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Dapagliflozin has no effect on markers of bone formation and resorption or bone mineral density in patients with inadequately controlled type 2 diabetes mellitus on metformin

Ö. Ljunggren[1], J. Bolinder[2], L. Johansson[3,4], J. Wilding[5], A. M. Langkilde[4], C. D. Sjöström[4], J. Sugg[6] & S. Parikh[6]

[1]*Osteoporosis Research Unit, Department of Medical Sciences, Uppsala University, Uppsala, Sweden*
[2]*Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital Huddinge, Karolinska Institute, Stockholm, Sweden*
[3]*Department of Radiology, Oncology and Radiation Science (ROS), Section of Radiology, Uppsala University, Uppsala, Sweden*
[4]*AstraZeneca, Mölndal, Sweden*
[5]*Department of Obesity & Endocrinology, University of Liverpool, Liverpool, UK*
[6]*AstraZeneca, Wilmington, DE, USA*

Aims: Dapagliflozin, a selective sodium-glucose cotransporter 2 (SGLT2) inhibitor, reduces hyperglycaemia in patients with type 2 diabetes (T2DM) by increasing urinary glucose excretion. Owing to its mechanism of action, dapagliflozin could potentially affect the renal tubular transportation of bone minerals. Therefore, markers of bone formation and resorption and bone mineral density (BMD) were evaluated in patients with T2DM after 50 weeks of dapagliflozin treatment.

Methods: This international, multi-centre, randomized, parallel-group, double-blind, placebo-controlled study (ClinicalTrials.gov NCT00855166) enrolled patients with T2DM (women 55–75 years and men 30–75 years; HbA1c 6.5–8.5%; BMI $\geq$ 25 kg/m^2; body weight $\leq$ 120 kg) whose T2DM was inadequately controlled on metformin. One hundred and eighty-two patients were randomly assigned 1:1 to receive dapagliflozin 10 mg/day or placebo added to open-label metformin for a 24-week double-blind treatment period followed by a 78-week site- and patient-blinded extension period. At week 50, serum markers of bone formation (procollagen type 1 N-terminal propeptide; P1NP) and resorption (C-terminal cross-linking telopeptides of type I collagen; CTX), bone mineral density (BMD) as assessed by standardized Dual-Energy X-ray Absorptiometry (DXA) measurements and adverse events of fracture were evaluated as safety objectives.

Results: One hundred and sixty-five patients (90.7%) completed the first 50 weeks. Compared with placebo, no significant changes from baseline in P1NP, CTX or BMD were identified over 50 weeks of dapagliflozin treatment, with no significant treatment-by-gender interactions. No fractures were reported.

Conclusions: Dapagliflozin had no effect on markers of bone formation and resorption or BMD after 50 weeks of treatment in both male and post-menopausal female patients whose T2DM was inadequately controlled on metformin.

Keywords: body composition, clinical trial, renal glucose handling, SGLT2 inhibitor, type 2 diabetes

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Introduction

Dapagliflozin, a highly selective inhibitor of sodium-glucose cotransporter 2 (SGLT2) [1], increases urinary glucose excretion and improves glycaemic control in patients with type 2 diabetes mellitus (T2DM) when used as monotherapy [2] or when added to metformin [3,4], glimepiride [5], pioglitazone [6] or insulin [7,8].

Given the renal tubular mechanism of action of the SGLT2 inhibitor class, dapagliflozin may potentially alter calcium and phosphate homeostasis and bone mineral density (BMD). Preliminary clinical data have shown no changes in serum calcium or vitamin D but small increases in serum magnesium, phosphate and parathyroid hormone with dapagliflozin treatment compared with placebo [4,9]. Furthermore, dapagliflozin-induced urinary caloric loss produces weight loss [2–5,7,10], which may also potentially reduce BMD [11].

In addition to these SGLT2-specific issues, there is heightened awareness of the increased risk of fracture posed by T2DM itself, especially in older patients with a long duration of diabetes [12], and of the potential for glucose-lowering drugs to enhance this fracture risk [13].

Low BMD, as measured by Dual-Energy X-ray Absorptiometry (DXA), is a major risk factor for osteoporotic fractures in the general population of older adults [14]. However, BMD in many patients with T2DM is increased, which may be secondary

Correspondence to : Östen Ljunggren, MD, PhD, Department of Medical Sciences, Uppsala University, 751 85 Uppsala, Sweden.
E-mail: osten.ljunggren@medsci.uu.se

to increased body mass index (BMI) [15], insulin resistance [16] and/or low bone turnover [17,18]. The apparent paradox of increased BMD accompanied by increased fracture risk in these patients [19–21] may be explained by two pertinent findings. First, within patients with T2DM a lower femoral neck BMD T-score is still associated with an increased risk of fractures [22] as is the case in individuals without T2DM [14]. Second, when compared with individuals without T2DM, patients with T2DM have a higher risk of fracture for any given BMD T-score and age [22]. Thus, although low BMD remains an important risk factor for fractures in patients with T2DM, fracture risk is higher for any given BMD in these patients.

