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Serum adipokine zinc α2-glycoprotein and lipolysis in cachectic and noncachectic heart failure patients: relationship with neurohormonal and inflammatory biomarkers

Stefano Tedeschi[a], *Elisabetta Pilotti*[a], *Elisabetta Parenti*[a], *Vanni Vicini*[a], *Pietro Coghi*[a], *Alberto Montanari*[b], *Giuseppe Regolisti*[a], *Enrico Fiaccadori*[a], *Aderville Cabassi*[a,*]

[a] *Clinical Physiology Section, Cardiorenal Research Unit, Department of Internal Medicine and Health Prevention, University of Parma Medical School, 43126 Parma, Italy*

[b] *Heart Failure Clinic, Department of Clinical Sciences, University of Parma Medical School, Via Gramsci 14, 43126 Parma, Italy*

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ABSTRACT

Chronic heart failure is often complicated by the development of cachexia with the loss of fat mass. Zinc α2-glycoprotein (ZAG) is a serum adipokine with lipolytic effects in cancer cachexia. We evaluated in patients with advanced heart failure with (CxHF) or without cachexia (nCxHF) the relationship of ZAG with circulating free fatty acid (FFA), as an index of lipolysis, and with other neurohormonal and inflammatory biomarkers. Two groups, nCxHF (n = 46) and CxHF (n = 18), the latter having a documented, involuntary, edema-free loss of body weight of at least 7.5% in the previous 6 months, underwent plasma determination of FFA, ZAG, norepinephrine (NE), tumor necrosis factor–α, and natriuretic peptide levels (atrial natriuretic, B-type natriuretic peptide). The patients were compared with age-matched healthy controls (CTR) (n = 21). Zinc α2-glycoprotein, atrial natriuretic peptide, B-type natriuretic peptide, and tumor necrosis factor–α circulating levels were similarly greater in CxHF and nCxHF than in CTR. Free fatty acid and NE were higher in CxHF than in nCxHF. A positive correlation between FFA and NE was found in both CxHF ($r = 0.73$, $P < .01$) and nCxHF ($r = 0.48$, $P < .01$) but only in CxHF between ZAG and FFA ($r = 0.54$, $P = .02$) and between ZAG and NE ($r = 0.70$, $P < .01$). No correlations between natriuretic peptides and ZAG were found. Serum ZAG levels are increased in advanced heart failure patients compared with CTR, without differences between CxHF and nCxHF. Only in CxHF, ZAG levels are directly correlated to circulating levels of FFA and NE, suggesting a close interaction of ZAG with sympathetic-mediated lipolysis.

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* *Corresponding author*. Tel.: +390521033194; fax: +390521033185.
E-mail address: aderville.cabassi@unipr.it (A. Cabassi).

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1. Introduction

Chronic heart failure is often complicated by the development of cachexia, a powerful mortality risk predictor [1]. In the last 2 decades, different thresholds, ranging from 15% to 5% of edema-free body weight loss, have been used to define patients with cachexia [1,2], until a recent experts' Consensus Conference proposed a limit of 5% [3]. Loss of fat, lean, and bone mass characterizes body weight loss in cardiac cachexia [4]. The loss of lean and bone tissue affects quality of life because of muscle atrophy, osteoporosis, and early fatigue, whereas fat mass loss implies poorer survival [5,6]. Neuroendocrine, inflammatory, and immunological disturbances, found in advanced heart failure patients, could contribute to the imbalance between lipolytic and lipogenetic pathways in cardiac cachexia, leading to progressive triglyceride breakdown and adipose tissue loss [2,7,8]. In addition to activation of the sympathetic and renin-angiotensin systems, neurohormonal disturbances are manifested by increased plasma levels of natriuretic peptides whose recently discovered lipolytic effects in humans may potentially contribute to fat mass loss in heart failure [9-11]. Zinc α2-glycoprotein (ZAG) [12-14], a 42-kd circulating protein released by mature adipocytes [15] and by other types of epithelial cells, has been attributed an emerging role in fat wasting as a local lipolytic mediator in human cancer cachexia [16,17] and as a lipid metabolism modulator in healthy and obese subjects [18]. To date, no data on ZAG levels in chronic heart failure patients with or without cachexia are available. Therefore, the present study focused on the influence of ZAG on lipolysis, expressed by circulating free fatty acid (FFA), and on its interactions with neurohormonal and inflammatory biomarkers in 2 groups of advanced heart failure patients with (CxHF) or without cachexia (nCxHF) compared with an age-matched group of healthy controls (CTR).

