INTRODUCTION

Chronic inhalation of cigarette smoke is the main etiological factor in the development of lung emphysema. This has been proven in a huge number of studies in humans and animals (1). The pathophysiological mechanisms underlying cigarette smoke-induced emphysema have mainly remained unresolved. However there are some hypotheses that focus on the way in which damage is inflicted on the lung architecture and which have received the most attention in the last decades. Firstly, the protease/antiprotease theory (2), secondly the oxidant/antioxidant theory (3) and thirdly, the contribution of the immune system have received much attention (4). A different approach is focussing on the failure to repair lung tissue damage (5). Retinoic acid (RA) has long been known to be essential for proper lung development in the embryonal stage (6). Later on, a role was suggested for retinoic acid in the perinatal formation of alveoli (7). It has been shown that administering RA to adult rats and mice in which lung development had been artificially impaired in early life, stimulated alveolar wall formation (8, 9). This remarkable effect of exogenous RA has been attributed to “reawakening” of the gene cascades normally active during embryonic development (10). However, it should be noted that retinoids can have both detrimental and beneficial effects: on one hand they are teratogens and on the other hand have tumour-suppressive capacity (11). RA is one of a range of vitamin A metabolites (retinoids), each with different biological functions. All-trans retinol, the transport form of vitamin A, can be reversibly converted into retinyl esters (mostly retinyl palmitate) which serve as storage form, mainly in liver but also in lungs (12). The 11-cis, 13-cis, and all-trans retinoic acid, retinol and retinyl palmitate were measured in plasma, liver and right lung lobe. The left lung lobe was used to assess mean linear intercept (Lm), as a measure of smoke-induced lung damage. Average feed intakes were not different between treatment groups. We show that both retinol and retinyl palmitate levels were dramatically decreased in the storage organs of mice on the low vitamin A diet (retinol 2-fold in both lung and liver, and retinyl palmitate 5-fold in lung) which shows that the depletion was successful. However, this treatment did not result in the development of lung emphysema. However, smoke exposure led to a significant increase in Lm in mice with a low vitamin A status compared to the room air-breathing controls. Lung levels of acid retinoids were similar in all mice, irrespective of diet or smoke exposure. Concluding, a low vitamin A status increases the susceptibility to the development of cigarette smoke-induced lung emphysema, possibly because of decreased anti-oxidant capacity in the lungs due to locally reduced retinol and retinyl palmitate levels. These observations indicate that human populations with a low vitamin A status and a high prevalence of smoking may be at increased risk of developing lung emphysema.

Key words: cigarette smoke, lung emphysema, vitamin A, chronic obstructive pulmonary disease
through their ability to activate two families of nuclear hormone receptors; the retinoic acid receptors (RAR) and the retinoid X receptors (RXR). The natural ligands for the RARs are ATRA and its stereoisomers 9-cis RA and 13-cis RA, whereas RXRs are activated by 9-cis RA only (13). In this way, these retinoids control development and homeostasis, by regulating cell differentiation, proliferation and apoptosis in virtually every vertebrate tissue (14). Vitamin A deficiency has a range of deleterious effects. In its most extreme form, animals suffer from stunted growth, blindness, keratinisation of epithelial linings, defective development of testis and atrophy of central and peripheral lymphoid organs (15). Vitamin A deficiency causes lung damage in rats and enhance ozone-induced lung injury in mice (16, 17). On the otherhand, vitamin A toxicity or overdoses can be induced by different causes in human and animals. Research on vitamin A toxicity has been carried out primarily in animals, and most studies have been short-term and have focused on acute effects (18, 19). Besides bone abnormalities in human may be induced by hypervitaminosis A (20, 21).

Exposure to cigarette smoke decreases the levels of retinol in lungs of rats (22) and of RA in lung tissue of ferrets, through increased expression of cytochrome P450 enzymes (23). It also cannot be excluded that cigarette smoke itself induces oxidative degradation of retinol or other beta carotenes (24-26).

