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ORIGINIAL CONTRIBUTION

Implication of Vitamin A deficiency on vascular injury related to inflammation and oxidative stress. Effects on the ultrastructure of rat aorta

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Abstract
Background Vitamin A deficiency induces activation of NF-kB and impairs activities of antioxidant enzymes in aorta.
Aim of the study We study the effect of vitamin A deficiency on the aorta histoarchitecture and the possibly contribution of its prooxidant and inflammatory effects to artery alterations.
Methods Twenty-one-day-old Wistar male rats were fed during 3 months with vitamin A-deficient diet (−A, $n = 8$) or the same diet containing 8 mg of retinol palmitate/kg of diet (+A, control, $n = 8$). In aortas, thiobarbituric reactive substances and reduced glutathione levels were measured by spectrophotometry. Expressions of TNF-alpha, NOX-2, VCAM-1, and TGF-beta1 were assessed by RT–PCR and Western Blot. The morphology of aorta was examined by light and transmission electron microscopy.
Results In −A rats, high levels of TBARS in serum and aorta and low levels of GSH in aorta were found. An increased expression of TNF-alpha, NOX-2, VCAM-1, and TGF-beta1 in aorta from −A rats was observed. Examination of the intimal layer by light microscopy indicated the presence of an irregular surface in −A aortas. TEM studies showed large vacuoles and multivesicular bodies along the endothelium and also multivesicular bodies in the subendothelial space of aortas from −A rats. Furthermore, the histological appearance of internal elastic lamina was different from control. Small vesicles in the medial layer were observed in aortas from vitamin A-deficient rats.
Conclusions Vitamin A deficiency produces histoarchitectural alterations in aorta, which can be associated, at least in part, to the oxidative stress and inflammation induced by vitamin A deficiency.

Keywords Vitamin A · Aorta histoarchitecture · TNF-alpha · NOX-2 · VCAM-1 · TGF-beta1

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Introduction

In the intact vasculature, the endothelium forms a continuous and semipermeable barrier that varies in integrity and control for different vascular beds [1]. The endothelium, like the epithelium, has adherent and tight junctions, both of which are critical for maintaining a restrictive barrier. The increased endothelial permeability or loss of barrier function is a feature of endothelial dysfunction that may be particularly important in determining the severity of a vascular disorder. It has been established that chronic inflammation and oxidative stress play crucial roles in endothelial dysfunction [2]. Reactive oxygen species (ROS), like other edemagenic mediators (e.g., thrombin, histamine, and TNF-alpha) induce morphological features implicated in the increase of vascular permeability [3]. Among the many enzymatic sources of vascular ROS, the nonphagocytic NOX family of NADPH oxidases is

particularly important [4]. On the other hand, in response to various inflammatory stimuli, endothelial cells selectively express specific adhesion molecules. An important example of this process is the localized endothelial expression of VCAM-1 and selective recruitment of mononuclear leukocytes to the vascular lesions observed in early atherosclerosis [5]. Factors commonly found in inflammatory vascular lesions, such as the cytokine TNF-alpha, induce the concurrent expression of VCAM-1 in cultured endothelial cells [6]. In the early atherogenic lesion, oxidative stress results in the oxidative modification of low-density lipoprotein (LDL) [7]. Reduced glutathione (GSH) is a tripeptide crucial in the antioxidant protection of vascular cells for its ability to react with oxidizing species, to breakdown inorganic and organic peroxides and to counteract LDL oxidation [8]. The modified LDL is retained in vessels by proteoglycans. Transforming growth factor (TGF)-beta1 has been identified in atherosclerotic vessels and has been shown to modulate the biosynthesis of proteoglycans by vascular smooth muscle cells (VSMC) in a manner that promotes binding to LDL [9].

Vitamin A (all-*trans* retinol) and its metabolites, all-*trans*- and *cis*-retinoic acid (ATRA), and 11-*cis*-retinal are involved in processes such as vision, reproduction, growth development, and immune function. ATRA and 9-*cis*-retinoic acid are potent regulators of gene transcription and play important roles in regulating cell proliferation and differentiation. In addition, ATRA has been reported to possess anti-inflammatory properties [10]. The endothelial cells are exposed to high concentration of circulating ATRA, express retinoid receptors, and play a significant role in ATRA metabolism compared with other cell types [11]. It has been communicated that ATRA can reduce the degree of lipid peroxidation and regulate the expression of antioxidant enzymes [12, 13]. In a previous work, we reported that rats that received during 3 months a free-vitamin A diet showed the activation of transcription factor NF-kB and impaired activities of antioxidant enzymes Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase (CAT) in aorta [14]. Nevertheless, it has not been demonstrated yet if vitamin A deficiency modifies the aorta histoarchitecture and whether the prooxidant environment [14], or some other effects altering the lipid metabolism in rat aortas [15], following vitamin A nutritional deprivation, are associated to alteration in the morphology of this artery.

The aim of this work was to study the effect of vitamin A deficiency on the aorta histoarchitecture and the possibly contribution of its prooxidant and inflammatory actions to the artery alterations. For this purpose, by means of light and transmission electron microscopy (TEM), measurements of the expression levels of cytokines (TNF-alpha, TGF-beta1), an adhesion molecule related to endothelial dysfunction (VCAM-1), and an enzyme involved in superoxide anion production (NOX-2), together with determination of oxidative stress parameters (TBARS and GSH levels), were carry out in aorta.

