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ORIGINAL ARTICLE

Bone turnover response is linked to both acute and established metabolic changes in ultra-marathon runners

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Abstract Bone and energy metabolisms regulation depends on a two-way street aimed at regulating energy utilization. Mountain ultra-marathons are highly demanding aerobic performances that deeply affect the whole body homeostasis. In this study we aimed to investigate and characterize the metabolic profile (in terms of hormones involved in energy metabolism), the inflammatory adipokines, and the bone turnover; in particular the osteocalcin-mediated response has been compared in experienced mountain ultra-marathons runners versus control subjects. Serum concentrations of specific markers of bone turnover (pro-collagen type I N-terminal propeptide, carboxylated/undercarboxylated osteocalcin), measured by enzyme-linked immunosorbent assay, and metabolic hormones (C-peptide, insulin, glucagon, glucagon-like peptide, gastric-inhibitory peptide, ghrelin, leptin, resistin, and visfatin), measured by fluorescent-based multiplex assay, were compared before and after a 65 km mountain ultra-marathons in 17 trained runners and 12 age-matched controls characterized by a low physical activity profile. After the mountain ultra-marathons, runners experienced a reduction in pro-collagen type I N-terminal propeptide, though it remained higher than in controls; while carboxylated osteocalcin remained unchanged. Among the metabolic hormones, only glucagon and leptin were different between runners and controls at rest. C-peptide and leptin decreased after the mountain ultra-marathons in runners; while glucagon, glucagon-like peptide 1, resistin, and visfatin were all increased. Uncarboxylated osteocalcin (and uncarboxylated/carboxylated osteocalcin ratio) was decreased and this highly correlated with insulin and C-peptide levels. In conditions of high energy expenditure, homeostasis is maintained at expenses of bone metabolism. Changes in the uncarboxylated osteocalcin clearly mark the global energy needs of the body.

Keywords Fatigue · Osteocalcin · Bone turnover · Energy metabolism · Mountain ultra-marathon

Introduction

Endurance running has been the basis of human activities over an extended period of time, determining the natural selection of the metabolic profiles specifically addressed at satisfying the energetic requirements [1]. Very recently

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(on an evolutionary timescale) lifestyle has become sedentary leading to the exponential increase in the prevalence of a wide panel of diseases [e.g., metabolic disorders, type 2 diabetes (T2D), obesity, cardiovascular diseases, cancers, and neurodegenerative disorders]; all recognized to be associated with suboptimal physical activity (PA) or inactivity. In 2009, the World Health Organization indicated physical inactivity as the 4th potential cause-of-death (5.5 % of deaths, globally) immediately after hypertension (12.8 %), smoke (8.7 %), and hyperglycemia (5.8 %), and just before obesity and overweight (4.8 %) [2]. Accordingly, aerobic exercise is a recognized therapeutic strategy against metabolic syndrome and T2D, since it reduces the metabolic risk factors of insulin resistance [3]. Aerobic exercise mimics the effects of insulin by increasing glucose uptake into skeletal muscles and liver via insulin-independent membrane translocation of glucose transporter (GLUT)-4. Further, it induces the expression in muscles of myokines which are known regulators of the energy metabolism [4].

Another key target of exercise is the adipose tissue. Indeed, regular exercise decreases the size of adipocytes and increases their insulin sensitivity [5, 6]. Through the secretion of adipokines, adipocytes exert endocrine and autocrine functions aimed at modifying insulin sensitivity at the liver and skeletal muscle level, as well as in the adipose tissue itself [5]. By modulating adipokines expression PA is a powerful lipolytic stimulus in the visceral adipose tissue (VAT), contributing also to reduce the central adiposity, thus improving the systemic inflammation [4–7]. Besides the benefits of low-to-high intensity PA on metabolism, it is currently emerging that excessive PA can be deleterious due to the instauration of a pro-inflammatory profile [8].

PA, particularly strength training and impact loading, is also one of the main determinants, among the few modifiable factors, of bone mass and bone metabolism [9, 10]. Exercise-induced gain in bone mass is mainly due to the increased mechanical strain. However, several endocrine factors play a critical role in this context. Accordingly, runners who train using large and repetitive loads of the lower limbs, which is a key factor in stimulating bone anabolism [4], have a higher bone mass than athletes performing not weight-bearing endurance activities (i.e., cycling), who instead experience a more prominent bone resorption [11–13].

