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Influence of norbixin on plasma cholesterol-associated lipoproteins, plasma arylesterase/paraoxonase activity and hepatic lipid peroxidation of Swiss mice on a high fat diet

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Abstract

Effects of the carotenoid norbixin (7.7 and 92.8 mg/kg) added to drinking water were studied in male Swiss mice fed with a high fat diet for 4 weeks. High fat diet decreased, by 55.7%, the levels of high-density lipoprotein (HDL)-cholesterol ($P < 0.05$) increased, by 167%, the levels of very low-density lipoprotein (VLDL)-cholesterol/low-density lipoprotein (LDL)-cholesterol ($P < 0.0001$). When the high fat diet was ingested with norbixin, HDL-cholesterol and VLDL/LDL-cholesterol remained near control levels while plasma triglycerides were not altered. High fat diet decreased the activity of plasma arylesterase/paraoxonase (EC3.1.8.1) by 46% ($P < 0.0001$), a reduction that was also counteracted by norbixin. Basal levels of thiobarbituric acid-reactive substances (TBARS), measured in liver homogenates, were not increased by high fat diet. However, there was a significant increase in TBARS levels ($P < 0.001$) when liver homogenate from mice submitted to high fat diet was incubated with Fe^{2+} and H_2O_2. Under the same oxidative conditions, liver homogenate was much less responsive to peroxidation ($P < 0.0001$) when high fat diet was associated with norbixin. Thus, norbixin, a common carotenoid food additive, alters plasma lipid levels and paraoxonase activities in Swiss mice fed with an atherogenic diet. Further studies should be performed in order to determine the lowest effective norbixin concentration as this carotenoid is ingested with industrialized food in very tiny amounts.

Keywords: Norbixin; Cholesterol; Lipoproteins; Paraoxonase; Peroxidation

1. Introduction

Plasma cholesterol and triglycerides above normal levels are closely related to the incidence of atherosclerosis and coronary artery disease, which arise from alterations of lipoprotein metabolism. Increased levels of low-density lipoprotein (LDL) and/or diminished levels of high-density lipoprotein (HDL) are associated with the pathogenesis of the inner layer of the arteries. Oxidized LDL particles have been deeply implicated in the development of atherosclerosis, and dietary intake of antioxidant molecules is believed to diminish its incidence (Diaz, Frei, Vita, & Keaney, 1997; Diplock et al., 1998; Heinecke, 1998; Zyriax & Windler, 2000).

Antioxidants, such as carotenoids and tocopherols (vitamin E), are found associated with lipoproteins, particularly with LDL. One of the most recent epidemiological surveys of plasma levels of several dietary carotenoids (α- and β-carotene, lutein, lycopene, zeaxanthin, β-cryptoxanthin), vitamin A and vitamin E, and atherosclerosis, indicated that a high intake of α- and β-carotene has a protective role in early atherogenesis (D'Odorico et al., 2000). Antiatherogenic potential of β-carotene has been demonstrated in vitro and in animal models (Dugas, Morel, & Harrison, 1998; Shaish, Daugherty, O'Sullivan, Schonfeld, & Heinecke, 1995). However, plasma levels of vitamins E and A did not seem to correlate with atherosclerosis (D'Odorico et al., 2000), although it was experimentally demonstrated that vitamin E has antiatherogenic properties (Diaz et al., 1997; Chen, 1998). Earlier epidemiological reports also indicated a good correlation of vitamin E intake

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Nomenclature

BHT	butylated hydroxytoluene
H_2O_2	hydrogen peroxide
HDL	high-density lipoprotein
LDL	low-density lipoprotein
MDA	malondialdehyde
nBix	norbixin
TBARS	thiobarbituric acid-reactive substances
TCA	trichloroacetic acid
VLDL	very low-density lipoprotein
TRIS	tris (hydroxymethyl) aminomethane

with lower risk of cardiovascular disease (Diplock et al., 1998; Rimm, Stampfer, Ascherio, Giovannucci, Coldtiz, & Willett, 1993). Divergences among independent epidemiological studies may be ascribed to factors such as type of population evaluated, dietary habits, and differences in the protocols adopted.