In addition to the general increase in fracture risk associated with T2DM itself, certain glucose-lowering therapies may further exacerbate this risk. For example, the thiazolidinediones inhibit osteoblast activity and are associated with an increased risk of fracture in patients with T2DM [13]. Consequently, for SGLT2 inhibitors with their specific renal tubular mechanism of action, it is especially important to evaluate the potential effect of this new class of glucose-lowering therapy on bone metabolism.

Therefore, the long-term effects of dapagliflozin on serum markers of bone formation and resorption, BMD and other biochemical parameters of relevance to bone metabolism were investigated after 50 weeks of treatment in a randomized, placebo-controlled trial in patients whose T2DM was inadequately controlled on metformin.

Materials and Methods

Study Design

This was a 24-week, international, multi-centre, randomized, parallel-group, double-blind, placebo-controlled phase III study with a 78-week site- and patient-blinded extension period conducted from 13 February 2009 and on-going at 40 sites in Bulgaria, Czech Republic, Hungary, Poland and Sweden. Primary efficacy, safety and tolerability results at 24 weeks have been presented elsewhere [10]. Here, changes in markers of bone turnover, BMD, and other biochemical parameters of relevance to bone metabolism at week 50 are presented as safety objectives. Figure 1 shows the disposition of the patients recruited to the study. The study complied with the Declaration of Helsinki and the International Conference on Harmonization/Good Clinical Practice Guidelines, was approved by regulatory authorities and/or institutional review boards or independent ethics committees for participating centres, and is registered with ClinicalTrials.gov (NCT00855166). All participants provided informed consent before entering the study.

Patients

Inclusion criteria were as follows: patients with T2DM; women aged 55–75 years who were post-menopausal for a period of at least 5 years or men aged 30–75 years; haemoglobin A1c (HbA1c) 6.5–8.5%; fasting plasma glucose (FPG) $\leq$ 13.2 mmol/l; body mass index (BMI) $\geq$ 25 kg/m^2; body weight $\leq$ 120 kg (due to limitations imposed by DXA equipment); and treatment with metformin at a stable dose of $\geq$1500 mg/day for $\geq$12 weeks prior to enrolment.

In order to reliably ascertain the effect of dapagliflozin on changes in BMD it was necessary to recruit patients with a stable rate of BMD change. Thus, in order to avoid the possibility of including women who were perimenopausal, and who would be expected to show an unstable rate of BMD change, women < 55 years of age were not recruited for participation. In addition, women had to have been postmenopausal (or have had an oophorectomy) for at least 5 years prior to consenting to participate. Patients with BMD T-scores less than −2.0 at lumbar spine, femoral neck or total hip regions at the baseline DXA measurement were also not recruited for participation so as not to expose patients with low BMD at risk of osteoporosis to an investigational product with unknown effects on bone metabolism. Patients with poor glycaemic control (HbA1c > 8.5%) were excluded to maximize patient retention without need for rescue therapy in order to evaluate dapagliflozin-specific effects on long-term BMD. Patients receiving treatments known to significantly influence bone metabolism (e.g. bisphosphonates, calcitonin, corticosteroids or hormone replacement therapy) were excluded. However, patients taking vitamin D and/or calcium supplements at enrolment could continue to do so, but were instructed to keep the doses unchanged throughout the entire study. For detailed exclusion criteria see Appendix S1.

Treatments and Interventions

Eligible patients entered a 2-week single-blind placebo lead-in period. Patients were randomized in a 1:1 ratio to double-blind treatment with either dapagliflozin 10 mg or placebo (taken once daily in the morning just before or together with a meal) as add-on therapy to continuing open-label metformin.

Patients with inadequate glycaemic control during the treatment period remained in the trial, but received open-label rescue therapy with sitagliptin 100 mg, an agent unlikely to affect bone metabolism [23]. The progressively stricter criteria defining inadequate glycaemic control and eligibility for rescue therapy were FPG > 13.2 mmol/l during weeks 4–7, FPG > 11.1 mmol/l during weeks 8–24 and HbA1c > 8.0% during weeks 25–50. Patients could be discontinued due to inadequate glycaemic control at the discretion of the study investigator. As metformin therapy is contraindicated with renal impairment, patients were also discontinued at any point if calculated creatinine-clearance was <60 ml/min using the Cockcroft-Gault equation [24].