Bone affects energy metabolism through osteocalcin (OC), a hormone that works on both pancreatic β-cells [14] and adipocytes [15]. OC is a vitamin K-dependent protein mainly synthesized by osteoblasts which binds hydroxyapatite and regulates bone mineralization. OC, in both its carboxylated (Gla-OC) and undercarboxylated (Glu-OC) forms, regulates glucose metabolism, even though their relative efficacies are still unknown [16]. We previously demonstrated that in the absence of load a strenuous endurance activity (i.e., *Giro d'Italia*, a major cycling stage race) decreased total OC serum concentrations while it increased the relative Glu-OC fraction. Changes in Glu-OC were directly associated with modifications in adiponectin and inversely related to leptin and energy expenditure [11].

Mountain ultra-marathons (MUMs) consist of running on mountain trails over distance longer than those of a classical marathon (42.195 km) [17–19]. MUMs may thus offer a unique opportunity to investigate the human body's limits to the physiological and adaptive responses [20].

Though the scientific literature in this field is growing, studies concerning the integrated osteo-metabolic changes occurring during MUMs are still lacking. Accordingly, the aim of this study was to investigate and characterize the metabolic profile (in terms of hormones involved in energy metabolism), the metabolic inflammatory profile (in terms of adipokines), and the bone metabolism by comparing the OC-mediated response in experienced MUM runners, before and after a competition, with that of control subjects with a low PA profile.

Methods

Ethical approval

After being informed about the study aims, procedure, and risks, participants gave their written consent for data collection and use. The protocol was conducted fully according to the Declaration of Helsinki and approval was obtained from the local institutional Ethics Committee.

Subjects

The race organizer invited male runners to take part in the present study through an announcement posted on the event website (http://www.trentinotrailrunning.it/collaborazioni/vigolanatrail). Twenty experienced male ultra-marathon runners accepted to take part in the study. Seventeen out of the 20 (82.3 %) initially enrolled participants completed the MUM (EXP group, mean ± SD: age 38.8 ± 7.2 years, body mass 74.4 ± 7.5 kg, stature 1.79 ± 0.07 m, body mass index 23.3 ± 2.1 $kg \cdot m^{-2}$). Training history consisted of 3–4 weekly sessions of running for 8.0 ± 5.0 h and 58.5 ± 28.0 km. The last high-intensity training was performed ~5 days before the race, with a tapering strategy consisted of a 50 % training reduction in both volume and intensity [21].

In parallel, a control group (CTRL, $n = 12$, mean ± SD: age 39.3 ± 6.8 years, body mass 75.2 ± 7.3 kg, stature 1.79

± 0.07 m, body mass index 23.4 ± 2.0 kg·m^{-2}) participated in this study. Subjects enrolled as CTRL were not classified as moderately trained (i.e., aerobic activity performed for a minimum of 30 min, 5 times a week [22]).

Intervention

The international race (the Vigolana Trail®) took place in Vigolo Vattaro (Trento, Italia, EU) in June 2014. The course was 65-km long over rough terrain at moderate altitude (altitude range between 725 and 2100 m), with a cumulative elevation gain of + 4000 m (i.e., the sum of every gain in elevation throughout the entire MUM). Of the 204 males runners, 178 (85.4 %) completed the MUM. The time of the winner of this MUM race was 6.6 h and the average time of the study participants was 10.5 h (ranking 3rd–200th), with a delay from the winner between 18.5 min and 7.5 h. During MUM athletes were allowed to eat and drink ad libitum.

Blood sampling and biochemical determinations

Blood samples were collected in the morning, the day before the race (between 08:00 and 10:00 a.m.) both in EXP and CTRL, and only in EXP after the MUM by means of a standard antecubital venipuncture in *SST II Advance* serum tubes (Becton Dickinson & Co., Franklin Lakes, NJ, USA). Shortly after the subjects had crossed the finishing line, they were brought by car to the laboratory (~300 m away).

Blood samples were allowed to clot for 30 min at room temperature, centrifuged at 1500 g for 10 min at 4 °C, and then the serum was immediately aliquoted and stored at – 80 °C until assayed. Strict adherence to pre-analytical warnings [23] was applied.

