Clinical Investigations

Adiponectin Levels Are Elevated in Patients With Pulmonary Arterial Hypertension

Address for correspondence:
Mário Santos, MD,
Cardiology Department
Hospital Santo António,
Centro Hospitalar do Porto,
Largo Professor Abel Salazar
Porto 4099–001, Portugal
mad_ss@yahoo.com

Mário Santos, MD; Abílio Reis, MD; Fabienne Gonçalves, MD; Manuel J. Ferreira-Pinto, MD; Sofia Cabral, MD; Severo Torres, MD; Adelino F. Leite-Moreira, MD, PhD; Tiago Henriques-Coelho, MD, PhD

Department of Physiology and Cardiothoracic Surgery (Santos, Ferreira-Pinto, Leite-Moreira, Henriques-Coelho), Faculty of Medicine, University of Porto; and Cardiology Department (Santos, Cabral, Torres) and Pulmonary Vascular Disease Unit (Reis, Gonçalves), Hospital Santo António, Centro Hospitalar do Porto, Porto, Portugal

ABSTRACT

Background: In addition to insulin-sensitizing effects, adiponectin influences several mechanisms involved in pulmonary arterial hypertension (PAH) pathobiology. Insulin resistance has been associated with PAH, and elevated adiponectin levels have been described in left heart failure (HF) as a response to the increased metabolic stress. No studies have been performed in right HF or PAH patients.

Hypothesis: Compared to healthy controls, PAH patients have a different plasma adipocytokine profile, higher insulin resistance, and higher inflammatory systemic activation.

Methods: A case–control study was conducted in PAH patients individually matched for sex, age, and body mass index. We characterized the clinical features, functional status (6-minute walking test), and hemodynamic profile of cases (n = 25). We measured insulin resistance (homeostasis model assessment and high-density lipoprotein/triglycerides ratio), inflammatory systemic activation (high-sensitivity C-reactive protein), and plasma adipocytokine profile (adiponectin, leptin, visfatin, and resistin) in cases and controls.

Results: PAH patients had significantly higher adiponectin levels than controls (12.4 ± 6.9 vs 8.1 ± 4.5 μg/mL; $P < 0.05$) and higher high-sensitivity C-reactive protein (2.96 ± 3.2 vs 1.08 ± 1.1; $P < 0.05$). No statistically significant differences were found in plasma levels of leptin, visfatin, and resistin between groups.

Conclusions: Adiponectin levels are increased in PAH patients compared to controls. Further studies are needed to study the potential role of adiponectin as a PAH biomarker.

Introduction

Pulmonary arterial hypertension (PAH) is a pulmonary vasculopathy in which a progressive increase in pulmonary vascular resistance leads to right ventricle dysfunction, heart failure (HF), and a 15% annual mortality rate.[1] PAH pathogenic mechanisms are complex and involve abnormal vasoconstriction and cell proliferation, increased inflammation, and thrombosis in situ, amongst others.[2] This multifactorial pathobiology emphasizes the need to explore other pathways that could influence PAH.

Adiponectin is a protein produced almost exclusively by adipose cells, and it is the most abundant adipocytokine.[3] It has important central and peripheral insulin-sensitizing properties.[3] Apart from the metabolic role, adiponectin also influences several mechanisms involved in PAH pathobiology, such as vascular inflammation,[4] vascular smooth muscle cell proliferation,[5] vascular dilatation,[6] and myocardial protection.[7] In addition, several experimental studies have described a beneficial role of adiponectin in pulmonary vascular remodeling and in the attenuation of PAH in several animal models.[5,8,9] Patients with advanced left HF have increased levels of adiponectin that are independently associated with poor outcome.[10] These paradoxically increased levels are interpreted as a compensatory response to the increased metabolic stress that characterizes HF. The elevated circulating levels of adiponectin reflect an effort to overcome a functional adiponectin resistance.[11] The role of adiponectin in right HF or PAH patients is still unknown.

