Zoledronic acid does not affect insulin resistance in men receiving androgen deprivation therapy: a prespecified secondary analysis of a randomised controlled trial

Ada S. Cheung (iD), **Rudolf Hoermann** (iD), **Jasmine Zhu, Daryl Lim Joon, Jeffrey D. Zajac and Mathis Grossmann**

Abstract

Background: Animal studies suggest that undercarboxylated osteocalcin may improve insulin sensitivity *via* its effect on testicular testosterone production. Human studies have been conflicting. Men undergoing androgen deprivation therapy (ADT) for prostate cancer experience profound hypogonadism resulting in increased insulin resistance. In a randomised controlled trial (RCT) of zoledronic acid versus placebo in men commencing extended-duration ADT, we aimed to examine the effects on fat mass and glucose metabolism. We hypothesised that zoledronic acid, which reduces osteocalcin concentrations, would worsen ADT-induced insulin resistance.

Methods: This was a prespecified secondary analysis of an RCT designed to evaluate the effects of zoledronic acid on bone microarchitecture in 76 men with non-metastatic prostate cancer undergoing curative radiotherapy combined with adjuvant ADT ($n = 39$ randomised to a single dose of zoledronic acid 5 mg, $n = 37$ randomised to matching placebo). Oral glucose tolerance tests to determine Matsuda Index were performed at 0, 3, 12 and 24 months. Using a mixed model, mean adjusted differences [MAD (95% confidence interval)] between the groups over time are reported.

Results: Over 24 months of ADT, fat mass increased and lean mass decreased for both groups, with no significant between group difference [MAD 401 g (–1307; 2103), $p = 0.23$ and –184 g (–1325; 955), $p = 0.36$ respectively]. Bone remodelling markers C-telopeptide [MAD –176 ng/l (–275; –76), $p < 0.001$ and P1NP –18 mg/l (–32; –5), $p < 0.001$] as a surrogate for osteocalcin, remained significantly lower in the zoledronic acid group, compared with placebo. There was no mean adjusted between-group difference for homeostatic model assessment 2 insulin resistance (HOMA2-IR) [–0.2 (–0.6; 0.2), $p = 0.45$], HbA1c [–0.1% (–0.3; 0.1), $p = 0.64$] or Matsuda Index [0.8 (–1.1; 2.7), $p = 0.38$]. The Matsuda Index decreased in both groups consistent with worsening insulin resistance with ADT.

Conclusion: A single dose of zoledronic acid does not appear to influence glucose metabolism in men newly commencing ADT. Further study to evaluate the endocrine relationship between bisphosphonates, bone and glucose metabolism is required.

Trial Registration Number: [ClinicalTrials.gov identifier: NCT01006395].

Keywords: androgen deprivation, bisphosphonates, diabetes, insulin resistance, prostate cancer

Correspondence to:
Mathis Grossmann
Department of Medicine (Austin Health), The University of Melbourne, VIC, Australia

Department of Endocrinology, Austin Health, Studley Road, Heidelberg, VIC 3084, Australia
mathisg@unimelb.edu.au

Ada S. Cheung
Department of Medicine (Austin Health), The University of Melbourne, Victoria, Australia

Department of Endocrinology, Austin Health, Heidelberg, VIC, Australia

Rudolf Hoermann
Department of Medicine (Austin Health), The University of Melbourne, VIC, Australia

Jasmine Zhu
Department of Endocrinology, Austin Health, Heidelberg, VIC, Australia

Daryl Lim Joon
Department of Radiation Oncology, Austin Health, Heidelberg, VIC, Australia

Jeffrey D. Zajac
Department of Medicine (Austin Health), The University of Melbourne, VIC, Australia

Department of Endocrinology, Austin Health, Heidelberg, VIC, Australia

Introduction

The osteoblast-secreted molecule osteocalcin, in particular the undercarboxylated form, has been reported in animal studies to have endocrine actions that improve insulin sensitivity, insulin secretion, beta cell proliferation and energy metabolism.[1] The mechanism of this action is unclear. In addition to potentially regulating energy homeostasis, osteocalcin has been found to regulate male fertility in mice by enhancing testosterone production by Leydig cells.[2] It is possible that undercarboxylated osteocalcin may affect insulin sensitivity *via* its effect on the testis and testosterone concentrations. Studies in rats have suggested that bisphosphonates, which reduce undercarboxylated osteocalcin may increase fasting blood glucose and worsen insulin sensitivity.[3]