2. Methods

Sixty-four consecutive stable ambulatory advanced heart failure patients (New York Heart Association [NYHA] functional class III/IV, 44/20; 33 male and 31 female) were recruited from the Outpatient Clinic of the Department of Internal Medicine and Health Sciences of the University Hospital of Parma and separated into 2 groups: CxHF (n = 18) and nCxHF patients (n = 46). Cachexia was diagnosed in CxHF patients on the basis of an involuntary, edema-free loss of body weight of at least 7.5% in the previous 6 months. A recent statement by a panel of experts at the 2006 Cachexia Consensus Conference defined the principal criterion for cachexia, regardless of its etiology, as a loss of 5% of body weight [3]. However, a more stringent threshold (>7.5%) of body weight loss was used in our patient population. Twenty-one age-matched healthy subjects were recruited as CTR from patients reporting for a periodical checkup at the same department. On study entry, a complete medical history, a physical examination, basal laboratory tests (total blood count, serum creatinine, electrolytes, uric acid, lipid profile), an electrocardiogram, and an echocardiogram were obtained from all patients. Estimated glomerular filtration rate (eGFR) was calculated from the 4-component Model of Disease in Renal Disease equation incorporating age, race, sex, and serum creatinine level: estimated eGFR = 186 * [serum creatinine (in milligrams per deciliter)] – 1.154 * [age (in years)] – 0.203. For women, the product of the equation must be multiplied by a correction factor of 0.742 [19]. The diagnosis of chronic heart failure was based on symptoms and clinical signs according to guidelines issued by the European Society of Cardiology [20] and the American College of Cardiology [21]. The patients had no clinical or laboratory signs of acute infection, rheumatoid or other autoimmune diseases, primary cachectic states (cancer, thyroid disease, severe liver disease, severe chronic lung disease, HIV infections treated with highly active antiretroviral therapy), neuromuscular disorders, myocardial infarction within the previous 20 weeks, diabetes mellitus, or severe chronic renal failure (serum creatinine level >2.0 mg/dL, >177 μmol/L). All of the patients were clinically stable and on constant therapy at least 8 weeks before entering the study. The study was approved by the University of Parma Ethics Committee and complied with the Declaration of Helsinki, and all participants provided written informed consent.

2.1. *Venous blood sampling procedure and biomarker assay*

Biomarkers related to different biological domains (neurohormonal, inflammatory, metabolic, nutritional) including ZAG, norepinephrine (NE), epinephrine (EPI), atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), high-sensitivity C-reactive protein (Hs-CRP), tumor necrosis factor–α (TNF-α), albumin, prealbumin, FFA, and insulin were measured in morning venous blood samples collected from all enrolled patients between 8:00 AM and 9:00 FFA after a 12-hour fast. Samples were obtained after at least 30 minutes of supine rest from an indwelling catheter and collected in polypropylene tubes containing an ethylenediaminetetraacetic acid buffer (1.5 mg/mL) for all biomarkers analyzed except for ANP and BNP where a mix of protease inhibitors (phenylmethylsulfonyl fluoride, trypsin inhibitor, and aprotinin 500 U/mL) was added. They were immediately placed on ice and centrifuged at 4°C. Plasma samples were then stored at –80°C until assays were performed without any freeze-thaw cycles. All laboratory measurements were performed by investigators blind to the clinical data.

