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ORIGINAL RESEARCH

Bone Turnover Markers in Men and Women with Impaired Fasting Glucose and Diabetes

Kara L. Holloway-Kew[1] · Lelia L. F. De Abreu[1] · Mark A. Kotowicz[1,2,3] · Muhammad A. Sajjad[1] · Julie A. Pasco[1,2,3,4]

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Abstract

Bone turnover markers (BTMs) are reduced in diabetes, but whether BTM changes occur in impaired fasting glucose (IFG) is unknown. The aim of this study was to investigate whether BTMs are altered in IFG and diabetes compared to normoglycaemia. For men and women ($n = 2222$) in the Geelong Osteoporosis Study, IFG was defined as fasting plasma glucose (FPG) 5.5–6.9 mmol/L and diabetes as FPG ≥ 7.0 mmol/L, use of antihyperglycemic medication and/or self-report. Serum C-terminal telopeptide (CTx) and procollagen type 1 N-terminal propeptide (P1NP) were measured. After natural log transformation to normalise the data, multivariable regression was used to examine the relationship between glycaemia status and bone turnover markers (BTMs), before and after adjusting for other confounders. There were 643 men and 682 women with normoglycaemia, 355 men and 391 women with IFG and 97 men and 54 women with diabetes. Men with IFG or diabetes had lower adjusted ln(CTx) and ln(P1NP) compared to normoglycaemia (all $p < 0.05$). Women with IFG or diabetes had lower adjusted ln(CTx) and ln(P1NP) (all $p < 0.05$) except for ln(P1NP) when comparing diabetes with normoglycaemia, which showed a trend for lower ln(P1NP) ($p = 0.053$). In both sexes, an age * glycaemia interaction term indicated between-group differences in BTMs diminished with increasing age. No other confounders were identified. Bone turnover was lower in those with either IFG or diabetes compared to normoglycaemia.

Keywords Diabetes mellitus · Impaired fasting glucose · Bone turnover markers

Introduction

Diabetes affects 425 million people worldwide, and the prevalence is increasing due to an ageing population and high rates of obesity [1]. Diabetes is associated with a number of comorbidities including nephropathy [2], cardiovascular disease [3], retinopathy [4] and early mortality [5]. An intermediate between normoglycaemia and diabetes, known as impaired fasting glucose (IFG), is defined by the American Diabetes Association (ADA) as a fasting plasma glucose (FPG) level between 5.5 and 6.9 mmol/L (100–125 mg/dL) [6]. We have recently reported a prevalence for IFG of 33.8% and 6.5% for diabetes in Australian women aged ≥ 20 year [7].

Individuals with diabetes have an increased fracture risk despite higher or normal bone mineral density (BMD) [8]. However, no differences have been detected for BMD and fracture risk in those with IFG [9, 10]. Several reasons have been suggested to explain the increased fracture risk for individuals with diabetes, including increased fall risk, altered bone material properties, changes in bone microarchitecture and low bone turnover [8].

Bone turnover markers (BTMs) are measured to estimate bone resorption and/or formation [11]. Bone formation markers include osteocalcin, bone-specific alkaline phosphatase, alkaline phosphatase, procollagen type 1 amino terminal propeptide, and procollagen type 1 carboxyl terminal propeptide. Markers for bone resorption include N-terminal cross-linked telopeptide of type-I collagen and C-terminal cross-linked telopeptide of type-I collagen [12].

Two meta-analyses have reported that bone formation and resorption markers appear to be lower in diabetes [11,

✉ Kara L. Holloway-Kew
khollo@barwonhealth.org.au

1 Deakin University, Geelong, Australia

2 Department of Medicine–Western Campus, The University of Melbourne, St Albans, Australia

3 Barwon Health, Geelong, Australia

4 Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia

13]. However, another meta-analysis [14] showed that only osteocalcin and CTx were lower in individuals with diabetes and that other biomarkers were not different to individuals without diabetes. This meta-analysis also reported that there was substantial heterogeneity between studies. To date, there have been a number of studies investigating BTMs and diabetes [15, 16], but most focus on postmenopausal women. There are only a small number of studies investigating BTMs in men with diabetes [15, 17, 18] and two [18, 19] in men and women with IFG. This study aimed to investigate BTMs in Australian men and women with normoglycaemia, IFG and diabetes.