Methods

Diet and experimental design

Animal maintenance and handling was performed according to the NIH Guide for the Care and Use of Laboratory Animals (NIH publication No 86-23, revised 1985 and 1991) and the UK requirements for ethics of animal experimentation (Animals Scientific Procedures, Act 1986). Male Wistar rats were weaned at 21 d of age and immediately assigned randomly (eight per group) to either the experimental diet, devoid of vitamin A (vitamin A-deficient group), or the same diet with 4,000 IU of vitamin A (8 mg retinol as retinyl palmitate per kg of diet) (control group) for 3 months. Rats were housed in individual cages and kept in a 21–23 °C controlled environment with a 12-h light:dark cycle. They were given free access to food and water throughout the entire 3 months of the experimental period. Diets were prepared according to AIN-93 for laboratory rodents [16]. Body weight and food intake were registered daily.

Plasma retinol concentration analyses

Rats were killed by cervical dislocation at 09:00 h. Blood samples were collected in EDTA-coated tubes. To minimize photoisomerization of vitamin A, the plasma was taken under reduced yellow light and frozen in the dark at −70 °C until determination of retinol concentrations. Analyses were carried out within 1–3 weeks of obtaining the samples. Plasma retinol concentration was determined by high-performance liquid chromatography [17]. Retinoids were extracted from plasma (0.5 mL) into hexane containing 5 μg butylated hydroxytoluene/ml as antioxidant for analysis. Retinyl acetate was used as internal standard. Chromatography was performed on a Nucleosil 125 C-18 HPLC column with methanol:water (95:5, by vol.) as the mobile phase. Retinol was detected by UV absorbance at 325 nm (Model 440, Waters Associates), and peak areas were calculated by integration (Spectra Physics Analytical).

Thiobarbituric reactive substances and reduced glutathione determinations

TBARS were measured in serum and aorta as described by Jentzsch et al. [18]. Absorption was read at 535 and 572 nm to correct baseline absorption. Malondialdehyde

(MDA) equivalents were calculated using the difference in absorption at the two wavelengths and quantification was made with calibration curve using TMP (1,1,3,3-tetramethoxypropane) as standard. Reduced glutathione (GSH) was determined using an assay based on the reduction of 5,5′-dithiobis-2-nitrobenzoate (DTNB). The formation of 5-thio-2-nitrobenzoate (TNB) was followed spectrophotometrically at 412 nm (oxidative stress indicator) [19].

RNA isolation and RT–PCR analysis

Eight rats for experimental groups were killed, and their aortas were isolated, dissected, frozen in liquid nitrogen and stored at −80 °C, for less than a month, until RNA isolation. Total RNA from frozen aortas was isolated by using TRIzol (Life Technologies). All RNA isolations were performed as directed by the manufacturers. Gel electrophoresis and ethidium bromide staining confirmed the purity and integrity of the samples. Quantification of RNA was based on spectrophotometric analysis at 260/280 nm. Ten micrograms of total RNA was reverse transcribed with 200 units of MMLV Reverse Transcriptase (Promega Inc.) using random hexamers as primers in a 20-μL reaction mixture, following the manufacturer's instructions. PCR was performed in 35 μL of reaction solution containing 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1.25 U of Taq polymerase, 50 pmol of each rat-specific oligonucleotide primers, and RT products (1/10 of RT reaction). The samples were heated to 94 °C for 2 min, followed by 35 temperatures cycles. Each cycle consisted of three periods: (1) denaturation, 94 °C for 1 min; (2) annealing, 58 °C for β-actin, 60 °C for TNF-alpha and NOX-2, and 65 °C for VCAM-1 during 1 min; (3) extension, 72 °C for 1 min. After 35 reaction cycles, the extension reaction was continued for another 5 min. The sequence of the two TNF- alpha-specific primers was 5-AAGTTCCCAAATGGCCT CCCTCTCATC-3 (sense) and 5-GGAGGCTGACTTTC TCCTGGTATGAAA-3 (antisense) [20]. The sequence of the two NOX-2-specific primers was 5-CCAGTGTG TCGGAATCTCCT-3 (sense) and 5-ATGTGCAATGG TGTGAATGG-3 (antisense) [21]. The sequence of the two VCAM-1-specific primers was 5-CACCTCCCCCAAGAA TACAGA-3 (sense) and 5-GCTCATCCTCAACACCCA CAG-3 (antisense) [22]. The sequence of the two β-actin-specific primers was 5-CGTGGGCCGCCCTAGGC ACCA-3 (sense) and 5-TTGGCCTTAGGGTTCAGAG GGG-3 (antisense) [23]. The expected PCR product of TNF-alpha was 400 bp, for NOX-2 was 150 bp, for VCAM-1 was 473, and β-actin was 243 bp. The PCR products were electrophoresed on 2% (w/v) agarose gel with 0.01% (w/v) ethidium bromide. The image was visualized and photographed under UV transillumination. The intensity of each band was measured using NIH Image software and reported as the values of band intensity units.