Glu-OC and Gla-OC were measured by the means of two specific monoclonal antibody-based sandwich immunoassays (Undercarboxylated OC EIA kit and Gla-Type OC EIA kit, Takara Bio Inc., Otsu-Shi, SHG, Japan). The lower limit of detection (LLD) was 0.25 ng/mL for both assays. As reported by the manufacturer, intra-assay (CV_i) and inter-assay (CV_b) coefficients of variation were 4.58 % and 5.67 % for Glu-OC and 3.3 % and 1.0 % for Gla-OC, respectively. PINP (procollagen type I N-terminal propeptide) was measured, in sera, through a monoclonal-antibody based ELISA kit (Cloud-Clone Corp., Houston, TX, USA). LLD was 13.4 pg/mL and CV_i and CV_b were < 10 % and < 12 %, respectively. Absorbance readings were performed on a Victor X3 reader (Perkin Elmer, Waltham, MA, USA).

According to the manufacturer instructions, C-peptide, ghrelin, gastric inhibitory polypeptide (GIP), glucagon-like peptide (GLP)-1, glucagon, insulin, leptin, resistin, and visfatin were tested altogether through a magnetic fluorescently dyed microspheres-based multiplex assay on a Bio-Plex® Multiplex System (Bio-Rad Laboratories Inc., Hercules, CA, USA).

All samples were tested in duplicate.

Statistical analysis

In the descriptive analysis, data are shown as median (5th–95th percentile). Comparisons between EXP and CTRL were analyzed by means of the Mann–Whitney's test for unpaired samples; whereas, paired comparisons between pre-race EXP and post-race EXP were analyzed by means of the Wilcoxon's matched-pairs test. Data distribution is shown by box and whiskers plot. Comparisons with a p value < 0.05 were considered significantly different. Spearman's ranked test was applied to test the reciprocal correlations between different parameters. The existence of any correlation was tested separately in CTRL, pre-race EXP, and post-race EXP groups. Furthermore, in the whole EXP group, the correlations between the post-MUM vs. pre-MUM differences for every parameter (Δx) was tested. Calculating numerous correlations increases the risk of a type I error. To avoid this, the level of statistical significance of correlation coefficients has been adjusted according to Bonferroni's correction [24]. The applied p value was $p < 0.004$.

Results

Comparisons between control subjects and athletes

Osteoblast anabolic activity, revealed as PINP serum concentration (Fig. 1), was 50 % greater in EXP, at rest, compared to CTRL ($p = 0.001$). On the other hand, no

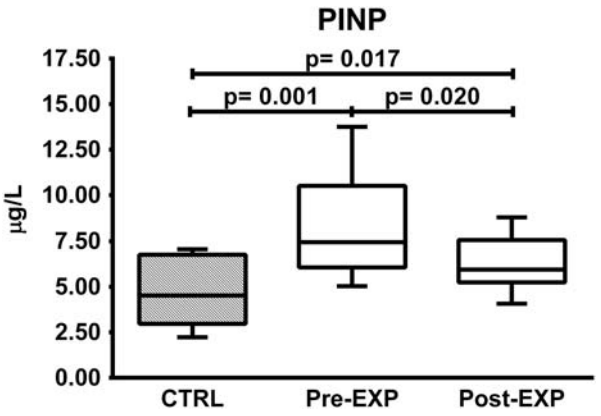

Fig. 1 Modification of serum PINP concentrations. Serum procollagen I N-terminal propeptide concentrations measured by ELISA in inactive control subjects (CTRL, oblique lines-filled boxes) and before (pre-EXP) and after (post-EXP) the race in experimental subjects (empty boxes). Significant changes are indicated by the corresponding p values

differences in Gla-OC, as well as in the distribution width of Gla-OC, were recorded between the two groups. Conversely, Glu-OC, the undercarboxylated form of OC ($p = 0.001$), and consequently the Glu-OC-to-Gla-OC ratio ($p = 0.001$), were significantly lower in EXP than in CTRL (Fig. 2a–c).

Among the metabolic markers tested, glucagon was higher in EXP ($p = 0.007$) and leptin was higher in CTRL ($p = 0.014$). C-peptide, insulin, GLP-1, resistin, visfatin, ghrelin, and GIP concentrations were all comparable between the two groups. Figure 3 illustrates the trend of the metabolic marker panel.