Methods

Participants

This study included men and women enrolled in the Geelong Osteoporosis Study (GOS). The GOS is a population-based, observational cohort study situated in south-eastern Australia and has been described previously [20]. The region has a large (~280,000), stable population with a range of social, cultural and geographical settings which are representative of the broader Australian population, making it suitable for epidemiological studies. This study used data from the baseline visit for women (1993–1997) and baseline or 5-year follow-up for men (2001–2006 or 2006–2010), depending on when a blood sample was collected for ascertainment of glycaemia status. Individuals taking glucocorticoids or bisphosphonates were excluded from the analysis, leaving 1095 men and 1127 women to be included in this study.

The study was approved by the HREC at Barwon Health. Participants provided written, informed consent.

Blood Samples

Venous blood samples were collected and FPG measured. Diabetes was classified as FPG $\geq$ 7.0 mmol/L (126 mg/dL) and/or a self-report of diabetes and/or use of antihyperglycaemic agents. IFG was classified if FPG was between 5.5 and 6.9 mmol/L (100–125 mg/dL); according to the 2003 ADA diagnostic criteria [6]. The CV for analysis of FPG was 1.98%. The blood samples were also analysed for serum C-terminal telopeptide (CTx) and procollagen type 1 N-terminal propeptide (P1NP). The samples were analysed using the automated Roche Modular Analytics E170 analyser (Roche Diagnostics, Mannheim, Germany). The serum CTx limit of detection was 10 ng/L with inter-assay coefficient of variations (CVs) of 6.5% at 361 ng/L, 3.8% at 816 ng/L and 3.4% at 3304 ng/L ($n = 10$). Serum P1NP inter-assay CVs were 4.9% at 73 µg/L, 2.6% at 392 µg/L, and 2.1% at 768 µg/L ($n = 10$) with a limit of detection of 5 µg/L [21].

Other Measurements

Weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. Femoral neck BMD was measured using DXA (Lunar DPX-L, Madison, WI, USA) for the women and the first 544 men at baseline; when the DPX-L became outmoded, male scans were performed using a GE-Prodigy (Prodigy; GE Lunar, Madison, WI, USA). There were no differences detected in lumbar spine or femoral neck BMD after performing cross-calibration [20]. Other variables were obtained using self-report. Physical activity (high/low) was determined by self-report; participants were considered to have ''high'' physical activity if they had an active lifestyle including light exercise or more undertaken several times per week. Alcohol consumption was determined using a Food Frequency Questionnaire [22] and dichotomised into <3 or ≥ 3 standard drinks of alcohol per day. Glucocorticoid and bisphosphonate use was ascertained by self-report.

Statistical Analysis

The ANOVA or Kruskal–Wallis test was used to determine differences for continuous variables according to glycaemia status. A Chi-Square test or Fisher's exact test was used for categorical variables.

Natural log transformation was applied to normalise CTx and P1NP values. The first analysis investigated the association between bone turnover markers and glycaemia status, without adjusting for other factors. Then, a multivariable regression was used to examine relationships between glycaemia status and BTMs, after adjusting for other confounders (age, weight, height, physical activity, smoking, alcohol intake). Variables that were not significant were not included in the final models. Following this analysis, interaction terms were assessed and included in the model if significant. Statistical analyses were performed using Minitab (version 17, Minitab, State College, PA, USA).

Results

There were 643 men and 682 women with normoglycaemia, 355 men and 391 women with IFG and 97 men and 54 women with diabetes. Table 1 shows the descriptive characteristics of the study participants.

Compared to normoglycaemia, men and women with IFG and diabetes were older, heavier, had shorter height and were more likely to have low physical activity level. Femoral neck BMD was lower in men and women with IFG or diabetes compared to normoglycaemia.

Table 1 Descriptive characteristics for men and women in the study, according to glycaemia status

