Do single or sequential measurements of leptin and adiponectin in plasma have prognostic value in pulmonary arterial hypertension?

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Abstract

Leptin (a neuroendocrine peptide that enhances metabolism and acts on the hypothalamus to suppress appetite) and adiponectin (a protein that has insulin-sensitizing, anti-inflammatory, and antiproliferative properties) are involved in the pathobiology of pulmonary arterial hypertension (PAH). We hypothesized that plasma leptin and adiponectin as well as the leptin/adiponectin ratio are abnormal in PAH patients and their levels track with disease severity and functional changes during follow-up. We tested this hypothesis in a cohort of patients included in the 16-week, international, multicenter, double-blind, placebo-controlled FREEDOM-C2 study. Blood was collected at baseline and week 16 in 178 out of 310 randomized patients with PAH. Baseline plasma leptin and adiponectin concentrations were 25 ± 31 ng/mL and 7.8 ± 6.1 µg/mL, respectively. Leptin, adiponectin, and leptin/adiponectin (mean ± SD) changes at 16 week were of small magnitude. Leptin at baseline was significantly associated with older age, higher BMI, higher Borg dyspnea index, and lower NT-pro BNP. Women had higher levels of leptin than men (30.5 ± 33.2 versus 7.2 ± 6.4 ng/mL), even when adjusting for background therapy and etiology (linear regression: β = 21.8, P < 0.001). Adiponectin was negatively associated with BMI and positively associated with NT-pro BNP. Changes in leptin, adiponectin, and leptin/adiponectin ratio adjusted for weight at 16 weeks did not predict functional class, distance walk in 6 min or survival at one, two, three, or four years. Plasma leptin and adiponectin at baseline and their change at 16-week do not appear to significantly impact prognosis in PAH.

Keywords

pulmonary arterial hypertension, pathobiology, leptin, adiponectin, treprostinil

To the editor

Although great advances have been made in understanding the complex pathophysiology of pulmonary arterial hypertension (PAH), many questions still remain. Our group1,2 and others3-5 showed that leptin (a neuroendocrine peptide that enhances metabolism and acts on the hypothalamus to suppress appetite) and adiponectin (a protein that has insulin-sensitizing, anti-inflammatory, and antiproliferative properties) are involved in the pathobiology of PAH.

We showed that plasma leptin levels are elevated in PAH patients and that lower levels were associated with worse survival.1 A recent study demonstrated that leptin plays a role in pulmonary vascular remodeling and increases the susceptibility to develop pulmonary hypertension (PH) in animal models.3 In addition, adiponectin levels are increased in PAH patients compared to controls6 and this adipose-derived hormone attenuates PH in animal models.5,7,8

We hypothesized that plasma leptin and adiponectin as well as the leptin/adiponectin ratio are abnormal in PAH patients.
patients and their levels track with disease severity and functional changes during follow-up. We tested this hypothesis in a cohort of patients included in the 16-week, international, multicenter, double-blind, placebo-controlled FREEDOM-C2 study. The primary endpoint of this study was the placebo-corrected change in 6-minute walk distance (6MWD) between baseline and week 16. Secondary endpoints included clinical worsening, Borg dyspnea score, World Health Organization (WHO) functional class, and N-terminal pro-brain natriuretic peptide (NT-pro BNP). Survival status was captured for four years.

Blood was collected at baseline and week 16 in 178 out of 310 randomized patients of the FREEDOM-C2 study. Leptin and adiponectin were determined in plasma (multiplexed immunoassay, Myriad-RBM, Austin, TX, USA). We calculated leptin/body mass index (BMI), adiponectin/BMI, and leptin/adiponectin ratios. Baseline characteristics of the cohort are shown in Table 1. Patients in this cohort were randomized either to oral treprostinil (n = 83) or placebo (n = 95).

Baseline plasma leptin and adiponectin concentrations were 25 ± 31 ng/mL and 7.8 ± 6.1 ug/mL, respectively. Leptin and adiponectin were above the laboratory reference range (middle 95%) for apparently healthy individuals (high range cutoffs = 38 ng/mL and 15 ng/mL) in 16 (9%) and 16 (9%) patients, respectively. Meanwhile, leptin and adiponectin were below the reference range (lower cutoffs = 0.85 ng/mL and 1.8 ng/mL) in four (2%) and six (3%) patients, respectively.