If RA is essential for the repair of damage in the adult lung, cigarette smoke could thus be a double-edged sword, causing damage while simultaneously depriving the lung tissue of the possibility of repair. Since low vitamin A intakes and even vitamin A deficiency continue to be widespread in developing countries (27, 28), while the smoking of tobacco is increasing in these areas (29, 30), this combination could have a major impact on the development of smoking-related lung diseases on a global scale. We, therefore, investigated the effect of cigarette smoke exposure on levels of the biologically active transport and storage forms of retinoids in lungs, plasma and liver and their relation with smoke-induced lung damage in mice with a normal and a reduced, but not deficient, vitamin A status.

MATERIALS AND METHODS

Chemicals

HPLC-grade acetonitrile, methanol, n-hexane and methyl tert-butylether were obtained from Biosolve BV (Valkenswaard, The Netherlands). 13-cis-RA, 9-cis-RA, acetic acid, acetone, ATRA, butyl methacrylate, chloroform, ethanol, methyl methacrylate, retinol, retinol acetate, retinyl palmitate and urethane of pro analysi quality were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands).

Animals

C57BL/6J breeding pairs were obtained from Charles River (Maasricht, The Netherlands) and were bred in the university breeding unit (Gemeenschapelijk Dieren Laboratorium, Universiteit Utrecht, The Netherlands) with the dams and their offspring on a pelleted, casein-based purified diet (Research Diets Services, Wijk bij Duurstede, The Netherlands). The diets contained either a normal (16,000 IU/kg) or low (200 IU/kg) amount of vitamin A, in the form of retinyl acetate (Table 1). These levels were chosen to reflect the vitamin A concentration in standard rodent chow, and a strongly decreased level which, while depleting the animal’s vitamin A stores, would not give rise to deficiency symptoms (31). Since mice are known to be very resistant to vitamin A depletion, and pups receive large amounts of vitamin A through maternal milk (31), the animals were bred for 3 generations on the special diet, before starting experiments. At 7 weeks of age, male animals of the fourth generation (i.e., 3 maternal progenitors had been on the special diet from weaning on) were transferred to the departmental animal unit. They were housed under a 12 h dark/12 h light cycle (lights on at 06:00), at 35-50% relative humidity and 20-22°C room temperature, on woodchips in polycarbonate cages, under filter-tops. Each treatment group consisted of 6 animals, stratified based on body weight. To allow easy and unambiguous identification of the animals, a radio frequency identification device (12×2 mm micro ID ISO transponder, UNO Roestvastaal BV, Zevenaar, The Netherlands) was implanted in the neck fat, while animals were under light isoflurane inhalation anaesthesia. The implantation wound was sealed using Histoacryl Blue (B. Braun AG, Melsungen, Germany). For each animal, the unique 16-digit code of the implanted transponder was linked to the treatment group it was assigned to; either low vitamin A diet – control, normal vitamin A diet – smoke, or normal vitamin A diet – control, or normal vitamin A diet – smoke. The transponders were read using the GES Reader 2S (UNO Roestvastaal BV, Zevenaar, The Netherlands). Animals received water and feed with normal or low vitamin A level ad libitum. Experiments were started when the animals were 11 weeks old. All experiments were conducted in accordance with the Animal Care Committee of Utrecht University.

Food intake and body weights

Food intake per group and individual body weights were measured every Monday morning, on a balance with an accuracy 1 g.

