



**HAL**  
open science

## **In patients with anorexia nervosa, myokine levels are altered but are not associated with bone mineral density loss and bone turnover alteration**

Laurent Maïmoun, Denis Mariano-Goulart, Helena Huguet, Eric Renard, Patrick Lefebvre, Marie-Christine Picot, Anne-Marie Dupuy, Jean-Paul Cristol, Philippe Courtet, Vincent Boudousq, et al.

### ► **To cite this version:**

Laurent Maïmoun, Denis Mariano-Goulart, Helena Huguet, Eric Renard, Patrick Lefebvre, et al.. In patients with anorexia nervosa, myokine levels are altered but are not associated with bone mineral density loss and bone turnover alteration. *Endocrine Connections*, 2022, 11 (5), pp.e210488. 10.1530/EC-21-0488 . hal-03669685

**HAL Id: hal-03669685**

**<https://hal.science/hal-03669685>**

Submitted on 16 May 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# In patients with anorexia nervosa, myokine levels are altered but are not associated with bone mineral density loss and bone turnover alteration

Laurent Maïmoun<sup>1,2</sup>, Denis Mariano-Goulart<sup>1,2</sup>, Helena Huguet<sup>3</sup>, Eric Renard<sup>4,5,6</sup>, Patrick Lefebvre<sup>4</sup>, Marie-Christine Picot<sup>3,5</sup>, Anne-Marie Dupuy<sup>7</sup>, Jean-Paul Cristol<sup>2,7</sup>, Philippe Courtet<sup>8,9</sup>, Vincent Boudousq<sup>10</sup>, Antoine Avignon<sup>11</sup>, Sébastien Guillaume<sup>8,9</sup> and Ariane Sultan<sup>2,11</sup>

<sup>1</sup>Département de Médecine Nucléaire, Hôpital Lapeyronie, Centre Hospitalier Régional Universitaire (CHU) Montpellier, Montpellier, France

<sup>2</sup>PhyMedExp, Université de Montpellier, INSERM, CNRS, Montpellier, France

<sup>3</sup>Unité de Recherche Clinique et Epidémiologie, Hôpital Lapeyronie, CHU Montpellier, Montpellier, France

<sup>4</sup>Département d'Endocrinologie, Diabète, Nutrition, Hôpital Lapeyronie, CHRU Montpellier, Montpellier, France

<sup>5</sup>CIC INSERM 1411, Hôpital Gui de Chauliac, CHU Montpellier, Montpellier Cedex 5, France

<sup>6</sup>Institut de Génomique Fonctionnelle, CNRS UMR 5203/INSERM U661/Université Montpellier, Montpellier, France

<sup>7</sup>Département de Biochimie, Hôpital Lapeyronie, CHU Montpellier, Montpellier, France

<sup>8</sup>Institut de Génomique Fonctionnelle, CNRS, INSERM Université Montpellier, Montpellier, France

<sup>9</sup>Département d'Urgence et Post-Urgence Psychiatrique, Hôpital Lapeyronie, CHU Montpellier, Montpellier, France

<sup>10</sup>Département de Médecine Nucléaire, Hôpital Carémeau, CHU Nîmes, Nîmes, France

<sup>11</sup>Département Endocrinologie, Nutrition, Diabète, Equipe Nutrition, Diabète, CHU Montpellier, Montpellier, France

Correspondence should be addressed to L Maïmoun: [l-maimoun@chu-montpellier.fr](mailto:l-maimoun@chu-montpellier.fr)

## Abstract

**Objectives:** The two-fold aim of this study was: (i) to determine the effects of undernutrition on the myokines in patients with restrictive anorexia nervosa (AN) and (ii) to examine the potential link between myokines and bone parameters.

**Methods:** In this study, 42 young women with restrictive AN and 42 age-matched controls (CON) (mean age, 18.5 ± 4.2 years and 18.6 ± 4.2 years, respectively) were enrolled. aBMD and body composition were determined with DXA. Resting energy expenditure (REEm), a marker of energy status, was indirectly assessed by calorimetry. Bone turnover markers and myokines (follistatin, myostatin and irisin) were concomitantly evaluated.

**Results:** AN patients presented low aBMD at all bone sites. REEm, bone formation markers, myostatin and IGF-1 were significantly lower, whereas the bone resorption marker and follistatin were higher in AN compared with controls. No difference was observed between groups for irisin levels. When the whole population was studied, among myokines, only myostatin was positively correlated with aBMD at all bone sites. However, multiple regression analyses showed that in the AN group, the independent variables for aBMD were principally amenorrhoea duration, lean tissue mass (LTM) and procollagen type I N-terminal propeptide (PINP). For CON, the independent variables for aBMD were principally LTM, age and PINP. Whatever the group analysed, none of the myokines appeared as explicative independent variables of aBMD.

**Conclusion:** This study demonstrated that despite the altered myokine levels in patients with AN, their direct effect on aBMD loss and bone turnover alteration seems limited in comparison with other well-known disease-related factors such as oestrogen deprivation.

## Key Words

- ▶ anorexia nervosa
- ▶ myokines
- ▶ bone loss
- ▶ irisin
- ▶ myostatin
- ▶ follistatin

## Introduction

It has been well documented that patients with anorexia nervosa (AN) present lower areal bone mineral density (aBMD) and altered bone remodelling compared with normal-weight women (1, 2, 3, 4, 5, 6). The alteration in these bone characteristics may explain the increased fracture risk observed in this population (7). Various endocrine and metabolic disturbances, including hypothyroidism (8), hypogonadism (1, 9, 10), hypercortisolism (2, 11) and IGF-1 deficiency (1, 12, 13), among others, as well as undernutrition effects due to calcium and vitamin D deficiency (12), might be implicated in the loss of bone mass. In parallel, a synchronization of the losses in both muscle and bone mass is generally observed in these patients (10). Moreover, aBMD appears to be better correlated with muscle mass measured as lean tissue mass (LTM) than with fat mass (FM), and of these two anthropometric factors, only LTM is independently linked to aBMD (10). In addition, LTM appears to be a better predictor of bone loss than a decrease in the BMI or FM (14). In other physiological conditions like immobilization, reduced physical activity or aging, the loss of skeletal muscle mass and function – which defines sarcopenia – is also often accompanied by a decrease in bone mass and microarchitecture deterioration – which defines osteoporosis (15, 16, 17). In this context, how do we explain the connection between the deterioration in skeletal muscle and the deterioration in bone tissue? Emerging evidence suggests that this concomitant deterioration is driven, at least in part, by bone and muscle crosstalk. The skeleton and skeletal muscle are closely linked anatomically and mechanically (18, 19). In addition, biochemical and metabolic connections between the two tissues have more recently been demonstrated (20). Thus, skeletal muscle can be classified as an endocrine organ that regulates target organs, including pancreas, liver and adipose tissue (21), through the synthesis and release of various cytokines or peptides in response to muscular contraction or various stimuli (22). These muscle-secreting factors are called ‘myokines’ and they also biochemically affect bone metabolism in both endocrine and paracrine manners (23, 24, 25). Among the several myokines identified, myostatin acts as an inhibitor of muscle growth and bone metabolism (26, 27), and the inhibition of the myostatin pathway induces massive muscle hypertrophy and an increase in bone turnover leads to an increase in bone mass (26, 28). In postmenopausal women and boys with Duchenne muscular dystrophy, the administration of ACE-031, a fusion protein that binds avidly to myostatin

and inhibits its biological effects by preventing signalling through the endogenous receptor, was found to decrease fat mass and increase total lean body mass and lumbar spine aBMD (29, 30). Follistatin is a myostatin-binding glycoprotein that antagonizes the myostatin-induced inhibition of myogenesis and consequently enhances skeletal muscle hypertrophy (31), increases mineralization and stimulates osteoblastogenesis (32). Irisin is produced primarily by muscles and is released into the circulation during physical activity and muscle shivering (33). Its production increases energy expenditure and oxidative metabolism, and it improves glucose metabolism (34). Studies on animal models have shown that irisin can also improve osteoblastogenesis and bone mass (35, 36). The data in humans are discrepant, however, because a positive association between irisin levels and aBMD was reported in soccer players and older adult patients (37, 38), whereas no association was reported in postmenopausal women (39).

Although myokines may be implicated in the variation of body composition components (i.e. muscle and bone) and physiological functions (i.e. thermogenesis) in patients with AN, the irisin, follistatin and myostatin profiles remain poorly evaluated in this disease (40, 41, 42). Yet, more in-depth study might well open new and fruitful fields of investigation.

The two-fold aim of this case-control study was: (i) to determine the effects of undernutrition on myokine levels in patients with restrictive AN compared to normal-weight subjects and (ii) to examine the potential link between myokines and bone parameters.

## Subjects and method

This study followed a case-control design. Study approval was obtained from the Regional Research Ethics Committee (Comité de Protection des Personnes Sud-Mediterranee IV, Montpellier, France; reference: 11 02 03), and permission for the clinical trial was granted by the French Medicines and Healthcare Products Regulatory Agency (Agence Française de Sécurité Sanitaire des Produits de Santé; AFSSAPS). Informed consent was obtained from all subjects, as well as from the parents of subjects <18 years old.

