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OBESITY INDEPENDENT EFFECTS OF HIGH FAT DIET ON PULMONARY ARTERIES REACTIVITY

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Abstract

Published data sustain the involvement of diet on pathogenesis of immune-mediated diseases.

Aim. To determine if the high fat diet (HFD), in the absence of obesity, could modulate the altered pulmonary arteries reactivity associated to allergic lung diseases. **Materials and Methods.** Obese resistant rats were divided into 2 groups: standard chow diet and HFD. Randomly chosen rats from both groups were sensitized against ovalbumin. The histological aspect and reactivity of pulmonary arteries were comparatively assessed. Taking into account the involvement of adipokines on obesity associated vascular reactivity alteration we also studied the vasomotor effects of few adipokines on pulmonary vessels.

Results. Lung histological examination revealed that HFD aggravated the remodelling of pulmonary arteries and inflammation of lung parenchyma. The HFD amplified the phenylephrine - induced contractions. Angiotensinogen amplified and apelin inhibited the Phe contractile effects on sensitized HFD fed rats.

Conclusion. These effects could be at least mediated, by both the alteration of adipokines vasomotor effects and inflammation associated to pulmonary allergic disease.

Key words: high fat diet, angiotensinogen, apelin, leptin, pulmonary artery, allergy, rat.

INTRODUCTION

Published data sustain that high-fat diet (HFD) could have important detrimental effects on arterial function. Saturated fat intake has been linked to a higher risk of cardiovascular disease by increasing concentrations of LDL cholesterol (1) and uptake of cholesterol in the arterial wall (2). Moreover, high-fat meals could lead to exaggerated cardiovascular reactivity in healthy, normotensive individuals (3). Furthermore, leading to excessive

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energy intakes, HFD was strongly linked to the increasing obesity that is a key risk factor for the development of vascular disease (4, 5). It is known that obesity is associated with various conditions such as chronic pro-inflammatory state, increased responses to allergy triggers and cardiovascular disease risk. The relationship might be, at least partially, the result of disturbance on adipose tissue endo(para)crine secretion of adipokines, some of them having well documented, obesity altered, vasoactive properties (6). Published data indicated that obesity is also associated with a higher risk of developing pulmonary hypertension (7 - 10).

In this study were used only obese resistant rats to underline obesity - independent HFD effects on vascular functions. On the other hand, taking into account both the role of obesity in the development of pulmonary hypertension and our data about adipokines contribution on pulmonary vessels reactivity on healthy and sensitized rats (11) we studied the effects of some adipokines (leptin, apelin, angiotensinogen) on contractile responses of pulmonary arteries from obese resistant rats fed with a HFD. Our aim was to assess the interaction between HFD and adipokines on isolated pulmonary arteries from healthy and ovalbumin sensitized rat, in the absence of obesity.

MATERIAL AND METHODS

Rats. Obese resistant rats (OR rats; Charles River Laboratories International Inc., Wilmington, MA) were developed from a line of Crl:CD(SD) rats. This model is supposed not to become obese when fed high-fat diet. Rats were divided into 2 groups. Group I was fed a standard rat chow diet (12% of calories as fat). Group II was fed a HFD (32.5% of calories as fat). After 4 weeks, rats from both groups were randomly selected to become OVA sensitized as previously described (12). There was no significant difference between the body weight of rats from both groups (Fig. 1). All the experiments described here were performed in compliance with the European Communities Council Directive 86/609/EEC and Ordinance No. 37/2002 of the Romanian Government.