Table 1. Composition of the purified diets.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg</th>
</tr>
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<tbody>
<tr>
<td>Casein</td>
<td>200.0</td>
</tr>
<tr>
<td>Corn starch (native)</td>
<td>299.2</td>
</tr>
<tr>
<td>Dextrose</td>
<td>350.0</td>
</tr>
<tr>
<td>Soy Oil</td>
<td>50.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.0</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>12.4</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>10.5</td>
</tr>
<tr>
<td>NaH₂PO₄·2H₂O</td>
<td>3.4</td>
</tr>
<tr>
<td>MgCO₃</td>
<td>1.4</td>
</tr>
<tr>
<td>KCl</td>
<td>1.1</td>
</tr>
<tr>
<td>Mineral premix*</td>
<td>10.0</td>
</tr>
<tr>
<td>Vitamin premix*#</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* The mineral premix provided the following mineral concentrations per kg diet: 174 mg FeSO₄·7H₂O, 16 mg CuSO₄·5H₂O, 79 mg MnO, 33 mg ZnSO₄·H₂O, 0.2 mg KJ, 0.3 mg Na₂SeO₃·5H₂O, 0.3 mg NiSO₄·6H₂O, 2.0 mg NaF, 1.5 mg CrCl₃·6H₂O, 1.9 mg SnCl₂·2H₂O, 0.2 mg NH₄VO₃.

# The vitamin premix provided the following vitamin concentrations per kg diet: cholecalciferol 1,000 IU, DL-α-tocopherol 30 mg, menadione 0.05 mg, thiamin 4 mg, riboflavin 3 mg, pyridoxine-HCl 6 mg, cyanocobalamin 0.05 mg, niacin 20 mg, D-panthotenic acid 8 mg, cholin chloride 1,000 mg, folic acid 1 mg, biotin 2 mg. For the Normal diet: 16,000 IU Retinyl acetate. For the Low diet: 200 IU Retinyl acetate.
of 0.1 gram (CP2201, Sartorius, Göttingen, Germany) using the averaging function to correct for animal movement.

Smoke exposure

Cigarette smoke was generated by the burning of commercially available Lucky Strike™ cigarettes without filter with nicotine - 0.9 mg, Tar - 12 mg (British-American Tobacco, Groningen, The Netherlands), using the TE-10z smoking machine (Tague Enterprises, Davis, CA, USA), which is programmed to smoke cigarettes according to the Federal Trade Commission protocol (35-ml puff volume drawn for 2 seconds once per minute) as described before (32). Briefly, before starting smoke exposure, mice were accustomed to exposure tubes by gradually prolonging their stay in these over the course of two weeks. Then, smoke exposure was started with 1×1 cigarette, increasing the dosage to 2×3 cigarettes within two weeks. Mice were exposed nose-only to the diluted main- and side-stream smoke of 3 simultaneously burning cigarettes, twice every weekday for 3 months using the In-Tox 24-port nose-only exposure chamber (In-Tox Products Inc., Albuquerque, NM). Control mice underwent the same procedures, but were allowed to breathe room air throughout the whole exposure period.

Tissue preparation

Twenty-four hours after the last smoke exposure, animals were sacrificed. To minimise retinoid degeneration, section was performed under red light. Following injection of an overdose of pentobarbital (Nembutal™, Ceva Santé Animale, Naaldwijk, The Netherlands), blood was obtained by cardiac puncture, immediately mixed with 150 units of heparin (Leo Pharma, Weesp, The Netherlands) and centrifuged (600 g for 5 min) in a table-top centrifuge to obtain plasma. The liver was dissected free, separated from the gall bladder and rinsed in PBS. A cannula was inserted into the trachea and fixed with a ligature. The right branch of the trachea was closed with a ligature, and the right lung lobes were cut free. Right lung lobes, liver and plasma were snap-frozen in liquid nitrogen before storage at -70°C, awaiting processing. The left lung lobe was cut free from the heart and inflated via the cannula with Carnoy’s fixative (edanol:chloroform:acetic acid 60:30:10) at a fluid pressure of 25 cm for 5 min. Fixation was continued for at least 4 hours (edranol:chloroform:acetic acid 60:30:10) at a fluid pressure of -70°C, awaiting processing. The left lung lobe was immersed in fresh fixative for at least 24 hrs, after which it was embedded in paraffin. After paraffin embedding, 5 µm sections were cut and stained with hematoxylin/eosin (H&E). Lung morphometry was determined using digitised images of representative fields not containing vessels or bronchi as described previously (33-35). Briefly, mice (n=4-5), used for morphometric analysis, were sacrificed by an i.p. injection with an overdose of pentobarbital. After excision, the volume of the fixed lungs was measured by fluid displacement. Then, the left lung was immersed in fresh fixative for at least 24 hrs, after which it was embedded in paraffin. After paraffin embedding, 5 µm sections were cut and stained with hematoxylin/eosin (H&E) according to standard methods. Morphometric assessment of emphysema, included determination of the average inter-alveolar distance, was estimated by the mean linear intercept (Lm) analysis. The Lm was determined by light microscopy at a total magnification of 100×. Grids of 8 horizontal and 10 vertical lines were superimposed on the images, and the number of intersections with alveolar walls was counted using the Image Pro™ 4.0 software package and a custom-written macro. Per section two digitised images were captured each in the cranial, medial and caudal region, at 5x magnification. By dividing total grid length by the number of intersections, the mean linear intercept (Lm) was calculated. In total, 30 fields were used per lung to calculate the average Lm.