### Subjects

Eighty-four adolescents and young women with ages ranging from 14.5 to 33.5 years (mean, 18.5 ± 4.2) were enrolled in this study. All were Caucasian. Forty-two of them had been diagnosed with AN. Patients were recruited from

the Endocrinology Department at Montpellier University Hospital Centre in France. They fulfilled the criteria for the diagnosis of restrictive AN as defined by the DSM-5, that is restriction of energy intake relative to requirements leading to a significantly low BMI fear of gaining weight and alteration in body size perception (43). Only patients with the pure restrictive form of AN and with BMI <18 kg/m<sup>2</sup> were included in this study to limit potential bias due to a mix of the eating/purging and pure restrictive AN types. The control group (CON) was recruited from advertisements in local newspapers and from the staff and students in the Departments of Nuclear Medicine, Endocrinology and Psychiatry of Lapeyronie Hospital, Montpellier University Hospital Centre. The group composed of 42 non-obese healthy adolescents and young women with 18 < BMI < 30 kg/m<sup>2</sup> and no history of eating disorders or other psychiatric illness (as determined by the SCOFF questionnaire (44) and the Mini International Neuropsychiatric Interview (45)). All CON presented normal menstrual cycles and performed only leisure physical activities. Neither control subjects nor anorexic patients presented with primary amenorrhoea or were taking any medications that were known to affect bone metabolism, particularly prolonged glucocorticoid use (>3 months) or oestrogen, vitamin K, vitamin D, calcium, bisphosphonate, selective oestrogen receptor modulator or teriparatide use. None used contraceptives. CON and patients with AN were age-matched ( $\pm$ 6 months).

## Methods

### Anthropometric measurements

Standing height was measured with a stadiometer to the nearest 0.1 cm. Weight was determined using a weight scale with a precision of 0.1 kg. BMI was calculated as weight (kg) divided by the square of height (m). Height standard deviation score (height SDS) and weight standard deviation score (weight SDS) were calculated according to the French standard curves.

### Medical and menstrual histories

Each subject responded to a medical questionnaire designed to assess the general medical and menstrual (age of menarche, menstrual function) and disease histories (age of AN onset, duration of AN, weight variations).

### Assays

Fasting blood samples (25 mL) were collected in the morning (08:30–09:30 h) in chilled sterile tubes by standard

venipuncture technique. The samples were allowed to clot at room temperature and were then centrifuged at 1509 g for 10 min at 4°C. Plasma samples were stored at –80°C until analysis. All samples were run in duplicate and, to reduce inter-assay variation, the plasma samples were analysed in a single session. The date of the last menses was not recorded for CON, and hormonal values were thus obtained at an unsynchronized menstrual stage. Serum osteocalcin, procollagen type I N-terminal propeptide (PINP), type I-C telopeptide breakdown products (CTX), insulin-like growth factor (IGF-1), myostatin, follistatin and irisin were evaluated.

OC, PINP, CTX and insulin were assayed by Cobas 6000 (Roche Diagnostic). The inter- and intra-assay coefficients of variation (CVs) for the three latter parameters were lower than 7%.

The intra- and inter-assay CVs for IGF-1 (Immuline 2000<sup>®</sup> IGF-1, Siemens Healthcare Diagnostics) were lower than 6%.

The determination of myokines was performed by ELISA assays. Follistatin and myostatin were measured with R&Dsystems kits. The intra-assay CV was <2.7% and <5% and inter-assay CV was from 7 to 9% and <6% for follistatin and myostatin, respectively. Lower limit of detection (manufacturer's data) was <29 and 2.25 pg/mL, respectively. Irisin was measured with Biovendor kits (Karasek, Czech Republic) with intra- and inter-assay CVs of <8 and <10%, respectively. Lower limit of detection (manufacturer's data) was <1 µg/mL.

For all biological parameters analysed in this study, the CVs for the intra- and inter-assay variations were given by the manufacturer.

### Areal bone mineral density, body fat and fat-free soft tissues

DXA (Hologic QDR-4500A, Hologic, Inc., Waltham, MA) measured the areal bone mineral density (aBMD; g/cm<sup>2</sup>) of the whole body and at specific bone sites: the anteroposterior lumbar spine (L1–L4), the dominant arm radius, hip and femoral neck (FN). The soft tissue body composition (fat mass (FM, kg), percentage of body fat mass (% FM) and LTM (kg)) was derived from the whole-body scan. All scanning and analyses were performed by the same operator to ensure consistency, after following standard quality control procedures. Quality control for DXA was checked daily by scanning a lumbar spine phantom consisting of calcium hydroxyapatite embedded in a cube of thermoplastic resin (DPA/QDR-1; Hologic x-caliber anthropometric spine phantom). The CVs given

by the manufacturer were 0.8% for spine and radius, 1.1% at the hip and <1% for LTM and FM.

### Resting energy expenditure measurements

Measured resting energy expenditure (REEm) was assessed in the patients with AN over a period of at least 30 min by indirect calorimetry (Quark RMR, Cosmed, Rome, Italy) after an overnight fast. Predicted REE values (%; REEp) were calculated for AN and CON from the equation of Harris and Benedict modified by Roza and Shizgal (46) as follows:  $REEp = 667.051 + 9.74 \times (\text{weight}) + 1.729 \times (\text{height}) - 4.737 \times (\text{age})$ .

### Statistical analysis

The characteristics of the patients are described with proportions for categorical variables and with mean  $\pm$  s.d. for quantitative variables. Comparisons between lean controls and patients with AN were performed using Student's *t*-test for quantitative variables or the Mann-Whitney *U* test when the distribution of variables was non-Gaussian. For qualitative variables, the chi-square test or Fisher's exact test was used. To determine which parameters differed most between lean controls and patients with AN, Cohen's *d* was calculated (difference between the means of the two groups divided by a pooled standard deviation) for each parameter, which is a standardized measure of the effect of a factor (here AN) independent of the scale of the parameter under consideration (47). As an indication, a Cohen's *d* of 0.2 corresponds to a small effect of the factor, 0.5 to a medium effect, 0.8 to a large effect, 1.2 to a very large effect and 2 to a huge effect (48).

Correlations between the anthropometric, gynaecological, aBMD, disease-related parameters or energy expenditure and myokines were analysed by Pearson or Spearman coefficient.

To determine the independent factors that could influence aBMD in the whole population, in patients with AN and in CON, multivariate linear regressions were performed. All clinically relevant variables were included in the model. The best method to select variables was determined using leave-one-out cross-validation (LOO). Finally, a stepwise selection based on the Bayesian Information Criterion (BIC) was applied. No collinearity between variables was detected with variance inflation factors. The normality of residues in the final model was tested using the Shapiro-Wilk test.

The statistical significance was set at 0.05 and analyses were performed using software SAS Enterprise Guide, version 7.13 (SAS Institute, Cary, NC, USA).

### Results

The anthropometric characteristics and gynaecological data of the 42 patients with AN and the 42 CON are summarized in Table 1. The age distribution ranged from 14.5 to 33.5 years and was comparable between the two groups. In patients, the mean age of the disease onset was  $16.1 \pm 2.3$  years (range: 12.5 to 26.2 years) and the mean duration was  $2.5 \pm 3.1$  years (range: 0.5 to 16.9 years). There were no

**Table 1** Clinical characteristics of adolescent and young adult women with anorexia nervosa and controls.

Parameters	Controls	Patients with AN	P-value
Number of subjects	<i>n</i> = 42	<i>n</i> = 42	
Age, years	18.5 $\pm$ 4.2	18.6 $\pm$ 4.2	0.78
Anthropometric data			
Weight, kg	58.8 $\pm$ 8.2	43.8 $\pm$ 6.0	<b>&lt;0.01</b>
Weight, SDS	0.9 $\pm$ 1.3	-1.5 $\pm$ 1.0	<b>&lt;0.01</b>
Height, cm	164.1 $\pm$ 6.2	165.2 $\pm$ 6.7	0.42
Height, SDS	0.3 $\pm$ 1.1	0.5 $\pm$ 1.2	0.34
BMI, kg.m <sup>-2</sup>	21.8 $\pm$ 2.5	16.0 $\pm$ 1.7	<b>&lt;0.01</b>
Lowest BMI, kg.m <sup>-2</sup>	21.4 $\pm$ 1.8	15.0 $\pm$ 1.9	<b>&lt;0.01</b>
WB fat mass, %	27.1 $\pm$ 6.1	15.7 $\pm$ 5.0	<b>&lt;0.01</b>
WB fat mass, kg	16.0 $\pm$ 6.0	7.7 $\pm$ 4.7	<b>&lt;0.01</b>
WB fat-free soft tissue, kg	40.5 $\pm$ 4.0	35.6 $\pm$ 4.7	<b>&lt;0.001</b>
REEm, kcal/day	-	1086.5 $\pm$ 195.5	-
REEp, kcal/day	1435.6 $\pm$ 86.2	1291.6 $\pm$ 72.9	<b>&lt;0.001</b>
Predicted REE values, %	-	-17.9 $\pm$ 12.5	-
Characteristics of the pathology			
Age of AN onset, years	-	16.1 $\pm$ 2.3	-
Duration of AN, years	-	2.5 $\pm$ 3.1	-
Gynaecological data			
Age of menarche, years	12.7 $\pm$ 1.6	12.8 $\pm$ 1.1	0.86
Menstrual disorders, <i>n</i> (%)	12 (28.6)	37 (88.1)	<b>&lt;0.001</b>
Duration of amenorrhoea, months	-	20.1 $\pm$ 34.3	-

Values are presented as mean  $\pm$  s.d.