Comparisons between pre-race and post-race in athletes

The metabolic effort of the MUM was suddenly translated into a slight 15 % decrease in PINP ($p = 0.020$). However, it remained higher than in CTRL ($p = 0.017$), as shown by Fig. 1. Moreover, while Gla-OC was unchanged (Fig. 2a), Glu-OC was 50 % lower after the MUM ($p = 0.020$) and its serum concentrations were reduced by two-thirds compared to CTRL ($p = 0.017$). The Glu-OC/Gla-OC ratio follows the same trend as Glu-OC (Fig. 2b, c).

In general, MUM participation caused important metabolic changes involving almost all the parameters considered (Fig. 3a–i). C-peptide and insulin were lower after the MUM although only C-peptide reached the significance ($p = 0.005$). For both the markers, the post-MUM results were lower than CTRL ($p = 0.036$, for both) (Fig. 3a, b). Conversely, glucagon and GLP-1 increased in the EXP group after the race ($p = 0.002$ and $p = 0.005$, respectively) and the post-race concentrations were higher than those observed in CTRL ($p < 0.001$ and $p = 0.004$, respectively) (Fig. 3c, d). Resistin and visfatin showed the same trend. Here, both the parameters were 100 % higher after the MUM and compared to CTRL ($p < 0.001$) (Fig. 3e, f). Leptin was halved compared to the pre-MUM values ($p < 0.001$) and reduced to a quarter compared to CTRL ($p < 0.001$) (Fig. 3g).

Finally, Ghrelin and GIP were not modified by the intervention. However, contrarily to GIP and compared to CTRL, post-MUM ghrelin concentrations results were slightly increased ($p = 0.044$) (Fig. 3h, i).

Correlation analysis

The main finding from the correlation analysis is that, besides a few obvious associations (i.e., insulin vs. C-peptide, Glu-OC/Gla-OC vs. Glu-OC), which were highly significant in both groups (CTRL, post-race vs. pre-race EXP (ΔEXP)), many correlations were peculiar of either CTRL or EXP, or they were modified by the effort. The results from the correlation analysis are summarized in Table 1 and Supplementary Table 1.

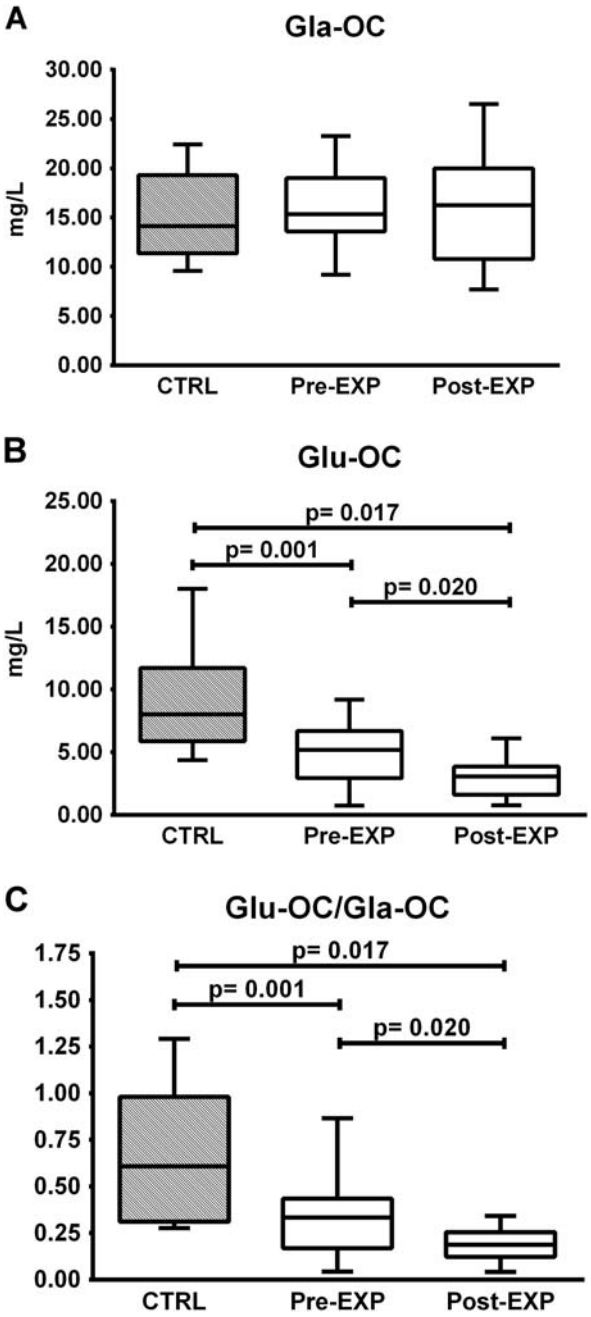

Fig. 2 Modification of serum OC forms concentrations. Serum carboxylated OC (Gla-OC, **a**) and undercarboxylated OC (Glu-OC, **b**) concentrations measured by ELISA in inactive control subjects (CTRL, oblique lines-filled boxes) and before (pre-EXP) and after (post-EXP) the race in experimental subjects (empty boxes). The calculated ratio between Glu-OC and Gla-OC is reported in **c**. Significant changes are indicated by the corresponding *p* values