All patients received diet and lifestyle counselling for T2DM, including advice on exercise, according to usual clinical routine, commencing during the lead-in period and continuing throughout the study.

Allocation Concealment and Blinding

Randomization was performed in two strata, men and women, and was done within balanced block sizes of four to ensure approximately equal numbers of patients across the treatment groups and within each stratum. Patients were allocated to study treatments according to a predefined computer-generated

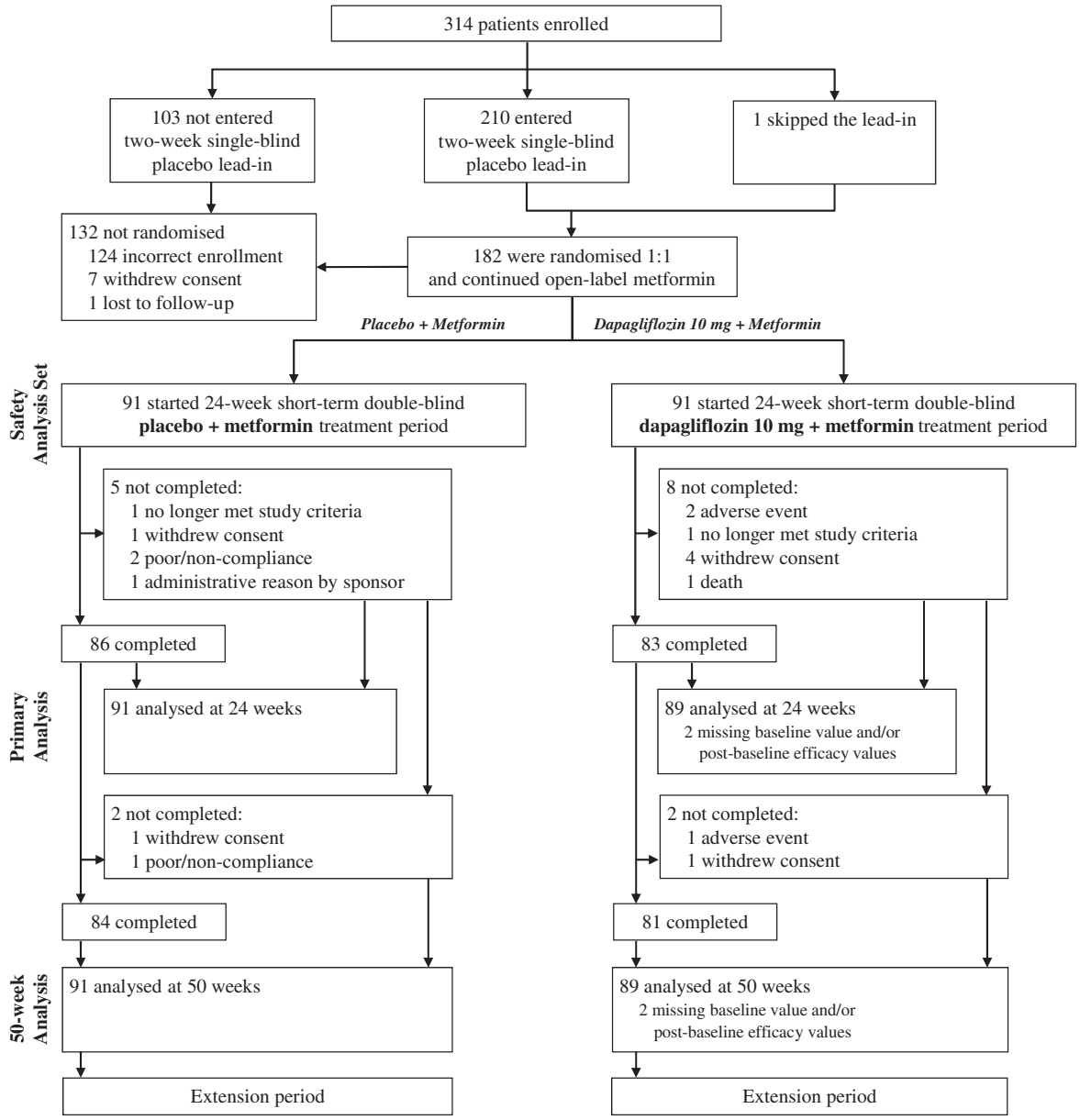

Figure 1. Trial profile. The term 'incorrect enrolment' was defined as patients not meeting inclusion criteria or meeting exclusion criteria.

randomization scheme provided by AstraZeneca. No patients, investigators or personnel at AstraZeneca or Bristol-Myers Squibb had access to the randomization codes during the 24-week double-blind treatment period. During the 78-week extension period, patients, investigators and study monitors continued to have no access to the randomization codes.

