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Effect of high fat diet on pulmonary expression of parathyroid hormone-related protein and its downstream targets

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Abstract

Aims: Parathyroid hormone-related protein (PTHrP) is involved in lung development and surfactant production. The latter one requires a paracrine interaction between type II alveolar cells and lipofibroblasts in which leptin triggers PTHrP-induced effects. Whether increased plasma leptin levels, as they occur in high fat diet, modify the expression of PTHrP remains unclear. Furthermore, the effect of high fat diet under conditions of forced pulmonary remodelling such as response to post myocardial infarction remains to be defined. **Materials and methods:** C57 bl/6 mice were randomized to either normal diet or high fat diet at an age of 6 weeks. Seven months later, the mice were euthanized and the lung was removed and frozen in fluid nitrogen until use. Samples were analyzed by real-time RT-PCR and western blot. Leptin deficient mice were used to investigate the effect of leptin on pulmonary expression of PTHrP more directly. A subgroup of mice with and without high fat diet underwent *in vivo* ischemia (45 min) and reperfusion (4 weeks). Finally, experiments were repeated with prolonged high-fat diet.

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Key findings: High fat diet increased plasma leptin levels by 30.4% and the pulmonary mRNA expression of PTHrP (1,447-fold), PTH-1 receptor (4.21-fold), and PTHrP-downstream targets ADRP (7.54-fold) and PPARγ (5.27-fold). Pulmonary PTHrP expression was reduced in leptin deficient mice by 88% indicating leptin dependent regulation. High fat diet further improved changes in pulmonary adaptation caused by ischemia/reperfusion (1.48-fold increased PTH-1 receptor protein expression). These effects were lost during prolonged high fat diet.
Significance: This study established that physiological regulation of leptin plasma levels by high fat diet affects the pulmonary PTHrP expression and of PTHrP downstream targets. Modification of pulmonary expression of PTH-1 receptors by high fat diet after myocardial infarction suggests that the identified interaction may participate in the obesity paradox.

Keywords: Medicine, Physiology

1. Introduction

Parathyroid hormone-related protein (PTHrP) and its corresponding receptor, parathyroid hormone (PTH)-1 receptor, are essential for regular foetal lung development and are required to maintain regular lung function [22, 23]. The PTHrP pathway participates in the regulation of type II alveolar cell proliferation, differentiation, apoptosis, and surfactant formation [21]. Type II alveolar cells release PTHrP that acts on adjacent lipofibroblasts via stimulation of the PTH-1 receptor-mediated cAMP pathway. This leads to an induction of the expression of adipocyte differentiation-related protein (ADRP) and peroxisome proliferator-activated receptor (PPAR)-γ in lipofibroblasts. Both factors improve lipid uptake, formation of surfactants, and attenuate transdifferentiation of lipofibroblasts into myofibroblasts [23]. Furthermore, lipofibroblasts produce leptin that then acts as a positive feedback modulator on the expression of surfactant proteins by type II alveolar cells. Transdifferentiation of lipofibroblasts into myofibroblasts is a major problem in lung disease and it is also inhibited by PTHrP [23]. On the other side, PTHrP deficiency or defect in PTH-1 receptor activation attenuate proliferation and differentiation of type II alveolar cells and instead of alveolarization, angiotensin II is produced that further damages the lung [21, 26, 28].

In this study we focus on the role of leptin in the regulation of pulmonary PTHrP expression. Although leptin has been identified as a paracrine factor in the lung that participates in PTHrP-dependent regulation of surfactants, the relationship between obesity, plasma leptin concentrations, and the role of leptin as a paracrine factor in pulmonary function and differentiation has to be clarified. In general, obesity is associated with a high risk to develop lung disease and also associated with high plasma levels of leptin [10]. Under conditions of high fat diet (HFD) leptin plasma levels increase but it remains unclear whether this will be sufficient to affect

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pulmonary expression of PTHrP and of its downstream targets ADRP and PPARγ. It is important to clarify this point for an understanding of the relationship between obesity and pulmonary disease. This is specifically interesting in conditions of pulmonary stress as it occurs after myocardial infarction.