Specifically, in CTRL bone markers did not show any correlation with either metabolic or metabolic-inflammation markers, while in ΔEXP PINP was fairly correlated with insulin and C-peptide and Glu-OC was directly correlated

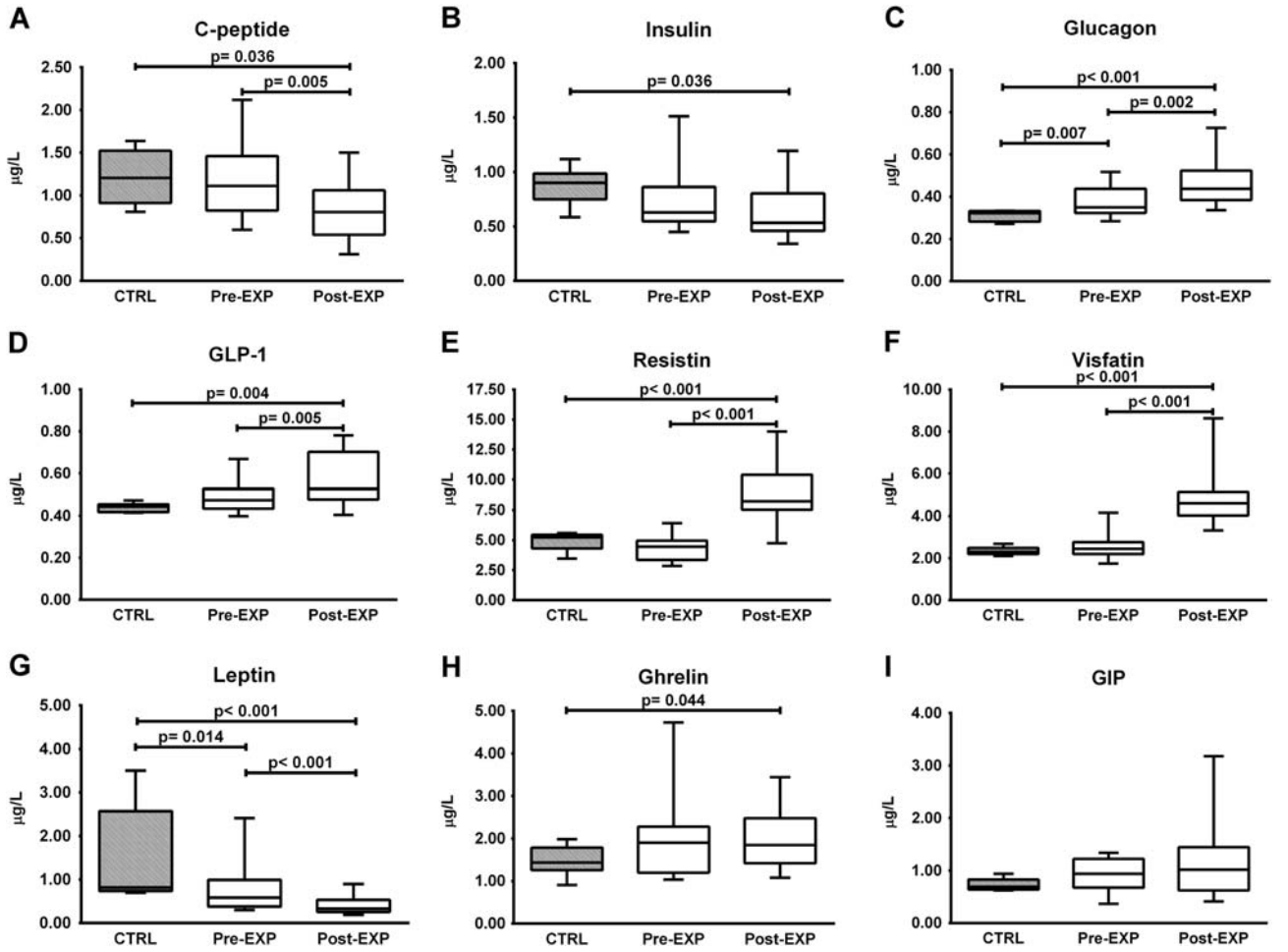

Fig. 3 Modification of serum concentrations of metabolic and metabolic inflammation markers. C-peptide (**a**), insulin (**b**), glucagon (**c**), glucagon-like peptide (GLP)-1 (**d**), resistin (**e**), visfatin (**f**), leptin (**g**), ghrelin (**h**), and gastric inhibitory polypeptide (GIP, **i**) measured by a multiplex assay, in inactive control subjects (CTRL, oblique lines-filled boxes) and before (pre-EXP) and after (post-EXP) the race in experimental subjects (empty boxes). Significant changes are indicated by the corresponding *p* values

with leptin and inversely with visfatin (fair correlations), although following Bonferroni's correction these associations were lost.

The strong correlations between GLP-1 and ghrelin, and visfatin and glucagon in CTRL were lost in ΔEXP, as well as the good-to-strong correlations between leptin and insulin/C-peptide, and resistin and GIP although these latter resulted not significant following Bonferroni's correction.

On the contrary in ΔEXP, but not in the CTRL, we found good correlation in the following comparisons: visfatin vs. C-peptide, visfatin vs. leptin (indirect), GLP-1 vs. GIP, resistin vs. glucagon, PINP vs. Glu-OC/Glu-OC-to-Gla-OC ratio. Visfatin and GIP, which showed an inverse good correlation in CTRL (before Bonferroni's correction), became directly correlated in ΔEXP.

Finally, resistin vs. visfatin, Glu-OC and Glu-OC/Gla-OC vs. C-peptide were correlated only in ΔEXP.

Discussion