Human studies, however, have been conflicting. Cross-sectional studies suggested serum total osteocalcin may be inversely associated with insulin resistance in both men and women,[4,5] but the association between undercarboxylated osteocalcin and insulin secretion remains unclear.[6,7]

Bisphosphonates, especially potent intravenous formulations such as zoledronic acid, are anti-resorptive medications that reduce bone remodelling markers including total osteocalcin and undercarboxylated osteocalcin by >50%.[8,9] Prospective studies assessing the effects of bisphosphonate therapy on glucose metabolism are lacking.

Men with prostate cancer receiving adjuvant androgen deprivation therapy (ADT) experience profound hypogonadism, and the resultant severe sex steroid deficiency leads to an increase in insulin resistance. This is likely mediated by metabolically adverse changes in body composition, including loss of muscle mass and gain of fat mass.[10,11] Over time, increased insulin resistance leads to an increased risk of incident diabetes.[12]

Men undergoing ADT are therefore an attractive population to study effects of bisphosphonates on glucose metabolism because, as they experience an increase in insulin resistance, an adverse effect of bisphosphonate on glucose metabolism may be augmented. Therefore, as bisphosphonate therapy reduces osteocalcin concentrations,[13,14] we hypothesised that zoledronic acid given to men with prostate cancer receiving ADT, would worsen ADT-induced insulin resistance as measured by the Matsuda Index.[15] The Matsuda Index is based upon an oral glucose tolerance test — rather than insulin measurements as used in the homeostatic model assessment (HOMA) index – was chosen given the ease of calculation for serial monitoring over time and its close approximation to whole-body insulin sensitivity relative to the gold standard hyperinsulinemic euglycemic clamp.[15] In this prespecified secondary analysis of a randomised controlled trial (RCT) designed to evaluate the effects of zoledronic acid on bone microarchitecture in men with non-metastatic prostate cancer undergoing curative radiotherapy combined with adjuvant ADT,[16] we prospectively examine the effects of extended-duration ADT on fat mass and glucose metabolism using a detailed assessment of glucose metabolism, including determination of Matsuda Index.

Material and methods

Study design

We conducted a double-blind, randomised, parallel group, placebo-controlled trial to compare the effects of a single dose of zoledronic acid treatment with placebo in men with prostate cancer newly commencing ADT. The primary study outcome was bone microarchitecture measured by high resolution-peripheral quantitative computed tomography (HR-pQCT) over 24 months following administration of the research trial medication, which has been reported previously.[16] We hereby report a prespecified secondary outcome of fat mass and insulin resistance estimated with the Matsuda Index. The trial was approved by Human Research Ethics Committee at Austin Health (H2010-03833) and was registered [ClinicalTrials.gov identifier: NCT01006395]. All participants provided written and informed consent.

Participants and setting

All participants were men with non-metastatic prostate cancer (T1-3 Nx M0), about to commence treatment with gonadotrophin releasing hormone (GnRH) agonists to suppress androgen production, in whom treatment with ADT was intended for at least 2 years and were able and willing to comply with the study protocol requirements. GnRH agonists were selected by treating clinicians and comprised of either goserelin, triptorelin or leuprorelin at standard doses. Localised prostate cancer is not expected to affect glucose