In addition to adiponectin, the secretion of other adipocytokines such as leptin, visfatin, and resistin are altered in many cardiovascular diseases.[12] Leptin has proinflammatory, proliferative, and prothrombotic actions.[13] It is overexpressed in endothelium of PAH patients and could have a role in its pathogenic mechanisms.[14] Resistin is produced almost exclusively in adipose tissue and influences endothelial function by promoting the secretion of endothelin-1 and proinflammatory cytokines.[15] Similarly, visfatin is associated with insulin-resistant conditions and can potentiate vascular inflammation and vasoconstriction.[16]

The present study assessed serum adipocytokine profiles in PAH patients in comparison to controls.

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Methods

Study Design and Population

A cross-sectional, matched case–control study was designed. Outpatients from the Pulmonary Vascular Disease Unit at Hospital Santo António in Porto, Portugal were recruited between February and December of 2011. Inclusion criteria were (1) diagnosis of group 1 of Dana Point PAH classification and (2) clinical stability for at least 3 months. The diagnosis of PAH was according to European Society of Cardiology criteria.[17] Exclusion criteria were (1) recent hospitalization (<3 months), (2) recent clinically infectious condition, and (3) glucocorticoid treatment.

Clinical evaluation included medical history, physical examination with measurement of systemic arterial pressure, heart rate, and transcutaneous oxygen saturation. Patients were characterized by PAH etiology, and demographic and anthropometric features. Functional assessment was done using New York Heart Association (NYHA) classification and by the 6-minute walking test (6MWT). 6MWT was performed in a 30-m-long corridor under the same environmental conditions and at approximately the same time of the day. Healthy control subjects matched for age, sex, and body mass index (BMI) were recruited. In all control subjects, a medical history was taken with a complete physical examination. The local ethics committee approved the study, and written consent was obtained from all participants. The investigation conforms with the principles outlined in the Declaration of Helsinki.[18]

Hemodynamic Assessment

Hemodynamic assessment was performed by right heart catheterization using the right femoral vein. Pulmonary artery, right atrial, and pulmonary capillary wedge pressures were recorded at the end of a quiet respiratory cycle. Cardiac output was obtained with the Fick principle using table-derived oxygen consumption values. Pulmonary vascular resistance indexes were calculated using the standard formulas.[19]

Blood Sample Collection and Laboratory Measurements

All subjects underwent fasting venous blood sampling drawn from an antecubital vein. All samples were processed immediately. Hemoglobin, glucose, insulin, lipid panel, creatinine, pro-brain natriuretic peptide (proBNP), and high-sensitivity C-reactive protein (hsCRP) were measured on the day of blood sampling using the standard protocol of our hospital laboratory. The insulin resistance was estimated by 2 methods: the homeostasis model assessment (HOMA) and the high-density lipoprotein (HDL)/triglycerides ratio.[20]

Blood samples for adiponectin, leptin, resistin, and visfatin measurement were immediately centrifuged; the serum was aliquoted and stored at −80°C until analysis. Measurements were performed using commercially available magnetic bead-based assay kits from Bio-Rad Laboratories (Hercules, CA; Bio-Plex kit). The adiponectin assay had a sensitivity of 32.7 pg/mL, and the intra- and interassay coefficients of variation (CVs) were 4% and 2%, respectively. The leptin assay had a sensitivity of 3.1 pg/mL, and the intra- and interassay CVs were 3% and 4%, respectively. The resistin assay had a sensitivity of 1.3 pg/mL, and the intra- and interassay CVs were 3% and 4%, respectively. The visfatin assay had a sensitivity of 32.7 pg/mL, and the intra- and interassay CVs were 4% and 3%, respectively.

Statistical Analysis

Results are expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). Categorical variables are presented as counts and proportions. Comparisons between groups were performed using paired parametric or nonparametric tests, as appropriate. Correlations between variables were assessed using Pearson coefficient of correlation for normally distributed variables and Spearman coefficient of correlation when at least 1 of the variables in the analysis had a skewed distribution. The statistical analysis was conducted using SPSS version 19.0 (SPSS, Chicago, IL). P values < 0.05 were considered to be statistically significant.