Western blot analysis for TGF-beta1 and TNF-alpha

Aortas were homogenized in Tris–HCl 50 mM (pH 7.8) containing protease inhibitors (Pepstatin A and phenylmethylsulfonyl fluoride, PMSF, 1X). Protein was measured by the method of Lowry et al. [24] using bovine serum albumin as standard. Forty milligrams of proteins was mixed with 10 mL of sample buffer (125 mM Tris–HCl, pH 6.8, 4% SDS, 3.5 mM DTT, 0.02% bromophenol blue, and 20% glycerol), boiled for 2–3 min and loaded into a 10% SDS–PAGE gel. Protein molecular mass markers were always loaded on each gel. Separated proteins were transferred to PVDF membranes (Polyscreen NEF 1000 purchased from NEN Life Science Products) using a blot transfer system (BioRad Laboratories, Hercules, CA). After being blocked with 5% BSA-TBS solution (20 mM Tris, 500 mM NaCl, pH 7.5) overnight at 4 °C, membranes were incubated with rabbit anti-TGF-beta1 or goat anti-TNF-alpha polyclonal antibody solution (Santa Cruz Biotechnology) (1:1,000 and 1:500 dilution, respectively) for 1 h, at room temperature. The expression levels of β-actin (rabbit anti-β-actin, 1:500 dilution, Santa Cruz Biotechnology) were also analyzed to show the amount of protein loading. After washing three times with TTBS (0.1% Tween 20, 100 mM Tris–HCl, pH 7.5, 150 mM NaCl), membranes were incubated with an anti-rabbit or anti-goat IgG secondary antibody (1:5,000 dilution, Santa Cruz Biotechnology) linked to biotin for 1 h at room temperature. Membranes were washed again, and the color was developed using a Vectastain ABC detection system.

Histological studies

Light microscopy

The aortas were extracted and fixed in Bouin's solution. The samples were dehydrated in graded series of ethanol and embedded in paraffin. All sections were obtained from the same thoracic section of the artery. Sections of 5–6 μm thickness were obtained using a Porter Blum Hn40 microtome and stained with hematoxylin–eosin. Photographs were obtained with a Leitz Dialux microscope equipped with a Leica camera.

Electron microscopy

The aortas were fixed in glutaraldehyde 2% (v/v, final concentration) buffered in PBS (PBS: phosphate buffer saline, pH 7.2). The samples were dehydrated in graded

series of ethanol-acetone and embedded in Epon 812 (Pelco). Thin sections were obtained using a Ultracut Leica ultramicrotome. Microphotographies were obtained with a transmission electron microscope Zeiss 900.

Statistical analyses

Data are presented as means ± SEM. They were analyzed by Student's *t* test. Statistical significance was accepted at $p < 0.05$.

Results

Body weight and plasma retinol concentration

The initial body weight (g) of the animals of the two dietary groups was 55 ± 2.6. At the time of killing, the body weight of rats fed the vitamin A-deficient diet was significantly lower than that of control (381.43 ± 14.18 vs. 441.61 ± 10.38, $p < 0.01$). The lower body weight of vitamin A-deficient rats has also been shown by other authors [25]. In addition, we have previously demonstrated that deprivation of vitamin A for 3 months does not affect the daily food intake in relation to control rats [26]. Vitamin A deficiency was determined by the content of retinol in plasma. The plasma retinol concentrations (μmol/L) of rats fed the vitamin A-deficient diet were significantly lower (0.55 ± 0.013 vs. 1.80 ± 0.01, $p < 0.01$) than those of controls.

Vitamin A deficiency on thiobarbituric reactive substances and reduced glutathione levels

Serum (11.24 ± 1.17 vs. 8.15 ± 0.7; $p < 0.01$, $n = 4$) and aorta (13.26 ± 0.02 vs. 10.73 ± 0.01; $p < 0.05$, $n = 4$) TBARS (pmol MDA/mg protein) were significantly higher in vitamin A-deficient rats in comparison with control animals.

The GSH level (nmol/mg protein) in aorta from vitamin A-deficient rats was significantly lower (1.03 ± 0.07 vs. 1.95 ± 0.12; $p < 0.01$, $n = 4$) than the control.

Vitamin A deficiency on the levels of mRNA expression of TNF-alpha, NOX-2, and VCAM-1 in aorta

As shown in Fig. 1, the expression of TNF-alpha, NOX-2, and VCAM-1 mRNA was significantly higher in aorta from vitamin A-deficient rats when compared to control group ($p < 0.001$ for TNF-alpha and $p < 0.01$ for NOX-2 and VCAM-1).

Evaluation of TGF-beta1 and TNF-alpha expressions in aortas of vitamin A-deficient rats

Western blot analysis showed an increased expression of TGF-beta1 in vitamin A-deficient rats ($p < 0.05$) in relation to controls. Immunoblotting for the study of TNF-alpha protein levels are in concordance with analysis of mRNA expression by RT–PCR ($p < 0.001$) (Fig. 2).