Although a few epidemiological studies have indicated an association of high serum levels of β-carotene with reduced risk of atherosclerosis (Kohlmeier & Hastings, 1995; Street, Comstock, Salked, Schuep, & Klag, 1994), results from the Alpha-Tocopherol Beta-Carotene (ATBC) trial and from the β-Carotene and Retinal Efficacy Trial (CARET) demonstrated a need for caution in the ingestion of β-carotene supplements (ATBC, 1994; Omenn et al., 1996; Redlich, Chung, Cullen, Blaner, Van Bennekum, & Berglund, 1999). The ATBC and CARET participants who received β-carotene and were heavy smokers had a significantly higher incidence of lung cancer and mortality than participants receiving placebo. Additionally, β-carotene supplementation resulted in increased cardiovascular disease incidence or mortality in both studies. The lack of success of the clinical trials of β-carotene in both ATBC and CARET studies indicates the need for more solid scientific knowledge about this carotenoid. Alternatively, more attention has been given to other carotenoids, in an effort to find molecules with high antioxidant properties and low levels of collateral effects.

Endogenous antioxidant activity has been ascribed to arylesterase/paraoxonase in artery diseases, since this enzyme is associated to the ability of HDL to decrease lipid peroxidation in LDL (Aviram, 1999; Aviram, Rosenblat, Bisgaier, Newton, Primo-Parmo, & La Du 1998; Mackness, Durrington, & Mackness, 2000). Classically, human serum paraoxonase plays a role in the detoxification of organophosphorus insecticides (such as paraoxon), nerve agents, and a number of aromatic carboxylic esters.

Norbixin is a water-soluble carotenoid originating from the hydrolytic removal of a methyl ester group of bixin, the major carotenoid found in the waxy surrounding material of *Bixa orellana* seeds. Almost no information is available on the biological properties of bixin and norbixin, possibly because they are not usually found in dietary fruits and vegetables. Nonetheless, both carotenoids (commercially known as annatto or E160b) are quite commonly found in a variety of industrialized food, as colorants. We have recently characterized the antioxidant potential of norbixin in vitro (Kovary et al., 2001). To elucidate the biological role that norbixin might have in vivo we investigated its effects on the metabolism of plasma lipids, as well as on plasma activity of arylesterase/paraoxonase, under conditions of high fat intake. In addition, studies were also performed to evaluate the efficacy of norbixin in preventing the formation of hepatic lipid peroxides under the same dietary conditions.

2. Materials and methods

2.1. Norbixin and chemicals

Bixin was isolated from fresh annatto seeds, as described elsewhere (Kovary et al., 2001). The grade of purity of bixin was greater than 98% based on high-performance liquid chromatography (HPLC) analysis. Norbixin was prepared by saponification of bixin in aqueous-sodium hydroxide solution (bixin:NaOH, 1:3) by agitation for several hours at 37 °C, followed by water evaporation to dryness at 50 °C. Saponification was monitored by HPLC analysis until the conversion of bixin to norbixin was higher than 98%. Norbixin was kept at −20 °C until further use. All other reagents used were of the highest purity, and purchased from Sigma (St. Louis, MO), Merck (Rio de Janeiro, Brazil) or GibcoBRL (Gaithersburg, MD).

2.2. Animals and diet

Three-month-old male Swiss mice (average weight 28–36 g) were purchased from a local laboratory animal supplier (Rio de Janeiro, Brazil) and were housed with free access to food and water for at least 1 week prior to the experiments. Mice on a control diet were fed with a commercial standard rodent diet (Nuvilab Ltd, Curitiba, Brazil), which contained 4% fat. Experimental mice, treated with a semi-synthetic high fat diet (Nishina, Verstuyft, & Paigen 1990; Nishina, Wang, Toyofuku, Kuypers, Ishida, & Paigen 1993) were fed with house-made pellets consisting of 15% commercial hydrogenated fat, 1.25% cholesterol, 0.3% cholic acid, and 83.45% Nuvilab standard diet. Mice were fed ad libitum with either control diet or high fat diet and