REEm, resting energy expenditure measured by calorimetry; REEp, resting energy expenditure predicted from the equation of Harris and Benedict modified by Roza and Shizgal (41); SDS, standard deviation score; WB, whole body. Controls presented only minor alterations in the duration of menstrual cycles (~28 days).

Bold indicates statistical significance between groups.

**Table 2** Areal bone mineral density at various bone sites and biological variables in patients with anorexia nervosa and controls.

Parameters	Controls	Patients with AN	Cohen's d <sup>a</sup>	P-value
Areal bone mineral density (g cm <sup>-2</sup> )				
Lumbar spine	0.974 ± 0.122	0.850 ± 0.127	-0.892	<0.01
Lumbar spine Z-score (s.d.)	-0.133 ± 1.163	-1.238 ± 1.243	-0.838	<0.01
Hip	0.943 ± 0.121	0.825 ± 0.139	-0.831	<0.01
Hip Z-score (s.d.)	0.068 ± 0.997	-0.969 ± 1.140	-0.873	<0.01
Femoral neck	0.861 ± 0.112	0.765 ± 0.132	-0.733	<0.01
Radius	0.540 ± 0.035	0.517 ± 0.049	-0.535	<b>0.02</b>
Biological parameters				
CTX, ng/mL	0.65 ± 0.26	0.96 ± 0.45	0.775	<0.01
PINP, ng/mL	129.8 ± 115.0	63.8 ± 30.3	-0.734	<0.01
OC, ng/mL	42.3 ± 21.6	24.3 ± 10.7	-0.937	<0.01
IGF-1, ng/mL	333.3 ± 91.4	209.8 ± 155.1	-0.975	<0.01
Irisin, µg/mL	19.5 ± 12.9	21.0 ± 16.7	0.101	0.96
Myostatin, pg/mL	1494.5 ± 510.3	920.4 ± 397.4	-1.066	<0.01
Follistatin, ng/mL	1537.7 ± 1356.7	2159.3 ± 1541.7	0.421	<b>0.02</b>

Values are presented as mean ± s.d.

<sup>a</sup>(mean AN–mean CONT)/s.d.<sub>pooled</sub>

CTX, type I-C telopeptide breakdown products; IGF-1, insulin-like growth factor-1; OC, osteocalcin; PINP, procollagen type I N-terminal propeptide; Z-score, the number of s.d. above or below the mean for the patient's age, sex and ethnicity.

Bold indicates statistical significance between groups.

significant differences between the two groups with regard to height, whereas, as expected due to undernutrition, weight, BMI, lowest BMI, body LTM and body FM (kg and %) were significantly lower in AN compared with CON ( $P < 0.01$ ). Moreover, when weight SDS and height SDS were calculated according to the French standard curves, AN again presented low values for weight ( $-1.5 \pm 1.0$  s.d.) and normal values for height ( $0.5 \pm 1.0$  s.d.).

The values of REEm ( $1086.5 \pm 195.5$  kcal/day) indicated hypometabolism in AN. REEp was significantly lower ( $P < 0.001$ ) in AN patients compared with CON. A mean significant difference of 205.1 kcal/day was observed between REEm and REEp in AN, which corresponded to an average variation of 17.9%.

Concerning the gynaecological profile (Table 1), the age of menarche was not different between groups (12.7 ± 1.6 years for CON vs 12.8 ± 1.1 years for AN). Menstrual disorders were more frequent in patients with AN than in CON (88.1% vs 28.6%,  $P < 0.001$ ). In patients, the mean duration of amenorrhoea was 20.1 ± 34.3 months. Controls with menstrual disorders had only minor variations in cycle duration (~28 days), but no cases of secondary amenorrhoea were encountered in this group. None of the patients or controls presented primary amenorrhoea.

## Bone characteristics

### Areal bone mineral density

Table 2 presents the aBMD for the two groups at various bone sites. Compared with CON, patients with AN

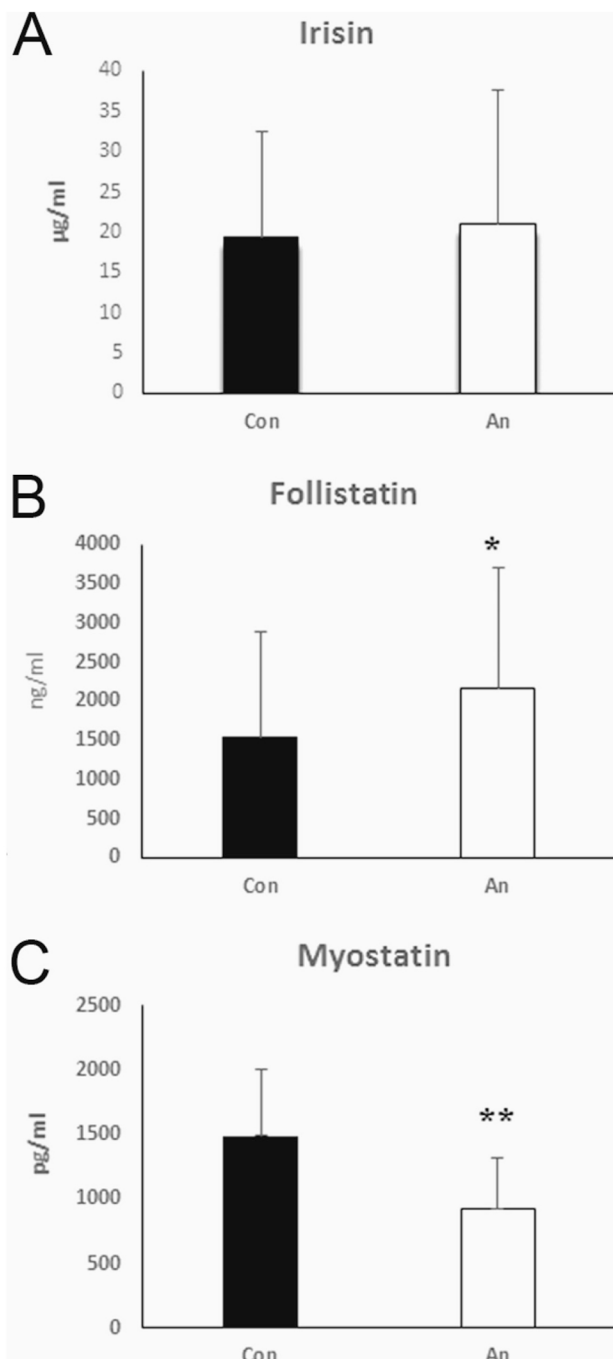
presented significantly lower values at the femoral neck ( $P < 0.01$ ), hip ( $P < 0.01$ ), lumbar spine ( $P < 0.01$ ) and radius ( $P = 0.02$ ), but the difference was greater at the lumbar spine (Cohen's  $d = -0.892$ ), hip (Cohen's  $d = -0.831$ ) and femoral neck (Cohen's  $d = -0.733$ ), compared with radius (Cohen's  $d = -0.535$ ). Concomitantly, the Z-score for aBMD was also lower in patients with AN at all bone sites compared with the reference data, ranging from  $-0.97$  to  $-1.24$  s.d. Controls group presented normal Z-score values.

### Biological parameters

Markers of bone turnover are described in Table 2. Markers of bone formation (OC and PINP) were significantly lower in patients with AN ( $P < 0.01$ ), whereas the marker of bone resorption (CTX) was significantly higher than in CON ( $P < 0.01$ ). Cohen's  $d$  values showed that markers of bone formation and bone resorption were affected with the same magnitude (Cohen's  $d$  ranging from 0.734 to 0.937).

Regarding myokines (Fig. 1 and Table 2), follistatin values were higher ( $P = 0.02$ ) and myostatin levels were lower ( $P < 0.01$ ) in patients with AN compared with controls. A large effect of AN was observed for myostatin (Cohen's  $d = -1.066$ ) and a small-medium effect for follistatin (Cohen's  $d = 0.421$ ). No significant difference was observed between groups for irisin levels ( $P = 0.96$ ; Cohen's  $d = 0.101$ ).

IGF-1 levels were significantly lower ( $P < 0.01$ , Cohen's  $d = -0.975$ ) in patients compared with controls.



**Figure 1**  
Myokine levels in controls (CON) and in patients with anorexia nervosa (An). (A) Irisin, (B) follistatin and (C) myostatin. Data are presented as mean  $\pm$  s.d.. \*significant difference between CON and patients with AN for  $P < 0.05$  and \*\* $P < 0.01$ .

