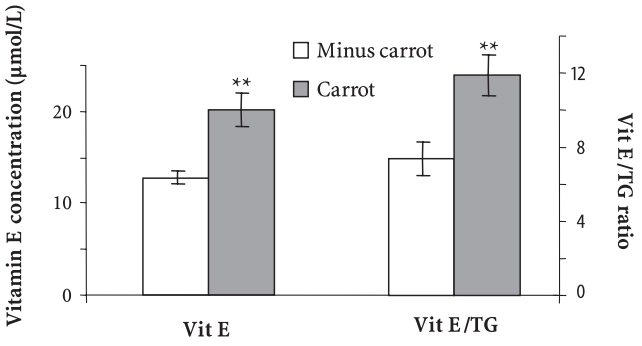

Fig. 4 Vitamin E status and vitamin E/triglycerides ratio in rats fed carrot diet or control diet for 21 days

Discussion

Hypocholesterolemic effects of dietary fibers are well documented; however, little is known about the impact of complex plant food such as vegetables. Protective effects against cardiovascular diseases might be mediated by complex carbohydrates and other micronutrients. The aim of this work is to show that 3-week carrot diet intake can affect positively lipid metabolism and antioxidant protection in rats with dietary conditions, in which the macronutrients supply was relatively well equilibrated. In the present study, carrot consumption exerts a moderate lowering cholesterol effect (–12 % decrease). A significant 11 % reduction of cholesterolemia has been observed in human subjects by Robertson et al. [21], whereas an absence of effect was reported by Wisker et al. [22]. In this work, the carrot diet seems to act mainly by lowering cholesterol absorption.

Some viscous fibers have the capacity to bind bile acids and therefore impair their reabsorption in the small intestine, especially the ileum [23, 24] but this was not apparent on the fecal balances in the present experiment. It is frequently observed that a greater excretion of steroids with viscous fibers or plant foods is chiefly dependent on a greater elimination of neutral sterols [25, 26]. However, the decrease of apparent cholesterol absorption should lead to a reduction of the fecal bile acids losses. These losses were maintained in rats fed carrot diet, which means that a greater proportion of the apparently absorbed cholesterol was eliminated by fecal bile acids excretion (63 % in rats fed carrot diet *vs* 45 % in the control rats).

The decrease of digestive cholesterol absorption by the carrot diet results in a highly significant decrease of hepatic cholesterol and triacylglycerol levels. These results are in agreement with previous ones which showed that pectins are the most effective fibers to decrease the cholesterol level in tissues with differences according to the plant origin of pectins [27, 28]. The role of fermentation end-products, especially that of propionate, has frequently been involved in the effects of fiber on lipid metabolism. The enlargement of the cecal SCFA pool in the present work was limited and made unlikely marked alterations of liver metabolism. However, butyrate which significantly increases upon carrot intake (123 %) is an interesting feature since butyric acid is considered as protective against colonic cells hyperproliferation [29], especially if butyrate producing colonic ecosystem is maintained [30]. Butyrate was also demonstrated on Caco-2 cells to regulate apoA-IV secretion and therefore to reverse cholesterol transport resulting in a cholesterol efflux [31].

Carrot carotenoids could also contribute to reduce the cholesterolemia. It has been previously described that a supplementation of twice the amount of β-carotene (125 mg/kg of diet) consumed in this study reduced plasma and lipoprotein (LDL and VLDL) cholesterol in hypertensive rats fed cholesterol enriched diet [32].