Patients and investigators were blinded to study treatment. All investigational products (dapagliflozin 10 mg and matching placebo) were identical in appearance, smell and taste, and packaged into identical bottles.

Efficacy Evaluation

The primary endpoint, change from baseline at week 24 in total body weight (TBW), and key secondary endpoints, change from baseline at week 24 in waist circumference and total fat mass, and the proportion of patients achieving a body weight reduction $\geq$5% at week 24, have been previously reported [10]. Here, changes from baseline over 50 weeks for the exploratory efficacy variables TBW and HbA1c are reported.

Safety Evaluation

Changes from baseline over 50 weeks were analysed for the following safety variables: (i) serum markers of bone formation and resorption; (ii) BMD as measured by DXA and (iii) other biochemical variables of relevance to bone metabolism.

Markers of Bone Formation and Resorption. The serum marker of bone formation reported in the current study was procollagen type 1 N-terminal propeptide (P1NP), which is released into the blood after cleavage from procollagen 1 during the process of type I collagen incorporation into bone matrix. P1NP was measured by an electrochemiluminescence immunoassay, the Roche Elecsys® (Roche Diagnostics, Basel, Switzerland) total P1NP using the cobas® (Roche Diagnostics, Basel, Switzerland) e411 fully automated analyser, which has low within-run (1.8–2.9%) and between-run (2.3–3.7%) coefficients of variation [25], and which detects elevated levels of P1NP in patients with osteoporosis and is sensitive to changes in P1NP in response to treatment [26]. The serum marker of bone resorption reported in this study was C-terminal cross-linking telopeptides of type I collagen (CTX), which is released from type I collagen degradation during the process of bone resorption. CTX was measured by an electrochemiluminescence immunoassay, the Roche Elecsys® β-CrossLaps/serum using the cobas® e411 fully automated analyser, which has low within-run (1.0–4.6%) and between-run (1.6–4.7%) coefficients of variation [27], and which predicts the rate of bone loss and fracture risk in postmenopausal women [28]. All samples for markers of bone turnover were obtained in the morning in the fasting state, were frozen immediately after collection and then processed in a single central laboratory.

DXA Assessment of Bone Mineral Density. DXA estimation of BMD at baseline and change from baseline in BMD after a 1-year time interval was performed for all three anatomical regions – lumbar spine, femoral neck and total hip – as recommended by the International Society for Clinical Densitometry [29]. Patients attended diabetes clinics for most assessments but were referred to specialist centres for DXA examinations. DXA was performed at 15 separate DXA-sites in the participating countries using Hologic (3 sites), Lunar Prodigy (11 sites), and Lunar DPX-L (1 site). DXA measurements and quality control of equipment were performed according to manufacturer protocol and supervised by personnel from the central DXA core laboratory (Uppsala Osteoporosis Research Unit, Uppsala University, Sweden). Prior to the study, personnel from the core laboratory visited all 15 DXA sites to ensure that local technicians positioned patients and employed manufacturer quality control procedures in accordance with the routines conducted at the core laboratory. In addition, a DXA spine-phantom was circulated to all 15 DXA sites in order to calibrate all DXA equipment prior to study start. Furthermore, individual patients were always scanned using the same DXA equipment. Scans were sent to the core laboratory for central reading and analysis when all patients had completed the 50-week treatment period.

Adverse Events. Adverse events (AEs) of fractures were collected using a pre-defined list of preferred terms derived from the Medical Dictionary for Regulatory Activities (MedDRA version 13.0). General AEs from this study at 6 months have been reported elsewhere [10] and will be further reported at 2 years.

Statistical Analysis

Two analysis sets were defined: the safety analysis set, consisting of all randomized patients who received ≥1 dose of investigational product; and the full analysis set, consisting of all randomized patients who received ≥1 dose of investigational product and who had both a baseline and ≥1 post-baseline efficacy value for ≥1 efficacy variable.

The evaluation of TBW and HbA1c used the full analysis set. Adjusted mean changes from baseline value and 95% CI were derived from a longitudinal repeated-measures mixed model using the SAS PROC MIXED procedure with terms for treatment, gender, week and week-by-treatment interaction as fixed effects, and terms for baseline value and week-by-baseline value as fixed covariates. For TBW, observations after the initiation of rescue therapy were included and an additional rescue term was introduced into the mixed model. For HbA1c, observations after the initiation of rescue therapy were excluded.