Retinoid extraction from tissue and plasma

To minimise retinoid loss and degradation, all procedures were carried out under red light, on ice, using glass labware. First, tissue was weighed. Then, a 25% (v/v) suspension was prepared in 0.5% acetic acid, using a Polytron homogeniser (Kinematica, Lucerne, Switzerland). Plasma was mixed with 3 volumes of 0.5% acetic acid. Then, four parts of tissue homogenate or plasma mixture were mixed with 6 parts of acetonitrile, vortexed vigorously for 1 minute and centrifuged for 5 minutes at 3490 × g. The liquid between pellet and floating cake was transferred to a new tube, mixed with 4 ml of n-hexane, vortexed vigorously for 1 minute and centrifuged for 5 minutes at 3490 × g. The hexane layer was transferred to a new tube and evaporated under a stream of nitrogen at 37°C. Residues were dissolved in 110 µl of a 50:50 methanol:water mixture and transferred to 300 µl inserts inside amber HPLC vials (Omnilabo, Breda, The Netherlands).

Retinoid separation and detection using HPLC/MS/MS

Retinoid separation and detection was performed as described before (36). Briefly, after addition of 10 µl retinyl acetate (1.2 pmol/µl in 50:50 methanol:water) as internal standard to the samples, analytes were resolved on a 1.5 µm analytical reversed-phase C18 column (Micro NPS ODS-1, Eprogen, Darien, IL, USA) at a flow rate of 350 µl with a gradient of solvent A (water/methanol/acetic acid 50:50:0.5) and solvent B (methanol/methyl tert-butylether/acetic acid 50:50:0.5) using a micro LC pump and an autosampler cooled at 4°C (to minimise sample degradation) (2000 Series, Perkin Elmer, Boston, MA, USA). Injected sample volume was 100 µl. The gradient was as follows: 0 to 1 minutes; 30%B, 1 to 7 minutes; 30 to 75%B, 7 to 7.1 minutes; 75% to 90%B, 7.1 to 10 minutes; 90% to 10%B, 10 to 10.1 minutes; 10 to 30%B, 10.1 to 16 minutes; 30%A. The eluent was passed (through fused silica tubing, to avoid loss of retinoids) to a triple quadrupole mass spectrometer (API 3000, MDS Sciex/Applied Biosystems, Foster City, CA, USA) equipped with an atmospheric pressure chemical ionisation probe for detection of the retinoids. To detect 13-cis RA, 9-cis RA and ATRA, in selected reaction monitoring mode the transition from m/z 301.2 to 205 was followed, and for retinol, retinyl acetate and retinyl palmitate the transition from m/z 269.2 to 93, with a dwell time of 400 ms. Source temperature was set at 400°C. Standards consisted of 0.1, 0.05, 0.01, 0.005 and 0.001 pmol/µl mixtures of 13-cis RA, 9-cis RA, ATRA, retinol and retinyl palmitate. Chromatograms were analysed and analytes quantified using Analyst software version 1.4.1 (Applied Biosystems, Foster City, CA, USA). The limit of quantification (signal-to-noise ratio of 10:1) was calculated at 0.04 pmol for ATRA, 13-cis RA, retinol and retinyl palmitate, and 0.08 pmol for 9-cis RA.