Sensitization. The rats were sensitized against OVA by s.c. and i.p. injection of 0.2 mL physiological saline, containing 100 mg OVA and 8 mg aluminium hydroxide. The protocol was repeated 2 weeks later (11,12). *In vitro*

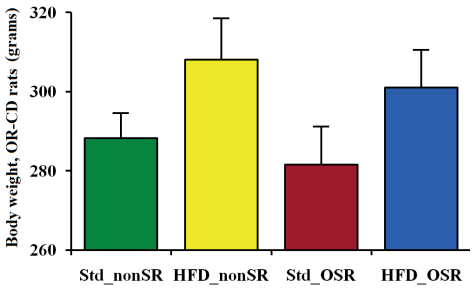

Figure 1. The body weight (mean ± SEM) in male obese resistant rats fed standard chow diet (Std) or high fat diet (HFD). Either high fat diet (HFD) or ovalbumin sensitization did not significantly modify the weight of male obese resistant rats in this study. Std_nonSR =standard fed non sensitized rats (green bar); HFD_nonSR = high fat diet fed non sensitized rats (yellow bar); Std_OSR = standard fed ovalbumine sensitized rats (red bar); HFD_OSR = high fat diet fed ovalbumine sensitized rats (blue bar).

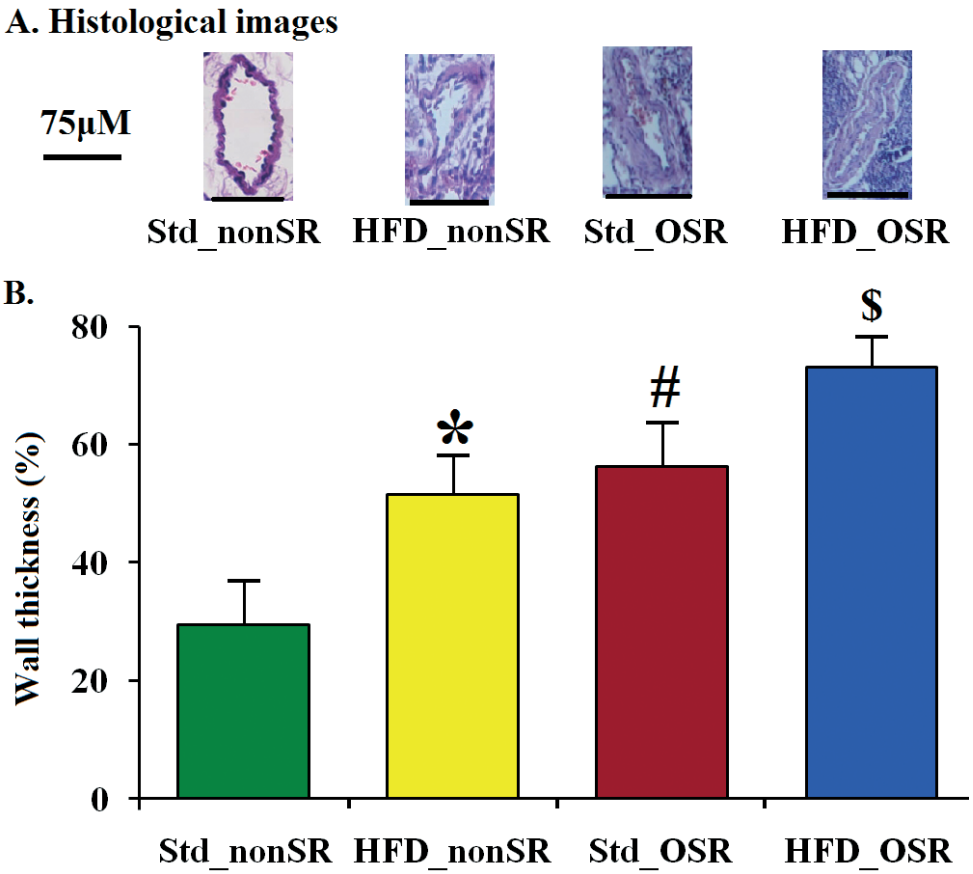

Figure 2. Effects of high fat diet on pulmonary arteries structure in non-sensitized (nonSR) orovalbumin sensitized (OSR) rats. (A): Muscularized pulmonary arteries (with an external diameter lower than 150 µm) from standard chow diet (Std)/ high fat diet (HFD) fed non-sensitized (nonSR)/ovalbumin sensitized (OSR) rats. Histological examination reveals increase in muscularization of small pulmonary arteries and perivascular inflammatory infiltrate induced by HFD feeding and /orovalbumine sensitization. Scale bars represent 75µM. (B): The wall thickness (%) was calculated as described in the text (mean±SEM). Both, HFD and ovalbumin sensitization increased the wall thickness of pulmonary arteries as compared with standard chow fed non sensitized rats.Std_nonSR =standard fed non sensitized rats (green bar); HFD_nonSR = high fat diet fed nonsensitized rats (yellow bar); Std_OSR = standard fed ovalbumine sensitized rats (red bar);HFD_OSR = high fat diet fed ovalbumine sensitized rats (blue bar).*: $p<0.05$ when compared HFD fed non sensitized rats with Standard chow fed non sensitized rats (HFD_nonSR vs. Std_nonSR). #: $p<0.05$ when compared standard chow fed ovalbumin sensitized rats with non sensitized rats (Std_OSR vs. Std_nonSR). $: $p<0.05$ when compared HFD fed ovalbumin sensitized rats with non sensitized rats (HFD_OSR vs. HFD_nonSR).

challenge: After 7 days, before starting the administration of the studied substances, vascular rings were pre-treated with OVA (100 mg/mL).

