Eur J Appl Physiol (2004) 92: 227–230
DOI 10.1007/s00421-004-1092-7

SHORT COMMUNICATION

L. James · G. Onambele · R. Woledge · D. Skelton
D. Woods · K. Eleftheriou · E. Hawe · S. E. Humphries
F. Haddad · H. Montgomery

IL-6–174G/C genotype is associated with the bone mineral density response to oestrogen replacement therapy in post-menopausal women

Accepted: 24 February 2004 / Published online: 9 April 2004

Abstract A reduction in interleukin-6 (IL-6) activity may contribute to the beneficial effects of hormone replacement therapy (HRT) on the menopausal decline in bone mineral density (BMD). We have examined this hypothesis using a genetic strategy. The –174C (rather than G) IL-6 gene variant is associated with lower IL-6 expression. As such, we might anticipate the C allele to be associated with a greater response to HRT. We have tested this hypothesis. Mean three-site [spine (L1-L4), neck of femur, and Ward's triangle] BMD was measured in 65 women in a 1-year randomised controlled trial of HRT with 0.625 mg oestrogen/day and 0.15 mg norgestrel ($n = 30$). Baseline BMD was genotype-independent for both the control and HRT group. In the control group, the percentage change in BMD after 1 year was similar between genotypes ($P = 0.45$). In contrast, in the HRT group, the rise was genotype-dependent. Those homozygous for the G allele showed a 3.62 (2.14)% increase in BMD compared with 10.44 (4.68)% for the C-homozygous group. Heterozygotes had an intermediate BMD increase of 5.6 (2.82)% [$P = 0.006$ (P value for interaction between HRT and genotype was 0.04)] Although the study was limited by its small sample size, these are the first data to demonstrate the importance of IL-6 genotype in determining response to oestrogen therapy, rather than its physiological withdrawal.

Keywords Interleukin-6 · Polymorphism · Bone remodelling · Hormone replacement therapy · Bone mineral density

L. James · K. Eleftheriou · F. Haddad
Department of Orthopaedic Surgery,
University College London NHS Trust,
Mortimer Street, London, W1N 8AA, UK

E. Hawe · S. E. Humphries · H. Montgomery (✉)
Department of Medicine,
Centre for Cardiovascular Genetics,
The BHF Labs,
RF & UCL Medical School,
5 University Street,
London, WC1E 6JF, UK
E-mail: rmhahum@ucl.ac.uk
Tel.: +44-0207-6796965
Fax: +44-0207-6796212

G. Onambele
Department of Sport and Exercise,
Centre for Biophysical and Clinical Research into Human Movement, Manchester Metropolitan University,
Alsager, Cheshire, ST7 2HL, UK

G. Onambele · R. Woledge · D. Skelton
UCL Institute of Human Performance,
Royal National Orthopaedic Hospital Trust,
Brockley Hill, Stanmore,
Middlesex, HA7 4LP, UK

D. Woods
Department of Medicine,
Freeman Hospital, Newcastle upon Tyne,
NE7 7DN, UK

Introduction

Post-menopausal bone resorption appears dependent on the increased production of the pro-inflammatory cytokine interleukin-6 (IL-6) by bone cells (Manolagas and Jilka 1995). A functional G/C polymorphism at position −174 in the IL-6 gene promoter has been identified where the C allele is associated with lower in vitro transcriptional activity and lower circulating IL-6 levels (Fishman et al. 1998). Consistent with a role for IL-6 in mediating bone resorption, GG genotype is associated with accelerated bone demineralisation in post-menopausal women (Ferrari and Garnero 2001). Meanwhile, hormone replacement therapy (HRT) with supplemental oestrogen is protective, and may indeed partially reverse such demineralisation. One year of treatment with Prempak C (0.625 mg oestrogen/day and 0.15 mg norgestrel) was associated with a rise in bone mineral density (BMD) in post-menopausal women (Skelton et al. 1999). However, whether this response is partly mediated through regulation of IL-6 is not known. We have

addressed this issue by examining the influence of IL-6 genotype on the BMD response to HRT in this group.