Statistics

Effects of dietary vitamin A status and smoke exposure were tested using the Univariate General Linear Model. Comparisons
between groups were made using two-way ANOVA, followed by Bonferroni’s correction for multiple comparisons. All calculations were performed using SPSS 13 for Windows. A confidence level of $p<0.05$ was considered significant.

RESULTS

Effects of dietary vitamin A and smoke exposure on food intake and body weights

There was no effect of dietary vitamin A level or smoke exposure on average food intake between the ages of 8 and 25 weeks (Fig. 1A). In all groups, body weights increased sharply between 8 and 11 weeks of age, after that the increase levelled off (Fig. 1B). Body weights were similar in all groups at all times, except for week 11, when the smoke-exposed group on a normal vitamin A diet weighed significantly less than the smoke-exposed group on low vitamin A diet.

Effects of dietary vitamin A and smoke exposure on retinoid profiles of tissues

1. Lungs

Neither dietary vitamin A level nor smoke exposure had an effect on the concentration of any of the acid retinoids measured in the lung (Fig. 2A). Retinol levels were affected by dietary vitamin A level, but not by smoke exposure; they were decreased in animals on the low, compared to the animals on the normal diet, but only significantly ($p=0.0001$) in smoke-exposed mice (Fig. 2B). Both dietary vitamin A and smoke exposure had an effect on retinyl palmitate levels. Again, levels were decreased in animals on the low, compared to those of animals on the normal diet, but this was only significant ($p=0.001$) in smoke-exposed mice. Additionally, in smoke-exposed animals fed the normal vitamin A diet, retinyl palmitate levels were significantly increased, compared to room air-breathing controls on the same diet ($p=0.02$). In animals on the low vitamin A diet, concentrations were below the limit of quantification.

2. Plasma

Dietary vitamin A levels had no significant effect on the plasma concentrations of any of the acid retinoids (Fig. 3A). Smoke exposure affected both ATRA and 13-cis RA levels. It increased 13-cis RA significantly in mice on the low diet ($p=0.04$) and tended to increase ATRA. Levels of 13-cis and 9-cis RA were below the limit of quantification in the room air breathing control group on a low vitamin A diet. Retinol levels were unaffected by dietary vitamin A level and tended to increase upon smoke exposure (Fig. 3B). Retinyl palmitate levels were below the limit of quantification in all mice.

3. Liver

Both dietary vitamin A levels and smoke exposure affected values of 9-cis RA, but not of 13-cis RA or ATRA, in liver (Fig. 4A). Levels of 9-cis RA were below the limit of quantification in mice fed a diet with normal vitamin A levels, irrespective of smoking. However, room-air breathing controls on a low vitamin A diet had detectable liver 9-cis RA levels (higher than normal vitamin A diet, $p=0.002$), but these decreased below the quantification limit upon smoking ($p=0.001$). Both retinol and retinyl palmitate concentrations in liver were affected by dietary vitamin A, but not by smoke exposure (Fig. 4B). Retinol levels were decreased in mice fed the low vitamin A diet, compared to animals on the diet with a normal level of vitamin A, to a significant degree in both room air-breathing controls ($p=0.0001$) and smoke exposed animals ($p=0.0003$). Retinyl palmitate concentrations were below the limit of quantification in mice on the diet containing a low level of vitamin A, irrespective of smoking, and decreased when compared to the normal vitamin A diet animals, but only significantly between the control groups ($p=0.009$).