Norepinephrine and EPI concentrations were measured by high-pressure liquid chromatography coupled to electrochemical detection as previously described [22]. The detection limit of the assay for NE and EPI was 11 and 5 pg/mL, respectively. The intra- and interassay coefficients of variation for NE and EPI were 6% and 8%, and 5% and 10%, respectively. Plasma natriuretic peptides levels (α-human ANP and 1-32 BNP) were measured using a commercially available immunoradiometric assay kit (Shiono RIA ANP assay kit; Shionogi; Shiono RIA BNP Kit, IRMA type, CIS Bio International, Gif-sur-Yvette, France) The intra- and interassay coefficients of variation were 7% and 9%, respectively, for ANP and 8% and 13%, respectively, for 1-32 BNP. The detection limit of the assay was 5 pg/mL for ANP and 2.0 pg/mL for 1-32 BNP. High-sensitivity CRP was measured using the Dade Behring N Highly Sensitive CRP assay (Dade Behring Diagnostics, Deerfield, IL) on the BN 100 Nephelometer. Intra- and interassay

Table 1 – Clinical characteristics of CxHF, nCxHF, and CTR

	CxHF (n = 18)	nCxHF (n = 46)	CTR (n = 21)
Age (y)	77 (7)	76 (10)	74 (11)
Sex (M/F)	8/10	25/21	9/12
Body mass index (kg/m^2)	22 (3)†	24 (4)	24 (4)
Systolic blood pressure (mm Hg)	121 (20)*	127 (19)	136 (21)
Diastolic blood pressure (mm Hg)	70 (12)	72 (15)	78 (12)
Heart rate (beat/min)	81 (12)	76 (11)	80 (12)
Ischemic etiology (%)	78	80	-
LVEF (%)	33 (10)*	39 (11)*	67 (6)
NYHA class	3.4(0.5)	3.4(0.4)	
eGFR (mL/min)	45 (11)*	44 (13)*	73 (24)
Sodium (mmol/L)	137 (5)*	138 (6)*	141 (4)
Drug therapy			
Furosemide (% of patients) dose (mg/[kg d])	100 1.66 (1.06-2.19)	100 1.83 (0.81-2.50)	-
ACE-I or ARB (%)	79	76	-
β-Blocker (%)	72	67	-
Spironolactone (%)	42	33	–

Values are presented as mean (SD). LVEF indicates left ventricular ejection fraction; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor 1 blocker.
* $P < .05$ vs CTR by 1-way analysis of variance followed by Bonferroni post hoc test.
† $P < .05$ vs nCxHF by 1-way analysis of variance followed by Bonferroni post hoc test.

variations were 4% and 5%, respectively. Plasma levels of TNF-α were measured using highly sensitive commercially available immunoassays (Quantikine HS; R&D System, Minneapolis, MN). The intra- and interassay coefficients of variation were 7% and 9% for TNF-α, and analytical sensitivity was 0.12 pg/mL. Free fatty acids were measured using an in vitro enzymatic colorimetric assay (NEFA C Kit; Wako Chemicals USA, Richmond, VA), whereas plasma insulin levels were measured using the radioimmunoassay Human Insulin Specific RIA Kit (Linco Research, St Charles, MO). Intra- and interassay coefficient of variations for FFA were 3.5% and 8.2%, whereas for insulin, the limit of sensitivity was 2 μU/mL and the intra- and interassay coefficients of variation were 8.5% and 10%.

To estimate an index of insulin resistance, the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as previously described [23].

The ZAG serum concentrations were assessed using a commercially available enzyme-linked immunosorbent assay (Biovendor, Modrice, Czech Republic EU) according to the manufacturer's instructions. In brief, serum samples, after being diluted (1:5000), were incubated in microplate wells precoated with polyclonal anti-human ZAG antibody. After a 1-hour incubation and subsequent washing, polyclonal anti-human ZAG antibody conjugated with horseradish peroxidase was added to the wells and incubated for 1 hour with captured ZAG. Following another washing step, the remaining horseradish peroxidase conjugate was allowed to react with the substrate tetramethylbenzidine. The reaction was then stopped by addition of an acidic solution. The absorbancies were measured to determine the concentrations against a standard curve. The intra- and interassay coefficients of variation were 5% and 7%, respectively; and the analytical sensitivity was 1.25 ng/mL.