Methods

The study received ethical approval from the UCL & UCH Ethics Committee. Written informed consent was obtained from all participants.

Subjects

Subjects were drawn from a randomised trial of HRT in post-menopausal women, described in detail by Skelton et al. (1999). In brief, changes in BMD were assessed during 12 months of treatment with Prempak C, consisting of 0.625 mg oestrogen/day and 0.15 mg norgestrel for 12 consecutive days in each cycle, for 13 cycles of 4 weeks, and compared to untreated controls receiving placebo. Women entering the trial had oestrogen levels below 150 pmol l^{-1}, confirming their post-menopausal hormonal status. We were able to contact, and retrospectively genotype, 65 of the original group of 122 women who had taken part in the study. Genotyping was carried out by individuals blind to the initial study results.

DNA extraction and IL-6 genotype assessment

DNA was extracted from a 10 ml sterile 0.9% saline mouthwash sample and IL-6 –174 G/C genotype was determined by polymerase chain reaction (PCR) as previously described (Fishman et al. 1998). The 190 base pair product was digested overnight at 37°C with the restriction endonuclease *Nla*III to yield DNA fragments of 143 and 47 base pairs in the case of the C allele. These were visualised using an 8% MADGE (microplate array diagonal gel electrophoresis) gel stained with ethidium bromide.

Bone mineral density

BMD was recorded at regular intervals (baseline, weeks 26 and 52) in the spine (L1-L4), total hip, femur neck, and Ward's triangle using dual energy X-ray absorptiometry (QDR-4500A; Hologic, Inc.), and mean BMD was calculated. Change in mean BMD between baseline and week 52 was calculated. As reported in the original study, analysis was performed in an a priori manner to assess global (not individual) measure of skeletal BMD. All sites but the femur neck responded to HRT (Skelton et al. 1999). Data are thus presented for the three responding sites.

Statistics

Statistical analysis was conducted by the senior statistician at The Centre for Cardiovascular Genetics, using intercooled STATA version 7.0 (College Station, Texas). Differences in baseline variables by IL-6 genotype and HRT were considered using ANOVA. BMD changes in controls and those randomised to HRT were considered via paired *t*-tests. Differences in percentage change in BMD by IL-6 and HRT were considered using ANOVA. In view of the low number of C-allele homozygotes, statistical analysis was also carried by pooling the ≥1 C allele individuals (i.e. CC and GC) and comparing this pooled group with the GG homozygotes. There was no evidence to suggest deviations from normality for either percentage change or baseline BMD, nor was there any evidence to suggest heteroscedacity between groups (Bartlett's test). Values are presented as mean (SD) unless otherwise stated. Throughout, a P value of < 0.05 was considered significant.

Results

DNA was available and genotyping successful in 65 women, all of whom had completed the trial. The overall genotype distribution (25 GG, 33 GC, 7 CC) did not differ significantly from Hardy-Weinberg equilibrium (Fishman et al. 1998). Of these, 30 were in the treatment group (12 GG, 16 GC, 2 CC) and 35 were controls (13 GG, 17 GC, 5 CC). Baseline characteristics of age 60.7 (0.01) years, height 162.9 (0.01) cm, body mass 64.0 (0.02) kg, body mass index (BMI) 24.1 (0.01)kg m^{-2}, mean three-site BMD 0.75 (0.01) g.cm^{-2} were independent of genotype or treatment group, and were no different from those for whom genotypes were not available.