drinking water containing two doses of purified norbixin that ranged from 3 to 5.4 mg% (dose 1) and 30 to 54 mg% (dose 2). Solutions of drinking water containing norbixin were always freshly prepared and changed every day. Volume of consumed experimental solution was recorded daily in order to calculate the average amount of norbixin ingested per mouse per day. After 4 weeks of treatment, mice were fasted for 16 h, lightly anaesthetized with ether and sacrificed by cardiac puncture. Plasma was collected immediately by centrifuging heparinized blood (2.5 U of heparin per ml of blood) at 10,000 rpm for 5 min and kept either at 4 °C or −20 °C for further biochemical determinations. Animal care and experimental protocols complied with the "Guidelines for the Ethical Use of Laboratory Animals" in effect at the Rio de Janeiro State University (Rio de Janeiro, Brazil).

2.3. Biochemical analytical determinations

Plasma total cholesterol, HDL-cholesterol, and triglycerides were determined by commercial colorimetric enzymatic assays (BioMérieux, France). VLDL/LDL-cholesterol was calculated as the difference between plasma total cholesterol and HDL-cholesterol. Protein was determined by the method described by Petersen (1977), with bovine serum albumin as protein standard. All control and experimental mice had their plasma evaluated individually.

2.4. Plasma arylesterase/paraoxonase activity assay

Enzymatic activity was determined using phenylacetate as substrate. Plasma samples (10 μl) were assayed in a reaction medium consisting of 4.0 mM phenylacetate, 20 mM Tris–HCl pH 8.0, 0.5 M NaCl, and 2.0 mM $CaCl_2$. Phenol production was recorded at 270 nm, over a 180-s period. A unit of arylesterase/paraoxonase activity is defined as 1 μmol of phenol produced per min per 100 ml plasma. Similar results were obtained using paraoxon as substrate and the product *p*-nitrophenol was determined at 400 nm.

2.5. Basal and induced lipid peroxidation levels

Both basal and induced lipid peroxidation were determined in whole liver homogenates. Liver homogenates were prepared in five volumes of ice-cold 50 mM Tris–HCl, pH 7.4, using a Teflon® Potter-Elvehjem homogenizer. For determination of basal lipid peroxidation, 10 mg protein of whole homogenate were incubated for 1 h, at 37 °C, in 50 mM Tris–HCl, pH 7.4, in a total volume of 500 μl. For determination of lipid oxidative susceptibility, 5 mg of protein homogenate were treated with 50 μM ferrous iron [stock solution: 100 μM $Fe(NH_4)_2(SO_4)_2$ in 0.05 N HCl] and 50 μM H_2O_2, in 500 μl of 50 mM Tris–HCl, pH 7.4, for 1 h, at 37 °C. After incubation, samples were processed for TBARS determination.

2.6. Determination of thiobarbituric acid-reactive substances (TBARS)

Lipid peroxidation was estimated by the TBARS assay, which detects mainly malondialdehyde (MDA), which is an end product of the peroxidation of polyunsaturated fatty acids (Esterbauer & Cheeseman, 1990). Briefly, to 500 μl of each oxidative reaction, 500 μl of 20% TCA and 10 μl of 2% butylated hydroxytoluene (BHT) were added. After centrifugation at 3,000 × ***g*** for 5 min, 900 μl of each supernatant and 100 μl of 10% TCA were added to 1 mL of 0.67% thiobarbituric acid in water. After incubating for 1 h, at 98 °C, the absorbance at 532 nm was determined. Quantification of MDA was obtained from a calibration curve of MDA, obtained by acid hydrolysis of

Table 1
Effects of norbixin on daily liquid intake and body and liver weights in mice submitted to control or high fat diet[a]