Morphological changes induced by vitamin A deficiency in aorta

The intima of the aortas of control animals was composed of a continuous layer of endothelial cells. In the tunica media, several elastic fibers were seen to be lying parallel to each other with VSMC interposed between them (Fig. 3a). Irregular luminal layers of endothelial cell linings were noted in aortas of vitamin A-deficient group (Fig. 3b). Light microscopy revealed no apparent structural changes in the tunica media and adventitia of the aortas of vitamin A-deficient rats. By electron microscopy, the endothelial cells of control group were seen located close to the internal elastic laminae (IEL), leaving a relatively narrow subendothelial space with the least amount of connective tissue fibers. In addition, several pinocytotic vesicles were present in the cytoplasm. The apical membrane was regular (Fig. 4a). Endothelial cells of vitamin A-deficient rats showed cytoplasmic processes projecting into the lumen (Fig. 4b), and large vacuoles were observed inside the endothelium (Figs. 5, 6). At the subendothelial space, can be observed small vesicles mixed with dark bodies, multivesicular bodies and collagen fibers (Fig. 7). In vitamin A-deficient aortas (Fig. 8b), the electron-dense appearance of the IEL was different from the control ones (Fig. 8a). Ultrastructural changes in the tunica media were only noticeable in the aortas of vitamin A-deficient rats. Small vesicles mostly clear and some filled with granular material were observed in that layer (Fig. 9b).

Discussion

In this study, we demonstrated for the first time that the histoarchitecture of rat aorta is sensitive to circulating vitamin A levels, which can be associated to redox imbalance and inflammation.

It had been demonstrated that vitamin A modulates growth, differentiation, and morphology of the endothelial cells [11]. Inflammation and oxidative stress play a critical role in vascular injury [27]. In the present study, the vitamin A-deficient group had significantly higher MDA levels in serum and aorta and lower GSH levels in aorta compared to control rats. In addition, an increase in the aorta mRNA

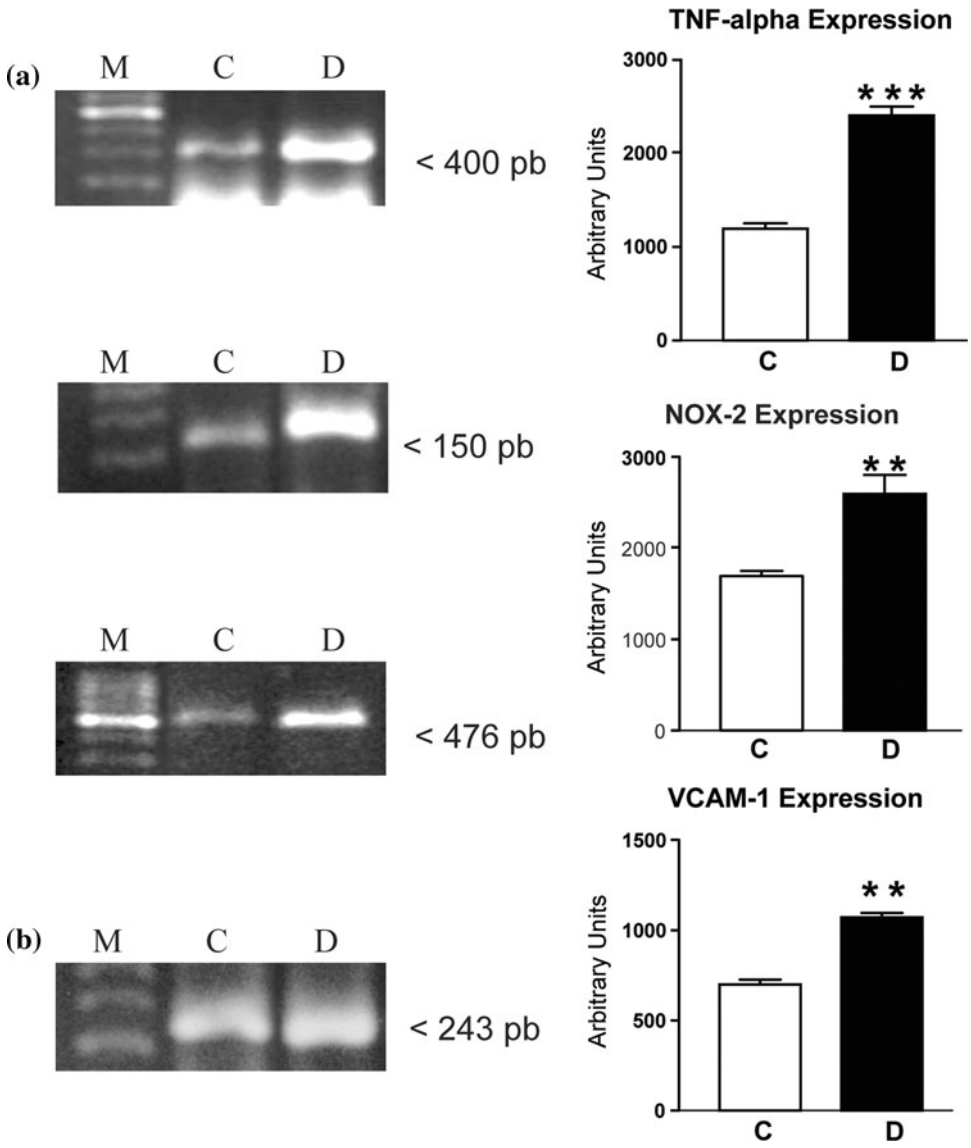

Fig. 1 Effect of Vitamin A deficiency on TNF-alpha, NOX-2, and VCAM-1 mRNA expression. Representative RT–PCR analysis for: **a** TNF-alpha, NOX-2, and VCAM-1 and **b** β-actin, used as an internal control. *M* molecular weight marker. On the side, quantification of the intensity of the fragment bands. Identical results were obtained in four independent experiments; **($p < 0.01$) and ***($p < 0.001$) indicate differences when Vitamin A-deficient rats (*D*) were compared to control (*C*)