metabolism.[17] Participants were living independently in the community, ambulant, fully active, unrestricted in their physical activities with normal performance status (Eastern Co-operative Oncology Group performance status 0) and had no evidence of metastases on bone scan or CT abdomen/pelvis at entry to the study. Men were excluded if they had evidence of androgen deficiency (baseline total testosterone <10 nmol/l, the lower limit of the reference range at our laboratory), known metabolic bone disease prior to study entry (such as osteoporosis, osteomalacia, parathyroid abnormalities or any condition with an abnormality of calcium, phosphate, magnesium or vitamin D homeostasis), had active liver, renal or thyroid disease, had previously used any antiresorptive therapy, had a history of long-term glucocorticoid therapy of at least 3 months at a dose of at least 7.5 mg per day of prednisolone or equivalent), had contraindications to zoledronic acid (atrial fibrillation, renal impairment, vitamin D deficiency <50 nmol/l, active dental disease) or had major depression, recreational drug use, alcohol dependence, known HIV/AIDS or any disease which was likely lead to serious illness or death within the study period. Participants were recruited from an outpatient clinic for men with prostate cancer at a tertiary referral hospital (Austin Health, Melbourne, Australia). Two men (both in the zoledronic acid group) started osteoporosis therapy, a prespecified protocol violation leading to their exclusion from the analyses.

Intervention
Blinded research trial medication (zoledronic acid 5 mg or matching placebo) was administered intravenously over 15 min as a single dose within 6 weeks of commencement of ADT.

Outcomes
All biochemical tests were drawn fasting between 8 a.m. and 10 a.m. at 0, 3, 6, 12 and 24 months after administration of the research trial medication. Insulin resistance was calculated from fasting glucose and fasting insulin as HOMA2-IR (HOMA2-IR calculator, http://www.dtu.ox.ac.uk/homacalculator/) as described.[18] HbA1c (%) was measured by turbidometric inhibition immunoassay (Roche Cobas Integra 800), which had an inter-assay variation of 3.4% at HbA1c 5.5% and 2.2% at HbA1c 9.7%. A 2-h oral glucose tolerance test (OGTT) according to Matsuda's

protocol was performed at 0, 3, 12 and 24 months after administration of research trial medication to determine the Matsuda index.[15] Matsuda's index of whole-body insulin sensitivity is highly ($r = 0.73$, $p < 0.0001$) correlated with the rate of whole-body glucose disposal during the euglycemic insulin clamp.[15] Higher Matsuda indices indicate higher insulin sensitivity (or less insulin resistant). Each parameter was analysed on the same platform, with no change in assay methodology in any of the parameters during the course of this study. Fasting serum total testosterone was determined using an immunometric testosterone assay (Access, Beckman Coulter, Inc.) with a minimum detection limit of 0.4 nmol/l and an inter-assay variation of 5.7% at 4.7 nmol/l. The reference range was 10.0–27.6 nmol/l, derived from an independent reference panel of healthy reproductively normal young men.[19] Bone remodelling markers procollagen type 1 amino-terminal propeptide (P1NP) and beta carboxyl-terminal type I collagen telopeptide (CTX) were measured as a surrogate for osteocalcin by electrochemiluminescence on Roche Cobas C8000 (Roche Diagnostics). The lower limit of detection for P1NP is 5 µg/l, with an inter-assay variation of 5.4% at 73.5 µg/l. The inter-assay variation for P1NP was 2.8% at 34 µg/l and 2.6% at 205 µg/l, and for CTX was 1.3% at level of 334 ng/l and 1.3% at 764 ng/l.

Fat and lean mass
Body composition including fat mass and lean tissue mass was measured by dual X-ray absorptiometry (DXA) at 0, 6, 12 and 24 months (Prodigy version 7.51; GE Lunar, Madison, WI, USA). Coefficient of variation was <2% for repeated scans.[20]

Randomisation and blinding
Subjects were randomised with equal probability to the two treatments using randomly permuted blocks of size 2, 4 or 6 (also randomly chosen) stratified by age (<72 or >72 years-old). Identical ampoules were dispatched by the Austin Health pharmacy, using subject number to ensure blinding.

Statistical methods
Data were partly non-normally distributed and were presented as median and interquartile

range (IQR). Statistical analyses of treatment effects on primary and secondary endpoints followed the intention-to-treat principle, whereby all randomised subjects were included as assigned, and all had received a single dose of the research trial medication, either zoledronic acid or placebo. The treatment effect of bisphosphonate therapy on body composition and glucose metabolism markers were tested *via* repeated measures mixed effects models including main effects for the starting level (baseline), bisphosphonate group, time points and the interaction of time point by group and random effects at the subject level. The latter represents the treatment effect, quantified as the mean adjusted between-group difference (MAD); 95% confidence intervals (CI) for the MADs were profiled. Statistical significance testing relied on a single p value over all time points (p overall). Within-variation in a single group was only used for assessing time-sensitive biological effects, not for any treatment effect. A two-sided p value of <0.05 was considered indicative of statistical significance; as structural bone parameters are inter-correlated, no adjustments for multiple testing were made. Statistical analyses were performed using R statistical package (version 3.6.2 for Mac) together with lme4 1.1–21.[21,22]