Myocardial infarction leads to a transient reduction of cardiac pump function thereby producing a nonsufficient support of each organ with oxygenated blood. In this critical phase of ischemia, often also during the first period of successful reperfusion, it is important that the remaining blood flow can efficiently be oxygenated in the lung. However, symptoms of dyspnoea are common due to reduced blood supply of organs including the respiratory muscles that compensate for this imbalance of oxygen supply by forced respiration. Subsequently, lung remodelling and pulmonary hypertension are common complications following myocardial infarction [11, 12]. The pulmonary paradox in cardiology describes a situation in which obese people have a higher prevalence to suffer from myocardial infarction, but a better chance to survive such an event [2, 16]. The mechanisms behind this paradox have not been fully understood and the interaction between fat intake and cardioprotection remains elusive. PTHrP is a key player in pulmonary remodelling by inhibiting the transdifferentiation of lipofibroblasts into myofibroblasts and supporting surfactant formation and it is regulated by leptin. Therefore, we hypothesized that HFD will improve pulmonary remodelling. Nevertheless, it is not yet known whether modifications of plasma leptin levels by HFD are sufficient to modify the pulmonary expression of PTHrP. Therefore, the current study was designed to clarify this point.

Our current understanding about the role of PTHrP for the onset of surfactant production and release depends largely on previous studies that were performed in models with newborn rodents [23, 24]. However, although pulmonary expression of PTHrP is necessary to induce the formation of surfactants and inhibits transdifferentiation of lipofibroblasts into myofibroblasts, it is less known whether PTHrP exerts a similar role for pulmonary adaptation in adults.

In summary this study is therefore aimed to clarify the following points: Does HFD increase plasma leptin levels significantly enough to affect pulmonary expression of PTHrP, does myocardial infarction affect pulmonary expression of PTHrP, and finally whether this is modified by HFD. In conclusion, this study is aimed to clarify the role of extra-pulmonary leptin on pulmonary expression of PTHrP.

2. Materials and methods

2.1. Animals

Animal studies were approved by Research Commission on Ethics of the Hospital Vall d'Hebron. All animal procedures conformed to EU Directive 2010/63EU and

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Recommendation 2007/526/EC regarding the protection of animals used for experimental and other scientific purposes, enforced in Spanish law under Real Decreto1201/2005.

C57 bl/6 mice of either sex were used at the age of 6 weeks and fed with either a standard diet (24% kcal from protein, 18% kcal from fat and 58% kcal from carbohydrate) obtained from Harlan Iberica (Barcelona) or a high fat diet (HFD; 20% kcal from protein, 60% kcal from fat and 20% kcal from carbohydrate) obtained from Research Diets (New Brunswick, NJ) for 6 months. Thereafter, animals were anesthesized (2% isofluorane), orally intubated and mechanically ventilated (Inspira ASV, Harvard Apparatus). Mice were subjected to either a sham procedure or 45 min myocardial ischemia via ligation of the left anterior descending coronary artery (LAD) followed by reperfusion. Four weeks later the mice were sacrificed and the lung was excised and quickly frozen in liquid nitrogen until use. In additional set of experiments, C57 bl/6 mice were fed with standard diet or HFD for 12 months before being subjected to sham or ischemia/reperfusion protocol as described. Infarct sizes were significantly smaller in the HFD group (16.1 ± 4.4% IS/AAR vs. 35.3 ± 8.2% IS/AAR). Infarct sizes were quantified as follows and described before [17]: at the end of the experimental protocol, mice were killed with an overdose of pentobarbital sodium and the hearts were perfused for 5 min and fixed overnight with 4% paraformaldehyde and embedded in paraffin. The heart was cross sectioned from base to apex at 200 μm intervals and four serial 4 μm sections were obtained for each interval. Sections were stained with Picrosirius red (Sigma-Aldrich, MO, USA), scanned and evaluated using ImagePro Software (Image Pro-Plus, Media Cybernetics, MD, USA).

B6.V-Lep^{ob}/J mice were used as a genetic model of leptin deficiency. Mice homozygeous for the Lep^{ob} mutation developed obesity. Mice used here were 3–5 months old.

2.2. Determination of leptin concentration

Leptin was analyzed in plasma obtained from blood samples collected before HFD feeding and at the end of the experimental protocol using a commercial multiplexed bead-based immunoassay (Milliplex MAP, Millipore Iberica) and processed in a Luminex 200 instrument (Luminex Corp., Austin, TX).

2.3. Real-time RT-PCR

In order to quantify the pulmonary expression of genes of interest quantitative real-time RT-PCR was used. Tissue samples were prepared to analyze the steady state mRNA levels of proteins of interest according to the previously described method [4]. Briefly, total RNA was isolated from the lungs using peqGoldTriFast (peqlab, Biotechnology GmbH, Germany) according to the manufacturer's protocol.