MUMs are extreme aerobic activities that presuppose an extensive training as well as a certain degree of natural selection [25]. Accordingly, MUM runners experience both the beneficial effects of the endurance training (e.g., improved metabolic, inflammatory, cardiovascular, and bone turnover profile) as well as the dangers due to overtraining and overuse (e.g., inflammation, muscle damage, metabolic acidosis, bone resorption) [8, 9]. Thus, MUMs are interesting models for investigating how the homeostatic response intervenes to regulate the energy expenditure and to preserve the homeostasis [20].

Our results show that runners experienced an increased bone formation rate, both before and after the race, than their control counterparts, as marked by PINP levels (Fig. 1), though the effort of the race slightly, but significantly, counteracted it. Instead, no differences were

Table 1 Correlation analysis

			CTRLs		ΔEXP	
			r	*p*	*r*	*p*
GIP	vs.	C-peptide	n.s.		n.s.	
GLP-1	vs.	Ghrelin	− 0.94	< 0.001	n.s.	
	vs.	GIP	n.s.		0.60	< 0.001
Glucagon	vs.	GLP-1	n.s.		0.71	< 0.001
Insulin	vs.	C-peptide	0.97	< 0.001	0.83	< 0.001
	vs.	GIP	n.s.		n.s.	
	vs.	GLP-1	n.s.		n.s.	
Leptin	vs.	C-peptide	n.s.		n.s.	
	vs.	Insulin	n.s.		n.s.	
Resistin	vs.	GIP	n.s.		n.s.	
	vs.	GLP-1	n.s.		n.s.	
	vs.	Glucagon	n.s.		0.57	< 0.001
	vs.	Leptin	n.s.		n.s.	
Visfatin	vs.	C-peptide	n.s.		0.61	0.001
	vs.	GIP	n.s.		0.55	< 0.001
	vs.	GLP-1	n.s.		0.54	0.001
	vs.	Glucagon	− 0.86	0.001	n.s.	
	vs.	Insulin	n.s.		n.s.	
	vs.	Leptin	n.s.		− 0.53	0.001
	vs.	Resistin	n.s.		0.55	< 0.001
PINP	vs.	C-peptide	n.s.		n.s.	
	vs.	Insulin	n.s.		n.s.	
Glu-OC	vs.	C-peptide	n.s.		0.65	0.002
	vs	Insulin	n.s.		0.65	0.004
	vs.	Leptin	n.s.		n.s.	
	vs.	Visfatin	n.s.		n.s.	
	vs.	PINP	n.s.		0.51	0.003
Glu/Gla	vs.	C-peptide	n.s.		0.68	0.001
	vs.	PINP	n.s.		0.69	< 0.001
	vs.	Glu-OC	0.85	< 0.001	0.84	< 0.001