Effects of dietary vitamin A and smoke exposure on Lm

A three months smoke exposure did not result in lung emphysema in mice with normal vitamin A levels (Fig. 5).
addition, no effect was observed on the Lm in the offspring of 3 generations of air exposed mice (Fig. 2A) that were fed a purified diet containing low levels of vitamin A and showed significant lower levels of retinol and retinyl palmitate levels in liver and lungs. In contrast, a significant increase in Lm was observed when the combination of vitamin A deficient mice with cigarette smoke was used (p=0.03) (Fig. 2B and Fig. 6B).

**DISCUSSION**

In this study we investigated whether vitamin A status influenced the susceptibility of male C57BL/6J mice to cigarette smoke-induced lung emphysema. To this end, we exposed mice with a normal or reduced dietary vitamin A status to cigarette smoke. Since laboratory mice are bred on diets with very high vitamin A levels, it is difficult to deplete their body vitamin A stores (37). Therefore, we bred mice for 3 generations on a purified diet containing low levels of vitamin A to obtain mice with a reduced, but not deficient, vitamin A status. The observation that both retinol and retinyl palmitate levels were dramatically decreased in the storage organs of mice on the low vitamin A diet (retinol 165-fold in both lung and liver, and retinyl palmitate 424-fold in lung and 73-fold in liver), shows that the depletion was successful. It did not result in deficiency, since retinol levels in plasma were only 3-fold reduced, while a reduction to <10% of normal levels has been reported in deficient mice (38). Furthermore, the mice did not show any of the prototypical signs of vitamin A deficiency (15).

The low vitamin A status per se did not lead to emphysematous changes in lung structure, as has been described before for vitamin A deficient rats (16) and transretinoic acid did not cause a reversal of lung emphysema in mice (39). However, vitamin A depletion induced by cigarette smoke is associated with the development of emphysema in rats (58). In addition, mice with a low vitamin A status, unlike the ones with a normal status, were susceptible to the induction of lung emphysema after 3 months of exposure to cigarette smoke. Since the mice with the emphysematous changes had similar lung levels of acid retinoids as the smoke-exposed, ‘healthy’ mice with a normal vitamin A status, and since acid retinoids are thought to be essential for alveolar wall regeneration (9), the smoke-induced emphysematous changes in the mice with a low vitamin A status are apparently not due to reduced repair because of a lack of these retinoids. Lack of repair, however, cannot be excluded, for instance because oxidative stress that originates from smoke exposure could have reduced the expression of RAR (40), or the
RAR/RXR–induced transcription of genes (41, 42) that are essential for repair processes. Interestingly, lung levels of the storage retinoids, retinol and retinyl palmitate, were considerably lower in the lungs of animals with a reduced vitamin A status. Since the latter retinoids are more potent antioxidants than acid retinoids (43), and much more abundant in the lungs, a reduction of their levels may result in more oxidative damage upon smoke exposure and decreased repair because of reduced retinoid signalling. Our observation that smoke exposure did not decrease retinoid levels in the lung was unexpected, since cigarette smoke contains numerous aryl hydrocarbon receptor ligands (44), which have been found to cause depletion of retinoids by inducing cytochrome P450 enzymes that catabolise retinoids (23, 45, 46). Retinoid levels, however, were assessed 24 hrs after smoke exposure and may have been replenished during that period.

Fig. 4. Acid retinoid and retinoid levels in livers of mice fed a diet with different vitamin A levels and exposed to cigarette smoke. Mice were fed a diet containing a normal (16,000 IU/kg) or low (200 IU/kg) amount of vitamin A, and were exposed daily to smoke or room air from 13 to 25 weeks of age. Twenty-four hours after the last smoke exposure, livers were isolated and snap-frozen. After extraction, retinoid levels (A) 13-cis RA; white bars, 9-cis RA; light grey bars, ATRA; dark gray bars. (B) retinol; white bars, retinyl palmitate; light gray bars. In tissue were determined by HPLC with MS detection. Bars represent means ±S.E.M. of 4-6 animals per group. * significantly different from control, p<0.05. #: significantly different from normal diet, p .<0.05.