NOX-2, enzyme involved in superoxide anion production, was observed with the vitamin A deficiency. In agreement, a decrease in the activities of antioxidant enzymes (SOD and GPx) in aortas from vitamin A-deficient rats, which was restored after vitamin A refeeding to control values, has been previously shown [14]. By contrast, retinoic acid (RA) has been shown to increase the peroxisome proliferator-activity binding to the peroxisome proliferator-response element that participates in the induction of the SOD gene and to increase the activity of CAT, SOD, and glutathione reductase, suggesting that RA may improve the antioxidant defense system [28]. All these findings indicate that vitamin A deficiency modifies the enzymatic and non-enzymatic antioxidant defenses in aorta. Thus, vitamin A could protect the artery against pro-oxidative environment. In addition, our group has previously shown a strong association of vitamin A deficiency with increased oxidative stress in heart, liver, and hippocampus [29–31]. The prooxidant effect of vitamin A deficiency in those tissues is also supported by the fact that incorporation of vitamin A into the diet of vitamin A-deficient rats reverted the increase of serum and heart TBARS and the decrease of heart GSH/GSSG ratio induced by vitamin A deficiency (unpublished results), and it also restored the antioxidant enzyme expressions in hippocampus to control levels [31].

On injury, endothelial cells are capable of producing various cytokines that participate in inflammatory reactions in the arterial wall. In the present study, the expression levels of TNF-alpha and VCAM-1 in aortas from vitamin A-deficient rats were increased, compared to control. It is known that TNF-alpha, derived from both inflammatory and endothelial cells, induces the cellular synthesis of ROS [32]. The enhanced TNF-alpha mRNA and protein expressions could contribute to the increased oxidative

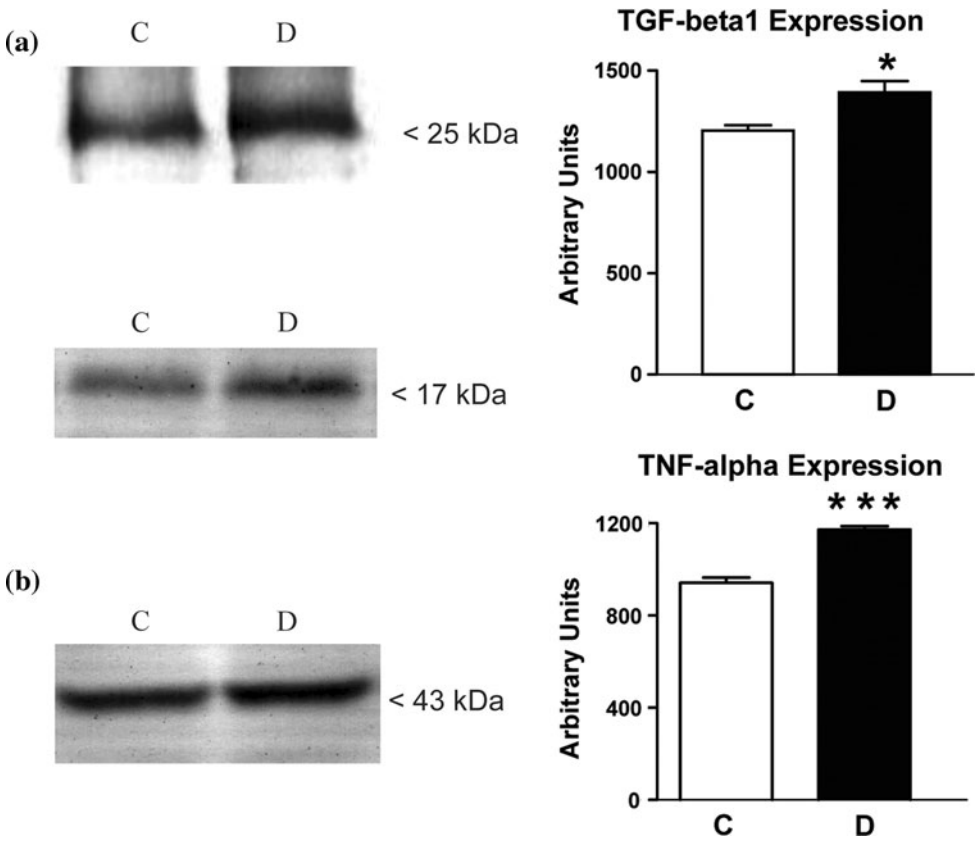

Fig. 2 Effect of Vitamin A deficiency on the expression of cytokines TGF-beta1 and TNF-alpha. Immunoblot analyses of: **a** TGF-beta1 and TNF-alpha and **b** β-actin, used as an internal control. On the side, quantitative analysis. Identical results were obtained in four independent experiments; *($p < 0.05$) and ***($p < 0.001$) indicate differences when Vitamin A-deficient rats (*D*) were compared to control (*C*)