The effect of the carrot diet on the carotenoid status was examined. Carrot contains three main carotenoids lutein, α-carotene and β-carotene (1.4, 8.5 and 45.6 mg/100 g of carrot dry matter, respectively). All these carotenoids were recovered in the plasma of rats fed carrots and not detected in controls. Beta-carotene (and α-carotene) can be converted to vitamin A in contrast to lutein. With a high dose of carotenoids ingestion, as in present study, and with a dietary supply of vitamin A, a large part of β-carotene could escape the conversion into vitamin A. Thus, the percentage of carotenoids recovered in plasma did not reflect exactly their respective bioavailability. However, the relatively high level of lutein in plasma and low level in feces as compared to the carotenes indicate a higher bioavailability, in agreement with previous studies on human subjects [33, 34]. This could be explained by either a higher gut absorption of lutein or a higher stability as compared to carotenes which are converted to vitamin A [35]. A higher affinity of carotenes for fibers known to inhibit their absorption could also explain the higher bioavailability of lutein [36].

It is difficult to extrapolate results obtained from rats studies to humans not only because of the different metabolic response but also because of a higher food ingestion in the rat model. In our model, the diet contained 7 mg of total carotenoids/100 g dry weight. Humans consume in mean 500 g of dry matter, which could correspond to a consumption of 35 mg of carotenoids (a level from 1 to 2 mg/100 g of carotenoids assumes a mean intake of 5 to 10 mg of carotenoids per day). We observed in human studies that with a lower intake of carotenoides (from 3 to 13 mg/d) [37] the plasma concentrations were 4-fold higher. This could be related to the amount and the repartition of lipoproteins fraction, especially LDL fractions [38], which are an important fraction in human plasma in contrast to rats.

We observed also that carotenoids have reached target tissues (liver, heart, lung and kidney). The difference of carotenoid distribution in those tissues may be due to the specific lipid composition of each organ. β-Carotene is transported by LDL and accumulated in high levels in liver because of its high number of LDL receptors. β-Carotene was also detected in heart, but the concentrations were more than 160-fold lower than in liver (57 *vs* 0.35 μg/organ). These contents corroborate with β-carotene supplementation in rats [39] or in mice [40].

In rat model, the increase of carotenoids in tissues seems to contribute to protect them against oxidative stress. They are known to protect LDL from oxidation [41, 42] and may consequently limit cardiovascular risk [43, 44]. In the present work, the oxidative stress was reduced by the carrot diet as seen by a decrease of the TBARS content in both urine and heart tissue and a slight increase of the FRAP values in plasma. A 10-day β-carotene supplementation was also shown to reduce the TBARS content in urine in mice [40]. In man, the consumption of 330 mL of carrot juice for 2 weeks had no effect on plasma TBARS content but diminished the rate of *ex vivo* oxidation of LDL lipids [38]. The discrepancy in the TBARS results between the present study and former human studies could be explained by the higher intake of carotenoids (5.4 *vs* 0.48 mg/day/kg b. w.) as compared to the human study. It has also been reported that β-carotene could not contribute to the antioxidant activity when determined by the FRAP modified method [45]. This could explain the lack of significant differences in hepatic model.

The increase of carotenoid level and the reduction of susceptibility to lipid peroxidation in heart tissue with the carrot diet might be particularly relevant to reduce the cardiovascular risk. A link between cardiac dysfunction and oxidative stress in myocardial tissues was observed in rabbits and both functions were improved by supplementation of the diet with antioxidants (β-carotene, ascorbic acid and α-tocopherol) [46].

Plasma level of α-tocopherol was markedly increased in rats fed carrot diet. It is well recognized that L-ascorbic acid has the ability to regenerate the activity of lipid-soluble antioxidants, such as α-tocopherol and β-carotene, by interacting with biological membranes at the aqueous-lipid interface essentially in an *in vitro* model [47]. The increase of the plasma vitamin E concentration and vitamin E/TG ratio upon carrot feeding could also be explained by an increase of vitamin E intake as compared to the control diet (carrot contains 513 μg vitamin E/100 g).

In conclusion, our data show that carrot consumption may enhance antioxidant defenses and improve the lipid status. Both effects could be largely explained by the high carotenoid and fiber content of this vegetable. Carrot consumption may thus significantly contribute to the protective effects of fruit and vegetable consumption against cardiovascular diseases in human.

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