Men	Normoglycaemia ($n=643$)	IFG[a] ($n=355$)	Diabetes ($n=97$)	p value
Age (years)	56.0 (39.0–72.0)	65.0 (51.0–76.0)	71.0 (63.3–80.0)	<0.001
Weight (kg)[b]	81.6 ± 13.9	85.6 ± 15.1	85.5 ± 13.1	<0.001
Height (cm)[b]	175.0 ± 7.3	174.1 ± 7.1	172.4 ± 6.7	0.002
Low physical activity	129 (20.1)	110 (31.0)	33 (34.0)	<0.001
Smoking (y)	86 (13.4)	45 (12.7)	9 (9.3)	0.529
High alcohol intake	135 (21.0)	85 (23.9)	15 (15.5)	0.178
Femoral neck BMD (g/cm^2)	1.000 ± 0.156	0.978 ± 0.142	0.958 ± 0.142	0.023
Women	Normoglycaemia ($n=682$)	IFG[a] ($n=391$)	Diabetes ($n=54$)	p value
Age (years)	42.1 (31.0–57.7)	56.5 (45.0–69.4)	65.2 (61.3–74.6)	<0.001
Weight (kg)	66.7 ± 13.0	72.6 ± 15.8	75.5 ± 19.1	<0.001
Height (cm)	161.9 ± 6.2	160.8 ± 6.7	156.7 ± 6.1	<0.001
Low physical activity	139 (20.4)	125 (32.0)	34 (63.0)	<0.001
Smoking (y)	118 (17.3)	55 (14.1)	11 (20.4)	0.275
High alcohol intake[c]	11 (1.6)	10 (2.6)	0 (0.0)	–
Femoral neck BMD (g/cm^2)	0.941 ± 0.154	0.913 ± 0.162	0.900 ± 0.143	0.007

Data presented as mean ± SD, median (IQR) or n (%)

[a]Impaired fasting glucose

[b]Missing data: weight and height; men: $n = 20$; femoral neck BMD; men: $n = 269$, women: n=4

[c]Too few to conduct statistical analysis

Table 2 Values for ln(CTx) and ln(P1NP) in men and women with normoglycaemia, impaired fasting glucose and diabetes

Men	Normoglycaemia ($n=643$)	IFG* ($n=355$)	p value[†]	Diabetes ($n=97$)	p value[†]
Unadjusted					
ln(CTx) (ng/L)	5.83 (5.79–5.87)	5.71 (5.65–5.76)	<0.001	5.56 (5.45–5.66)	<0.001
ln(P1NP) (µg/L)	3.67 (3.63–3.71)	3.57 (3.52–3.62)	0.002	3.43 (3.33–3.53)	<0.001
Adjusted					
ln(CTx) (ng/L)	5.81 (5.77–5.85)	5.52 (5.35–5.70)	0.011	4.94 (4.36–5.51)	<0.001
ln(P1NP) (µg/L)	3.64 (3.61–3.68)	3.35 (3.19–3.52)	0.001	2.93 (2.38–3.47)	0.012
Women	Normoglycaemia ($n=682$)	IFG* ($n=391$)	p value*	Diabetes ($n=54$)	p value[†]
Unadjusted					
ln(CTx) (ng/L)	5.73 (5.67–5.79)	5.68 (5.61–5.76)	0.341	5.59 (5.38–5.79)	0.189
ln(P1NP) (µg/L)	3.57 (3.53–3.61)	3.58 (3.52–3.63)	0.776	3.33 (3.18–3.48)	0.003
Adjusted					
ln(CTx) (ng/L)	5.74 (5.68–5.80)	5.23 (5.01–5.44)	<0.001	4.44 (3.21–5.67)	0.040
ln(P1NP) (µg/L)	3.56 (3.52–3.60)	3.37 (3.22–3.52)	0.008	2.67 (1.77–3.56)	0.053

Data presented as mean (95% CI)

Adjusted model for men and women includes age and an age/diabetes interaction term

CTx C-terminal telopeptide, *P1NP* procollagen type 1 N-terminal propeptide, *IFG* Impaired fasting glucose

* p value for difference between IFG and normoglycaemia

[†]p value for difference between diabetes and normoglycaemia

Men

In an unadjusted model (Table 2), both mean ln(CTx) and ln(P1NP) were lower for IFG and diabetes compared to normoglycaemia. These relationships were sustained after adjustment for age. No other confounders were identified. The reduction in CTx was 5.0% and 15.0% for those with IFG and diabetes, respectively. For P1NP, men with IFG had a reduction of 8.0% and those with diabetes, 19.5%.

Women

Unadjusted mean ln(CTx) was not different in women with diabetes compared to the normoglycaemia group (Table 2). There was also no difference between IFG and normoglycaemia groups. For ln(P1NP), women in the diabetes group had lower values compared to normoglycaemia, but no differences were observed for IFG.

After adjustment for age, there was a difference detected between IFG and normoglycaemia for both ln(CTx) and ln(P1NP) (Table 2). Women with diabetes had lower ln(CTx) than women with normoglycaemia, and there was a trend for ln(P1NP) to be lower in women with diabetes. The reduction in CTx was 8.9% and 22.6% for IFG and diabetes, respectively. For P1NP, these values were 5.3% and 25.0%.