Myography. The second order rat pulmonary arteries were rapidly removed, cleaned and cut into 1-2 mm wide rings. Individual rings were then mounted between tungsten (50μm) wires organ bath. Individual rings were then mounted in a MYO-01 MYOGRAPH SYSTEM (Experimetria LTD., Budapest, Hungary) and changes in vessel tension were recorded and analyzed by ISOSYS data acquisition system (Experimetria LTD., Budapest, Hungary). The tissue organ bath contained the Krebs–Henseleit solution containing (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.6, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.5. The Krebs-Henseleit buffer was maintained at 37°C, and bubbled continuously with a mixture of 95% O_2 and 5% CO_2 (pH=7.2–7.4). A resting tension of 0.3g for PA was applied to each ring and then allowed to equilibrate for 45-60 minutes before starting the experiment as previously described (11). The bathing medium was renewed every 15 minutes. After the equilibration period, vessel rings were initially stimulated with 40 mM KCl as a standard stimulus. The functional integrity of the endothelium was assessed by testing the degree of relaxation produced by adding 10 μM acetylcholine (ACh) to 1 μM phenylephrine (Phe) pre-contracted rings. The rings that produced less than 50% relaxation in response to ACh were discarded. After re-equilibration in Krebs solution for 45 min, we administered leptin (LEP, 10 ng/mL), angiotensinogen (Aogen, 10 μM) and apelin 13 (AP13, 1 μM) in doses that did not modify the basal tone (11, 12). After 10 minutes, 1 μM Phe was administered and the contraction was measured on plateau phase as percentage from control contraction 40 mM KCl. To assess the mechanism involved in Aogen and AP13 effects were used losartan (LOS, 1 μM, a blocker of AT1 receptors) and apelin13 [ALA13] (F13A, 1 μM, apelin-specific antagonist) administered with 10 minutes before Phe. Results are expressed as mean ± S.E.M (n=6). The statistical significance was tested using one-way analysis of variance (ANOVA), completed by the Bonferroni method (SigmaStat software, Jandel Corporation). $p<0.05$ was considered statistically significant.

Histology. After removal of pulmonary arteries, the lungs were embedded in paraffin. Lung parenchyma sections were stained with haematoxylin-eosin. Pulmonary vascular remodelling was examined (as previously described) (11) in 10 (per animal) muscularized pulmonary arteries (with an external diameter of 100-150μm). Random circular vessel profiles were selected; the external and the internal diameter were measured and reported as wall thickness (%) = (external diameters – internal diameter)/external diameter x 100.

Leptin, angiotensinogen, losartan, phenylephrine were all obtained from Sigma-Aldrich Inc. (St Luis, MO, US). Apelin 13 and the antagonist Apelin-13[Ala13] were purchased from Phoenix Europe GmbH (Germany). All the other compounds used were of analytical grade.