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To remove genomic DNA contamination, isolated RNA samples were treated with 1 U DNase per mg RNA (Invitrogen, Karlsruhe, Germany) for 15 min at 37 °C. On μg of total RNA was used in 10 μl reaction to synthesize cDNA using superscript RNaseH reverse transcriptase (200 U/μg; Invitrogen) and oligo dTs (Roche, Mannheim, Germany) as primers. Reverse transcriptase reactions were performed for 60 min at 37 °C. Real-time PCR was performed using the Icycler IQ detection system (Bio-Rad, Munich, Germany) in combination with IQ SYBR Green real-time supermix (Bio-Rad). A complete list of all primers used in this study is given in Table 1. Data are normalized to Hypoxanthine Phosphoribosyltransferase (HPRT) expression that was used as a house-keeping gene in this study. Preliminary experiments with β2 microglobulin, which was alternatively considered as housekeeping gene, revealed similar results but higher variability. The relative change in expression was quantified by the 2 $[-\Delta\Delta C_T]$ method [14].

2.4. Western Blots

Tissue samples from lungs stored at −80 °C were used as described previously and prepared for standard SDS gel electrophoresis. Protein was extracted as described before [4]. The extraction buffer contained (mmol/L): Mops 5, sucrose 300, EGTA 1, bovine serum albumin (0.015) and 0.01% (v/v) protease inhibitor cocktail (Sigma, Taufkirchen, Germany). The homogenate was centrifuged at 1,000 g at 4 °C for 10 min and the supernatant was used for protein detection by Western blotting. Protein samples were loaded on NuPAGE Bis-Tris Precast gels (10%; Life Technology, Darmstadt, Germany) and subsequently transferred onto nitrocellulose membranes. Primary antibodies directed against PTHrP, PTH-1 receptor and α-actin (loading control) were used as described before [20].

Table 1. List of primers used in this study.

Gene	Forward	Reverse
PTHrP	GAG ATC CAC ACA GCC GAA AT	CGT CTC CAC CTT GTT GGT TT
PTH-1 receptor	TTG CCT CCC TCA CCG TGG CT	CGG CGC GCA GCA TAA ACG AC
ADRP	CCC GCA ACC TGA CCC AGC AG	CGC CTG CCA TCA CCC CCA AG
PPARγ	GCC TTG CTG TGG GGA TGT	TCA GCG GGA AGG ACT TTA TGT
Elastin	CTG CTG CTA AGG CTG CTA AG	CCA CCA ACA CCA GGA ATG
TGF-β_1	GTC CTT GCC CTC TAC AAC CA	GTT GGA CAA CTG CTC CAC CT
Fibronectin	ACA GAG CTC AAC CTC CCT GA	TGT GCT CTC CTG GTT CTC CT
β_2-Microglobulin	GCT ATC CAG AAA ACC CCT CAA	CAT GTC TCG ATC CCA GTA GAC GGT

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2.5. Statistics

The results are expressed as means ± S.D. or box plots as indicated in the legends to the figures. Statistical comparisons were performed by two-side ANOVA and Student-Newman-Keuls post hoc analysis. Levene test was used to check the normal distribution of the samples. A p value of 0.05 was considered as statistical significant.

3. Results

3.1. Effect of high fat diet on pulmonary expression of PTHrP in adult mice

Compared to age-matched control mice, body weight in mice fed with HFD for 6 months increased from 35.8 ± 0.9 g to 46.7 ± 0.8 g and plasma leptin levels increased from 4.95 ± 0.83 ng/ml to 15.91 ± 2.41 ng/ml (each $p < 0.001$, $n = 6–8$). The pulmonary expression of PTHrP mRNA increased significantly in mice fed with HFD (Fig. 1). Similarly, the pulmonary mRNA expression of PTH-1R, ADRP, and PPARγ increased (Fig. 1). Collectively these data are in agreement with the hypothesis that leptin stimulates the pulmonary expression of PTHrP under basal conditions. The relevance of this observation was confirmed in mice with leptin deficiency due to a mutation in the leptin gene (ob-mice). Ob-mice had also increased body weight (50.5 ± 3.8 g vs. 26.0 ± 3.2 g, $n = 4$, $p < 0.001$). In these mice the pulmonary expression of PTHrP was significantly reduced whereas the pulmonary expression of PTH-1R, ADRP, and PPARγ remained unchanged (Fig. 2).

Interestingly, the effect of HFD on the pulmonary expression of mRNA PTHrP, PTH-1R, and PTHrP downstream targets C57 bl/6 mice was lost when feeding was further extended for another 6 months (Fig. 3). Tissue levels of PTHrP and the

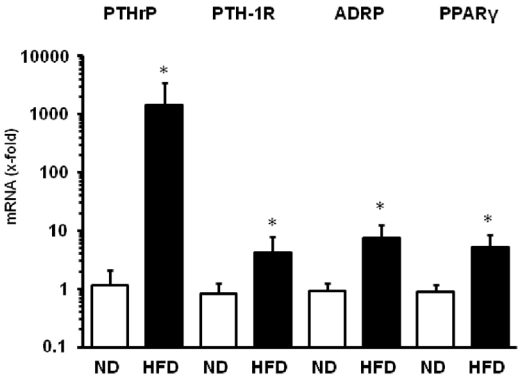

Fig. 1. Pulmonary mRNA expression of PTHrP, PTH-1 receptor, ADRP, and PPARγ in seven months old mice that were fed with either normal diet (ND, n = 12) or high fat diet (HFD, n = 7). Data are expressed as means ± S.D. *, $p < 0.05$ vs. ND.