Treatment	Average daily liquid intake (ml)[b]	Average daily exposure dose (mg/kg)	Body weight (g)			Wet liver weight (g/100 g body weight)
			Initial	Final	Net gain	
Control diet ($n=10$)	8.8±0.3	–	30.7±1.1	32.8±0.9	2.1	5.31±0.27
Dose 1 nBix ($n=5$)	9.3±0.2	7.7	35.4±1.8	37.8±1.5	2.4	5.53±0.17
Dose 2 nBix ($n=5$)	9.4±0.2	77.7	35.8±1.0	37.3±0.9	1.5	5.95±0.13
High fat diet ($n=6$)	5.9±0.3	–	32.2±0.9	33.7±1.5	1.5	6.31±0.13*
Dose 1 nBix ($n=6$)	4.3±0.2	7.7	29.7±1.9	30.4±1.8	0.7	6.29±0.19*
Dose 2 nBix ($n=6$)	5.4±0.2	92.8	30.5±1.5	32.4±1.7	1.9	6.55±0.30*

[a] Mice were treated for 4 weeks with either the control or high fat diet, in the presence or absence of norbixin added to drinking water. Mice not treated with norbixin received tap water only. Data are expressed as mean±S.E.M.

[b] Average values correspond to the liquid intake per animal of the corresponding group.* $P < 0.05$ (student's *t*-test), when compared to control diet group.

1,1,3,3-tetraethoxy propane. TBARS were calculated as nmol of MDA equivalents per mg of protein.

2.7. Statistical analysis

Statistical significance was evaluated by the unpaired Student's *t*-test, using InStat (GraphPad Software). The difference between each experimental group and control group was considered statistically significant at $P < 0.05$.

3. Results

3.1. Alterations in liquid consumption, body weight and liver weight

As shown in Table 1, male Swiss mice fed with a high fat diet during 4 weeks consumed nearly 33% less liquid per day than mice fed with control diet. No obvious differences were detected in liquid consumption between water/control and water/norbixin groups, regardless of the type of diet ingested. Based on the average daily intake of liquid, two doses of norbixin were tested: 7.7 mg/kg (for both diets) and 77.7 mg/kg (control diet) or 92.8 mg/kg (high fat diet). Mice on the high fat diet received a somewhat higher dose of norbixin due to variations in their liquid intake. No gross changes were noticed among final body weight gains. However, the livers from mice fed with the high fat diet weighed significantly more than those from control animals ($P < 0.05$). Ingestion of norbixin by itself also induced a dose-related enlargement of liver, but without statistical significance.

3.2. Influence of norbixin on plasma lipids

Plasma levels of lipids from mice fed with either the normal or high fat diet are shown in Table 2. Feeding mice for four weeks with the high fat diet did not significantly change the plasma levels of triglycerides and these levels were not significantly altered by the simultaneous ingestion of norbixin. Nevertheless, mice on high fat intake showed a 52.7% increase in total plasma cholesterol ($P < 0.05$), associated with a 55.7% reduction in HDL-cholesterol ($P < 0.05$) and a 167% increase in VLDL/LDL-cholesterol ($P < 0.001$). On the other hand, plasma levels of total cholesterol were not significantly reduced by norbixin, a pattern also observed in mice submitted to control diet and norbixin. However, under conditions of high fat intake and 92.8 mg/kg of norbixin, there was a considerable redistribution of cholesterol among lipoprotein particles. The levels of HDL-cholesterol and VLDL/LDL-cholesterol of these mice closely resembled those observed in mice submitted to control diet ($P < 0.05$ and $P < 0.0001$ respectively). Ingestion of approximately 10 times less norbixin (7.7 mg per kg) also significantly counteracted the lipoprotein changes induced by high fat intake ($P < 0.05$). Norbixin per se induced minor alterations of the levels of cholesterol-associated lipoproteins, as observed in mice fed with control diet (Table 2).

3.3. Effect of norbixin on plasma arylesterase/paraoxonase activity

Mice fed with high fat diet showed nearly two times less arylesterase/paraoxonase activity in their plasma than control mice ($P < 0.0001$; Fig. 1). The combined ingestion of high fat diet and norbixin induced a recovery in arylesterase/paraoxonase activity to near control levels (dose 2, $P < 0.05$). Under conditions of control diet, norbixin did not influence the plasma levels of this activity.