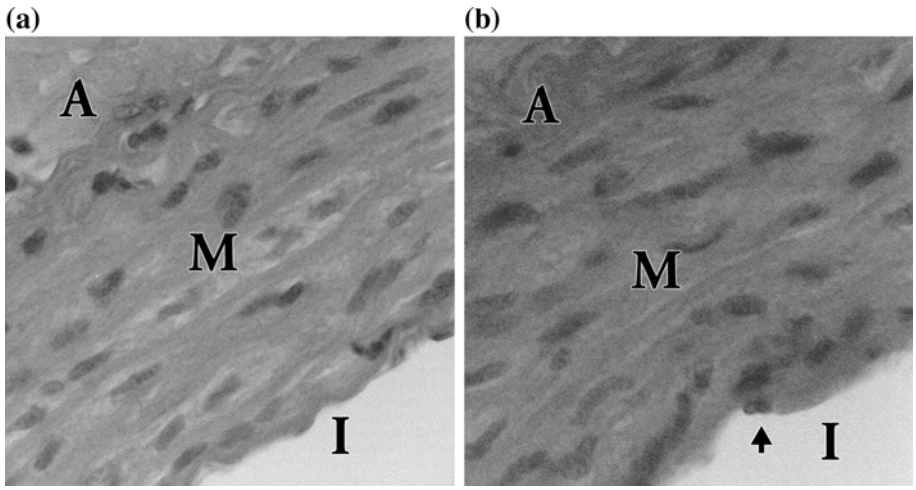

Fig. 3 Light photographies of the aortas of **a** Control and **b** Vitamin A-deficient rats. Note the irregular endothelial layer in Fig. 3**b** (*arrow*). *I* intimal layer; *M* medial layer; *A* adventitia. Hematoxylin-Eosin stain (×100)

stress found in aorta with vitamin A deficiency. It is also known that oxidative environment regulates VCAM-1 expression in endothelial cells [33]. Studies of the VCAM-1 promoter suggest that TNF-alpha activation of VCAM-1 transcription in endothelial cells is dependent, at least in part, on the activation of NF-kB [34]. It has been previously shown that the binding activity of the transcriptional activator NF-kB, considered as a "sensor" of oxidative stress, was increased in aorta of vitamin A-deficient rats and associated with coordinated expressions of pro-inflammatory iNOS and COX-2 [14]. The incorporation of vitamin A to the diet of vitamin A-deficient rats considerably improves the redox and inflammatory changes [14]. Vascular inflammation regulated by TNF-alpha is involved in endothelial permeability regulation [35]. The increased vascular permeability caused by endothelial damage may allow inflammatory cells, lipoproteins, other proteins, and plasma fluid to enter the subendothelial space [36]. In the vitamin A-deficient aortas, the presence of multivesicular bodies (MVB) in the subendothelial

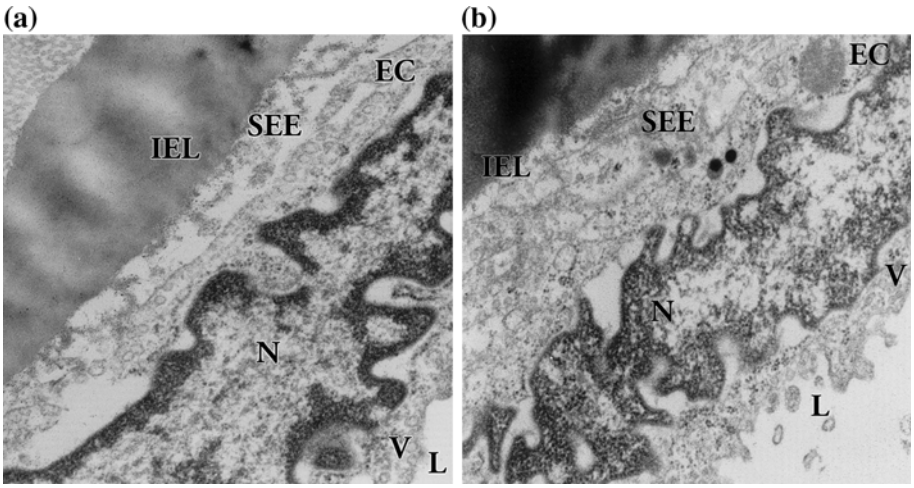

Fig. 4 Electron micrographs of aortas of **a** Control and **b** Vitamin A-deficient rats. Note the presence of the cytoplasmic processes in Fig. 4**b**. *L* lumen; *EC* endothelial cell; *V* pinocytic vesicles; *N* nuclei; *SES* subendothelial space; *IEL* internal elastic laminae (×20,000)

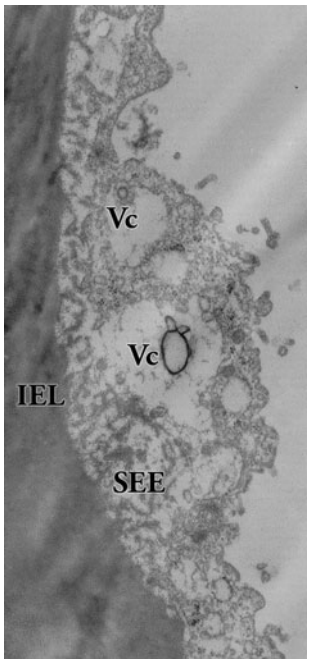

Fig. 5 Electron micrographs of aortas of Vitamin A-deficient rats. Note the presence of large vacuoles along the aortic endothelium. *Vc* vacuoles; *SES* subendothelial space; *IEL* internal elastic laminae (×12,000)