Results

We studied 25 patients, 13 with idiopathic PAH and 12 with congenital heart disease (CHD)-associated PAH. Control subjects were well matched for age, sex, and BMI. Clinical and hemodynamic features and laboratory parameters for the 50 participants in the study are displayed in Table 1. The mean age of patients was 35 ± 13 years, and 60% were female. Eighty percent of patients were classified as NYHA functional class II or III, and the mean distance on the 6MWT was 460 ± 108 m. The hemodynamic characterization showed an elevated mean pulmonary artery pressure (55 mmHg) and a mean cardiac index of 2.9 L/min/m^2. Nineteen patients (76%) were on combination therapy; 14 patients (56%) were on endothelin receptor antagonists and phosphodiesterase inhibitors, and 5 patients (20%) were on triple vasodilator therapy with intravenous prostanoids. The patient group had higher hemoglobin and proBNP levels than the control group. All the participants in the study had normal values of fasting glycemia.

PAH patients had significantly higher adiponectin levels than controls (12.4 ± 6.9 vs 8.1 ± 4.5 μg/mL; $P < 0.05$) and higher hsCRP (1.4 ± 4.9 vs 1.0 ± 1.1 mg/L; $P < 0.05$; Table 2). No significant correlation was found between these 2 variables ($r = 0.30$; $P = 0.25$). We found a statistically significant difference ($P = 0.005$) between adiponectin levels when we compared the median values for the idiopathic PAH patients (8.6 μg/mL, IQR = 3.7), CHD-PAH patients (14.7 μg/mL, IQR = 11.8), and controls (6.8 μg/mL, IQR = 4.8). The HOMA index (0.9 ± 0.7 vs 0.9 ± 0.3, $P = 0.60$) and the HDL/triglycerides ratio (2.0 ± 1.2 vs 2.0 ± 2.5, $P = 0.98$) were not different between groups.

No correlation between adiponectin and proBNP levels was found ($r = 0.2$; $P = 0.90$). No correlations between adiponectin and cardiac index ($r = 0.10$; $P = 0.8$), mean pulmonary artery pressure ($r = 0.34$; $P = 0.20$), or 6MWT ($r = -0.2$, $P = 0.32$) were found. Patients with NYHA class III or IV had higher levels of adiponectin than patients with lower NYHA classes (15.0 ± 8.6 vs 10.4 ± 4.9 μg/mL; $P = 0.05$). No statistical significant differences were found in

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Table 1. Patient and Control Group Characteristics

Variable	PAH Patients, n = 25	Controls, n = 25	P
Age, y, mean (SD)	35 (13)	34 (13)	0.06
Female gender, No. [%]	15 [60]	15 [60]	1.00
BMI, mean (SD), kg/m²	24.5 (5.0)	23.3 (3.6)	0.10
Etiology			
Idiopathic, No. [%]	13 [52]	NA	
CHD-associated, No. [%]	12 [48]	NA	
6MWT, m, mean (SD)	460 (108)	NA	
NYHA functional class, No. [%]			
I	4 [16]	NA	
II	11 [44]	NA	
III	9 [36]	NA	
IV	1 [4]	NA	
Hemodynamic parameters, mean (SD)			
mPAP, mmHg	55 (21)	NA	
CI mean, L/min/m²	2.9 (0.5)	NA	
PVR mean, Woods units	11.3 (5.2)	NA	
Laboratory data, mean (SD)			
Hemoglobin, g/dL	16.3 (3.7)	14 (1.5)	0.04
Glucose, mg/dL	71 (25)	80 (11)	0.10
Insulin, µg/mL	7.4 (5.4)	7.5 (2.7)	0.90
HDL, mg/dL	47 (11)	58 (12)	0.01
TG, mg/dL	86 (41)	99 (75)	0.41
Creatinine, mg/dL	0.8 (0.2)	0.8 (0.1)	0.35
ProBNP, ng/mL, median {IQR}	381 {1026}	26 {21}	0.001
Specific PAH medication [%]			
Ca2+ channel blocker	4 [16]	NA	
Phosphodiesterase inhibitors	14 [56]	NA	
Endothelin receptor antagonists	23 [92]	NA	
Prostanoids	5 [20]	NA	