Evaluation of bone markers and BMD used the safety analysis set and included observations after the initiation of rescue therapy. Adjusted mean (bone marker) and adjusted mean percent (BMD) changes from baseline at week 50, 95% CIs and nominal p values were derived from an ANCOVA model with terms for treatment and gender as fixed effects and baseline value as fixed covariate. For each ANCOVA model, the significance of treatment-by-gender interaction was tested. For analysis of proportions of patients with ≥3% decrease from baseline in BMD, logistic regression with adjustment for baseline BMD and gender as described by Zhang et al. [30] was employed.

Missing data were not imputed in any of the current analyses. While imputation is often used to avoid over-estimation of treatment effects for efficacy variables, the goal in this study was not to be conservative in assessing possible treatment effects for the bone safety endpoints. Thus, we analysed observed data only. For BMD, this involved data at baseline and week 50, as 1 year is suggested in international guidelines as the minimum assessment interval needed to demonstrate meaningful change [29]. For bone markers, data was analysed at the same time-point and used the same statistical model (ANCOVA) to correspond with the BMD analyses. Additionally for bone markers, where meaningful changes can occur earlier than 1 year, longitudinal repeated-measures mixed models analysed change from baseline data at all collected time-points (weeks 8, 24 and 50). For the exploratory efficacy analyses of TBW and HbA1c, observed data were analysed. Imputation, namely last observation carried forward, was used for the previously reported primary analysis of efficacy [10].

Sample size was calculated to detect a difference of 2% for mean percent change in BMD at week 50. Assuming a standard deviation of 3.2%, 55 evaluable subjects per treatment group were required to detect a 2% difference between treatment groups with 90% power for a two-sided test with a 0.05 significance level. An exclusion rate of 20% was estimated for BMD endpoints, due to potential difficulties in collecting and reading DXA scans. Given the planned sample size of 86 patients per group for this study based on considerations for the primary efficacy endpoint, the anticipated number of evaluable subjects for BMD endpoints was 73 patients per group. This number would provide 96% power to detect a 2% difference in mean percent change in BMD, and would provide a 95% CI

for the difference with a width of approximately ±1.0% from the point estimate.

Results

Patients

Figure 1 shows the disposition of the patients, with 90.7% of randomized patients completing the 50-week site- and patient-blinded extension period. The most common reasons for discontinuation were withdrawal of consent and adverse events. Demographic and baseline characteristics were balanced across treatment groups (Table 1).

Efficacy Evaluation

In the dapagliflozin group, mean change in TBW from baseline showed a faster decline over the first few weeks followed by a more gradual decline to week 24 (primary endpoint) that had not plateaued by week 50 (figure 2A). At week 50, the difference in TBW adjusted mean change from baseline between dapagliflozin and placebo was −2.37 kg (95% CI −3.41 to −1.32). Baseline values in the dapagliflozin and placebo groups were 92.1 and 90.9 kg, respectively.

In the dapagliflozin group, mean change in HbA1c from baseline showed an initial drop by 8 weeks, which was sustained over 50 weeks (figure 2B). At week 50, the difference in HbA1c adjusted mean change from baseline between dapagliflozin and placebo was −0.40% (95% CI −0.56 to −0.23). Baseline values in the dapagliflozin and placebo groups were 7.19 and 7.16%, respectively. Two patients in the dapagliflozin and six patients in the placebo group received rescue therapy for failing to achieve prespecified glycaemic targets over 50 weeks. No patients discontinued due to inadequate glycaemic control.

Safety Evaluation

Markers of Bone Formation and Resorption. Compared with placebo, no significant changes in markers of bone formation or resorption were evident with dapagliflozin treatment at week 50 (Table 2) or over time (figure 3). In addition, there were no dapagliflozin effects on these markers in either men or women, with p values for treatment-by-gender interaction terms for P1NP and CTX being 0.5404 and 0.3166, respectively.

Bone Mineral Density. No significant differences between dapagliflozin and placebo in BMD adjusted mean percent change from baseline at week 50 were found in any of the three regions: 0.1 (95% CI −0.83 to 1.04; p = 0.8318) for lumbar spine; −0.62 (95% CI −1.54 to 0.32; p = 0.1926) for femoral neck; and 0.22 (95% CI −0.79 to 1.24; p = 0.6713) for total hip (Table 3A). Furthermore, there were no dapagliflozin effects on BMD in either men or women, with p values for treatment-by-gender interaction terms for lumbar spine, femoral neck and total hip regions being 0.968, 0.295 and 0.729, respectively. Similarly, there were no significant differences between dapagliflozin and placebo in adjusted proportions of patients with ≥3% decrease in BMD from baseline at week 50, being 3.8% (95% CI −7.0 to 14.6%) for lumbar spine, 2.5% (95% CI −9.3 to 14.3%) for femoral neck, and 3.3% (95% CI

Table 1. Demographic and baseline characteristics of the full analysis set.