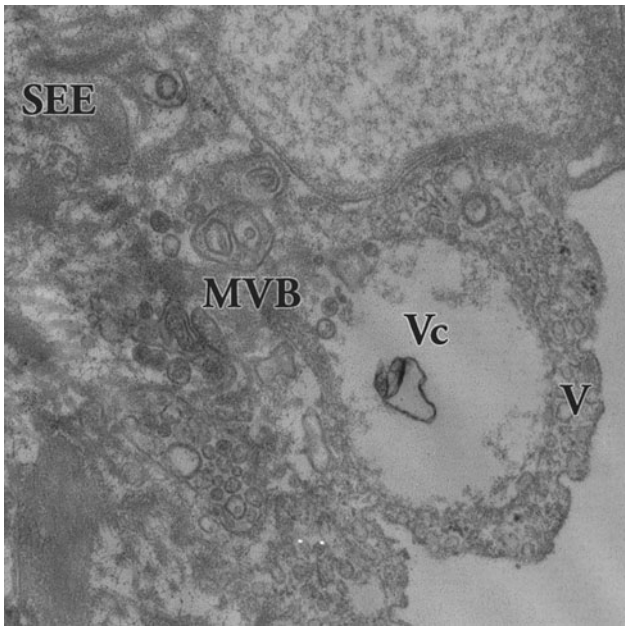

Fig. 6 Electron micrographs of aortas of Vitamin A-deficient rats. Note the presence of multivesicular bodies (*MVB*) and large vacuoles. *Vc* vacuoles; *V* pinocytic vesicles; *SES* subendothelial space (×20,000)

space was observed, in relation to control. The presence of MVB has been demonstrated by ultrastructural studies of injured vessels in the endothelium [37]. The MVB has been also observed in liver biopsies from alcoholic and nonalcoholic subjects, and it was negatively correlated with hepatic vitamin A level [38]. The MVB have been implicated in the traffic of lipids to the lysosomes and are associated to the endocytosis of LDL in endothelial cells [39]. The high serum and aorta TBARS levels found in this study, along with the decreased activity of PON-1 in serum and the increased expression of LOX-1 in aorta previously reported from vitamin A-deficient rats [15], lead us to propose that LDL could be modified by vitamin A deficiency and consequently could interact with the endothelium inducing aorta injury. Furthermore, the retention of lipoproteins by the intima could be promoted by the increase in the content of extracellular matrix components [40]. Vitamin A deficiency affects parenchyma and the expression of extracellular matrix proteins in liver, predisposing to fibrosis [41]. Although in this work we have not determined extracellular components expression, we noticed that vitamin A deficiency increased the TGF-beta1 protein expression in aorta. TGF-beta1 is the most potent cytokine in the pathogenesis of tissue

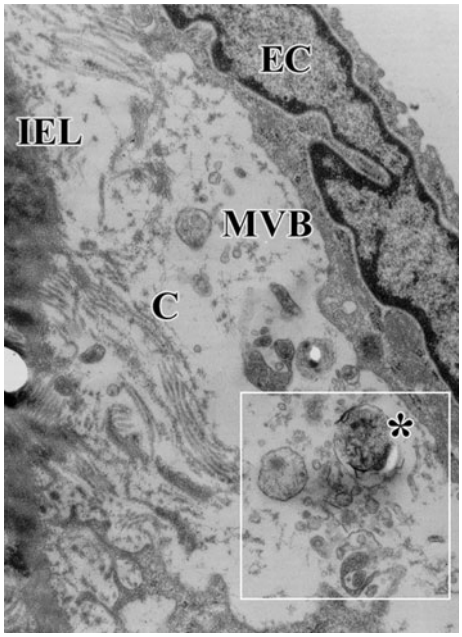

Fig. 7 Electron micrographs of aortas of Vitamin A-deficient rats. Note the thickened subendothelial space and the presence of multivesicular bodies (*MVB*). The *asterisk* indicates an electron-dense body. Small vesicles are locked up inside the box. *EC* endothelial cell; *C* collagen fibers; *IEL* internal elastic laminae (×12,000)

fibrosis, activating fibroblasts to secrete collagen and therefore, it provides the structural framework of stroma [42]. By contrast, it has been demonstrated that treatment with ATRA significantly decreased histological damage and TGF-beta expression [43].

The aorta of vitamin A-deficient rats showed large vacuoles inside the endothelium. Several models of vascular injury have been associated to the presence of vacuoles in arteries. Young-Ramsaran et al. [44] reported vacuolization of endothelial cells in a model of cardiac transplant-related accelerated arteriosclerosis. Similar results were informed by Huag et al. [45], who observed that plasma membrane was swelling with profuse intracellular edema and some vacuoles were seen in cytoplasm of endothelial cell in the presence of oxidized cholesterol. In addition to the oxidative stress observed in our experimental model, we have previously shown that vitamin A deficiency produces an increase in the aorta cholesterol content compared to control, which is reversed by vitamin A refeeding [15]. The cholesterol increase could contribute to the alteration in the histoarchitecture of the aorta internal elastic lamina (IEL) observed in animals fed on vitamin A-deficient diet. It is known that excess of cholesterol in coronary arteries induced ultrastructural changes of the IEL [46]. On the other hand, the presence of vesicles filled with granular material observed in the medial layer of aorta from vitamin A-deficient rats have been found by Kuwahara et al. [47] in a model of atherosclerosis.