Abbreviations: 6MWT, 6-minute walking test; BMI, body mass index; CHD, congenital heart disease; CI, cardiac index; HDL, high-density lipoprotein; IQR, interquartile range; mPAP, mean pulmonary arterial pressure; NA, not applicable; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; ProBNP, pro-brain natriuretic peptide; PVR, pulmonary vascular resistance; SD, standard deviation; TG, triglycerides.
Data are described as mean (SD) or median (IQR) for continuous variables and counts (%) for categorical values.

plasma levels of leptin, visfatin, or resistin between patients and controls.

Discussion

Understanding the role of neurohumoral changes in HF pathophysiology led to a significant therapeutic development. In recent years, abnormalities in adipocytokine signaling, insulin sensitivity changes, and proinflammatory status have all been ascribed to this syndrome.[11,21]

In advanced left HF, increased levels of adiponectin are associated with poor prognosis.[10] These higher levels are interpreted to be an adipose tissue counter-regulatory response to overcome the adiponectin functional resistance. This resistance state is supported by preclinical and clinical studies that documented a reduction in adiponectin

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Table 2. Serum Concentrations of Adipocytokines, hsCRP, and Insulin Resistance Indexes

Variable	PAH Patients, n = 25	Controls, n = 25	P
HOMA index, mean (SD)	0.9 (0.7)	0.9 (0.3)	0.60
TG/HDL ratio, mean (SD)	2.0 (1.2)	2.0 (2.5)	0.98
Adiponectin, μg/mL, mean (SD)	12.4 (6.9)	8.1 (4.5)	0.02[a]
Leptin, ng/mL, median [IQR]	6.7 [8.9]	4.9 [8.8]	0.24
Visfatin, ng/mL, median [IQR]	1.1 [0.8]	1.0 [0.6]	0.14
Resistin, ng/mL, mean (SD)	5.7 (1.6)	5.3 (2.2)	0.44
hsCRP, mg/L, median [IQR]	1.4 [4.9]	1.0 [1.1]	0.01[a]

Abbreviations: HDL, high-density lipoprotein; HOMA, homeostasis model assessment; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; PAH, pulmonary arterial hypertension; SD, standard deviation; TG, triglycerides.
Data are described as mean (SD) and median [IQR] for continuous variables.
[a]Statistically significant.

receptor (adipoR1) expression in myocardial and skeletal muscle, and a reduction in adipoR1 downstream signaling.[22] Interestingly, left ventricle unloading is associated with attenuated adiponectin resistance and lower levels of this adipocytokine.[22] In the present study, we have demonstrated a significant difference in adiponectin levels in PAH patients compared to matched healthy control subjects, with higher levels in the PAH group. Although clinically stable with specific vasodilator therapies, our patients still had an increased right ventricle afterload elevated pulmonary vascular resistance (PVR) and HF symptoms. Right HF pathophysiology has some particularities; however, its mechanisms are assumed to be generally similar to left HF.[23] Our results support this hypothesis, as we describe concordant changes in circulating adiponectin in right HF patients, when compared to previous studies of left HF.[22,24] Circulating natriuretic peptides increase adiponectin secretion both in healthy subjects and in HF patients.[25] These results corroborate similar findings in in vitro studies.[26] We did not find any correlation between proBNP and adiponectin. Given the large variance of our proBNP values (SD = 971 ng/mL), we would need a larger sample to accurately study the correlation between these 2 variables. We did not find significant correlations between adiponectin levels and some validated surrogate markers for PAH patients such as hsCRP, cardiac index, and 6MWT. Our limited sample size could be an explanation for these results. Interestingly, we found higher levels of adiponectin in PAH patients with worse functional status (NYHA class III or IV) when compared with patients with NYHA class I or II. The higher plasma levels of adiponectin found in the CHD-PAH patients could also be explained by the higher proportion of patients with NYHA class III or IV within this group than in idiopathic PAH (50% vs 31%). Further studies are needed to define the putative role of adiponectin as a prognostic marker in PAH.