	Placebo + metformin	Dapagliflozin 10 mg + metformin
Number of patients	91	89
Gender, n (%)		
Men	51 (56.0)	49 (55.1)
Women	40 (44.0)	40 (44.9)
Age, years, mean ± s.d.	60.8 ± 6.9	60.6 ± 8.2
Race, n (%)		
White	91 (100.0)	89 (100.0)
Physical measurements		
Weight, mean ± s.d.	90.9 ± 13.7	92.1 ± 14.1
BMI, kg/m^2, mean ± s.d.	31.7 ± 3.9	32.1 ± 3.9
Waist circumference, cm, mean ± s.d.	104.5 ± 12.3	105.6 ± 10.1
Duration of T2DM, years, mean ± s.d.	5.5 ± 5.3	6.0 ± 4.5
HbA1c, %, mean ± s.d.	7.16 ± 0.53	7.19 ± 0.44
FPG, mmol/l, mean ± s.d.	8.3 ± 1.4	8.2 ± 1.4
Diabetes-related diseases, n (%)		
Neuropathy	4 (4.4)	7 (7.9)
Retinopathy	4 (4.4)	1 (1.1)
Nephropathy	1 (1.1)	0
Microalbuminuria	3 (3.3)	4 (4.5)
Prior history of CVD*, n (%)	26 (28.6)	21 (23.6)
Hypertension, n (%)	75 (82.4)	77 (86.5)
Dyslipidaemia, n (%)	53 (58.2)	51 (57.3)
Estimated GFR†, n (%)		
<30 ml/min/1.73 m^2	0	0
≥30 and <60 ml/min/1.73 m^2	5 (5.5)	1 (1.1)
≥60 and <90 ml/min/1.73 m^2	56 (61.5)	54 (60.7)
≥90 ml/min/1.73 m^2	30 (33.0)	34 (38.2)
Concomitant medications potentially increasing bone mineral density‡		
Calcium and vitamin D_3 supplementation	1 (1.1)	0
Diuretic, thiazide	12 (13.2)	17 (19.1)
Estradiol	0	1 (1.1)
Multivitamin supplementation	0	3 (3.3)
Vitamin D_3 supplementation	2 (2.2)	3 (3.3)
Concomitant medications potentially decreasing bone mineral density‡		
Anticoagulant	4 (4.4)	2 (2.2)
Aromatase inhibitor	0	1 (1.1)
Diuretic, loop	4 (4.4)	3 (3.3)
Proton pump inhibitor	6 (6.6)	7 (7.8)
Serotonin reuptake inhibitor	1 (1.1)	1 (1.1)
Systemic corticosteroid§	1 (1.1)	2 (2.2)
Thyroxine replacement therapy	5 (5.5)	6 (6.6)

BMI, body mass index; CVD, cardiovascular disease; GFR, glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; OAD, oral antidiabetic drug; T2DM, type 2 diabetes mellitus.

*Does not include patients with a cardiovascular history of hypertension only.

†Calculation of GFR based upon the Modification of Diet in Renal Disease formula; eGFR (mL/min/1.73 m^2) = 186 × (serum creatinine [mg/dL])$^{-1.154}$ × (Age)$^{-0.203}$ × (0.742 if female) × (1.21 if black).

‡Patient numbers based on safety analysis set.

§Short-term (<7 days) treatment for respiratory infection or local injection for epicondylitis.