Bone mineral density

Baseline BMD was independent of IL-6 genotype both in the control and HRT groups ($P = 0.13$ and 0.23 respectively; see Table 1). Overall, BMD rose by 5.13 (3.1)% with HRT ($P < 0.00005$ by paired *t*-test), and to a lesser extent, by 1.23 (2.4)%, with placebo ($P = 0.004$) Table 1 compares BMD between HRT and controls. Amongst controls, BMD change was 0.59 (2.10)% for GG, 1.73 (2.29)% for GC and 1.20 (3.54)% for CC genotype ($P = 0.45$) and was independent of genotype. However, the response to HRT was IL-6 genotype-dependent (Fig. 1). Those homozygous for the G allele showed a 3.62 (2.14)% BMD increase compared with 10.44 (4.68)% for the C-homozygous group. The heterozygous group had an intermediate rise of 5.6 (2.82)% ($p = 0.006$). Comparison between the GG-homozygous group and the pooled ≥1 C allele group also showed a similar relationship (Fig. 1). The interaction between HRT and genotype was statistically significant ($P = 0.04$).

Discussion

We show here, for the first time, that IL-6 may play a role in dictating the magnitude of the BMD response to

Table 1 Baseline, post-treatment and percentage increase in bone mineral density (BMD) according to IL-6 genotype for control vs hormone replacement therapy (HRT) groups [values are mean (SD)]. Baseline BMD was genotype-independent for both the control and HRT group (*$P = 0.13$ and 0.23 respectively). There was no evidence of a difference in the percentage change for BMD between the IL-6 genotypes amongst the controls ($^{\P}P = 0.45$), but in the HRT group the rise was genotype dependent ($^{\P}P = 0.006$). A similar effect was seen when the GG group was compared with the ≥ 1C pooled group ($^{\forall}P = 0.22$ and 0.03 respectively)

Genotype	Control group			HRT group		
	Baseline BMD (g/cm^2)	Post BMD (g/cm^2)	BMD increase (%)	Baseline BMD (g/cm^2)	Post BMD (g/cm^2)	BMD increase (%)
All	0.74 (0.08)	0.75 (0.08) ($n = 35$)	1.23 (2.40)	0.76 (0.12)	0.80 (0.13) ($n = 30$)	5.13 (3.10)
GG	0.72 (0.09)	0.73 (0.09) ($n = 13$)	0.59 (2.10)	0.78 (0.13)	0.81 (0.13) ($n = 12$)	3.62 (2.14)
GC	0.77 (0.07)	0.78 (0.07) ($n = 17$)	1.73 (2.29)	0.76 (0.12)	0.80 (0.12) ($n = 16$)	5.60 (2.82)
CC	0.71 (0.07)	0.72 (0.09) ($n = 5$)	1.20 (3.54)	0.61 (0.03)	0.68 (0.06) ($n = 2$)	10.44 (4.68)
	$P = 0.13$*		$P = 0.45^{\P}$	$P = 0.23$*		$P = 0.006^{\P}$
≥1C	0.76 (0.07)	0.77 (0.08) ($n = 22$)	1.61 (2.54)	0.75 (0.12)	0.79 (0.12) ($n = 18$)	6.14 (3.28)
			$P = 0.23^{\forall}$			$P = 0.03^{\forall}$

oestrogen replacement. Such a putative role for IL-6 is supported by other data. Osteoporosis is the result of an imbalance in the levels of bone formation (by osteoblasts) and bone resorption (by osteoclasts). Interleukin-6 has known stimulatory effects on cells of the osteoclast lineage (Roodman 1992). IL-6 is thus implicated in the pathogenesis of bone loss associated with oestrogen deficiency (Ferrari and Garnero 2001), as well as in the bone resorption of systemic-onset juvenile chronic arthritis (Fishman et al. 1998). However, it remains unclear whether the effects observed in these studies, as in ours, are mediated through alterations in local (bone-synthesised) or systemic (circulating) IL-6. Certainly, circulating IL-6 levels do rise with acute exercise (Northoff et al. 1994) and are associated with a rise in markers of bone resorption. Systemic IL-6 levels are also known to correlate with rates of post-menopausal bone loss (Scheidt-Nave and Bismar 2001). Conversely, local bone IL-6 may play an important role; local IL-6 is implicated in microgravity-associated bone resorption (Kumei et al. 1996). The implied role of IL-6 across different age ranges, sexes (Ferrari and Garnero 2001) and diverse disease states (Fishman et al. 1998) suggests a fundamental role for IL-6 in driving bone resorption.