3.4. Effect of norbixin on hepatic lipid peroxidation

Both basal and induced lipid oxidative susceptibilities were determined in whole liver homogenate as an

Table 2
Plasma levels of triglycerides, total cholesterol, HDL-cholesterol and VLDL/HDL-cholesterol of mice treated with control diet or high fat diet, supplemented or not with norbixin[a]

Treatment	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	VLDL/LDL-cholesterol (mg/dl)
Control diet	104±13.9	67.6±5.2	44.5±6.3	30.3±6.0
+7.7 mg/kg nBix	113±25.1	81.3±7.0	30.1±2.4	47.2±9.0
+77.7 mg/kg nBix	111±15.7	76.5±8.8	35.0±5.0	41.0±5.8
High fat diet	83.2±11.0	103±8.2[b]	19.7±2.6[c]	81.0±8.1[e]
+7.7 mg/kg nBix	87.3±19.4	87.0±7.1[b]	25.9±2.3[d]	61.4±4.2[e,f]
+92.8 mg/kg nBix	80.6±8.2	87.3±6.0[b]	44.5±9.1[d]	42.2±4.4[g]

[a] Data are expressed as mean±S.E.M.
[b] $P < 0.05$ versus control diet.
[c] $P < 0.05$ versus control diet.
[d] $P < 0.05$ versus high fat diet.
[e] $P < 0.001$ versus control diet.
[f] $P < 0.05$ versus high fat diet.
[g] $P < 0.0001$ versus high fat diet.

Table 3
Hepatic TBARS levels of mice fed with control diet or high fat diet, supplemented or not with norbixin (nBix)

Treatment	% Lipid peroxidation[a]	
	Basal	Induced
Control diet	100	100
+7.7 mg/kg nBix	98.3±7.3	98.5± 5.0
+77.7 mg/kg nBix	84.3±6.4	82.8±4.3
High fat diet	139±13.3	193±8.3[b]
+7.7 mg/kg nBix	133±15.3	83.3±3.4[c]
+92.8 mg/kg nBix	101±7.1	58.5±7.2[d,e]

[a] Basal and induced levels of TBARS were calculated as described in Section 2. Results are expressed as % of control diet (basal or induced) taken as 100% (mean±S.E.M).
[b] $P < 0.001$ versus control diet.
[c] $P < 0.0001$ versus high fat diet.
[d] $P < 0.05$ versus control diet.
[e] $P < 0.0001$ versus high fat diet.

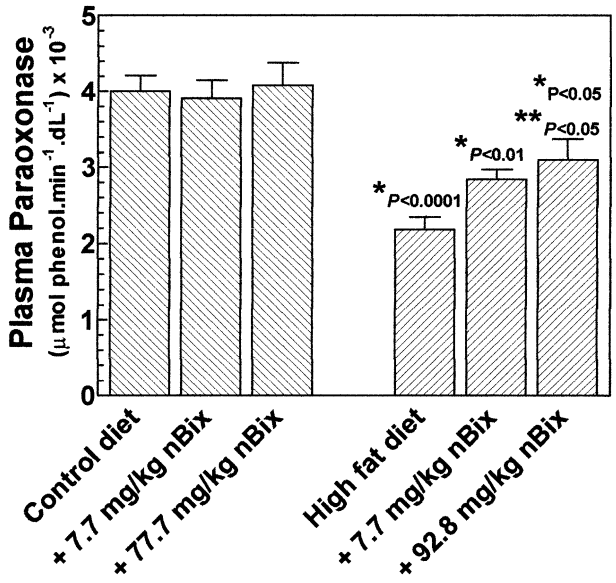

Fig. 1. Arylestease/paraoxonase activity in plasma from male Swiss mice fed with control or high fat diet, supplemented or not with norbixin (nBix). Enzymatic activity was determined using phenylacetate as substrate according to the procedure described in Section 2. Four mice from each experimental or control group have their plasma analysed and the results shown represents mean±S.E.M. * Significantly different from control group; ** Significantly different from high fat diet group.