Further adjustment for other variables did not affect the relationship between glycaemia status and BTMs in women.

Interaction Terms

In adjusted models for both ln(CTx) and ln(P1NP), in men and women, there was an interaction term for glycaemia status and age and this term was included in the model. No other interaction terms were identified in any of the models.

The impact of the age interaction term is shown in Fig. 1. In a younger age group (defined as < 65 year according to the Australian Institute of Health and Welfare [23]), the impact of diabetes on BTM values appears to be more pronounced than in the older age group (65+ year).

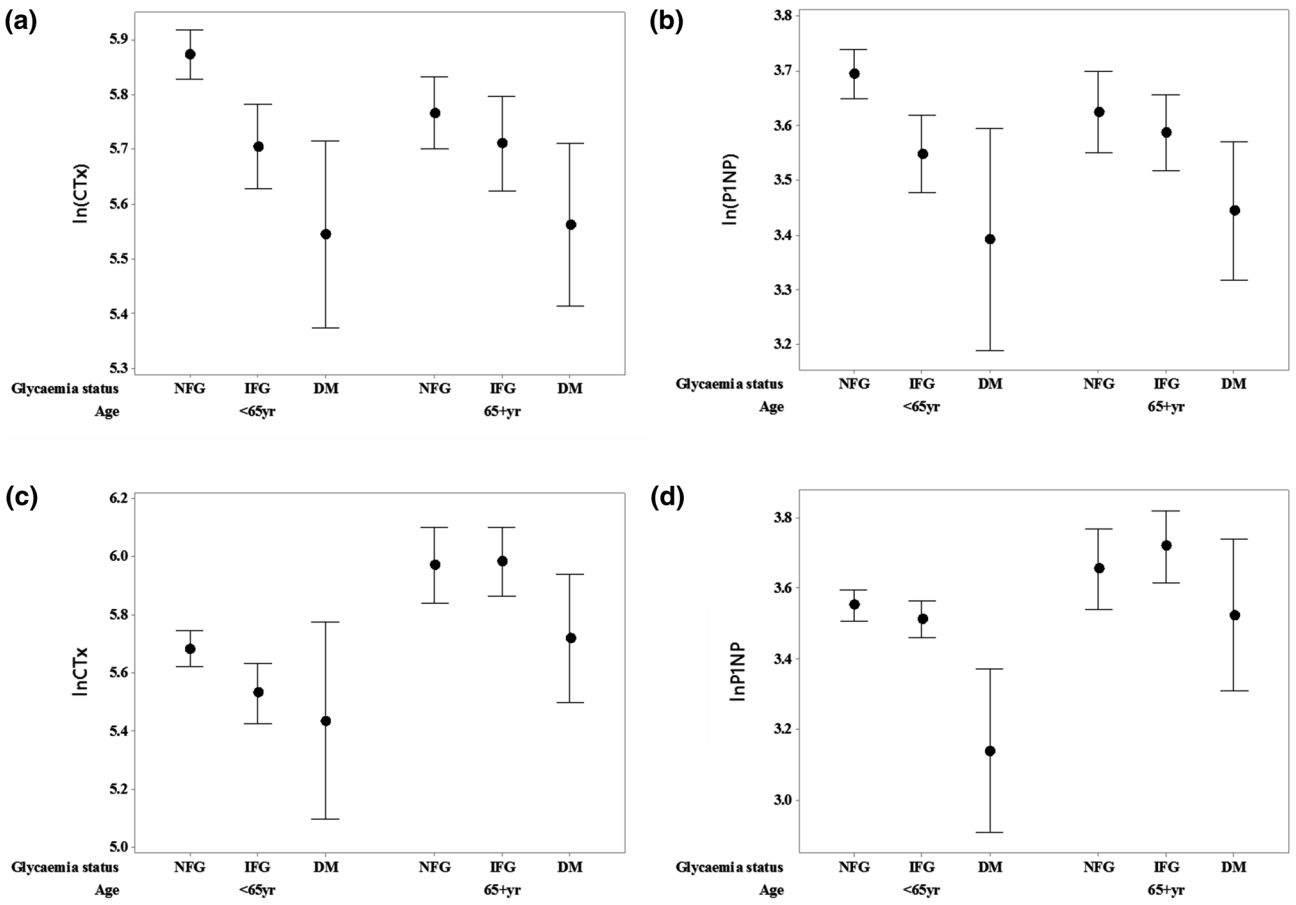

Fig. 1 Unadjusted bone turnover marker values for **a** ln(CTx) men, **b** ln(P1NP) men, **c** ln(CTx) women, and **d** ln(P1NP) women. *NFG* normoglycaemia, *IFG* impaired fasting glucose, *DM* diabetes, *yr* years