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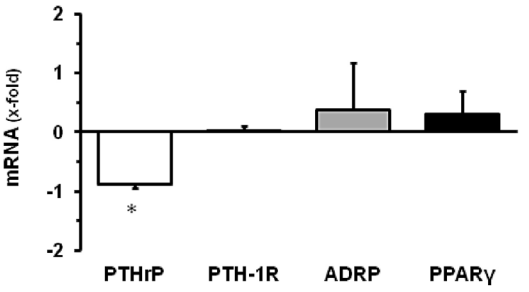

Fig. 2. Pulmonary mRNA expression of PTHrP, PTH-1 receptor, ADRP, and PPARγ in leptin deficient mice ($Lep^{ob/ob}$). Data are expressed as means ± S.D. and normalized to wild-type littermates. n = 4 mice each. *, $p < 0.05$ vs. wild-type littermates.

PTH-1R protein expression were not affected by HFD at any of the two time points (Fig. 4).

3.2. Effect of ischemia/reperfusion on PTHrP and PTH-1 receptor protein expression

In mice subjected to ischemia/reperfusion the pulmonary mRNA expression of PTHrP increased in the group fed with normal diet four weeks after infarction while no additive effect was observed in the HFD group (Fig. 5). The mRNA expression of the PTH-1 receptor remained unchanged in mice undergoing ischemia/reperfusion (Fig. 5). The pulmonary mRNA expression of two PTHrP downstream targets, ADRP and PPARγ were also up-regulated after ischemia/reperfusion but only the pulmonary mRNA expression of PPARγ was further increased by HFD (Fig. 5). These effects were toned down in mice with advanced age (Fig. 6). A significant increase of pulmonary PTHrP protein expression was observed after ischemia/reperfusion (Fig. 4). However, no additive effects of HFD were observed. In contrast, PTH-1 receptor protein expression was significantly

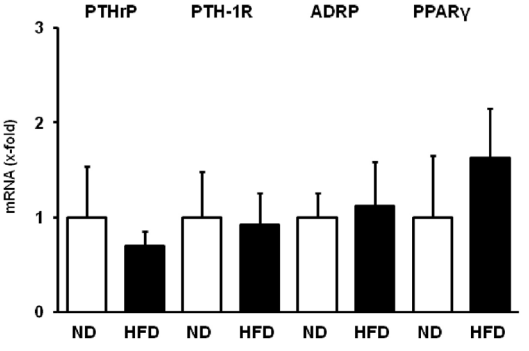

Fig. 3. Pulmonary mRNA expression of PTHrP, PTH-1 receptor, ADRP, and PPARγ in thirteen months old mice that were fed with either normal diet (ND, n = 6), high fat diet (HFD, n = 5). Data are expressed as means ± S.D. all values $p > 0.05$ vs. ND.