RESULTS

Lung histological examination revealed that HFD increased the sensitization – induced remodelling of pulmonary arteries (Fig. 2A and 2B). Both HFD feeding or ovalbumine sensitization increased the wall thickness and perivascular inflammatory infiltrate (Fig. 2A) as compared with standard chow fed non-sensitized rats. There was a significant increase of the wall thickness on HDF fed rats as compared with standard chow fed rats (51.49 ± 6.54% *vs.* 29.52 ± 7.11%; (Fig 2B and Table 1) only on non sensitized rats (Fig. 2B). The ovalbumin sensitization increased the wall thickness rats fed with either standard chow (56.33 ± 7.78% *vs.* 29.52 ± 6.11%) or HFD (73.08 ± 5.39% *vs.* 51.49 ±6.54%). The HFD increased the Phe -induced vasoconstriction only on non sensitized rats (83.96 ± 3.01% *vs*. 69.27 ± 4.43%). On the other hand, the sensitization amplified the Phe effects in pulmonary arteries of rats fed with either standard chow (86.25 ± 2.90% *vs.* 69.27 ± 4.43%) or HFD (93.08 ± 1.88% *vs.* 83.96 ± 3.01%) (Fig. 3A). In pulmonary artery rings pre-treated with LEP the HFD increased the Phe -induced vasoconstriction on both non sensitized (88.48 ± 4.80% *vs.* 68.57 ± 4.60%) and sensitized rats (115.58 ± 9.39% *vs.* 90.33 ± 5.59) (Fig. 3B).

The LEP pre-treatment did not induce any significant variation of Phe – induced contraction on our experimental conditions. Phe alone had higher contractile effects on HFD fed as compared with standard chow fed rats non-sensitized rats; sensitization increased Phe – induced contraction on both standard chow or HFD fed rats. In non sensitized rats, the Aogen pre-treatment significantly increased the contractile effects of Phe only on pulmonary arteries from HFD fed rats (102.22 ± 6.35% as compared with 83.96 ± 3.01% obtained in the absence of Aogen). The pulmonary arteries from sensitized rats fed with either standard chow or HFD contracted powerfully as response to Phe in the presence as compared with the absence of Aogen (100.02 ± 4.47% and 112.53 ± 4.16%, Fig. 3C). At the same time, on the HFD fed group, after the Aogen pre-treatment, there was no more a significant difference in the Phe effects on non-sensitized as compared with sensitized rats.

The AP13 pre-treatment reduced both the difference between contractile responses to Phe of pulmonary arteries from HFD (80.76 ± 5.30%) *vs*. standard chow fed rats (66.89 ± 4.93%) and the sensitization increased the Phe contractile effects only on standard chow fed group (Fig. 3E). So, the AP13 effects on Phe – induced contractions were opposite as compared with Aogen on HFD-induced increase of Phe contractile effects. Taking into account the Aogen and AP13 effects on Phe-induced contractions we included in our study the losartan (LOS, a blocker of AT1 receptors) and F13A (blocker of APJ receptors).

The significant difference between HFD and standard chow fed non sensitized rats was still maintained even in the presence of LOS (82.36 ± 3.89% *vs.* 63.88 ± 4.37%) only on non-sensitized rats. LOS pre-treatment prevented the sensitization associated