The table shows the correlation coefficient (*r*) and the *p* value (*p*) for the correlation analysis in the control group (CTRL) and the post-race vs. pre-race experimental group (ΔEXP). Following application of the Bonferroni's correction the significance has been set at $p < 0.004$

n.s. not significant

The complete version of this table, presenting all the calculated and "*r*" coefficients and relative "*p* values", can be found in the supplementary material

found in the three evaluation series for Gla-OC (Fig. 2a). Although considered, for a long time, a bone formation marker [26], OC is now recognized as an hormone involved in the endocrine signaling from the bone to the other tissues [16].

Acutely, the slowdown in bone formation observed in this study may represent the prompt metabolic response of bone to the increased energy demands of the muscles. Similarly Kerschan-Schindl K et al., in the two consecutive studies on a 246-km-long ultramarathon, not only confirmed the transient suppression of the bone formation, but also highlighted the acute increase in bone resorption [27, 28]. Chronically, MUM runners seem to experience a prevalent bone formation, when compared to subjects with a low PA profile, confirming previous data on different populations [9]. This is likely due to the constant load applied to the skeleton during both training and competitions. Further, the current study supports the hypothesis that chronic muscular traction alone on the bone is an insufficient stimulus for bone anabolism in absence of load [12], as already deduced by studies on comparable strenuous but not weight-bearing endurance activities, such as cycling

stage race, in which bone metabolism is shifted towards resorption [11, 29]. Moreover, we confirm that, compared to OC, PINP is much more suitable as a marker for monitoring the bony acute effects of exercise [9] as well as more useful than the common radiological analyses (bone mineral density, bone mineral content, and dualenergy X-ray absorptiometry) [30].

Surprisingly, the metabolic profiles of resting MUM runners and controls only differ for glucagon and leptin, which are respectively lower and higher in CTRL than in EXP (Fig. 3a–i). Serum leptin is inversely correlated with training status and performance in marathoners; that is, the better the athlete is trained and ranked, the lower is leptin concentration [31]. On the contrary, the differences in glucagon could be due to the peculiar diet followed by these athletes who, generally, increase the carbohydrates intake the week before the race along with decreased training volume and intensity [32]. Further, the post-race behaviors of C-peptide, insulin, and glucagon clearly mark the reduced concentration of glucose into the bloodstream due to its usage by muscles (and brain), derived from liver glycogenolysis and adipose tissue lipolysis. Specifically, the decrease in C-peptide, which more sensibly than insulin reflects the glucose-dependent response of β-cells [33], is linked to improved peripheral insulin sensitivity [34, 35]. The increase of the intestinal incretin GLP-1, though not accompanied by the increase of GIP, is aimed at contrasting the decline of insulin. Indeed, incretins enhance β-cells' insulin secretion and β-cells' viability and proliferation [36].

The stability of ghrelin [37], together with the evident decrease in leptin [38], indicates an attempt to restore insulinemia. Indeed, leptin is an adipokine involved in energy intake regulation and energy expenditure which has also a key role in regulating glucose and lipid metabolism [39]. Serum leptin is affected by exercise but how this happens is still debated. However, long-lasting continuous exercises (i.e., ultramarathons, sky races) decrease leptin concentrations dependently to the energy expenditure [39, 40]. As discussed above, OC, and possibly Glu-OC, are inducers of insulin release by β-cells [16]. The decrease in the Glu-OC and in the Glu-OC-to-Gla-OC ratio after the race clearly supports the hypothesis of the key (direct or indirect) role of OC in glucose metabolism. Since the whole set of metabolic markers here studied are energy consumption-dependent, and almost all these changes correlated with Glu-OC and Glu-OC-to-Gla-OC ratio (Table 1, Supplementary Table 1), it can be argued that Glu-OC modifications mark the energy expenditure. The correlation analysis (Table 1), though very complex and not exhaustively defining the intricate hormone network sustaining the homeostasis, clearly indicates that the canonical hormonal control (here depicted in CTRL) is completely subverted in athletes both at rest and after the strenuous exercise, as we previously demonstrated in different aerobic endurance activities [11, 12, 29, 41]. Despite the improvement of the post-race overall metabolic status in runners, compared to pre-race and controls, the metabolic inflammatory markers (i.e., resistin and visfatin) are two-fold increased. Visfatin (Nampt) is primarily expressed by VAT as both the intracellular nicotinamide phosphoribosyltransferase (Nampt [EC2.4.2.12]) form acting in NAD biosynthesis, and the extracellular form acting as an insulin-mimetic, pro-inflammatory/immuno-modulating adipokine. Visfatin blood concentrations are associated with obesity/VAT accumulation, insulin resistance, and energy-bone crosstalk [40, 42]. Resistin, also produced by adipose tissues, is involved in insulin resistance, adipogenesis and inflammation. Though both hormones shared insulin resistance and pro-adipogenic effects, in this study their prevalent role seems to be pro-inflammatory and/or energy deficit signallers [40].