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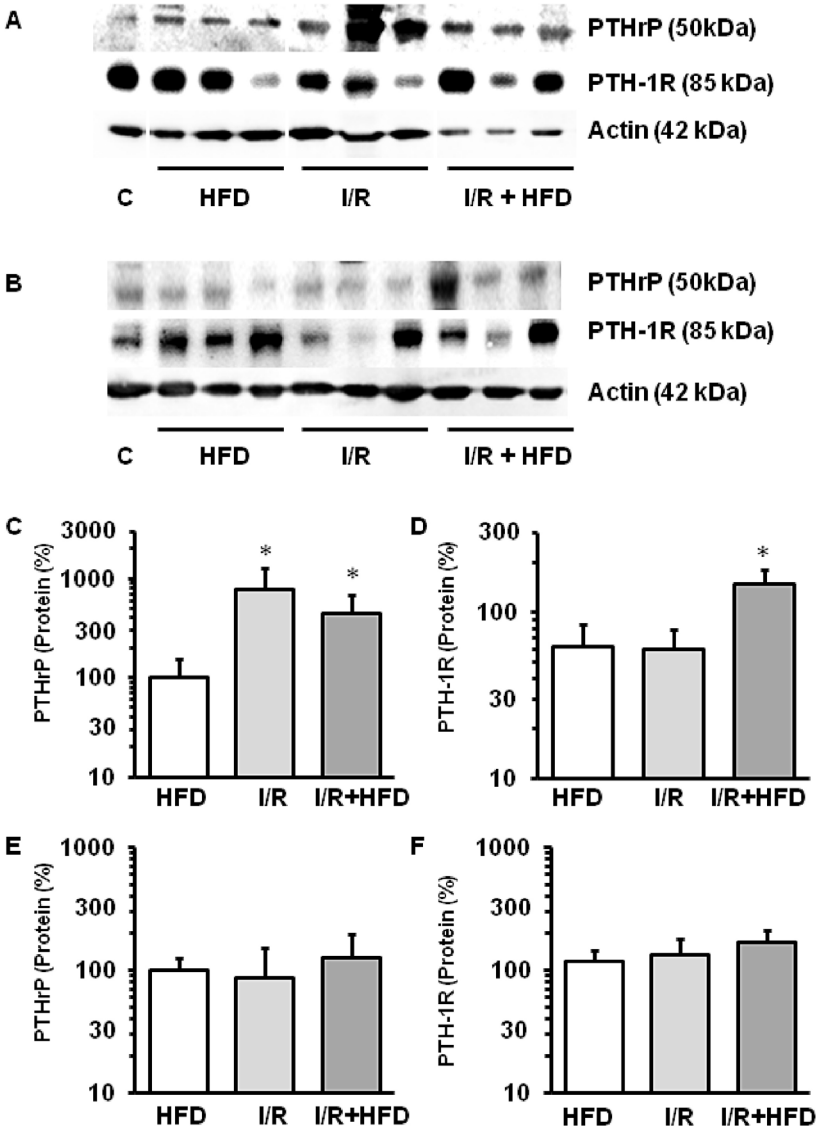

Fig. 4. Pulmonary protein expression of PTHrP and the corresponding PTH-1 receptor in mice. A + B) Representative western blots from seven months old (A) or thirteen months old (B) mice; HFD = high fat diet; I/R = ischemia/reperfusion; The original full size blots are given in the supplementary material as Fig. 4a-PTHrP, Fig. 4a-PTH-1R, Fig. 4a-Actin, Fig. 4b-PTH-1R, Fig. 4b-PTHrP; and Fig. 4b-Actin; C-F) Mean protein expression of PTHrP and PTH-1 receptor in seven months old mice (C,D) or thirteen months old mice (E,F). Data are expressed as means ± S.D. and normalized to age-matched mice under normal diet (always n = 3); *, $p < 0.05$ vs. normal diet.

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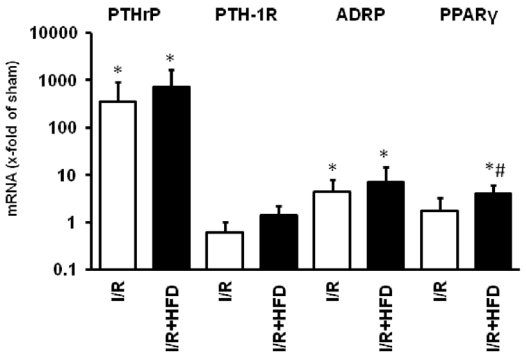

Fig. 5. Pulmonary mRNA expression of PTHrP, PTH-1 receptor, ADRP, and PPARγ in seven months old mice that underwent ischemia/reperfusion (I/R) with normal diet (n = 9) or high fat diet (HFD), n = 10). Data are expressed as means ± S.D. and normalized to sham mice with normal diet *, $p < 0.05$ vs. sham, #, $p < 0.05$ vs. I/R.

elevated only in the HFD group undergoing ischemia/reperfusion (Fig. 4). Again, these effects did not maintain at later time points (Fig. 4).

3.3. Effect of high fat diet on pulmonary expression of fibrotic proteins

The coordinative up-regulation of PTHrP, ADRP, and PPARγ mRNA under HFD indicates a significant activation of this paracrine pathway by HFD. Pulmonary function depends on intact lung elasticity that is disturbed by transition of lipofibroblast into myofibroblasts. This process is attenuated by PTHrP. We therefore analyzed the pulmonary mRNA expression of pro-fibrotic proteins, such as TGF-β_1, elastin, and fibronectin under conditions that indicated increased expression of PTHrP namely HFD and ischemia/reperfusion (Fig. 1, Fig. 5). HFD increased the pulmonary mRNA expression of TGF-β_1 and fibronectin (Fig. 7a).