### Correlations between the anthropometric, gynaecological, aBMD, disease-related parameters or energy expenditure and myokines

Table 3 summarizes the correlation coefficients between the anthropometric, gynaecological, aBMD, bone marker,

IGF-1, disease-related parameters or resting energy expenditure and the myokine levels. When the whole population was studied, myostatin was negatively correlated only with CTX and positively correlated with BMI, lowest BMI, FM, aBMD at all bone sites, markers of bone formation (OC and PINP), IGF-1 and REEp.

When subgroup analyses were performed, in patients with AN, irisin was negatively correlated with weight, BMI and FM, while follistatin was negatively correlated with CTX. Myostatin was positively correlated with BMI, FM, IGF-1 and REEm and negatively with CTX. No correlation was found between myokines and disease-related parameters including the lowest BMI, duration of AN and duration of amenorrhoea.

In CON, follistatin was positively correlated with hip aBMD and myostatin was positively correlated with IGF-1.

### Multiple regression analysis

Multiple regression analyses were performed to determine the independent factors that could influence aBMD in the whole population, in patients with AN and in CON (Table 4). Our final models explained between 13.2 and 61% of the aBMD variance, depending on the bone site analysed. In the AN group, the independent variables for lumbar spine, hip and radius aBMD were principally amenorrhoea duration, LTM and PINP. For CON, the independent variables for aBMD were principally LTM, age and PINP. Whatever the group analysed (whole population, patients with AN or CON), none of the myokines appeared as explicative independent variables of aBMD.

### Discussion

In this study, we investigated the effects of AN on myokine levels and their potential interrelationships with bone metabolism and body composition in young women around the peak bone mass period. AN is well known to be associated with noticeable alterations in aBMD, bone remodelling and body composition, and we found that these patients also presented a specific myokine profile that tended to preserve muscle and bone mass.

This cross-sectional study confirmed that patients with AN present lower aBMD values and an alteration in bone remodelling characterized by an increase in bone resorption and a decrease in bone formation compared with young normal-weight women (1, 2, 3, 4, 5, 6). The study was designed to gain a deeper understanding of the biological modifications that act on aBMD in this population of

**Table 3** Correlations of body composition, areal bone mineral density (aBMD), biological parameters, resting energy expenditure and disease-related parameters with myokines in all subjects (All), in controls and in patients with anorexia nervosa.

Parameters	All			Controls			Patients with anorexia nervosa		
	Irisin	Follistatin	Myostatin	Irisin	Follistatin	Myostatin	Irisin	Follistatin	Myostatin
Body composition									
Weight, kg	−0.137	−0.100	<b>0.373<sup>a</sup></b>	−0.102	0.113	−0.067	<b>−0.334<sup>b</sup></b>	0.230	0.085
BMI, kg/m <sup>2</sup>	−0.132	−0.099	<b>0.462<sup>a</sup></b>	−0.171	0.231	−0.213	<b>−0.325<sup>b</sup></b>	0.253	<b>0.328<sup>b</sup></b>
Lowest BMI, kg/m <sup>2</sup>	<b>−0.295<sup>b</sup></b>	0.018	<b>0.420<sup>c</sup></b>	−0.100	0.300	0.149	−0.271	0.248	0.275
FM, kg	−0.182	−0.108	<b>0.400<sup>a</sup></b>	<b>−0.312<sup>b</sup></b>	0.040	−0.200	<b>−0.402<sup>c</sup></b>	0.163	<b>0.337<sup>b</sup></b>
LTM, kg	0.000	−0.002	<b>0.209<sup>b</sup></b>	0.175	0.239	0.043	−0.224	0.106	−0.041
aBMD (g/cm <sup>2</sup> )									
Lumbar spine	0.017	−0.033	<b>0.408<sup>a</sup></b>	0.142	0.118	0.151	−0.039	0.081	0.131
Lumbar spine Z-score	0.055	0.016	<b>0.417<sup>a</sup></b>	0.148	0.183	0.197	0.001	0.063	0.179
Hip	0.004	0.052	<b>0.358<sup>a</sup></b>	0.009	<b>0.345<sup>b</sup></b>	0.174	0.005	−0.027	0.137
Hip Z-score	−0.009	0.061	<b>0.422<sup>a</sup></b>	−0.045	<b>0.352<sup>b</sup></b>	0.259	0.018	0.003	0.260
Femoral neck	−0.024	−0.011	<b>0.310<sup>c</sup></b>	−0.048	0.194	0.217	−0.041	0.013	0.040
Radius	0.057	−0.007	<b>0.220<sup>b</sup></b>	0.146	0.156	−0.007	−0.025	−0.057	0.118
Biological parameters									
CTX, ng/mL	0.045	−0.014	<b>−0.330<sup>c</sup></b>	0.053	0.089	−0.004	0.108	<b>−0.323<sup>b</sup></b>	<b>−0.388<sup>b</sup></b>
PINP, ng/mL	−0.057	−0.106	<b>0.346<sup>c</sup></b>	−0.127	0.032	0.139	−0.013	0.010	0.252
OC, ng/mL	−0.049	−0.142	<b>0.351<sup>c</sup></b>	0.014	0.055	−0.043	−0.081	−0.053	0.255
IGF-1, ng/mL	0.023	0.018	<b>0.487<sup>a</sup></b>	−0.098	0.090	<b>0.334<sup>b</sup></b>	0.047	0.133	<b>0.652<sup>c</sup></b>
Energy metabolism									
REEm, kcal/day	−	−	−	−	−	−	0.190	−0.082	<b>0.433<sup>c</sup></b>
REEp, kcal/day	−0.143	−0.090	<b>0.355<sup>a</sup></b>	−0.147	0.151	0.033	−0.282	0.156	0.039
Disease-related parameters									
AN duration, years	−	−	−	−	−	−	−0.105	0.132	−0.257
Amenorrhoea duration, months	−	−	−	−	−	−	0.084	−0.049	−0.216

Data are presented as *r* (Pearson or Spearman coefficient correlation) and significant correlations are denoted by <sup>a</sup>*P* < 0.001, <sup>b</sup>*P* < 0.05 and <sup>c</sup>*P* < 0.01. aBMD, areal bone mineral density; AN, anorexia nervosa; CTX, type I-C telopeptide breakdown products; FM, fat mass; IGF-1, insulin-like growth factor-1; LTM, lean tissue mass; OC, osteocalcin; PINP, procollagen type I N-terminal propeptide; REEm, measured resting energy expenditure; REEp, predicted resting energy expenditure calculated according to the Harris Benedict equation modified by Roza and Shizgal (41); Z-score, the number of standard deviations (s.d.) above or below the mean for the patient's age, sex and ethnicity. Bold indicates statistical significance between groups.

patients, and it especially sought to determine the effects of undernutrition on myokine levels and to examine the potential link between myokines and bone parameters. Our results showed that the patients with AN presented a specific myokine profile characterized by comparable irisin levels, lower myostatin levels and higher follistatin levels compared with controls. Our data are original in that few studies have reported the effect of undernutrition on the myokine levels in AN patients (40, 41, 42) and none has concomitantly evaluated such a large panel of myokines with various and antagonist physiological functions.

Concerning irisin levels, we found comparable irisin values in the AN and CON groups. Our results are in accordance with those of Stengel *et al.* (41), who reported a non-significant reduction (−14%) in irisin levels in a limited number of patients with AN (*n* = 8) compared with a normal-weight group unmatched for age and gender.

Further, Hofmann *et al.* (40) reported no difference in irisin levels in a small number (*n* = 39) of young women across AN subtype groups (i.e. purging, restrictive and atypic), but in the absence of a normal-weight group, no conclusion on the specific effect of AN on irisin levels could be deduced. In this same study, the levels of physical activity did not modify the irisin levels and no correlation was observed between irisin levels and various parameters of energy expenditure (40). Similarly, in our study, the irisin level was not associated with REEm, REEp or LTM in patients with AN. In an experimental study on rats, Lee *et al.* (49) reported that, despite a substantial reduction in muscle mass induced by a 2-week starvation, no variation in the irisin levels was observed. In humans, another report suggested that there is no tight association between sarcopenia and the circulating irisin level (50). Our and previous results seem somewhat the opposite of what we expected. Indeed, irisin is known to



**Table 4** Univariate and multivariate regression analysis of factors influencing aBMD at various bone sites in the whole population, the controls and the patients with AN.