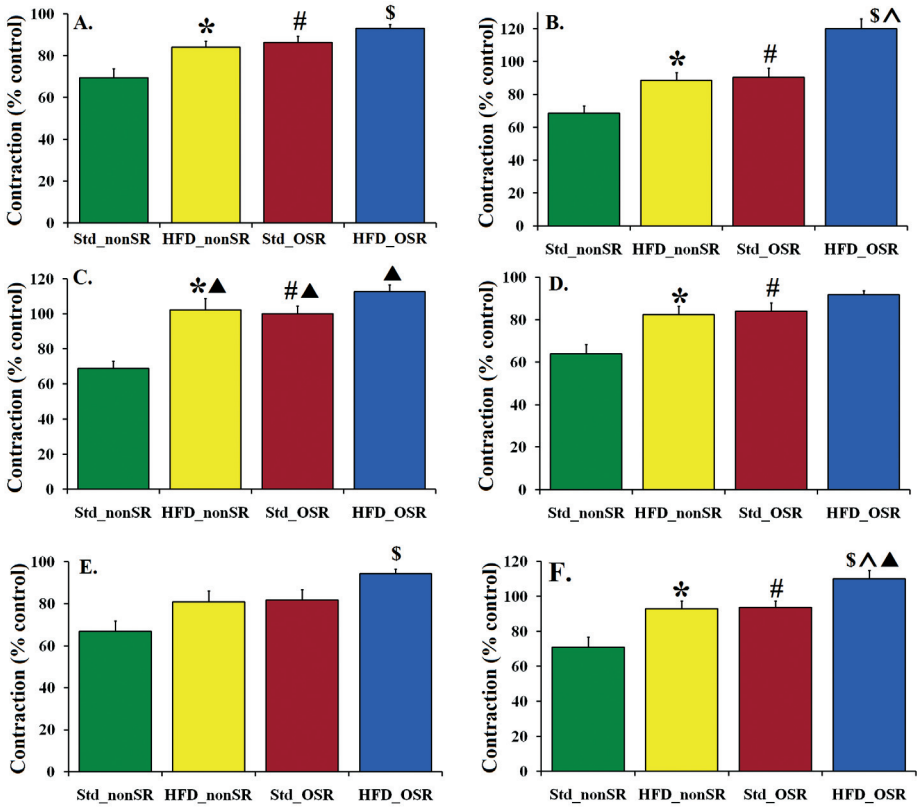

Figure 3. Phenylephrine (Phe)-. induced contraction of pulmonary arteries rings from non sensitized (nonSR) or ovalbumin sensitized (OSR) rats fed with standard chow diet (Std) or high fat diet (HFD). Contractile effects of Phe were recorded in the absence (A) or in the presence (B-F) of leptin (B), angiotensinogen (C), losartan (D), apelin 13 (E) and apelin-13[Ala13] (F). Results were expressed as percentage from control contraction elicited by 40 mM KCl (average ± SEM). Std_nonSR =standard fed non sensitized rats (green bar); HFD_nonSR = high fat diet fed non sensitized rats (yellow bar); Std_OSR = standard fed ovalbumine sensitized rats (red bar); HFD_OSR = high fat diet fed ovalbumine sensitized rats (blue bar).

*: $p<0.05$ when compared HFD fed non sensitized rats with Standard chow fed non sensitized rats (HFD_nonSR *vs*. Std_nonSR).

^: $p<0.05$ when compared HFD fed ovalbumin sensitized rats with Standard chow fed ovalbumin sensitized rats (HFD_OSR *vs.* Std_OSR).

#: $p<0.05$ when compared standard chow fed ovalbumin sensitized rats with non sensitized rats (Std_OSR *vs.* Std_nonSR).

$: $p<0.05$ when compared HFD fed ovalbumin sensitized rats with non sensitized rats (HFD_OSR *vs*. HFD_nonSR).

$^: $p< 0.05$ when compared the phenylephrine (Phe). induced contraction alone with results obtained in the same diet group, with the same sensitization protocol, but in the presence of studied substances (leptin, angiotensinogen, losartan, apelin 13 and Apelin-13[Ala13]).

Table 1. Differences between standard diet (Std) and high fat diet (HFD) fed obese resistant rats