Table 2 – Nutritional, neurohormonal, and inflammatory parameters in CxHF, nCxHF, and CTR

	CxHF (n = 18)	nCxHF (n = 46)	CTR (n = 21)
FFA (μmol/L)	1111 (346)*,†	813 (196)*	436 (98.4)
Albumin (g/dL)	3.3 (0.6)*	3.5 (0.7)	3.8 (0.5)
Prealbumin (mg/dL)	21.4 (9.3)*	23.8 (9.5)*	29.0 (5.7)
HOMA-IR	2.7 (1.0)*	2.9 (1.3)*	1.7 (0.9)
Fasting insulin (μU/mL)	11.9 (4.1)*	11.4 (4.4)*	8.7(3.9)
ZAG (μg/mL)	115 (41)*	110 (36)*	66 (23)
NE (pg/mL)	612 (264)*,†	425 (132)*	251 (72)
EPI[a] (pg/mL)	335 (112)*,†	130 (61)*	81 (34)
TNF-α (pg/mL)	5.7 (1.4)*	5.0 (2.4)*	2.9 (1.5)
Hs-CRP (mg/L)	25.8 (19.0)*	19.6 (19.5)*	3.8 (4.2)
ANP (pg/mL)	187 (121)*	144 (122)*	36 (14)
BNP (pg/mL)	298 (178)*	235 (168)*	40 (19)

Values are presented as mean (SD).
* $P < .05$ vs CTR by-1 way analysis of variance followed by Bonferroni post hoc test.
† $P < .05$ vs nCxHF by-1 way analysis of variance followed by Bonferroni post hoc test.
[a] Number of patients in the nCxHF group = 43.

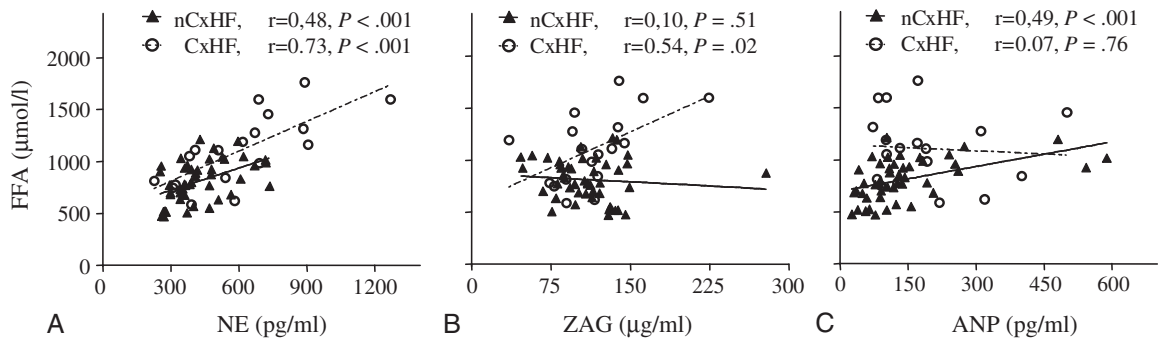

Fig. 1 – Relation between circulating FFA and NE (A), between FFA and ZAG (B), and between FFA and ANP (C) in CxHF (open circle, n = 18) and nCxHF (black triangle, n = 46) patients. *r* indicates Pearson correlation coefficient.

2.2. *Data analysis*

Values are presented as mean ± SD. Statistical analysis among groups of patients was based on a 1-way analysis of variance model followed by the Bonferroni post hoc test. Relations between parameters from different biological domains (neurohormonal, inflammatory, metabolic, nutritional) including NE, BNP, ANP, Hs-CRP, TNF-α, albumin, prealbumin, FFA, and ZAG, were analyzed by linear regression analysis using Pearson correlation coefficients. The D'Agostino-Pearson normality test was passed for all parameters, except Hs-CRP that was log transformed to create a normal distribution. *P* values less than .05 denote statistical significance.