Parameters	All			Controls			Patients with anorexia nervosa		
	Lumbar spine	Hip	radius	Lumbar spine	Hip	radius	Lumbar spine	Hip	radius
Univariate Analysis									
Age	-0.45 (±0.37) <sup>b</sup>	-0.94 (±0.36) <sup>a</sup>	0.01 (±0.12)	0.21 (±0.46)	-0.20 (±0.45)	0.003 (±0.130)	-1.10 (±0.45) <sup>b</sup>	-1.70 (±0.50) <sup>b</sup>	0.03 (±0.19)
Weight, kg	0.75 (±0.12) <sup>b</sup>	0.64 (±0.13) <sup>b</sup>	0.15 (±0.04) <sup>c</sup>	0.57 (±0.22) <sup>a</sup>	0.25 (±0.23)	0.12 (±0.06)	0.81 (±0.31) <sup>a</sup>	0.90 (±0.30) <sup>c</sup>	0.17 (±0.13)
Weight, SDS	4.58 (±0.77) <sup>b</sup>	3.99 (±0.84) <sup>b</sup>	0.85 (±0.28) <sup>c</sup>	3.43 (±1.41) <sup>a</sup>	1.61 (±1.48)	0.72 (±0.41)	4.57 (±1.81) <sup>a</sup>	5.00 (±2.00) <sup>a</sup>	0.76 (±0.74)
Height, cm	0.66 (±0.23) <sup>c</sup>	0.40 (±0.24)	0.12 (±0.08)	0.91 (±0.28) <sup>c</sup>	0.34 (±0.31)	0.15 (±0.08)	0.62 (±0.29) <sup>a</sup>	0.61 (±0.30)	0.14 (±0.12)
Height, SDS	3.71 (±1.30) <sup>c</sup>	2.41 (±1.37)	0.59 (±0.43)	4.99 (±1.54) <sup>c</sup>	2.21 (±1.69)	0.87 (±0.47)	3.42 (±1.67) <sup>a</sup>	3.40 (±1.80)	0.54 (±0.70)
BMI	1.80 (±0.38) <sup>b</sup>	1.71 (±0.39) <sup>b</sup>	0.35 (±0.13) <sup>c</sup>	0.79 (±0.75)	0.39 (±0.76)	0.24 (±0.21)	1.95 (±1.14)	2.72 (±1.21) <sup>b</sup>	0.38 (±0.45)
Lowest BMI <sup>d</sup>							2.04 (±1.03)	2.93 (±1.10) <sup>a</sup>	0.07 (±0.40)
WB fat mass, kg	0.001 (±0.0002) <sup>b</sup>	0.001 (±0.0002) <sup>c</sup>	0.0001 (±0.0001)	0.0003 (±0.0003)	-0.0001 (±0.0003)	0.0001 (±0.0001)	0.0003 (±0.0004)	0.0005 (±0.0005)	-0.0001 (±0.0002)
WB fat mass, %	0.55 (±0.18) <sup>c</sup>	0.44 (±0.19) <sup>a</sup>	0.06 (±0.06)	0.04 (±0.32)	-0.35 (±0.31)	-0.01 (±0.09)	-0.13 (±0.41)	0.03 (±0.44)	-0.24 (±0.15)
LTM	0.002 (±0.0002) <sup>b</sup>	0.001 (±0.0003) <sup>b</sup>	0.0004 (±0.00001) <sup>b</sup>	0.0015 (±0.0004) <sup>b</sup>	0.0011 (±0.0004) <sup>a</sup>	0.0003 (±0.0001) <sup>c</sup>	0.0012 (±0.0004) <sup>c</sup>	0.0013 (±0.0004) <sup>c</sup>	0.0003 (±0.0002) <sup>a</sup>
CTX	-1.16 (±3.86)	1.08 (±3.96)	-0.98 (±1.21)	-4.32 (±7.40)	-2.37 (±7.37)	-3.01 (±2.06)	9.27 (±4.22) <sup>a</sup>	11.60 (±4.50) <sup>a</sup>	1.21 (±1.70)
PINP	-0.01 (±0.02)	0.001 (±0.017)	-0.01 (±0.01)	-0.04 (±0.02) <sup>a</sup>	-0.03 (±0.02)	-0.02 (±0.01) <sup>b</sup>	-0.08 (±0.07)	-0.02 (±0.07)	-0.03 (±0.03)
OC	-0.02 (±0.08)	0.06 (±0.08)	-0.02 (±0.02)	-0.23 (±0.08) <sup>c</sup>	-0.15 (±0.08)	-0.08 (±0.02) <sup>c</sup>	-0.16 (±0.19)	0.09 (±0.20)	-0.03 (±0.07)
Irisin	-0.06 (±0.10)	-0.12 (±0.10)	-0.0002 (±0.03)	0.09 (±0.15)	-0.01 (±0.15)	0.05 (±0.04)	-0.12 (±0.12)	-0.11 (±0.10)	-0.04 (±0.05)
Myostatin	0.009 (±0.003) <sup>c</sup>	0.009 (±0.003) <sup>c</sup>	0.002 (±0.001)	0.004 (±0.004)	0.004 (±0.004)	-0.0001 (±0.0011)	0.004 (±0.005)	0.005 (±0.005)	0.001 (±0.002)
Follistatin	-0.001 (±0.001)	-0.001 (±0.001)	0.0001 (±0.0003)	0.0002 (±0.0014)	0.002 (±0.001)	0.0002 (±0.0004)	0.003 (±0.001)	-0.001 (±0.001)	0.0002 (±0.0005)
REE <sub>m</sub> <sup>d</sup>							0.01 (±0.01)	0.01 (±0.01)	0.001 (±0.004)
REEp	0.08 (±0.01) <sup>b</sup>	0.07 (±0.01) <sup>b</sup>	0.01 (±0.004) <sup>c</sup>	0.06 (±0.02) <sup>c</sup>	0.03 (±0.02)	0.01 (±0.01) <sup>a</sup>	0.08 (±0.02) <sup>c</sup>	0.10 (±0.03) <sup>b</sup>	0.01 (±0.01)
Age of AN onset <sup>d</sup>							-0.70 (±0.86)	-1.12 (±0.90)	0.48 (±0.32)
AN duration <sup>d</sup>							-1.62 (±0.60) <sup>a</sup>	-2.52 (±0.61) <sup>b</sup>	-0.22 (±0.25)
Amenorrhoea duration <sup>d</sup>							-0.20 (±0.05) <sup>b</sup>	-0.30 (±0.05) <sup>b</sup>	-0.04 (±0.02)
Multivariate analysis									
Age	-	-0.93 (±0.31) <sup>c</sup>	-	-	-	-0.21 (±0.12)	-	-	-
Height, cm	-	-	-	-	-	-	-	0.51 (±0.21) <sup>a</sup>	-
WB fat mass, %	0.53 (±0.24) <sup>c</sup>	-	-	-	-	-	-	-	-
LTM	-	0.001 (±0.0003) <sup>b</sup>	0.0004 (±0.0009) <sup>b</sup>	0.0015 (±0.0004) <sup>b</sup>	0.0011 (±0.0004) <sup>a</sup>	-	0.001 (±0.0003) <sup>c</sup>	-	0.0003 (±0.0002) <sup>a</sup>
PINP	-	-	-	-	-	0.018 (±0.004) <sup>b</sup>	-0.17 (±0.05) <sup>c</sup>	-	-
REE <sub>p</sub>	0.11 (±0.02) <sup>b</sup>	-	-	-	-	-	-	-	-
Age of AN onset <sup>d</sup>								1.85 (±0.73) <sup>a</sup>	-
Amenorrhoea duration <sup>d</sup>								-0.34 (±0.05) <sup>b</sup>	-
Final model	R <sup>2</sup> = 0.378	R <sup>2</sup> = 0.349	R <sup>2</sup> = 0.187	R <sup>2</sup> = 0.253	R <sup>2</sup> = 0.132	R <sup>2</sup> = 0.308	R <sup>2</sup> = 0.488	R <sup>2</sup> = 0.610	R <sup>2</sup> = 0.115

Data are presented as beta-estimate × 10<sup>2</sup> (±s.d. × 10<sup>2</sup>).

<sup>a</sup>P < 0.05, <sup>b</sup>P < 0.001 and <sup>c</sup>P < 0.001; <sup>d</sup>parameters unavailable in controls.

aBMD, areal bone mineral density; AN, anorexia nervosa; CTX, type I-C telopeptide breakdown products; FM, fat mass; IGF-1, insulin-like growth factor-1; LTM, lean tissue mass; OC, osteocalcin; PINP, procollagen type I N-terminal propeptide; SDS, standard deviation score; REEm, measured resting energy expenditure; REEp, predicted resting energy expenditure calculated according to the Harris Benedict equation modified by Roza and Shizgal (41).

increase energy expenditure by inducing the browning of s.c. white adipocytes, which are metabolically favourable for burning energy through thermogenesis (51). Depending on the experimental conditions (i.e. thermoneutral or cold), brown adipose tissue activity has been reported to be absent or drastically reduced in these patients (52, 53). This finding was interpreted as an adaptive response to compensate the chronic fuel deficiency caused by chronic restrictive eating behaviour (52, 53) and may partly explain the hypothermia generally observed in these patients (54). Consequently, we might logically expect lower irisin levels in our AN patients, in parallel to the reports in other situations of energy deficit and the likely hypogonadism in amenorrhoeic athletes compared with eumenorrhoeic athletes and non-athletes (55). In amenorrhoeic athletes, this phenomenon was interpreted as an adaptive response to conserve energy (55). However, compared with patients with AN who present a state of extreme energy deficit, amenorrhoeic athletes are in a state of subtle energy deficit, as indicated by their moderate reduction in fat mass and resting energy expenditure (55). In patients with AN, the decrease in irisin levels would accentuate the reduction of energy expenditure and body temperature. In fact, it seems that in this disease, a fragile equilibrium exists between low energy expenditure and whole-body temperature maintenance.