Results

Participant characteristics
A total of 76 men were randomised: 39 to zoledronic acid and 37 to matching placebo. Participant characteristics have been published previously but are reproduced in Table 1 for ease of reference.[16] At baseline, participants had a median age of 67.8 years, and had clinical characteristics typical of men with localised high-risk prostate cancer.[23] Approximately 20% had pre-existing diabetes mellitus. After commencement of ADT, in both groups, serum testosterone declined from a normal baseline concentration to castrate concentrations with no between group differences (Table 2).

Fat and lean mass
Whilst overall fat mass increased and lean mass decreased for both groups, there was no significant between group difference in body composition over time for men treated with zoledronic acid or placebo (Table 2).

Glucose metabolism
The mean adjusted between-group difference for HOMA2-IR, HbA1c and Matsuda Index was not significant in men who received a single-dose of zoledronic acid or placebo, with MADs (95% CI) being −0.2 (−0.6; 0.2), −0.1 (−0.3; 0.1) and 0.8 (−1.1; 2.7), respectively (Table 2). The Matsuda Index decreased in both groups consistent with worsening insulin resistance with ADT (Table 2).

Bone remodeling markers. As reported previously,[16] over 24 months, CTX and P1NP remained significantly lower in the zoledronic acid group, compared with the placebo group CTX −176 ng/l (−275; −76), $p < 0.001$ and P1NP −18 mg/l (−32; −5), $p < 0.001$ (Table 2).

Discussion
In this 2-year RCT of men with non-metastatic prostate cancer newly commencing ADT, we report that a single dose of zoledronic acid treatment given at ADT initiation did not significantly the change ADT-associated increase in insulin resistance over 2 years. This is in contrast to our hypothesis that suppression of bone remodelling (including osteocalcin) by bisphosphonates would worsen insulin resistance. While we did not measure osteocalcin given the negative outcome of our study, men receiving zoledronic acid had significantly lower mean concentrations of circulating P1NP and CTX over 24 months. These markers usually change in parallel with osteocalcin, which allows us to infer that osteocalcin concentrations should be reduced in in men assigned to zoledronic acid compared with placebo.

Since the initial design of our study in 2009 [ClinicalTrials.gov identifier: NCT01006395], retrospective cohort studies in the general population have reported that exposure to bisphosphonate therapy is associated with a significant reduction (up to 50%) in the risk of incident type 2 diabetes mellitus.[13,14] This is despite treatment with bisphosphonate therapy being associated with higher amounts of fat mass in postmenopausal women.[27] In contrast, prospective observational studies have reported that despite bisphosphonate treatment reducing osteocalcin, no associations with glucose metabolism or estimated insulin resistance were evident.[28] Moreover, RCTs have reported that treatment with the oral

Table 1. Baseline participant characteristics.

Characteristics	Placebo group (*n* = 36)	Zoledronic acid group (*n* = 38)
Age (years)	67.5 (65.2; 74.3)	68.8 (63.1; 73.2)
Body mass index (kg/m^2)	27.9 (25.3; 32.0)	28.8 (25.4; 31.1)
Prostate cancer Gleason score	8.00 (7.00; 9.00)	8.00 (7.00; 9.00)
Serum total testosterone (nmol/l)	12.7 (6.85; 18.3)	11.7 (7.90; 17.5)
PSA (µg/l)	4.35 (0.25; 15.8)	6.47 (0.17; 18.8)
Concurrent radiotherapy treatment *n* (%)	33 (94.3)	33 (94.3)
Smoking status		
Current (%)	4 (11.4)	2 (5.4)
Ex-smoker (%)	13 (37.1)	16 (43.2)
Never smoked (%)	18 (51.4)	18 (48.6)
Ethnic background (%)		
Caucasian (%)	38 (100)	35 (100)
Charlson medical co-morbidity index	2.00 (2.00; 3.00)	2.00 (2.00; 3.00)
Medical co-morbidities		
Ischaemic heart disease (%)	8 (22.9)	10 (26.3)
Diabetes mellitus (%)	7 (20.0)	7 (18.4)
Hypercholesterolaemia (%)	20 (57.1)	17 (44.7)
Hypertension (%)	24 (68.6)	19 (50.0)