Considering the marked insulin-sensitizing properties of adiponectin,[3] the functional resistance of this adipokine can contribute to an increased insulin resistance, which is commonly associated with HF.[27] Interestingly, several clinical studies have supported an association between conditions characterized by insulin resistance and PAH.[28,29] In our study, we did not find differences in insulin resistance between patients and the control group, measured by HDL/triglycerides ratio and HOMA index. Our results could be explained by the youth (mean age = 35 years) of the participants and their larger metabolic reserve and/or by the low sensitivity of these indexes when compared to the hyperinsulinemic-euglycemic clamp test, the gold standard method to assess insulin resistance.[20]

We did not find any difference in leptin, resistin, and visfatin between PAH patients and controls. Regarding leptin, our results were not concordant with a recent study finding that leptin plasma levels were higher in PAH and scleroderma-associated pulmonary hypertension.[14] In addition to the different pulmonary hypertension etiologies (scleroderma versus CHD), our study population was younger. In vitro studies established the influence of these adipocytokines (leptin, resistin, and visfatin) on several biological processes involved in pulmonary vascular remodeling. Despite this appealing biological plausibility, further studies are needed to ascertain whether they have a relevant role in the pathophysiology of PAH. When interpreting our negative findings on these adipocytokines, we also need to consider the low statistical power of our study, so we cannot fully exclude the possibility of a type II error.

The insight that systemic inflammation was associated with HF progression was already established in PAH patients. Quarck et al found elevated C-reactive protein in PAH patients and described the association between this inflammation marker and prognosis.[30] In our study, we used a more sensitive marker of systemic inflammation, hsCRP. Concordant with previous studies, we found that hsCRP was significantly elevated in PAH patients compared to controls.

Our study has several limitations. It was a single-center investigation with a small sample size. We did not evaluate the body composition with the gold standard method[31]; however, BMI is the most available and widely used method in the clinical field. We cannot extrapolate the conclusions to other etiologies of PAH, as we only included patients with idiopathic and CHD-associated PAH. However, given the similarities of the pathophysiology of these 2 etiologies of PAH and the low prevalence of other comorbidities,

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we studied a very homogeneous patient group with few confounding factors.

Conclusion

To the best of our knowledge, our study shows for the first time that circulating adiponectin levels are increased in PAH patients compared with controls individually matched for age, sex, and BMI. These findings warrant further investigation to establish the role of adiponectin as a biomarker or a modifier of PAH.