The patients with AN in our study presented lower myostatin and higher follistatin levels compared with normal-weight controls. Wu *et al.* investigated the effect of AN on myostatin secretion, and they also reported lower myostatin levels in a small group ( $n = 25$ ) of adolescent girls with AN (42). Due to the limited data available in this population of patients, it is difficult to draw parallels with the findings obtained in other conditions of energy deprivation. For example, follistatin levels increased in both males and females after 72 h of fasting (56, 57) and in women with hypothalamic amenorrhoea due to chronic energy deficiency (58). However, although these findings are concordant with our results, we must keep in mind that this situation represents a lower level of nutritional deprivation in comparison with those observed in AN patients. As follistatin acts as the regulator of myostatin through binding to the active form of myostatin and inhibiting the binding of myostatin to the activating IIB receptor (31), it was speculated that follistatin increases to protect muscle tissue from degradation via myostatin inhibition (31).

Given the undernutrition and muscle mass reduction in patients with AN, do myokine variations play a role in the alteration in aBMD and bone remodelling in these patients? Despite some experimental studies in the

literature showing that myokines may act on bone cell activity (59) and bone repair (60), data regarding myokine effects on bone tissue in humans are relatively scarce and contradictory. For example, a link between myostatin gene polymorphisms and peak bone mass acquisition has been demonstrated (61, 62), as has a link with fracture risk (63). Irisin levels were weakly correlated with BMD in subjects with osteoporosis (64) and positively associated with measures of areal and volumetric bone density and strength estimates in older adult patients (38), female athletes (55) and soccer players (37). We should note that this relationship was not reported in another group of soccer players (65), middle-aged male amateur runners (66) and postmenopausal women (39). Plasma irisin levels were associated with hip BMD in elderly Chinese men but not in women (67). In clinical studies, the inconsistent results concerning the relationship between irisin levels and aBMD may be related to the non-standardized technical assays for the quantification of irisin levels and, to date, the serum irisin levels measured by different manufacturers and ELISA kits are not comparable (68, 69, 70). Our results tended to show no noticeable effects of myokines on aBMD or bone remodelling markers in the patients with AN. To the best of our knowledge, only one study has investigated the potential role of myokines on bone alteration in a comparable population (42). In this study, Wu *et al.* (42) also found no relationship between myostatin (GDF 8) and aBMD in adolescent women with AN, whereas the level of growth differentiation factor-11 (GDF11), whose sequence is close to that of myostatin and whose values are higher in AN patients, was an independent negative predictor of aBMD. It is probable, as suggested by Wu *et al.* (42), that factors other than myokines have a greater impact on bone metabolism in this pathological state. In our study, among several factors, LTM, age of AN onset and longer amenorrhoea duration appeared as independent explicative variables for aBMD in this population. Nevertheless, our final model only explained between 11.5 and 61% of the aBMD variance, depending on the bone site evaluated. This suggests, as previously stated by Wu *et al.* (42), that other factors – and why not other myokines that remain thus far unidentified? – might influence aBMD.

This study had some limitations, particularly its cross-sectional design and the single measurements of both aBMD and the biological parameters, as well as the inclusion of a limited number of patients with AN. Indeed, it now seems important to further investigate the underlying mechanisms that link myokines and bone in a longitudinal study, particularly during weight loss or weight recovery in these patients. Also, evidence has shown

that physical activities can influence myokine levels (71), but this information was not available from our patients. It should be noted, however, that, as mentioned above, physical activities do not seem to modify myokine levels in patients with AN (40). Importantly, these limitations are mitigated by the wide age range for the two groups around the time of peak bone mass, the high degree of age-matching ( $\pm 6$  months) and the similar clinical profiles within groups. In addition, although we paid particular attention to collecting data on the history of the disease (age of AN onset, duration of AN and weight variations), these were self-reported, which might have induced a bias, particularly in this population of patients who present impairments in memory performance. To limit this bias, the data collected for patients followed for a long time in our department were compared with data from their medical files, whenever possible. Blood samples were obtained at an unsynchronized menstrual stage that may potentially have affected some of the biological parameters evaluated in the current study, particularly follistatin (58, 72). Other hormones that are produced by fat tissue also interact with bone tissue (13) and it will be interesting to evaluate the complex interactions between muscle, fat and bone tissues via myokine and lipokine analysis.

## Conclusion

This study confirmed that the presence of AN during the growth and young adult periods induces a deep alteration in bone mass and bone turnover. Although myokines are influenced by undernutrition, these biological parameters do not seem to be directly implicated in the bone metabolism alteration of AN patients. However, the lower myostatin/follistatin ratio would tend to preserve bone and muscle mass, and normal irisin values would tend to maintain a temperature compatible with life while limiting energy expenditure.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

### Funding

This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

### Author contribution statement

L M, H H, M C P, S G and A S are responsible for design; L M, D M G, E R, P L, A M D, J P C, M S, P C, V B and A A for writing; and H H and M C P for statistical analysis. All authors have read and approved the final version. S G and A S should be considered as having the same author position.

## References

- 1 Maimoun L, Guillaume S, Lefebvre P, Philibert P, Bertet H, Picot MC, Gaspari L, Paris F, Courtet P, Thomas E, *et al.* Role of sclerostin and Dickkopf-1 in the dramatic alteration in bone mass acquisition in adolescents and young women with recent anorexia nervosa. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E582–E590. (<https://doi.org/10.1210/jc.2013-2565>)
- 2 Misra M, Miller KK, Bjornson J, Hackman A, Aggarwal A, Chung J, Ott M, Herzog DB, Johnson ML & Klibanski A. Alterations in growth hormone secretory dynamics in adolescent girls with anorexia nervosa and effects on bone metabolism. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 5615–5623. (<https://doi.org/10.1210/jc.2003-030532>)
- 3 Singhal V, Sanchita S, Malhotra S, Bose A, Flores LPT, Valera R, Stanford FC, Slattery M, Rosenblum J, Goldstein MA, *et al.* Suboptimal bone microarchitecture in adolescent girls with obesity compared to normal-weight controls and girls with anorexia nervosa. *Bone* 2019 **122** 246–253. (<https://doi.org/10.1016/j.bone.2019.03.007>)
- 4 Schorr M, Fazeli PK, Bachmann KN, Faje AT, Meenaghan E, Kimball A, Singhal V, Ebrahimi S, Gleysteen S, Mickley D, *et al.* Differences in trabecular plate and rod structure in premenopausal women across the weight spectrum. *Journal of Clinical Endocrinology and Metabolism* 2019 **104** 4501–4510. (<https://doi.org/10.1210/jc.2019-00843>)
- 5 Gordon CM, Goodman E, Emans SJ, Grace E, Becker KA, Rosen CJ, Gundberg CM & Leboff MS. Physiologic regulators of bone turnover in young women with anorexia nervosa. *Journal of Pediatrics* 2002 **141** 64–70. (<https://doi.org/10.1067/mpd.2002.125003>)
- 6 Grinspoon S, Miller K, Coyle C, Krempin J, Armstrong C, Pitts S, Herzog D & Klibanski A. Severity of osteopenia in estrogen-deficient women with anorexia nervosa and hypothalamic amenorrhea. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 2049–2055. (<https://doi.org/10.1210/jcem.84.6.5792>)
- 7 Lucas AR, Melton LJ, 3rd, Crowson CS & O'Fallon WM. Long-term fracture risk among women with anorexia nervosa: a population-based cohort study. *Mayo Clinic Proceedings* 1999 **74** 972–977. (<https://doi.org/10.4065/74.10.972>)
- 8 Kiyohara K, Tamai H, Takaichi Y, Nakagawa T & Kumagai LE. Decreased thyroidal triiodothyronine secretion in patients with anorexia nervosa: influence of weight recovery. *American Journal of Clinical Nutrition* 1989 **50** 767–772. (<https://doi.org/10.1093/ajcn/50.4.767>)
- 9 Audi L, Vargas DM, Gussinye M, Yeste D, Marti G & Carrascosa A. Clinical and biochemical determinants of bone metabolism and bone mass in adolescent female patients with anorexia nervosa. *Pediatric Research* 2002 **51** 497–504. (<https://doi.org/10.1203/00006450-200204000-00016>)
- 10 Maimoun L, Garnero P, Mura T, Nocca D, Lefebvre P, Philibert P, Seneque M, Gaspari L, Vauchot F, Courtet P, *et al.* Specific effects of anorexia nervosa and obesity on bone mineral density and bone turnover in young women. *Journal of Clinical Endocrinology and Metabolism* 2020 **105** e1536–e1548. (<https://doi.org/10.1210/clinem/dgz259>)
- 11 Legroux-Gerot I, Vignau J, Collier F & Cortet B. Factors influencing changes in bone mineral density in patients with anorexia nervosa-related osteoporosis: the effect of hormone replacement therapy. *Calcified Tissue International* 2008 **83** 315–323. (<https://doi.org/10.1007/s00223-008-9173-y>)
- 12 Legroux-Gerot I, Vignau J, D'Herbomez M, Collier F, Marchandise X, Duquesnoy B & Cortet B. Evaluation of bone loss and its mechanisms in anorexia nervosa. *Calcified Tissue International* 2007 **81** 174–182. (<https://doi.org/10.1007/s00223-007-9038-9>)
- 13 Maimoun L, Guillaume S, Lefebvre P, Philibert P, Bertet H, Picot MC, Gaspari L, Paris F, Seneque M, Dupuys AM, *et al.* Evidence of a link between resting energy expenditure and bone remodelling, glucose homeostasis and adipokine variations in adolescent girls with anorexia nervosa. *Osteoporosis International* 2016 **27** 135–146. (<https://doi.org/10.1007/s00198-015-3223-x>)
- 14 Soyka LA, Misra M, Frenchman A, Miller KK, Grinspoon S, Schoenfeld DA & Klibanski A. Abnormal bone mineral accrual in adolescent girls with