Leptin at baseline was significantly associated with older age, higher BMI, higher Borg dyspnea index, and lower NT-pro BNP (Table 2). Women had higher levels of leptin than men (30.5 ± 33.2 versus 7.2 ± 6.4 ng/mL), even when adjusting for background therapy and etiology (linear regression: β = 21.8, P < 0.001). Adiponectin was negatively associated with BMI and positively associated with NT-pro BNP (Table 2). We did not find an association between plasma leptin or adiponectin levels and PAH etiology (idiopathic, connective tissue disease-associated or others), PAH treatment (endothelin receptor antagonist [ERA], phosphodiesterase type 5 inhibitor [PDE-5I], or both), 6MWD, or WHO functional class despite controlling for gender, age, and BMI.

Leptin, adiponectin, and leptin/adiponectin (mean ± SD) changes at 16 weeks were of small magnitude at +0.1 ± 12 ng/mL, −0.2 ± 3 ug/mL, and −0.2 ± 5 ng/ug, respectively. Only four of 178 (2.2%) patients met the criteria for clinical worsening at 16 weeks. Patients randomized to oral treprostinil had a significant larger decrease in leptin (β = −5.97, P = 0.0007) than patients receiving placebo, even when adjusted for BMI (β = −0.18, P = 0.0019). We did not find an association between change in leptin, adiponectin, or leptin/adiponectin and variations in 6MWD, WHO functional class, or NT-pro BNP, even after adjusting for age, gender, BMI, creatinine, and/or treatment allocation. Change in leptin at 16 weeks was positively associated with change in Borg dyspnea index (β = 0.07, P = 0.001). Changes in leptin, adiponectin, and leptin/adiponectin ratio adjusted for weight at 16 weeks did not predict survival at one, two, three, or four years; however, the event rate for each time period was low at ten, 16, 29, and 29 deaths, respectively.

In the present study, we confirmed that plasma leptin levels are elevated in patients with PAH, particularly women and patients with older age and higher BMI. The lack of correlation between 6MWD and leptin or adiponectin was similarly noted by others in patients with systemic sclerosis. Interestingly, higher leptin was weakly associated with more pronounced dyspnea during 6 MW test but lower levels of NT-pro BNP. Potential explanations include the observations that leptin is involved in the dysregulation of ventilation in chronic heart failure and its release is inhibited by atrial natriuretic peptide. Adiponectin was not significantly associated with 6 MW test results, but a higher plasma level corresponded to a higher NT-pro BNP. Cardiac natriuretic peptides activate lipolysis through cyclic GMP production, significantly stimulating adiponectin and inhibiting leptin release; findings that help explain why in our study, leptin was directly and inversely associated with adiponectin and leptin, respectively.

Table 1. Baseline characteristics.

| Characteristic | Mean ± standard deviation, n (%) |
|---------------|---------------------------------|
| n             | 178                             |
| Age (years)   | 51 ± 15                         |
| Female gender | 136 (76)                        |
| BMI (kg/m²)   | 27.3 ± 6.1                      |
| PAH etiology  |                                 |
| - Idiopathic or heritable | 110 (62) |
| - Connective tissue disease | 62 (35) |
| - Others*     | 6 (3)                           |
| WHO functional class† |                      |
| - II          | 41 (23)                         |
| - III         | 135 (76)                        |
| - IV          | 1 (1)                           |
| PAH-specific treatments at inclusion |            |
| - PDE-5I     | 79 (44)                         |
| - ERA        | 27 (15)                         |
| - PDE-5I and ERA | 72 (40) |
| Six-minute walk test |                |
| - Distance walked (m) | 333 ± 70 |
| - Borg dyspnea index | 3.8 ± 2.4 |
| NT-pro BNP (pg/mL) | 1429 ± 2091 |

*Others include congenital heart disease and HIV
†In one patient, the WHO functional class was not available.

BMI, body mass index; ERA, endothelin receptor antagonist; NT-pro BNP, N-terminal pro brain natriuretic peptide; PAH, pulmonary arterial hypertension; PDE-5I, phosphodiesterase type 5 inhibitor; WHO, World Health Organization.
Table 2. Baseline plasma levels of leptin and adiponectin and their association with selected surrogate markers of PAH severity.

|                      | Leptin | Adiponectin | Leptin/BMI | Adiponectin/BMI | Leptin/adiponectin |
|----------------------|--------|-------------|------------|-----------------|--------------------|
| **Age**              | R = 0.20, P = 0.006 | R = -0.01, P = 0.94 | R = 0.17, P = 0.02 | R = -0.09, P = 0.23 | R = 0.18, P = 0.02  |
| **BMI**              | R = 0.73, P < 0.0001 | R = -0.19, P = 0.01 | -           | -               | R = 0.67, P < 0.0001|
| WHO functional class | R = 0.10, P = 0.18  | R = -0.08, P = 0.29 | R = 0.09, P = 0.22 | R = 0.05, P = 0.54 | R = 0.03, P = 0.66  |
| 6MWD                 | R = -0.14, P = 0.06  | R = -0.01, P = 0.94 | R = -0.12, P = 0.10 | R = 0.05, P = 0.54 | R = -0.13, P = 0.07 |
| Borg dyspnea index   | R = 0.33, P < 0.0001 | R = 0.06, P = 0.40  | R = 0.29, P < 0.0001 | R = -0.05, P = 0.53 | R = 0.22, P = 0.003  |
| NT-pro BNP           | R = -0.22, P = 0.004 | R = 0.39, P < 0.0001 | R = -0.21, P = 0.004 | R = 0.38, P < 0.0001 | R = -0.34, P < 0.0001|