3. Results

The clinical characteristics of the 3 groups of patients are summarized in Table 1. No differences were observed between CxHF and nCxHF with regard to age, blood pressure levels, heart rate, mean NYHA functional class, cause of heart failure, left ventricular ejection fraction, eGFR, and plasma sodium concentration. Plasma FFA levels were higher in CxHF (+37%) compared with nCxHF; both groups showed higher plasma levels when compared with CTR ($P < .01$) (Table 2). The ZAG levels were similar in CxHF and nCxHF groups, but were almost doubled as compared with CTR (Table 2). No significant differences were found in inflammatory parameters levels such as TNF-α and Hs-CRP between CxHF and nCxHF, although they were higher than in the CTR group (Table 2). Plasma NE (+44%) and EPI (+157%) levels were higher in CxHF than in nCxHF (Table 2). Both ANP and BNP levels were significantly increased in CxHF and nCxHF groups compared with CTR; no difference was found between CxHF and nCxHF (Table 2). The CxHF and nCxHF groups had higher HOMA-IR than the CTR group. Fig. 1 depicts the relationship between FFA and NE (A), between FFA and ZAG (B), and between FFA and ANP (C) in CxHF and nCxHF patients; a close positive association was found between FFA and NE in CxHF ($r = 0.73$, $P < .01$) and in nCxHF ($r = 0.48$, $P < .01$) (Fig. 1A). Table 3 indicates a complete panel of linear regression analysis correlation coefficients between the analyzed biomarkers related to neurohormonal and inflammatory domains in the CxHF and nCxHF groups. A positive linear relationship was found in CxHF between ZAG and FFA ($r = 0.54$, $P = .02$) and NE ($r = 0.70$, $P < .01$) but not in the nCxHF group (Fig. 1B and Table 3). No correlation was found between ZAG and EPI levels (nCxHF: $r = 0.09$, $P = .61$; CxHF: $r = 0.26$, $P = .30$; CTR: $r = 0.32$, $P = .91$) in all groups of patients. In addition, no correlation was observed between ZAG and eGFR in all of the 3 studied groups (nCxHF: $r = 0.004$, $P = .98$; CxHF: $r = 0.33$, $P = .18$; CTR: $r = 0.20$, $P = .51$). Both ANP and BNP directly correlated with FFA in nCxHF (FFA vs BNP: $r = 0.41$, $P < .01$; FFA vs ANP: $r = 0.49$; $P < .01$), whereas such a relationship was not observed in CxHF (Fig. 1C and Table 3). The TNF-α levels were directly related to FFA in CxHF but not in HF; the NE plasma levels in both CxHF and nCxHF were significantly related to TNF-α ($r = 0.50$, $P < .05$; $r = 0.46$, $P < .05$,

Table 3 – Linear regression analysis (Pearson correlation coefficient *r*) between the biochemical parameters related to neurohormonal and inflammatory domains in CxHF and nCxHF

	FFA CxHF/nCxHF	NE CxHF/nCxHF	ZAG CxHF/nCxHF	BNP CxHF/nCxHF	TNF- α CxHF/nCxHF	Hs-CRP CxHF/nCxHF
FFA	–					
NE	**0.74**/**0.48**	–				
ZAG	**0.54**/0.10	**0.70**/0.01	–			
BNP	0.03/**0.41**	0.08/**0.54**	0.12/0.16	–		
TNF-α	**0.72**/0.22	**0.50**/**0.46**	0.45/0.001	0.11/**0.50**	–	
Hs-CRP	0.28/0.23	0.45/**0.52**	0.41/0.06	0.20/**0.39**	0.18/0.26	–
HOMA-IR	0.34/0.09	**0.59**/0.19	**0.57**/0.07	0.32/0.23	**0.58**/0.13	0.29/0.17

Numbers in bold denote a level of significance of $P < .05$.

respectively; Table 3). In the CxHF but not in the nCxHF group, the HOMA-IR was significantly correlated with NE ($r = 0.59$, $P < .01$), ZAG ($r = 0.57$, $P = .01$), and TNF-α ($r = 0.58$, $P = .01$) (Table 3).

4. Discussion

Our results show for the first time that serum levels of ZAG, a novel adipokine with a lipid mobilizing effect, are increased similarly in advanced heart failure patients with or without cachexia compared with CTR patients. We have also shown in the CxHF but not in the nCxHF group a close association between ZAG and FFA levels, raising the possibility that ZAG might be involved in active lipolysis, expressed by higher plasma FFA. An even closer positive linear relationship was also found between ZAG and NE but not with EPI levels, as well as between ZAG and HOMA-IR, once again only in the CxHF group.