- anorexia nervosa. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 4177–4185. (<https://doi.org/10.1210/jc.2001-011889>)
- 15 Paintin J, Cooper C & Dennison E. Osteosarcopenia. *British Journal of Hospital Medicine* 2018 **79** 253–258. (<https://doi.org/10.12968/hmed.2018.79.5.253>)
  - 16 Maimoun L, Fattal C, Micallef JP, Peruchon E & Rabischong P. Bone loss in spinal cord-injured patients: from physiopathology to therapy. *Spinal Cord* 2006 **44** 203–210. (<https://doi.org/10.1038/sj.sc.3101832>)
  - 17 Szulc P, Beck TJ, Marchand F & Delmas PD. Low skeletal muscle mass is associated with poor structural parameters of bone and impaired balance in elderly men – the MINOS study. *Journal of Bone and Mineral Research* 2005 **20** 721–729. (<https://doi.org/10.1359/JBMR.041230>)
  - 18 Burr DB, Robling AG & Turner CH. Effects of biomechanical stress on bones in animals. *Bone* 2002 **30** 781–786. ([https://doi.org/10.1016/s8756-3282\(02\)00707-x](https://doi.org/10.1016/s8756-3282(02)00707-x))
  - 19 Zhao LJ, Liu YJ, Liu PY, Hamilton J, Recker RR & Deng HW. Relationship of obesity with osteoporosis. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 1640–1646. (<https://doi.org/10.1210/jc.2006-0572>)
  - 20 Kawao N & Kaji H. Interactions between muscle tissues and bone metabolism. *Journal of Cellular Biochemistry* 2015 **116** 687–695. (<https://doi.org/10.1002/jcb.25040>)
  - 21 Giudice J & Taylor JM. Muscle as a paracrine and endocrine organ. *Current Opinion in Pharmacology* 2017 **34** 49–55. (<https://doi.org/10.1016/j.coph.2017.05.005>)
  - 22 Pedersen BK & Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nature Reviews: Endocrinology* 2012 **8** 457–465. (<https://doi.org/10.1038/nrendo.2012.49>)
  - 23 Hamrick MW, McPherron AC & Lovejoy CO. Bone mineral content and density in the humerus of adult myostatin-deficient mice. *Calcified Tissue International* 2002 **71** 63–68. (<https://doi.org/10.1007/s00223-001-1109-8>)
  - 24 Hamrick MW. Increased bone mineral density in the femora of GDF8 knockout mice. *Anatomical Record: Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology* 2003 **272** 388–391. (<https://doi.org/10.1002/ar.a.10044>)
  - 25 Hamrick MW, McPherron AC, Lovejoy CO & Hudson J. Femoral morphology and cross-sectional geometry of adult myostatin-deficient mice. *Bone* 2000 **27** 343–349. ([https://doi.org/10.1016/s8756-3282\(00\)00339-2](https://doi.org/10.1016/s8756-3282(00)00339-2))
  - 26 McPherron AC, Lawler AM & Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* 1997 **387** 83–90. (<https://doi.org/10.1038/387083a0>)
  - 27 Elliott B, Renshaw D, Getting S & Mackenzie R. The central role of myostatin in skeletal muscle and whole body homeostasis. *Acta Physiologica* 2012 **205** 324–340. (<https://doi.org/10.1111/j.1748-1716.2012.02423.x>)
  - 28 Bialek P, Parkington J, Li X, Gavin D, Wallace C, Zhang J, Root A, Yan G, Warner L, Seeherman HJ, et al. A myostatin and activin decoy receptor enhances bone formation in mice. *Bone* 2014 **60** 162–171. (<https://doi.org/10.1016/j.bone.2013.12.002>)
  - 29 Attie KM, Yang YS, Wilson DM, Haltom E & Sherman ML. Increased lean mass and muscle volume in healthy post-menopausal women treated with ACE-031 (soluble activin type IIB receptor), an inhibitor of myostatin and other negative regulators of muscle. In *Poster Presentation at the American Academy of Neurology Meeting*, 2011.
  - 30 Campbell C, McMillan HJ, Mah JK, Tarnopolsky M, Selby K, McClure T, Wilson DM, Sherman ML, Escobar D & Attie KM. Myostatin inhibitor ACE-031 treatment of ambulatory boys with Duchenne muscular dystrophy: results of a randomized, placebo-controlled clinical trial. *Muscle and Nerve* 2017 **55** 458–464. (<https://doi.org/10.1002/mus.25268>)
  - 31 Anthor H, Nicholas G, McKinnell I, Kemp CF, Sharma M, Kambadur R & Patel K. Follistatin complexes myostatin and antagonises myostatin-mediated inhibition of myogenesis. *Developmental Biology* 2004 **270** 19–30. (<https://doi.org/10.1016/j.ydbio.2004.01.046>)
  - 32 Eijken M, Swagemakers S, Koedam M, Steenbergen C, Derckx P, Uitterlinden AG, van der Spek PJ, Visser JA, de Jong FH, Pols HA, et al. The activin A-follistatin system: potent regulator of human extracellular matrix mineralization. *FASEB Journal* 2007 **21** 2949–2960. (<https://doi.org/10.1096/fj.07-8080com>)
  - 33 Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, Perron RM, Werner CD, Phan GQ, Kammula US, et al. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. *Cell Metabolism* 2014 **19** 302–309. (<https://doi.org/10.1016/j.cmet.2013.12.017>)
  - 34 Lombardi G, Sanchis-Gomar F, Perego S, Sansoni V & Banfi G. Implications of exercise-induced adipo-myokines in bone metabolism. *Endocrine* 2016 **54** 284–305. (<https://doi.org/10.1007/s12020-015-0834-0>)
  - 35 Colaiani G, Cuscito C, Mongelli T, Pignataro P, Buccoliero C, Liu P, Lu P, Sartini L, Di Comite M, Mori G, et al. The myokine irisin increases cortical bone mass. *PNAS* 2015 **112** 12157–12162. (<https://doi.org/10.1073/pnas.1516622112>)
  - 36 Zhang J, Valverde P, Zhu X, Murray D, Wu Y, Yu L, Jiang H, Dard MM, Huang J, Xu Z, et al. Exercise-induced irisin in bone and systemic irisin administration reveal new regulatory mechanisms of bone metabolism. *Bone Research* 2017 **5** 16056. (<https://doi.org/10.1038/boneres.2016.56>)
  - 37 Colaiani G, Notarnicola A, Sanesi L, Brunetti G, Lippo L, Celi M, Moretti L, Pesce V, Vicenti G, Moretti B, et al. Irisin levels correlate with bone mineral density in soccer players. *Journal of Biological Regulators and Homeostatic Agents* 2017 **31** (Supplement 1) 21–28.
  - 38 Colaiani G, Errede M, Sanesi L, Notarnicola A, Celi M, Zerlotin R, Storlino G, Pignataro P, Oranger A, Pesce V, et al. Irisin correlates positively with BMD in a cohort of older adult patients and downregulates the senescent marker p21 in osteoblasts. *Journal of Bone and Mineral Research* 2021 **36** 305–314. (<https://doi.org/10.1002/jbmr.4192>)
  - 39 Anastasilakis AD, Polyzos SA, Makras P, Gkiomisi A, Bisbinas I, Katsarou A, Filippaios A & Mantzoros CS. Circulating irisin is associated with osteoporotic fractures in postmenopausal women with low bone mass but is not affected by either teriparatide or denosumab treatment for 3 months. *Osteoporosis International* 2014 **25** 1633–1642. (<https://doi.org/10.1007/s00198-014-2673-x>)
  - 40 Hofmann T, Elbelt U, Ahnis A, Kobelt P, Rose M & Stengel A. Irisin levels are not affected by physical activity in patients with anorexia nervosa. *Frontiers in Endocrinology* 2014 **4** 202. (<https://doi.org/10.3389/fendo.2013.00202>)
  - 41 Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P & Klapp BE. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity – correlation with body mass index. *Peptides* 2013 **39** 125–130. (<https://doi.org/10.1016/j.peptides.2012.11.014>)
  - 42 Wu Y, Qu J, Li H, Yuan H, Guo Q, Ouyang Z & Lu Q. Relationship between serum level of growth differentiation factors 8, 11 and bone mineral density in girls with anorexia nervosa. *Clinical Endocrinology* 2019 **90** 88–93. (<https://doi.org/10.1111/cen.13871>)
  - 43 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed. Washington, DC, USA: American Psychiatric Press, 2013.
  - 44 Garcia FD, Grigioni S, Chelali S, Meyrignac G, Thibaut F & Dechelotte P. Validation of the French version of SCOFF questionnaire for screening of eating disorders among adults. *World Journal of Biological Psychiatry* 2010 **11** 888–893. (<https://doi.org/10.3109/15622975.2010.483251>)
  - 45 Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R & Dunbar GC. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry* 1998 **59** (Supplement 20) 22–33; quiz 4–57.
  - 46 Roza AM & Shizgal HM. The Harris Benedict equation reevaluated: resting energy requirements and the body cell mass. *American Journal of Clinical Nutrition* 1984 **40** 168–182. (<https://doi.org/10.1093/ajcn/40.1.168>)