Values are presented as median (IQR) or proportions *N* (%). There were no significant differences between groups for any characteristic. The Gleason score is a grading system for prostate cancer whereby a Gleason score of 6 is low grade, 7 is intermediate grade, and a score of 8–10 is high grade cancer.[24] PSA reference intervals used are age-specific for males, i.e. 60–64 years: <4.5 µg/l, 65–69 years: <5.5 µg/l, >70 years: <6.5 µg/l.[25] The Charlson comorbidity index is an objective measure of overall comorbidity (with a score assigned to 22 comorbid conditions) and was developed to predict 1-year mortality.[26] Higher scores reflect higher morbidity.
IQR, interquartile range; PSA, prostate specific antigen.

bisphosphonate alendronate was associated with improved fasting glucose and insulin indices compared with placebo in postmenopausal women.[29]

Strengths and limitations

This is the only RCT that has evaluated bisphosphonate therapy on glucose metabolism in men newly commencing ADT, which inherently worsens insulin resistance and predisposes to development of diabetes. Glucose metabolism was assessed as a pre-specified secondary outcome in a prospective fashion using several, well-validated established indices, including the Matsuda index, which correlates well with the euglycemic insulin clamp.[15] The observed lack of benefit questions underlying mechanisms between undercarboxylated osteocalcin and glucose metabolism and is consistent with recent evidence from preclinical studies.[30,31]

This study has several limitations. We administered only a single dose of zoledronic acid in this 2-year RCT. Therefore, the effects of zoledronic acid with repeated dosing, or more potent antiresorptive agents such as denosumab may be different. We did not directly measure undercarboxylated osteocalcin and infer only

Table 2. Mean adjusted difference in glucose metabolism indices and body composition.

	Placebo group (*n* = 36)	Zoledronic acid group (*n* = 38)	Mean adjusted difference (Zol *versus* Placebo)	*p* value
Total testosterone (nmol/l)				
0 months	12.7 (6.9; 18.3)	11.7 (7.9; 17.5)		
3 months	0.4 (0.4; 0.5)	0.4 (0.2; 0.6)	−0.3 (−2.7; 2.1)	
6 months	0.4 (0.2; 0.4)	0.4 (0.2; 0.5)	0.2 (−2.3; 2.7)	
12 months	0.4 (0.2; 0.5)	0.4 (0.3; 0.5)	−0.7 (−3.2; 1.8)	
24 months	0.4 (0.2; 0.5)*	0.4 (0.3; 0.6)*	0.6 (−2.2; 3.3)	0.84
C-telopeptide (ng/l)				
0 months	453 (278; 546)	377 (296; 496)		
3 months	618 (487; 806)	149 (98; 227)	−400 (−483; −317)	
6 months	770 (567; 886)	232 (185; 351)	−389 (−477; −300)	
12 months	688 (602; 962)	399 (328; 502)	−278 (−368; −187)	
24 months	679 (450; 1074)	465 (392; 588)	−176 (−275; −76)	<0.001
P1NP (mg/l)				
0 months	40 (35; 65)	44 (37; 54)		
3 months	46 (37; 60)	18 (15; 21)	−26 (−38; −15)	
6 months	59 (46; 73)	26 (20; 32)	−28 (−40; −16)	
12 months	73 (54; 102)	42 (34; 56)	−32 (−45; −20)	
24 months	71 (48; 89)	49 (38; 61)	−18 (−32; −5)	<0.001
Fasting glucose (mmol/l)				
0 months	5.35 (5.10; 6.10)	5.30 (5.00; 6.22)		
3 months	5.30 (5.00; 5.80)	5.40 (5.00; 6.20)	0.0 (−0.4; 0.4)	
6 months	5.65 (5.40; 6.27)	5.50 (5.00; 6.10)	0.0 (−0.4; 0.4)	
12 months	5.50 (5.10; 6.30)	5.55 (5.00; 5.93)	0.0 (−0.4; 0.4)	
24 months	5.30 (5.20; 5.80)	5.60 (5.10; 6.30)	0.1 (−0.3; 0.6)	0.97
HbA1c %				
0 months	5.90 (5.55; 6.20)	5.80 (5.55; 6.10)		
3 months	5.80 (5.60; 6.20)	5.75 (5.53; 6.10)	0.0 (−0.2; 0.2)	
6 months	5.80 (5.50; 6.07)	5.70 (5.40; 6.00)	−0.1 (−0.3; 0.1)	
12 months	5.75 (5.53; 6.07)	5.80 (5.50; 6.10)	0.0 (−0.2; 0.2)	
24 months	5.80 (5.60; 5.97)	5.80 (5.50; 6.18)	−0.1 (−0.3; 0.1)	0.65