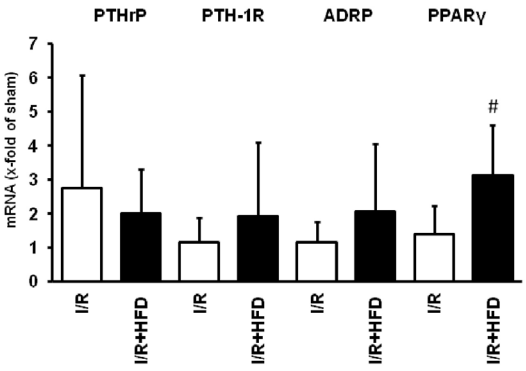

Fig. 6. Pulmonary mRNA expression of PTHrP, PTH-1 receptor, ADRP, and PPARγ in thirteen months old mice that underwent ischemia/reperfusion (I/R) with normal diet (n = 8) or high fat diet (HFD), n = 6). Data are expressed as means ± S.D. and normalized to sham mice with normal diet. #, $p < 0.05$ vs. I/R.

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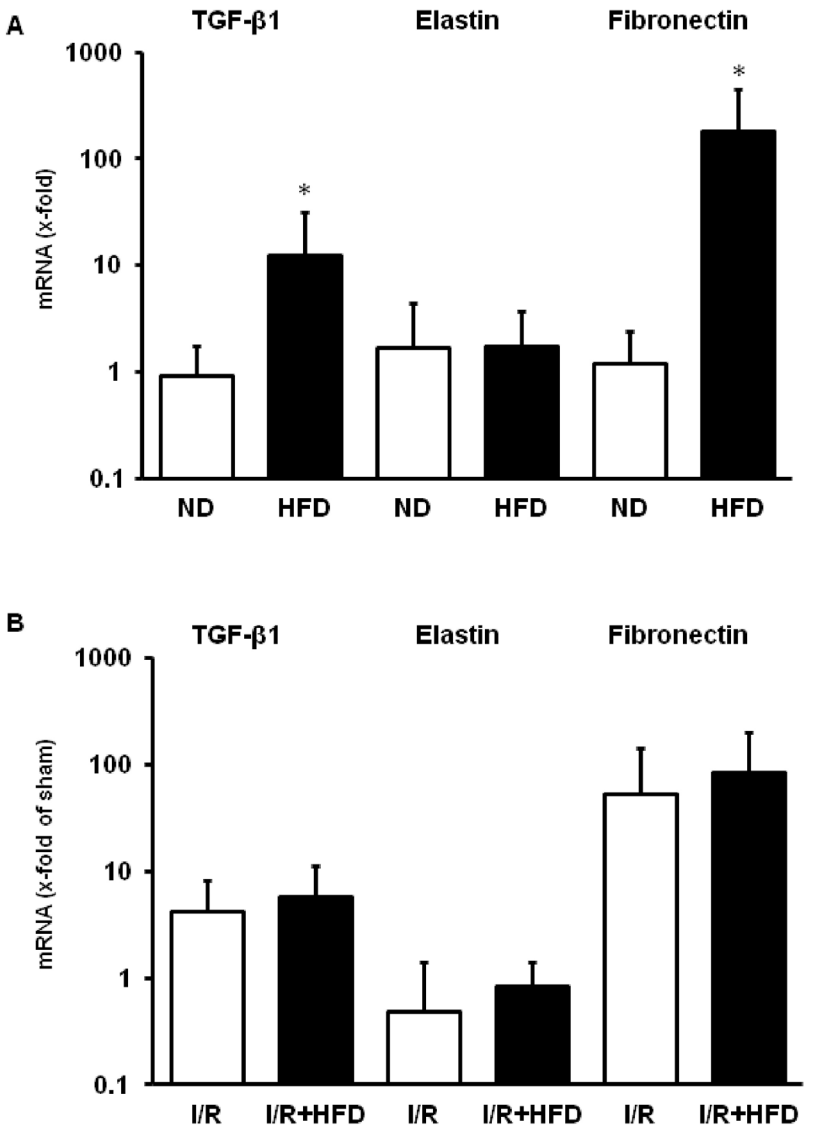

Fig. 7. Pulmonary mRNA expression of TGF-β_1, elastin and fibronectin in seven months old mice. (A) Comparison of the effect of nomrla diet (ND) versus high fat diet (HFD) (B) Comparison of the effect of ischemia/reperfusion (I/R) to sham I/R plus HFD. Data are expressed as means ± S.D. from n = 6–12 mice (see Fig. 1 and 5 for exact numbers) *, $p < 0.05$ vs. ND of seven months old mice.

However, it did not increase the expression of elastin (Fig.7a). Elastin mRNA slightly increased in HFD mice at a later time point when PTHrP expression was lower (data not shown). Ischemia/reperfusion and the combination with HFD produced similar changes in pulmonary expression of TGF-β_1 and fibronectin although large inter individual variation was strong. HFD did not modify this response and elastin expression was again not different (Fig. 7b).