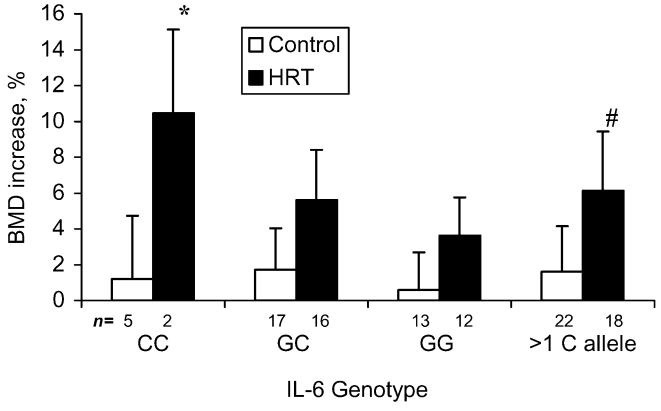

Fig. 1 Bone mineral density (BMD) percentage increase by IL-6 genotype in the control and hormone replacement therapy (HRT) group. Data are percentage changes for three-site BMD. The ≥1 C allele groups are made up of the CC and GC genotype individuals for each treatment group. *$P = 0.006$ for comparison of HRT group by individual genotype. $^{\#}P = 0.03$ for comparison of HRT group between the GG and ≥1 C allele genotypes. *Error bars* represent SD

The association of the IL-6 G allele with accelerated bone resorption is in keeping with the similar, previously reported associations with juvenile chronic arthritis (Fishman et al. 1998) and accelerated post-menopausal bone loss (Ferrari and Garnero 2001). Our data both support these observations, and extend them, showing a novel interaction of oestrogen *exposure* and IL-6 genotype, as opposed to *withdrawal* of oestrogen alone.

Our study was limited by its small sample size (e.g. only 5 and 2 C-homozygous individuals in the control and treatment group respectively). Thus, despite their statistical significance, even when pooling C-allele carriers, further studies are evidently warranted to confirm and extend these observations. In addition, those of different race should be studied. Nonetheless, these data offer an exciting insight into the mechanisms through which HRT may influence BMD, and confirm IL-6 as a potential therapeutic target in this regard.

Acknowledgements The original core study was funded by an educational grant from Wyeth Lederle. L.J. is funded as a research fellow by the charity Research into Ageing, UK. H.M. is funded by the Portex Endowment at the institute of Child health, London. The British Heart Foundation core funds The Centre for Cardiovascular Genetics, UCL. O.N.G.L. was funded by the Conquest Hospital in Hastings. The study received ethical approval from the UCL & UCH Ethics Committee in full compliance with the current U.K. laws. Written informed consent was obtained from all participants.

References

Ferrari S, Garnero P (2001) A functional polymorphic variant in the interleukin-6 gene promoter associated with low bone resorption in postmenopausal women. Arthritis Rheum 44:196–201

Fishman D, Faulds G, et al (1998) The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 102:1369–1376

Kumei Y, Shimokawa H, Katano H, et al (1996) Microgravity induces prostaglandin E2 and interleukin-6 production in normal rat osteoblasts: role in bone demineralization. J Biotechnol 47: 313–324

Manolagas SC, Jilka RL (1995) Bone marrow, cytokines and bone remodeling. N Engl J Med 332: 305–311

Northoff H, Weinstock C, Berg A (1994) The cytokine response to strenuous exercise. Int J Sports Med 15 [Suppl 3]:S167–171

Roodman G (1992) Interleukin-6: an osteotropic factor? J Bone Miner Res 7:475–476

Scheidt-Nave C, Bismar H (2001) Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. J Clin Endocrinol Metab 86: 2032–2042

Skelton DA, Phillips SK, et al (1999) Hormone replacement therapy increases isometric muscle strength of adductor pollicis in post-menopausal women. Clin Sci 96: 357–364