Discussion

Data from this study suggest that men and women with IFG and diabetes had lower BTMs than those with normoglycaemia, even after adjustment for confounders. This is one of the first studies to report on differences in BTMs for men and women with IFG. The reduction in CTx and P1NP and for individuals with dysglycaemia were similar. This may indicate that bone resorption and bone formation are both reduced, resulting in lower bone turnover overall.

Similar to our study, Furst et al. [16] showed that both CTx and P1NP were lower in postmenopausal women with diabetes compared to controls. Another study [17] involving Spanish men and women reported lower CTx in those with diabetes compared to those without, but markers of bone formation were not different. These results are different to what we report and could be explained by the different populations used (Spain vs. Australia) and different control groups; we used a population-based sample, whereas the Spanish study recruited controls from an osteoporosis screening clinic.

To our knowledge, there are only two studies to have considered individuals with IFG; only one of which included men. One of the studies reported that Swedish women with type 2 diabetes had lower crosslaps (CTx) than those with normoglycaemia [18]. However, there was no difference detected for IFG, defined using ADA criteria, similar to our study. These results are in contrast to ours, which report a difference in CTx for women with IFG, however differences in sample characteristics, such as lower mean age, may explain the differences observed. The Swedish study included participants with a mean age of 82 years, while our participants had a median age of 55.0 years. As bone turnover markers have been shown to decrease with age before increasing again in older age (70+ years [21]), our study and the Swedish study may show different results. The other study including individuals with IFG [19] reported that in Chinese postmenopausal women, those with IFG or diabetes had lower CTx and P1NP than those with normoglycaemia. Many individuals in China with diabetes are not overweight or obese and have lower insulin secretion and sensitivity compared to individuals from other countries such as Australia and the USA [19], which potentially limits the relevance of this study to these countries. Additionally, the Chinese study used the World Health Organization criteria for IFG (6.1–6.9 mmol/L), which is different to the criteria we used (ADA criteria: 5.5–6.9 mmol/L). The Chinese study also reported no difference in fractures between the IFG and normoglycaemia groups, despite differences in BTMs. We have previously reported no difference in trabecular bone score between IFG and normoglycaemia [24], or any differences in fracture risk [25].

This study has several strengths. We utilised a random selection process with a high participation, thus our sample of men and women is representative of the underlying population. We utilised a robust method of diabetes ascertainment including FPG, medication use and self-report. There are also some limitations, including that we did not differentiate between type 1 or type 2 diabetes. However, it is likely that most of our participants had type 2 diabetes. The results of this study may not be generalisable to other populations, as the study sample included mainly those of Caucasian ethnicity. However, our participants were representative of the broader Australian population and thus these results may be relevant to Australian men and women with IFG and diabetes. Our study was cross-sectional, and thus no causal inferences can be made. Further research should be completed, including longitudinal analyses, perhaps incorporating HbA1c values for the diagnosis of diabetes. Additionally, we were unable to assess the impact of lower bone turnover on fracture risk, however, in our previous work, we have shown no association between fractures and bone turnover markers in women with dysglycaemia [25]. This may indicate that lower bone turnover alone does not increase fracture risk in those with IFG or diabetes.

Conclusion

BTMs were lower in men and women with IFG or diabetes compared to normoglycaemia. This study is one of the first to report lower BTMs in IFG. Although we have not previously detected any differences between IFG and normoglycaemia in terms of fracture risk or trabecular bone score, it appears that disturbance of bone turnover occur in both men and women with elevated FPG, before diabetes has developed.

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Compliance with Ethical Standards

Conflict of interest Kara L. Holloway-Kew, Lelia L. F. De Abreu, Mark A. Kotowicz, Muhammad A. Sajjad, and Julie A. Pasco have no conflicts of interest.

Human and Animal Rights and Informed Consent All subjects signed informed consent. Ethical approval was obtained from the Barwon Health, Human Research Ethics Committee (ID 92/01 and ID 00/56).

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