- 47 Cohen J. *Statistical Power Analysis for the Behavioral Sciences*, p. 567. New York: Routledge, 1988.
- 48 Sawilowsky SS. New effect size rules of thumb. *Journal of Modern Applied Statistical Methods* 2009 **8** 597–599. (<https://doi.org/10.22237/jmasm/1257035100>)
- 49 Lee SR, Ko TH, Kim HK, Marquez J, Ko KS, Rhee BD & Han J. Influence of starvation on heart contractility and corticosterone level in rats. *Pflugers Archiv* 2015 **467** 2351–2360. (<https://doi.org/10.1007/s00424-015-1701-9>)
- 50 Choi HY, Kim S, Park JW, Lee NS, Hwang SY, Huh JY, Hong HC, Yoo HJ, Baik SH, Youn BS, *et al.* Implication of circulating irisin levels with brown adipose tissue and sarcopenia in humans. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 2778–2785. (<https://doi.org/10.1210/jc.2014-1195>)
- 51 Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, *et al.* A PGC1- $\alpha$ -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012 **481** 463–468. (<https://doi.org/10.1038/nature10777>)
- 52 Pasanisi F, Pace L, Fonti R, Marra M, Sgambati D, De Caprio C, De Filippo E, Vaccaro A, Salvatore M & Contaldo F. Evidence of brown fat activity in constitutional leanness. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 1214–1218. (<https://doi.org/10.1210/jc.2012-2981>)
- 53 Bredella MA, Fazeli PK, Freedman LM, Calder G, Lee H, Rosen CJ & Klubanski A. Young women with cold-activated brown adipose tissue have higher bone mineral density and lower Pref-1 than women without brown adipose tissue: a study in women with anorexia nervosa, women recovered from anorexia nervosa, and normal-weight women. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** E584–E590. (<https://doi.org/10.1210/jc.2011-2246>)
- 54 Birmingham CL, Hodgson DM, Fung J, Brown R, Wakefield A, Bartrop R & Beumont P. Reduced febrile response to bacterial infection in anorexia nervosa patients. *International Journal of Eating Disorders* 2003 **34** 269–272. (<https://doi.org/10.1002/eat.10189>)
- 55 Singhal V, Lawson EA, Ackerman KE, Fazeli PK, Clarke H, Lee H, Eddy K, Marengi DA, Derrico NP, Boussein ML, *et al.* Irisin levels are lower in young amenorrheic athletes compared with eumenorrheic athletes and non-athletes and are associated with bone density and strength estimates. *PLoS ONE* 2014 **9** e100218. (<https://doi.org/10.1371/journal.pone.0100218>)
- 56 Moraggianni VA, Aronis KN, Chamberland JP & Mantzoros CS. Short-term energy deprivation alters activin A and follistatin but not inhibin B levels of lean healthy women in a leptin-independent manner. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 3750–3758. (<https://doi.org/10.1210/jc.2011-1453>)
- 57 Vamvini MT, Aronis KN, Chamberland JP & Mantzoros CS. Energy deprivation alters in a leptin- and cortisol-independent manner circulating levels of activin A and follistatin but not myostatin in healthy males. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 3416–3423. (<https://doi.org/10.1210/jc.2011-1665>)
- 58 Perakakis N, Upadhyay J, Ghaly W, Chen J, Chrysafi P, Anastasilakis AD & Mantzoros CS. Regulation of the activins-follistatins-inhibins axis by energy status: impact on reproductive function. *Metabolism: Clinical and Experimental* 2018 **85** 240–249. (<https://doi.org/10.1016/j.metabol.2018.05.003>)
- 59 Hamrick MW, Shi X, Zhang W, Pennington C, Thakore H, Haque M, Kang B, Isaacs CM, Fulzele S & Wenger KH. Loss of myostatin (GDF8) function increases osteogenic differentiation of bone marrow-derived mesenchymal stem cells but the osteogenic effect is ablated with unloading. *Bone* 2007 **40** 1544–1553. (<https://doi.org/10.1016/j.bone.2007.02.012>)
- 60 Kellum E, Starr H, Arounleut P, Immed D, Fulzele S, Wenger K & Hamrick MW. Myostatin (GDF-8) deficiency increases fracture callus size, Sox-5 expression, and callus bone volume. *Bone* 2009 **44** 17–23. (<https://doi.org/10.1016/j.bone.2008.08.126>)
- 61 Zhang ZL, He JW, Qin YJ, Hu YQ, Li M, Zhang H, Hu WW, Liu YJ & Gu JM. Association between myostatin gene polymorphisms and peak BMD variation in Chinese nuclear families. *Osteoporosis International* 2008 **19** 39–47. (<https://doi.org/10.1007/s00198-007-0435-8>)
- 62 Yue H, He JW, Zhang H, Wang C, Hu WW, Gu JM, Ke YH, Fu WZ, Hu YQ, Li M, *et al.* Contribution of myostatin gene polymorphisms to normal variation in lean mass, fat mass and peak BMD in Chinese male offspring. *Acta Pharmacologica Sinica* 2012 **33** 660–667. (<https://doi.org/10.1038/aps.2012.12>)
- 63 Harslof T, Frost M, Nielsen TL, Husted LB, Nyegaard M, Brixen K, Borglum AD, Mosekilde L, Andersen M, Rejnmark L, *et al.* Polymorphisms of muscle genes are associated with bone mass and incident osteoporotic fractures in Caucasians. *Calcified Tissue International* 2013 **92** 467–476. (<https://doi.org/10.1007/s00223-013-9702-1>)
- 64 Zhou K, Qiao X, Cai Y, Li A & Shan D. Lower circulating irisin in middle-aged and older adults with osteoporosis: a systematic review and meta-analysis. *Menopause* 2019 **26** 1302–1310. (<https://doi.org/10.1097/GME.0000000000001388>)
- 65 Gaudio A, Rapisarda R, Xourafa A, Zanolli L, Manfre V, Catalano A, Signorelli SS & Castellino P. Effects of competitive physical activity on serum irisin levels and bone turnover markers. *Journal of Endocrinological Investigation* 2021 **44** 2235–2241. (<https://doi.org/10.1007/s40618-021-01529-0>)
- 66 Sliwicka E, Cison T, Pilaczynska-Szczesniak L, Ziemia A & Straburzynska-Lupa A. Effects of marathon race on selected myokines and sclerostin in middle-aged male amateur runners. *Scientific Reports* 2021 **11** 2813. (<https://doi.org/10.1038/s41598-021-82288-z>)
- 67 Wu LF, Zhu DC, Tang CH, Ge B, Shi J, Wang BH, Lu YH, He P, Wang WY, Lu SQ, *et al.* Association of plasma irisin with bone mineral density in a large Chinese population using an extreme sampling design. *Calcified Tissue International* 2018 **103** 246–251. (<https://doi.org/10.1007/s00223-018-0415-3>)
- 68 Anastasilakis AD, Polyzos SA, Makras P, Douni E & Mantzoros CS. Irisin: good or bad for the bone? A new path forward after the reported discovery of irisin receptor? *Metabolism: Clinical and Experimental* 2019 **93** 100–102. (<https://doi.org/10.1016/j.metabol.2019.01.013>)
- 69 Polyzos SA & Mantzoros CS. An update on the validity of irisin assays and the link between irisin and hepatic metabolism. *Metabolism: Clinical and Experimental* 2015 **64** 937–942. (<https://doi.org/10.1016/j.metabol.2015.06.005>)
- 70 Zhong X, Sun X, Shan M, Zhao X, Zhang R, Zhao Y & Yang Q. The production, detection, and origin of irisin and its effect on bone cells. *International Journal of Biological Macromolecules* 2021 **178** 316–324. (<https://doi.org/10.1016/j.ijbiomac.2021.02.181>)
- 71 Leal LG, Lopes MA & Batista ML, Jr. Physical exercise-induced myokines and muscle-adipose tissue crosstalk: a review of current knowledge and the implications for health and metabolic diseases. *Frontiers in Physiology* 2018 **9** 1307. (<https://doi.org/10.3389/fphys.2018.01307>)
- 72 Anastasilakis AD, Polyzos SA, Skouvaklidou EC, Kynigopoulos G, Saridakis ZG, Apostolou A, Triantafyllou GA, Karagiozoglou-Lampoudi T & Mantzoros CS. Circulating follistatin displays a day-night rhythm and is associated with muscle mass and circulating leptin levels in healthy, young humans. *Metabolism: Clinical and Experimental* 2016 **65** 1459–1465. (<https://doi.org/10.1016/j.metabol.2016.07.002>)