attempt to determine the mouse antioxidant status (Table 3). In spite of not being statistically significant, basal TBARS formation was still higher in liver homogenates from mice fed with high fat diet than from mice fed with control diet, a condition that was slightly reduced by simultaneous ingestion of 92.8 mg/kg of norbixin. In contrast, after being challenged with ferrous iron and H_2O_2, TBARS formation was much higher in livers from mice submitted to high fat intake ($P < 0.001$) than in livers from control mice. When the susceptibility to lipid oxidation was evaluated in livers from mice concomitantly treated with high fat diet and norbixin, their TBARS levels were drastically lowered to levels similar to those observed in control mice ($P < 0.0001$). On the other hand, supplementation of normal diet with norbixin did not induce any significant alteration of the susceptibility of hepatic lipids to peroxidation.

4. Discussion

We investigated the potential of norbixin in modulating the plasma levels of triglycerides, HDL-cholesterol and VLDL/LDL-cholesterol in mice fed with high fat diet, a condition that favours arterial diseases. Despite the large use of annatto pigments in industrialized food, little information is found on their metabolism in animal and human tissues. In 1980, the European Economic Community (EEC) surveyed the biological effects of the annatto pigments in rats, dogs, mice, and humans. This showed that annatto pigments were mainly metabolized in liver (EEC, 1980). Recent investigation, performed in humans, indicated that most of the ingested bixin is converted into norbixin by the intestinal tract and the bloodstream (Levy, Regalado, Navarrete, & Watkins, 1997). However, the means by which bixin and norbixin are absorbed in the gastrointestinal tract and how both are transported in plasma and distributed to the organism, remains to be clarified.

Male Swiss mice fed with high levels of cholesterol and high levels of hydrogenated fat showed levels of plasma lipids quite similar to those observed in mice strains that are classically known to be susceptible to atherogenesis (Nishina et al., 1993). Swiss mice on a high fat diet showed considerably higher levels of total cholesterol and non-HDL cholesterol, and lower levels of HDL-cholesterol than mice fed with control diet (Table 2). Experimental atherogenic diets are rich in saturated fat, capable of increasing LDL levels (Nishina et al., 1990, 1993). In our experiments we used commercial hydrogenated fat for the preparation of the semi-synthetic high fat diet. This kind of fat is one of the major sources of trans fatty acids present in our ordinary food. Ingestion of trans fatty acids not only increases LDL to a degree similar to that induced by saturated fats, but also reduces HDL, and therefore trans isomers are believed to be more atherogenic than saturated fatty acids (Lichtenstei, 2000). The simultaneous ingestion of high fat diet and norbixin considerably restored the levels of both HDL-cholesterol and VLDL/LDL-cholesterol, near to control levels (Table 2). Levels of total plasma cholesterol were barely reduced by norbixin, which suggests that norbixin neither influences the intestinal absorption of cholesterol nor affects its conversion into bile acids. Although norbixin did not work as a hypocholesterolemic agent, it

certainly favoured a substantial decrease in VLDL/LDL-cholesterol as well as an increase in HDL-cholesterol. It is noteworthy that a decrease in plasma LDL is associated with an increase in the activity of LDL receptor (Goldstein & Brown, 1984). As this activity is down-regulated by dietary cholesterol, an increase in plasma cholesterol would be followed by an increase in LDL levels. Therefore, norbixin might have influenced the redistribution of cholesterol between VLDL/LDL and HDL particles without significantly affecting the plasma levels of total cholesterol. As low levels of LDL-cholesterol and high levels of HDL-cholesterol are both associated with a diminished risk to cardiovascular diseases, norbixin may be useful for reducing the risk of these pathologies.