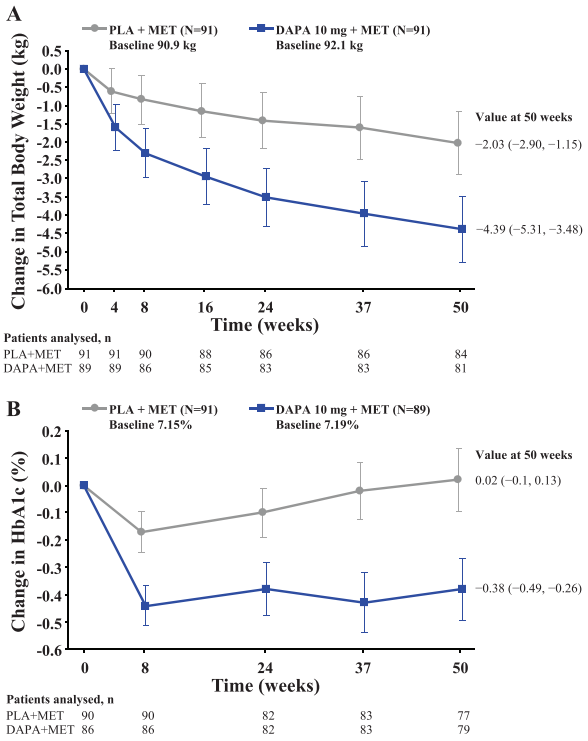

Figure 2. Changes in total body weight (A) and HbA1c (B) over time. Data are adjusted mean changes from baseline value and 95% CI derived from a longitudinal repeated-measures mixed model with fixed effects for treatment, gender, week and week-by-treatment interaction, and fixed covariates for baseline value and week-by-baseline value. The model for total body weight included an additional fixed effect of rescue. N is the number of patients in the full analysis set. n is the is the number of patients in the full analysis set with non-missing baseline and week-*t* values. DAPA, dapagliflozin; MET, metformin; PLA, placebo.

−6.1 to 12.6%) for total hip regions. No meaningful gender differences in these proportions were noted (Table 3B).

Laboratory Values of Interest. Dapagliflozin was associated with a large increase in urinary glucose excretion on commencement of therapy that was sustained over time as expected from its mechanism of action (Appendix S2). Also observed were small mean increases from baseline in serum magnesium (0.03 mmol/l) and inorganic phosphate (0.06 mmol/l) but with absolute values at week 50 that remained within the normal range for these parameters. No mean changes from baseline in serum calcium, 25-hydroxy vitamin D, parathyroid hormone or estimated glomerular filtration rate (eGFR) were evident (Table 4).

Adverse Events. No AEs of fracture were reported by week 50. Proportions of patients with at least one AE were similar in the dapagliflozin (58.2%) and placebo group (56.0%) by week 50. A higher proportion of patients in the dapagliflozin group experienced at least one serious adverse event (SAE) compared with placebo (12.2 vs 8.8%). SAEs reported in the dapagliflozin group were pneumonia (three events), vertigo (two events), breast cancer, prostate cancer, transient global amnesia, transient ischaemic attack, hypertension (two events), oesophageal variceal haemorrhage and spinal osteoarthritis; and in the placebo group, appendicitis, basal cell carcinoma, prostatic adenoma, back pain, acute myocardial infarction, goitre and ulcerative keratitis. The patient experiencing a transient ischaemic attack showed no meaningful change in haematocrit (baseline value, 42%; value before event, 40%; value after event 44%). One patient in the dapagliflozin group died during hospitalization for pneumonia due to oesophageal variceal haemorrhage. A higher proportion of patients in the dapagliflozin group were discontinued from the study due to an AE compared with placebo (6.6 vs 2.2%) or a SAE (2.2 vs 0%).

By week 50, no patient had reported 'major' hypoglycaemia (defined as a symptomatic episode requiring external assistance due to severe impairment in consciousness or behaviour with a capillary or plasma glucose value <3 mmol/l and prompt recovery after glucose or glucagon administration); four patients in the dapagliflozin and two patients in the placebo group had reported at least one 'minor' hypoglycaemic episode (defined as either a symptomatic episode with a capillary or plasma glucose measurement <3.5 mmol/l, regardless of the need for external assistance, or an asymptomatic capillary or plasma glucose measurement <3.5 mmol/l that did not qualify

Table 2. Bone formation and resorption markers, adjusted mean change from baseline to week 50 (safety analysis set).

	Procollagen type-1 N-terminal propeptide (µg/l)		C-terminal cross-linking telopeptides of type I collagen (µg/l)	
	PLA + MET(N = 91)	DAPA 10 mg + MET(N = 91)	PLA + MET(N = 91)	DAPA 10 mg + MET(N = 91)
n	77	78	76	78
Baseline mean (s.d.)	28.2 (13.5)	26.7 (10.5)	0.24 (0.13)	0.22 (0.12)
Adjusted mean change from baseline*	−3.38	−1.11	0.02	0.04
95% CI	−5.24 to −1.53	−2.94 to 0.73	−0.00 to 0.05	0.02 to 0.07
Difference from PLA + MET		2.28		0.02
95% two-sided CI of difference		−0.33 to 4.88		−0.02 to 0.05
p value of difference		0.0862		0.3338

DAPA, dapagliflozin; MET, metformin; N, number of patients in the safety analysis set; n, number of patients in the safety analysis set with non-missing baseline and week 50 values; PLA, placebo.