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4. Discussion

This study was aimed to investigate whether modification of plasma leptin levels as they occur by HFD can modify pulmonary expression of PTHrP. The first important finding of the present study is that HFD increases the steady state mRNA expression of PTHrP in the lung *in vivo*. This effect is most likely linked to elevated plasma leptin levels because HFD increased plasma leptin levels and leptin-deficient mice displayed reduced pulmonary PTHrP mRNA expression. Altered surfactant protein expression with excessive deposition of lipid droplets has been reported in different models of obesity [6, 9]. PTHrP has been identified as a main factor regulating surfactant production via a paracrine pathway involving leptin [23]. The new finding of our study is that extrapulmonary leptin, as it can be found under HFD, can modify pulmonary PTHrP expression. This increase in PTHrP mRNA expression by HFD led also to an increased expression of PTH-1 receptor, ADRP and PPARγ. The induction of classical PTHrP-downstream target genes suggests a physiological relevance of the observed increase in pulmonary PTHrP mRNA expression. However, a significant increase in PTHrP protein expression was not found. A possible explanation for the lack of proper protein expression may be that PTHrP is a paracrince factor. Extracellular PTHrP might have been washed out during preparation or alternatively be internalized and rapidly degraded by lipofibroblasts the natural target cells of PTHrP.

PTHrP is one of the main factors in the lung that regulates the epithelial-mesenchymal paracrine cross-talk. It is a main regulator for the formation of surfactants [23]. Epithelial cells form a positive feedback to type II alveolar cells via release of leptin [24]. In this physiological feedback mechanism PTHrP induces the release of leptin from epithelial cells that than trigger an improved expression and release of PTHrP [25]. We tested the hypothesis that increasing leptin levels by HFD can modify the expression of PTHrP as well. We found that HFD increased plasma leptin levels (as expected) and that pulmonary mRNA expression of PTHrP was also increased under these conditions. A causal link between plasma leptin levels and pulmonary expression of PTHrP was also confirmed in leptin deficient mice that displayed a lower pulmonary expression of PTHrP. Collectively, our study shows for the first time that plasma leptin levels affect pulmonary expression of PTHrP a mechanism that had been attributed to the paracrine effects of leptin derived from epithelial cells before.

The subsequent question is whether the increased pulmonary expression of PTHrP mRNA indicates an activation of the pulmonary PTHrP system. We were unable to find a significant increase in PTHrP protein concentration. However, as PTHrP can easily enter the circulation this does not necessarily tell us anything about an activation of the pulmonary PTHrP system. Therefore, we addressed this question by evaluating the expression levels of PTHrP-dependent regulated genes. As

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reported before, PTHrP released from type II alveolar cells acts on adjacent epithelial cells that express PTHrP receptors. Once PTHrP activates these receptors in increases the expression of ADRP and PPARγ in these cells [23]. We found indeed that under conditions of HFD, high plasma leptin levels, and increased pulmonary expression of PTHrP mRNA the well known down-stream targets of the pulmonary PTHrP pathway are induced. Both target genes, ADRP and PPARγ were upregulated.

In general a high activation of the PTHrP-driven epithelial-mesenchymal paracrine cross-talk is considered as a protective mechanism that allows better oxygenation of the blood in the lung [22, 23]. Several mechanisms are known by which PTHrP stabilizes lung function. These are its vasodilating effect that improves perfusion, its effect on surfactant formation that improves oxygen uptake, and its inhibitory effect on transdifferentiation of lipofibroblasts into myofibroblasts [8, 23]. We proved the latter assumption under conditions of HFD-dependent activation of the PTHrP system by studying the pulmonary expression of elastin. An upregulation of elastin indicates interstitial pulmonary fibrosis [3, 13, 15]. Our finding that elastin mRNA expression was not changed under conditions shown to increase pulmonary PTHrP mRNA expression is in fine agreement with the assumption that activation of the pulmonary PTHrP system attenuates transactivation of epithelial cells to myofibroblasts that favours pulmonary fibrosis. We have recently shown that the pulmonary expression of PTHrP is constitutively repressed by nitric oxide (NO) [1]. Inhibition of endothelial-derived NO formation significantly increased the expression of PTHrP, PTH-1 receptor, ADRP, and PPARγ. Under these conditions an induction of the pulmonary PTHrP system was not associated with increased surfactant production. A strict coupling between the pulmonary PTHrP system and surfactant formation in adult rodents has yet to be clarified as there are contrasting reports in the literature.