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References

1. Lourenco AP, Fontoura D, Henriques-Coelho T, et al. Current pathophysiological concepts and management of pulmonary hypertension. *Int J Cardiol.* 2012;155:350–361.
2. Archer SL, Weir EK, Wilkins MR. Basic science of pulmonary arterial hypertension for clinicians: new concepts and experimental therapies. *Circulation.* 2010;121:2045–2066.
3. Okamoto Y, Kihara S, Funahashi T, et al. Adiponectin: a key adipocytokine in metabolic syndrome. *Clin Sci (Lond).* 2006;110:267–278.
4. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol.* 2006;6:772–783.
5. Summer R, Fiack 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–L438.
6. Krenning G, Moonen JR, Harmsen MC. Pleiotropism of adiponectin: inflammation, neovascularization, and fibrosis. *Circ Res.* 2009;104:1029–1031.
7. Liao Y, Takashima S, Maeda N, et al. Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. *Cardiovasc Res.* 2005;67:705–713.
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. 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.
10. Kistorp C, Faber J, Galatius S, et al. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. *Circulation.* 2005;112:1756–1762.
11. Van Berendoncks AM, Garnier A, Ventura-Clapier R, et al. Adiponectin: key role and potential target to reverse energy wasting in chronic heart failure [published online ahead of print October 23, 2012]. *Heart Fail Rev*. doi:10.1007/s10741-012-9349-4.
12. Katagiri H, Yamada T, Oka Y. Adiposity and cardiovascular disorders: disturbance of the regulatory system consisting of humoral and neuronal signals. *Circ Res.* 2007;101:27–39.
13. Oda A, Taniguchi T, Yokoyama M. Leptin stimulates rat aortic smooth muscle cell proliferation and migration. *Kobe J Med Sci.* 2001;47:141–150.
14. Huertas A, Tu L, Gambaryan N, et al. Leptin and regulatory T-lymphocytes in idiopathic pulmonary arterial hypertension. *Eur Respir J.* 2012;40:895–904.
15. Verma S, Li SH, Wang CH, et al. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. *Circulation.* 2003;108:736–740.
16. Yamawaki H. Vascular effects of novel adipocytokines: focus on vascular contractility and inflammatory responses. *Biol Pharm Bull.* 2011;34:307–310.
17. Galie N, Hoeper MM, Humbert M, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). *Eur Heart J.* 2009;30:2493–2537.
18. Rickham PP. Human experimentation. Code of ethics of the World Medical Association. *Declaration of Helsinki. Br Med J.* 1964;2:177.
19. Hemnes AR, Forfia PR, Champion HC. Assessment of pulmonary vasculature and right heart by invasive haemodynamics and echocardiography. *Int J Clin Pract Suppl.* 2009;(162):4–19.
20. Antuna-Puente B, Disse E, Rabasa-Lhoret R, et al. How can we measure insulin sensitivity/resistance? *Diabetes Metab.* 2011;37:179–188.
21. Thierer J, Acosta A, Vainstein N, et al. Relation of left ventricular ejection fraction and functional capacity with metabolism and inflammation in chronic heart failure with reduced ejection fraction (from the MIMICA Study). *Am J Cardiol.* 2010;105:977–983.
22. Khan RS, Kato TS, Chokshi A, et al. Adipose tissue inflammation and adiponectin resistance in patients with advanced heart failure: correction after ventricular assist device implantation. *Circ Heart Fail.* 2012;5:340–348.
23. Bogaard HJ, Abe K, Vonk Noordegraaf A, et al. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. *Chest.* 2009;135:794–804.
24. Biolo A, Shibata R, Ouchi N, et al. Determinants of adiponectin levels in patients with chronic systolic heart failure. *Am J Cardiol.* 2010;105:1147–1152.
25. Birkenfeld AL, Boschmann M, Engeli S, et al. Atrial natriuretic peptide and adiponectin interactions in man. *PLoS One.* 2012;7:e43238.
26. Tsukamoto O, Fujita M, Kato M, et al. Natriuretic peptides enhance the production of adiponectin in human adipocytes and in patients with chronic heart failure. *J Am Coll Cardiol.* 2009;53:2070–2077.
27. Doehner W, Rauchhaus M, Ponikowski P, et al. Impaired insulin sensitivity as an independent risk factor for mortality in patients with stable chronic heart failure. *J Am Coll Cardiol.* 2005;46:1019–1026.
28. Pugh ME, Robbins IM, Rice TW, et al. Unrecognized glucose intolerance is common in pulmonary arterial hypertension. *J Heart Lung Transplant.* 2011;30:904–911.
29. Zamanian RT, Hansmann G, Snook S, et al. Insulin resistance in pulmonary arterial hypertension. *Eur Respir J.* 2009;33:318–324.
30. Quarck R, Nawrot T, Meyns B, et al. C-reactive protein: a new predictor of adverse outcome in pulmonary arterial hypertension. *J Am Coll Cardiol.* 2009;53:1211–1218.
31. Salamone LM, Fuerst T, Visser M, et al. Measurement of fat mass using DEXA: a validation study in elderly adults. *J Appl Physiol.* 2000;89:345–352.

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