Therefore, our results indicate that vitamin A deficiency induces histoarchitectural alterations in rat aorta and suggest that oxidative stress and inflammation are involved in these alterations. Vitamin A could protect aorta against the

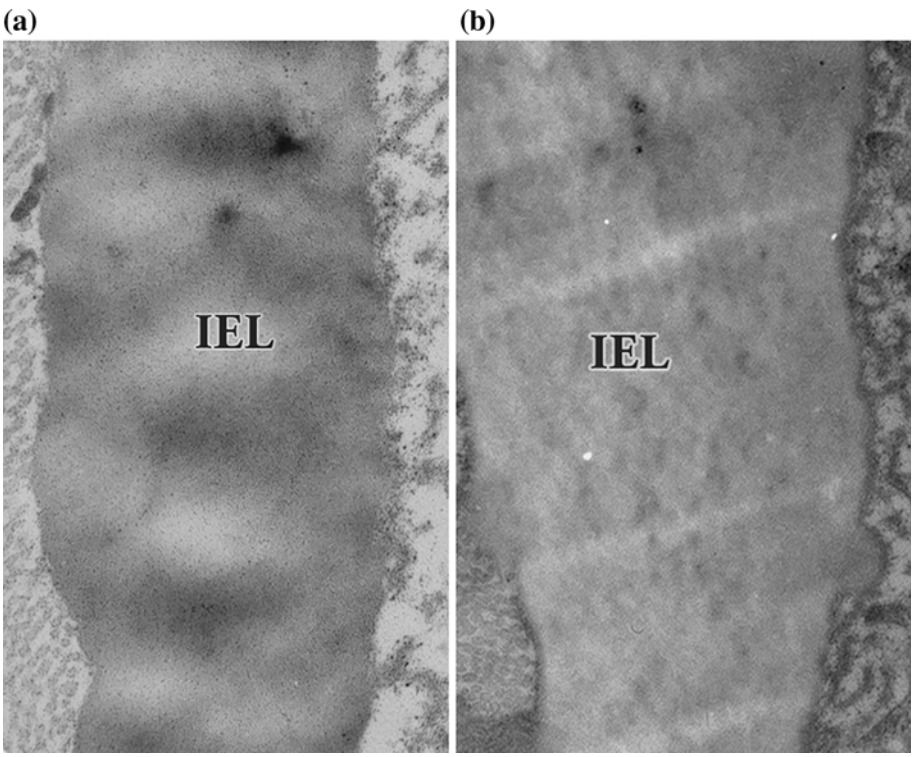

Fig. 8 Electron micrographs of aortas of **a** Control and **b** Vitamin A-deficient rats. Note the different histological feature in Fig. 8**b** when compared to Fig. 8**a**. *IEL* internal elastic laminae (×20,000)

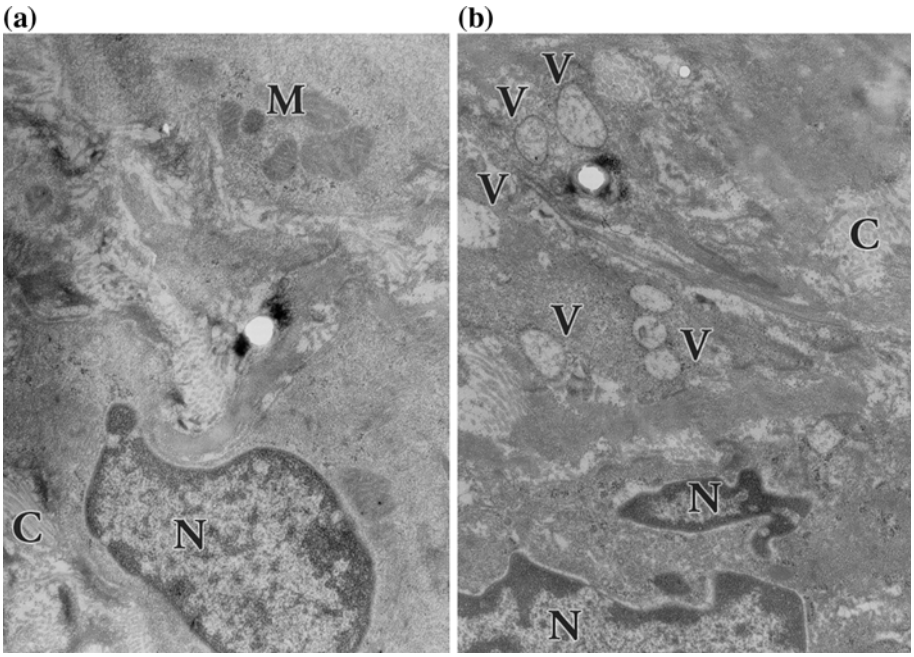

Fig. 9 Electron micrographs of aortas of **a** Control and **b** Vitamin A-deficient rats. Note the presence of several vesicles (*V*) in Fig. 9**b**. *N* nuclei; *C* collagen fibers; *M* mitochondria (×12,000)

endothelial dysfunction caused by the increment in cytokines (TNF-alpha, TGF-beta1) and adhesion molecules (VCAM-1), and associated redox changes (NOX-2 level and TBARS and GSH content), that can alter the maintenance of the aorta normal morphology. Knowing how vitamin A deficiency affects the aorta could provide some potential benefit in the prevention of vascular injury.

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