A wide variety of events can inhibit the protective role of PTHrP, including prematurity, barotrauma, oxytrauma, nicotine, and infection [5, 7, 18, 19]. Whether myocardial infarction also affects pulmonary PTHrP expression and function was unknown. However, myocardial infarction leads impairs left ventricular filling with subsequent congestion of the pulmonary venous circulation. Moreover, heart failure has been linked to endothelial dysfunction in the lung. Therefore, we were also interested to study the effect of myocardial infarction on the pulmonary expression of PTHrP. We found an increased expression of PTHrP protein. Former studies showed that lung structural remodelling after myocardial infarction can be attenuated by irbesartan, an angiotensin II receptor antagonist [12]. We have recently shown that captopril, an angiotensin converting enzyme inhibitor, can attenuate pulmonary induction of PTHrP, but not that of PTH-1 receptor, in a NO-deficiency model [1]. Collectively the data suggest that an infarct-dependent activation of the renin-angiotensin-system induces the expression of PTHrP and

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that this contributes to the formerly observed lung structural remodelling. We found indeed that mice undergoing myocardial infarction displayed induced pulmonary expression of TGF-β_1, fibronectin, and reduced expression of elastin alongside with increased expression of PTHrP.

The obesity paradox describes a situation in which obese patients tolerate ischemic events better than non-obese patients. Mice fed with HFD were used here as an experimental model of obesity. We hypothesized that these mice respond different to myocardial infarction with respect to the pulmonary PTHrP expression compared to those mice fed with normal diet. Indeed, pulmonary expression of PTHrP was high in mice with HFD undergoing myocardial infarction. Furthermore, PTHrP-1 receptor expression was elevated. However, in the post-infarct model, HFD caused a rather specific additive effect on the pulmonary expression of PPARγ that was independent from changes in PTHrP expression suggesting an PTHrP-independent mechanism on PPARγ expression. Collectively, these data suggest that HFD does not modify pulmonary expression and activation of the PTHrP system in the context of myocardial infarction.

Tissue adaption to stress is often reduced or even lost during ageing as shown for conditioning effects [27]. Therefore, all experiments were analyzed at two different time points: 6 months and 12 months after starting HFD. Older mice displayed higher steady state PTHrP mRNA levels und lower elastin levels. As mentioned above, this can be considered as a PTHrP-dependent repression of the transition of epithelial cells into myofibroblasts. HFD and ischemia/reperfusion seems to increase TGF-β_1 and fibronectin expression. TGF-β_1 is a classical angiotensin II target and therefore most likely regulated by activation of this pathway. In accordance to these findings myofibroblasts have been identified in post-infarct lungs after two weeks [12]. In general, the effects of HFD on the pulmonary expression of PTHrP and its downstream targets in mice at the age of twelve months were less pronounced than those on younger mice. The data show that pulmonary PTHrP mRNA expression can be modified by HFD mainly in young mice suggesting a desensitization of leptin receptors or activation of compensatory mechanisms.

In conclusion, the current study shows a remarkable effect of extrapulmonary leptin on the steady state level of PTHrP mRNA *in vivo.* HFD increases plasma levels of leptin that then acts on alveolar type II cells and increases the mRNA steady state level of PTHrP. Leptin deficient mice (ob mice) have reduced steady state levels of PTHrP. PTHrP acts in a paracrine way on alveolar interstitial fibroblasts (lipofibroblasts) and induces the expression of PTH-1 receptors, ADRP and PPARγ. At least in young mice the effect of HFD on pulmonary expression of PTHrP may contribute to some of the observations that are generally named the obesity paradox.

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In recent work, PTHrP had been identified as a main modulator that controls the alveolar type II epithelial cross-talk. The current study underlines the importance of leptin in this cell-cell communication. Despite this the current study mainly confirmed the suggested model how PTHrP acts on epithelial cells. However, we found specifically under conditions of myocardial infarction an activation of PPARγ in epithelial cells that was not associated with an activation of PTHrP. This point requires specific attention in the future, as it is specifically important for patients with impaired cardiac function.

5. Conclusion

HFD activates the pulmonary PTHrP/leptin cross-talk in healthy mice and this interaction contributes to lung remodeling. The beneficial effect of HFD on this pathway drops during ageing.

Declarations

Author contribution statement

Learta Oruqaj, Marcos Poncelas, Jordi Bañeras: Performed the experiments.

Svenja Forst: Performed the experiments; Analyzed and interpreted the data.

Rolf Schreckenberg: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Javier Inserte: Conceived and designed the experiments.

David Garcia-Dorado: Conceived and designed the experiments; Analyzed and interpreted the data.

Susanne Rohrbach: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Klaus-Dieter Schlüter: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

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Additional information

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