Given that bone remodeling is both an energetically highly expensive and a nonstrikingly essential process for short-term survival, it is limited in favor of lifesaving physiological activities when the homeostasis is profoundly perturbed. However, disorders of the energy metabolism (e.g., diabetes) have profound effects on bone [43]. On the other hand, being load the main determinant driving bone metabolism [11–13], and being bone the main mechanosensitive organ, bone cells first signal impelling increased metabolic energy needs (i.e., in exercise). Thus, it is consistent with the theory about the close relationship between bone and energy metabolisms [14, 16]. Our data suggest the following: acute energy usage due to the very strenuous effort decreases bone formation (PINP) and, in parallel, energy supply to the bone is reduced (marked by Glu-OC [16]). However, in experienced ultra-marathon runners, compared to controls, bone formation seems to be chronically increased. Noteworthy, despite a comparable metabolic profile at rest, skeletal requirements for energy (Glu-OC) are reduced in runners and we hypothesize that this is linked to its greater incorporation into bone matrix [16]. A possible confirmation of this latter hypothesis comes from the inverse behavior of Glu-OC in cyclists who, as explained above, perform a strenuous endurance activity but in absence of load, which causes bone resorption (and, thus, less incorporation of OC into bone matrix) [11]. Bone metabolism is regulated by several hormones, many of them are analyzed here. Insulin drives bone anabolism and a deregulated insulin physiology profoundly affects bone metabolism. Insulin increases OC synthesis in osteoblast, via Runx2 induction, but it also stimulates osteoclasts, which are involved in OC decarboxylation [16], by suppressing osteoprotegerin expression in osteoblasts. Hence, insulin stimulates bone turnover and Glu-OC release which, in turn, stimulates insulin sensitivity and secretion [43]. Also leptin positively regulates bone metabolism directly or

through neural circuits [44] and the observed reduction in leptin is consistent with this evidence. Ghrelin supports osteoblast differentiation and function, both directly and indirectly, through the GH–IGF (growth hormone-insulin-like growth factor) axis and its interaction with leptin [45].

Although the number of participants is limited, due to the really peculiar context the selection of subjects competing in MUMs naturally limits the inter-individual variability. This accounts for clearly delineated trends and, thus, gives univocal results. Noteworthy, the novelty of our results collides with the impossibility to compare them with those obtained in similar contexts, due to the scarce literature on this topic. Another possible limitation is represented by the fact that at least some of the changes observed could be due to exercise-dependent changes in plasma volume [46]. However, although its importance, previous studies, conducted over different seasons in wide cohorts of athletes, demonstrated that in such competitions there are no differences between pre-race and post-race hemoglobin and hematocrit [47, 48], need to calculate plasma volume changes, possibly due to the free access to beverages.

The present research, although far from explaining the hormonal changes governing the relationship between energy and bone metabolisms, clearly depicts the metabolic response to a strenuous prolonged physical effort. Here we show that the effort drives the homeostatic response towards the optimization of the energy usage possibly at the expenses, among other functions, of bone. Besides the metabolic inflammation induced by the race, the markedly metabolic improvement after the race, confirms, one more time, the positive effects of endurance.

Bone metabolism and the regulation of energy consumption are deeply related, even when homeostasis is hardly proved. The effort-dependent energy consumption drives the overall osteo-metabolic hormonal modifications, possibly to preserve life-saving functions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have conflict of interest. The results of the present study do not constitute endorsement by ACSM.

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