Moreover, ZAG levels in CxHF but not in nCxHF were also positively associated with inflammatory parameters, in particular TNF-α ($r = 0.45$, $P = .06$), although not significantly. We clearly recognize that significant correlations among these parameters do not imply direct causal relationships indicating that ZAG acts as a promoter of increased sympathetic activation, of insulin resistance, and of inflammation; but it could indicate that all these factors are strictly interacting to determine the marked activation of lipolysis that we observed in CxHF.

Heart failure is a clinical condition characterized by a reduced insulin sensitivity [24-26], as confirmed by the higher HOMA-IR levels in the nCxHF and CxHF groups than in CTR. It has already been reported in the literature that the increased inflammatory activation is associated with higher adrenergic activation and insulin resistance in both the nCxHF and CxHF patient populations [8,27]. Observations from in vitro studies demonstrated that long-lasting exposition of adipose cells to TNF-α induces the development of insulin resistance and white adipose catabolism with marked activation of lipolysis [28,29]. The close direct relationship between both TNF-α and NE with HOMA-IR in our CxHF group of patients suggests a negative modulatory action of these inflammatory and noradrenergic effectors on insulin sensitivity.

In the CxHF group, we also found a direct and close association between ZAG and HOMA-IR; such a positive association in cardiac cachexia is not in accordance with what was previously reported in a healthy population and in obese patients where a null [13] or a negative correlation [14,30] between these 2 parameters were found.

Insulin resistance represents a physiopathological pattern with a marked reduction of antilipolytic mechanisms; this situation of reduced insulin sensitivity coupled with higher sympathetic activation could be of major importance in the imbalance favoring lipolysis activation in the CxHF group of patients. Our results show an involvement of ZAG in the complex lipolysis mechanisms by the strict interplay between sympathetic activation, insulin resistance, and inflammation processes in CxHF, even if its exact role could not be determined on the basis of the present results. Notwithstanding, ZAG lipolytic effects on human adipocytes have never been demonstrated in in vivo situations. In a recent article, Mracek et al reported for the first time in an ex vivo situation (human preadipocytes culture from Simpson-Golabi-Behmel syndrome, a model for studying human fat cell metabolism) a direct lipolytic effect of physiologically relevant concentrations of recombinant ZAG protein [17].

No correlations between both natriuretic peptides and ZAG levels were observed in heart failure patients. A positive correlation was instead found between plasma natriuretic peptides (ANP and BNP) and circulating FFA, and NE and TNF-α in the nCxHF group and not in the CxHF group. Given that the 2 groups of nCxHF and CxHF had a similar class of heart failure, the contribution of natriuretic peptides on lipolysis in our CxHF group seems attenuated, whereas other prolipolytic mechanisms are prevailing.

Moreover, our patient population had reduced renal function, as shown by a reduced estimated GFR when compared with CTR; therefore, renal dysfunction might have accounted for the ZAG levels found in the heart failure and CTR groups. Higher serum ZAG levels have been reported in hemodialysis patients when compared with healthy subjects, suggesting that reduced renal clearance could affect ZAG elimination and increase its levels [30]. In our heart failure groups of patients, this might not be the case because, in both nCxHF and CxHF, no inverse correlations between ZAG levels and estimated GFR were observed.

Some limitations in the present study should be acknowledged. First, our observations derive from a reduced number of patients, in particular in the CxHF group; second, some conclusions are based on association studies that clearly do not imply a direct causal relationship; third, a surrogate static marker of insulin resistance such as HOMA-IR was used despite the dynamic, more accurate, but less suitable clamp technique–derived index. Despite these weaknesses, this is the first study reporting data on ZAG levels in advanced heart failure with or without cardiac cachexia and its relationships with other neurohormonal and inflammatory parameters. All these aspects may help clarify the mechanisms involved in lipolysis activation and fat loss in CxHF patients; this is rather pertinent because in patients developing cachexia (including cardiac cachexia), the reduction of fat mass represents a clinical element more closely related to mortality than the loss of lean tissue mass [31].

In summary, our study indicates that circulating adipokine ZAG levels are similarly greater in CxHF and in nCxHF patients as compared with those found in the CTR patients. Only in CxHF, ZAG levels are directly correlated to circulating levels of FFA and NE, suggesting an involvement of ZAG in sympathetic-mediated lipolysis.

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Conflict of Interest

The authors have nothing to disclose.

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