(Continued)

Table 2. (Continued)

	Placebo group (n = 36)	Zoledronic acid group (n = 38)	Mean adjusted difference (Zol *versus* Placebo)	*p* value
Matsuda index				
0 months	4.5 (2.9; 6.1)	4.4 (3.1; 6.4)		
3 months[a]	4.6 (2.7; 6.1)	3.2 (2.2; 6.3)	−0.7 (−2.4; 1.0)	
12 months	3.2 (1.7; 5.1)	3.3 (2.5; 6.0)	−0.7 (−2.5; 1.0)	
24 months	2.9 (1.2; 4.7)*	3.6 (2.5; 6.3)*	0.8 (−1.1; 2.7)	0.38
HOMA2-IR				
0 months	2.3 (1.5; 2.7)	2.3 (1.6; 2.7)		
6 months	2.8 (2.0; 3.3)	2.2 (1.7; 3.1)	−0.2 (−0.5; 0.2)	
12 months	2.7 (1.8; 3.7)	2.6 (1.7; 3.3)	−0.3 (−0.6; 0.1)	
24 months	2.6 (2.1; 4.0)	2.5 (1.8; 3.1)	−0.2 (−0.6; 0.2)	0.45
Fat mass (g)				
0 months	26066 (19641; 34620)	25203 (19261; 32645)		
6 months	27565 (22647; 36346)	28137 (22502; 35889)	1074 (−456; 2607)	
12 months	29198 (22690; 37593)	29425 (24201; 36045)	1561 (−4; 3124)	
24 months	33274 (24834; 39302)	29428 (23537; 38252)	401 (−1307; 2103)	0.23
Lean mass (g)				
0 months	54234 (50050; 60019)	54334 (50593; 59356)		
6 months	53376 (48956; 57377)	51199 (49456; 57560)	62 (−959; 1084)	
12 months	53949 (48514; 57305)	51893 (49219; 56271)	−809 (−1854; 235)	
24 months	53346 (47651; 57197)	49530 (48264; 56280)	−184 (−1325; 955)	0.36

Median (IQR) are reported.
[a]Matsuda Index available at timepoints where 2-hour glucose tolerance tests were performed at 0, 3, 12 and 24 months. Higher Matsuda Index indicates greater insulin sensitivity.
*Within group difference from 0 to 24 months statistically significant ($p < 0.05$).
HOMA2-IR, homeostatic model assessment 2 insulin resistance; IQR, interquartile range; P1NP, procollagen type 1 amino-terminal propeptide.

pharmacologically that bisphosphonate therapy would reduce osteocalcin based on previous studies and on our observed reduction in other bone remodelling markers (Table 2).[13,14]

In conclusion, a single-dose of zoledronic acid does not appear to influence glucose metabolism in men newly commencing ADT. Further studies are required to elucidate the endocrine interplay between bisphosphonates, bone and glucose metabolism.

Author contributions
ASC: Data curation; formal analysis; investigation; methodology; writing-original draft; writing-review and editing.

RH: Formal analysis; methodology; writing-review and editing.

JJZ: Data curation; formal analysis; writing-review and editing.

DLJ: Investigation; methodology; writing-review and editing.

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