6MWD, six-minute walk distance; BMI, body mass index; NT-pro BNP, N-terminal pro brain natriuretic peptide; R, Spearman correlation coefficient; WHO, World Health Organization.

Limitations of our study include: (1) echocardiographic and hemodynamic data were not available; (2) PAH patients were receiving ERA and/or PDE-5i at the time of enrollment and throughout the duration of the study; and (3) few patients met the criteria for clinical deterioration. Therefore, this outcome could not be considered in the study. However, we use one-, two-, three-, and four-year survival as an endpoint. Strengths of the study include the rigorous and prospective collection of data in the context of a well-designed study.

In conclusion, in this large cohort of PAH patients, plasma leptin and adiponectin at baseline and their change at 16 weeks do not appear to significantly impact prognosis in PAH. No significant associations between leptin, adiponectin, or leptin/adiponectin ratio and WHO functional class or 6MWD were noted, both at baseline and follow-up.

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Conflict of interest
The author(s) declare the following conflicts of interest: Wassim H. Fares is on the advisory board for United Therapeutics, Gilead, Actelion, and Bayer, and is in the Speakers bureau for United Therapeutics, Gilead, and Actelion; Youlan Rao and Xuan Zhou are employees of United Therapeutics Corporation.

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References
1. Tonelli AR, Aytekin M, Feldstein AE, et al. Leptin levels predict survival in pulmonary arterial hypertension. Pulm Circ 2012; 2: 214–219.
2. Aytekin M, Tonelli AR, Farver CF, et al. Leptin deficiency recapitulates the histological features of pulmonary arterial hypertension in mice. Int J Clin Exp Pathol 2014; 7: 1935–1946.
3. Huertas A, Tu L, Thuillet R, et al. Leptin signalling system as a target for pulmonary arterial hypertension therapy. Eur Respir J 2015; 45: 1066–1080.
4. Hansmann G and Rabinovitch M. The protective role of adiponectin in pulmonary vascular disease. Am J Physiol Lung Cell Mol Physiol 2010; 298: L1–2.
5. Summer R, Fiaack CA, Ikeda Y, et al. Adiponectin deficiency: a model of pulmonary hypertension associated with pulmonary vascular disease. Am J Physiol Lung Cell Mol Physiol 2009; 297: L432–438.
6. Santos M, Reis A, Goncalves F, et al. Adiponectin levels are elevated in patients with pulmonary arterial hypertension. Clin Cardiol 2014; 37: 21–25.
7. Medoff BD, Okamoto Y, Leyton P, et al. Adiponectin deficiency increases allergic airway inflammation and pulmonary vascular remodeling. Am J Respir Cell Mol Biol 2009; 41: 397–406.
8. Hansmann G, Wagner RA, Schellong S, et al. Pulmonary arterial hypertension is linked to insulin resistance and reversed by peroxisome proliferator-activated receptor-gamma activation. Circulation 2007; 115: 1275–1284.
9. Tapson VF, Jing ZC, Xu KF, et al. Oral treprostinil for the treatment of pulmonary arterial hypertension in patients receiving background endothelin receptor antagonist and phosphodiesterase type 5 inhibitor therapy (the FREEDOM-C2 study): a randomized controlled trial. Chest 2013; 144: 952–958.
10. Olewicz-Gawlik A, Danczak-Pazdrowska A, Kuznar-Kaminska B, et al. Circulating adipokines and organ involvement in patients with systemic sclerosis. Acta Reumatol Port 2015; 40: 156–162.
11. Wolk R, Johnson BD and Somers VK. Leptin and the ventilatory response to exercise in heart failure. J Am Coll Cardiol 2003; 42: 1644–1649.
12. Fain JN, Kanu A, Bahouth SW, et al. Inhibition of leptin release by atrial natriuretic peptide (ANP) in human adipocytes. Biochem Pharmacol 2003; 65: 1883–1888.
13. Tanaka T, Tsutamoto T, Sakai H, et al. Effect of atrial natriuretic peptide on adiponectin in patients with heart failure. Eur J Heart Fail 2008; 10: 360–366.