Studied parameters	Std_nonSR	HFD_nonSR	Std_ OSR	HFD_ OSR
Weight (g)	288.20 ± 6.37	308.02 ± 10.30	281.56 ± 9.38	301.00 ± 9.17
Wall thickness of pulmonary arteries [a]	29.52 ± 6.11	51.49 ±6.54*	56.33 ± 7.78$^{\#}$	73.08 ± 5.39$^{\$}$
Contractile effects of Phe alone [b]	69.27 ± 4.43	83.96 ± 3.01*	86.25 ± 2.90$^{\#}$	93.08 ± 1.88$^{\$}$
Contractile effects of Phe in the presence of LEP [b]	68.57 ± 4.60	88.48 ± 4.80*	90.33 ± 5.59$^{\#}$	115.98 ± 9.04$^{\$\wedge}$
Contractile effects of Phe in the presence of AOGEN [b]	68.92 ± 4.24	102.22 ± 6.35$^{*?}$	100.02± 4.47$^{\#?}$	112.53 ± 4.16$^{?}$
Contractile effects of Phe in the presence of LOS [b]	63.88 ± 4.37	82.36 ± 3.89*	83.92 ± 3.94$^{\#}$	91.71 ± 1.96
Contractile effects of Phe in the presence of AP13 [b]	66.89 ± 4.50	80.76 ± 4.83	81.59 ± 4.66	94.16 ± 1.99$^{\$}$
Contractile effects of Phe in the presence of F13A [b]	70.89 ± 5.81	92.76 ± 4.64*	93.59 ± 3.73#	110.04 ± 4.69$^{\$\wedge?}$

Std_nonSR =standard fed non sensitized rats; HFD_nonSR = high fat diet fed non sensitized rats; Std_OSR = standard fed ovalbumine sensitized rats; HFD_OSR = high fat diet fed ovalbumine sensitized rats.
Phe = phenylephrine; LEP = leptin; AOGEN = angiotensinogen; LOS = losartan; AP13 = apelin 13; F13A = apelin-13[Ala13]
*: p<0.05 when compared HFD fed non sensitized rats with Standard chow fed non sensitized rats (HFD_nonSR *vs.* Std_nonSR).
^: p<0.05 when compared HFD fed ovalbumin sensitized rats with Standard chow fed ovalbumin sensitized rats (HFD_OSR vs. Std_OSR).
#: p<0.05 when compared standard chow fed ovalbumin sensitized rats with non sensitized rats (Std_OSR *vs.* Std_nonSR).
$: p<0.05 when compared HFD fed ovalbumin sensitized rats with non sensitized rats (HFD_OSR *vs*. HFD_nonSR).
?: p< 0.05 when compared the phenylephrine (Phe) - induced contraction alone with results obtained in the same diet group, with the same sensitization protocol, but in the presence of studied substances (leptin, angiotensinogen, losartan, apelin 13 and Apelin-13[Ala13]).

increase of Phe contractile effects on HFD fed group (Fig. 3D).

In the presence of F13A, the Phe contractile effects were powerful on HFD group as compared with standard chow group for either non-sensitized (92.76 ± 4.64% *vs*. 70.89 ± 5.81%, p<0.05) or sensitized (110.04 ± 4.69% *vs*. 93.59 ± 3.73%) rats. In both diet groups, the sensitization significantly increased the Phe - induced contraction with more than 30% on the standard chow group and up to 20% on the HFD group (Fig. 3F). The APJ receptors blockade significantly amplified Phe-induced contraction on pulmonary arteries from HFD fed sensitized rats as compared with results obtained in the absence of F13A (Fig. 3E). All obtained data of the present study are completely presented in Table 1.

DISCUSSION

Our data suggested that HFD stimulated both the remodelling (Fig. 2) and the vasoconstrictor responses (Fig. 3a) of pulmonary arteries. The obtained results were in agreement with published data showing that dietary fat intake could modulate vascular reactivity in response to vasoconstrictors and vasodilators, as well as expression of receptors for vasomotor substances (13, 14). But the majority of experimental studies about impact of HFD on vascular function used experimental models that associated obesity and /or diabetes mellitus and analysed only systemic arteries.

The present study has demonstrated for the first time that HFD could increase the reactivity of pulmonary arteries on alpha 1 adrenergic agonists on rats even in the absence of obesity. In Fig. 1 there are presented the body weights of study groups and there was no significant difference between standard chow fed group and HFD fed group.

The ovalbumin induced sensitization is an experimental model which allows study of inflammation – dependent alteration of vascular function (15). In Fig. 3A there are comparatively presented the sensitization effects on standard chow fed and HFD fed rats. In both groups the sensitization induced an increase of contractile responses to Phe but, after sensitization there was no significantly difference between study groups suggesting an inflammatory component of HFD – dependent changes of vascular reactivity. Actually, the histological examination indicated an increased inflammatory infiltrate on perivascular space on HFD fed rats (data not shown).