Another positive aspect of norbixin metabolism, under conditions of high fat intake, was a partial recovery in plasma arylesterase/paraoxonase activity (Fig. 1), as it had dropped by nearly 46% in mice fed with a high fat diet. Decrease in this activity has been already observed after long-term (12 weeks) or short-term (up to 7 days) feeding of susceptible mice (C57BL/6J) with atherogenic diet (Hedrick et al., 2000; Shih et al., 1996). However, the mechanism responsible for such dietary regulation of arylesterase/paraoxonase activity is still unknown. In mice treated with high fat diet and norbixin, increase in arylesterase/paraoxonase activity, concomitantly with an increase in HDL-cholesterol, may have been simply due to a rise in the number of functional HDL particles, as paraoxonase has been described to be associated with this lipoprotein (Aviram, 1999; Aviram et al., 1998; Mackness et al., 2000). On the other hand, it has been recently shown that there is a close relationship between LCAT (lecithin:cholesteryl acyltransferase) and arylesterase/paraoxonase activities (Bielicki & Forte 1999; Forte et al., 1999). Working with LDL, Bielicki and Forte, (1999) isolated oxidized polar lipids that were able to strongly inhibit LCAT activity, suggesting that HDL-cholesterol transport could be impaired during early atherogenesis. The arylesterase/paraoxonase activity seems, therefore, to have a protective role in the mechanism of reverse cholesterol transport that is associated with HDL, by reducing the level of oxidized lipids in HDL that could inhibit LCAT activity. In addition, the interaction of a free sulfhydryl group (Cys 284) from arylesterase/paraoxonase with oxidized lipids present in LDL, results in its inactivation (Watson et al., 1995). Hence, under circumstances that favour an excess formation of oxidizing species, additional antioxidant factors may be necessary to prevent both the oxidation of cholesterol-rich lipoproteins and the decline of arylesterase/paraoxonase and LCAT activities.

It has been previously demonstrated that bixin functions as a quencher of singlet oxygen and as a scavenger of superoxide anion, peroxynitrite and hydroxyl radicals (Dimascio, Devasagayam, Kaiser, & Sies, 1990; Zhang, Cooney, & Bertram, 1991). More recently, we evaluated the antioxidant potential of norbixin under conditions that generate DNA oxidative damage (Kovary et al., 2001). Protective effects of bixin against lipid peroxidation have been demonstrated by in vitro studies performed in artificial phospholipid bilayers (Zhao, Han, Zhao, Hirota, Hou, & Xin, 1998) and in transformed cell lines (Zhang et al., 1991), while in vivo inhibition of serum and liver lipid peroxidation was demonstrated in rats submitted to gamma irradiation (Thresiamma, Mathews, & Kuttan, 1995). In our experiments hepatic lipids from Swiss mice fed with high fat were highly susceptible to peroxidation induced by iron and hydrogen peroxide, while dietary supplementation with norbixin rendered hepatic lipids strongly resistant to peroxidation (Table 3). Although TBARS determination is not the most accurate assay for determination of lipid peroxidation it still gives some information about lipid susceptibility to oxidation. TBARS results indicated that norbixin inhibits lipid peroxidation that occurs in vivo under conditions of oxidative stress induced by high fat intake. This could be one of the mechanisms involved in its putative role in handling plasma cholesterol levels, possibly by increasing arylesterase/paraoxonase, as lipid peroxidation interferes with reverse cholesterol transport (Bielicki & Forte, 1999). However, the presumed effective dose of norbixin is very high (greater than 7.7 mg/kg), which is above the tiny amounts that are ingested with industrialized food. Although mice and other non-human primates are not appropriate experiment models to clarify human atherosclerosis (Rudel, 1997), since mice have HDL-cholesterol as the major plasma lipoprotein fraction while humans have LDL-cholesterol as the major one, our data show the potentially important observation that norbixin alters plasma lipid levels and paraoxonase activities of Swiss mice fed with an atherogenic diet. Investigation is still needed to learn more about the biological effects and the safety of norbixin for humans, since the lowest dose we used here is at least a hundred times higher than the current acceptable daily intake (ADI) for this substance.

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