Notably, smoking tended to increase plasma levels of acid retinoids and retinol, both of which can replenish pulmonary retinoid levels (47). Such an increase may be indirectly indicative of increased retinoic acid turnover in the smokers’ lungs, since this is sensed by the body and results in release of retinoids from the liver (48). Nevertheless, smoking-induced retinoid depletion in the lungs can at most have been modest in our mice, since we did not observe marked depletion of liver retinoids in the smoke-exposed mice, except for a significant depletion of 9-cis RA in the mice with a low vitamin A status. Our observation that a reduced, but not deficient, vitamin A status potentiated smoking-induced lung damage may be relevant to the human situation. In agreement, very recently Hirayama et al. demonstrated that high levels of insoluble fiber, total dietary fiber, retinol and vitamin A intake were associated with reduced risk of COPD (49). Whereas in the western world vitamin A intake is generally above the recommended daily dose (50), a recent study showed that intake by young adults (19-24 year olds) in the UK can be below the recommended levels: even up to 16% lower (51). Moreover, it was previous reported that chronic ethanol consumption results in a significant drop in hepatic vitamin A in humans (52). In the same study, a toxic interaction between ethanol and β-carotene in nonhuman primates was reported (52).

In this regard, the percentage of smokers in this age group is higher than in any other, as is the percentage of excessive drinkers (53) while alcohol abuse is known to deplete hepatic vitamin A levels (49). Smoking by young adults with a low vitamin A and a high alcohol intake might thus lead to disproportionate lung damage at that age. This could lead to earlier manifestations of smoking-related lung disease if the unhealthy life style is continued. Although vitamin A intake is generally above recommended levels in western countries even in this area, there are groups at risk for low levels of retinol. These include women that have had multiple pregnancies (54) and possible obese individuals, a growing number in western countries.

Fig. 5. Distance between alveolar walls in lungs of mice fed a diet with different vitamin A levels and exposed to cigarette smoke. Mice were fed a diet containing a normal (16,000 IU/kg) or low (200 IU/kg) amount of vitamin A, and were exposed daily to smoke or room air from 13 to 25 weeks of age. Twenty-four hours after the last smoke exposure, lungs were isolated. The left lung lobe was fixed under pressure for morphometry. The mean distance between alveolar walls (Lm) was measured in digitised photomicrographs of haematoxylin & eosin stained lung sections (3 µm) using image-processing software. Bars represent means ±S.E.M. of 4 animals per group. * significantly different from each controls: low and normal diet p<0.05.

The mechanism underlying deficiency of vitamin A in obese people has not been established yet. However, conflicting reports of increased serum retinol-binding protein 4 in obese humans (54, 55) highlight the relevance of this question considering the reported expansion of intravascular volume (56) and the storage of vitamin A in adipose tissue (57). Thus, much more serious
consequences may be expected in the future in developing countries, especially (27) since cigarette smoking is still on the rise (21, 30). From the present results and a recent study by Herzog et al., it may be suggested to increase vitamin intake in patients suffering from lung diseases such as COPD. However caution should be taken since, Goodman et al. has reported that the beta-carotene and retinol increased the incidence of lung cancer and cardiovascular disease mortality (60).

Abbreviations: RA- retinoic acid; ATRA- all-trans retinoic acid; RAR- retinoic acid receptors; RXR- retinoid X receptors

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Conflict of interests: None declared.

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Fig. 6. Representative photomicrographs of hematoxylin and eosin stained lung tissue of air-exposed mice (A) or smoke-exposed mice (B). Mice were daily exposed to room air or cigarette smoke from 13 to 25 weeks of age as described in material and methods (original magnification ×200).


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