The experiments continued with incubation of pulmonary arteries rings with leptin, angiotensinogen or apelin 13, before administering Phe. We used these adipokines taking into account our previous data that allowed us to use low doses that did not modify pulmonary artery rings tone.

Adipokines could have a regulatory role in pathological situations. In agreement with literature published data, our previous results showed that LEP could have vasodilatatory effects on Phe pre-contracted pulmonary arteries from normal rats but not on sensitized rats (11). In this study the LEP was administered before Phe. Results are presented in Fig. 3B: the Phe - induced contractions were not significantly modified by LEP pre - incubation.

Aogen (the only known precursor of the angiotensin peptides) and all other components of renin angiotensin system (RAS) which were found on lungs confirmed existence of an intrapulmonary (local) RAS (16, 17). Activation of systemic or local (pulmonary) RAS could influence lung activity by increasing vascular permeability, airway reactivity, vascular tone and fibroblast activity or by reducing alveolar epithelial cell survival (16,18).

Inflammatory phenomenon associated to sensitization and challenge protocol activated the RAS on pulmonary vessels and increased the angiotensin II (Ang II) synthesis (19). In the present study, the Aogen amplified the Phe – induced contractions on

sensitized rats from the first group and on all HDF fed rats (Fig. 3C). These effects could be a result of reduced bioactivity of nitric oxide and an increased formation of reactive oxygen species associated with HFD demonstrated by Mundy AL and co-workers (2007) (13). But at the same time, taking into account the increased thickness of pulmonary artery from HFD fed rats, the Aogen effects could be a result of an accelerated Ang II synthesis associated to (pro) inflammatory state induced by HFD. To uncover the mechanism we used LOS. Pre-treatment of pulmonary arteries rings with LOS prevented the sensitization associated increased Phe contractile effects on first groups and the HFD – associated increased Phe on non-sensitized rats. On sensitized rats, there was a significant difference between HFD and standard chow fed rats (Fig. 3D). Taking into account the anti-inflammatory effects of AT1 receptors blockers on pulmonary allergic disease (20) we could suggest that HFD induced an activation of the RAS from pulmonary arteries in a sensitization similar manner.

Apelin is the endogenous ligand of recently de-orphanised APJ receptors with vasodilatatory and anti – inflammatory properties (21,22). Within the human vasculature, both APJ receptor-like immunoreactivity and apelin-like immunoreactivity are detectable in endothelial cells and vascular smooth muscle cells of human large conduit vessels including pulmonary vessels (23). In rat tissues, the highest expression of APJ mRNA was detected in the lung, suggesting that AP – APJ system plays an important role in the pulmonary system (24). The AP13 pre-treatment prevented the HFD – associated increase of Phe -induced contractions in non-sensitized rats and the sensitization protocol increased contractility of pulmonary arteries rings in HFD fed rats (Fig. 3E). Blockade of APJ receptors amplified the sensitization increased pulmonary artery reactivity on HFD fed rats (Fig. 3F). Taking into consideration the already documented anti -inflammatory effects of apelin/ APJ receptor system and the difference between AP13/ F13A effects on HFD as compared with standard chow we could suggest that HFD - associated increased Phe - induced contraction was the consequence, at least partially, of an inflammatory status induced by HFD.

In conclusion, our data suggested that HFD could induce either structural (the increase of vascular wall thickness) or functional (the increase of vasoconstrictor responses to Phe) changes on pulmonary arteries. These phenomena could be at least partly due to existence of pro-inflammatory status initiated by HFD. This status could amplify the vascular consequences of a pulmonary allergic disease. Furthermore, the adipokines influence on pulmonary arteries contractility, in this experimental condition (i.e. lack of obesity), sustain the possible involvement of adipose tissue on the HFD associated increased reactivity of pulmonary arteries.

Acknowledgements

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