* Data are adjusted mean changes from baseline derived from an ANCOVA model with terms for treatment and gender as fixed effects and baseline value as fixed covariate.

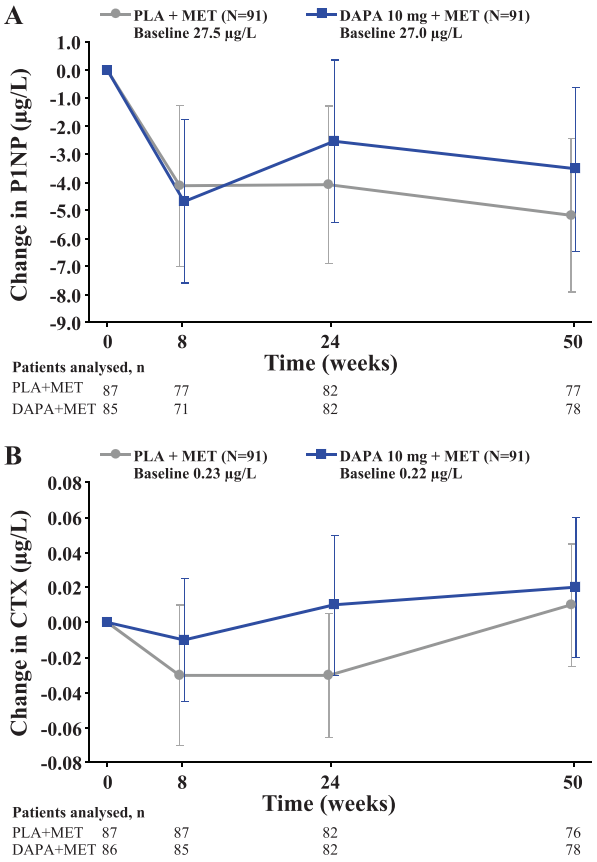

Figure 3. Change in markers of bone formation and resorption over time: (A) procollagen type-1 N-terminal propeptide (P1NP); and (B) C-terminal cross-linking telopeptides of type I collagen (CTX). Data are adjusted mean changes from baseline value and 95% CI derived from a longitudinal repeated-measures mixed model with fixed effects for treatment, gender, week, rescue and week-by-treatment interaction and fixed covariates for baseline value and week-by-baseline value. N is the number of patients in the safety analysis set. n is the is the number of patients in the safety analysis set with non-missing baseline and week-*t* values. DAPA, dapagliflozin; MET, metformin; PLA, placebo.

as a major episode) and one patient in the placebo group had reported at least one episode of 'other' hypoglycaemia (defined as a suggestive episode reported but not meeting the criteria for major or minor episodes). No patient was discontinued from the study or study medication due to a hypoglycaemic event.

Discussion

Compared with placebo, no relevant or significant changes in markers of bone formation and resorption or changes in BMD at any investigated anatomical region were observed with dapagliflozin treatment over 50 weeks. In addition, no significant gender differences were evident for these variables and no adverse events of fracture occurred. Dapagliflozin produced small increases in serum inorganic phosphate and magnesium, as has been noted in previous studies with dapagliflozin [4,8,9], although the clinical relevance of these changes is unclear. The mechanisms of the increases in serum phosphate and magnesium with dapagliflozin are not known. There were no clinically meaningful changes in serum calcium, 25-hydroxy vitamin D or parathyroid hormone.

Maintenance of the skeleton involves a continuous process of bone remodelling driven by osteoblasts, which form new bone, and osteoclasts, which resorb bone. Bone disease may occur if remodelling is accelerated or slowed or if an imbalance between bone formation and resorption occurs. For example, acceleration of bone turnover commonly occurs in osteoporosis and is associated with an increase in markers of bone formation and resorption [14].

T2DM is associated with an increase in BMD secondary to increased BMI [15], insulin resistance [16] and/or reduced bone turnover [17,18]. However, fracture risk in patients with T2DM is higher for any given BMD and age [22]. This increased fracture risk could arise from a number of mechanisms. First, older adults with T2DM have an increased risk of falls, and this risk is significantly associated with peripheral neuropathy, impaired renal function and low HBA1c with insulin therapy [31]. Second, T2DM may impair bone quality through influences on bone metabolism. For example, increased urinary pentosidine, an advanced glycation end product, is associated with an increased risk of fractures in patients with T2DM independent of BMD. This suggests that the accumulation of advanced glycation end products, which stiffen bone collagen, may account for reduced bone strength in T2DM [32]. In addition, chronic hyperglycaemia associated with single-gene mutations in the leptin gene or its receptor promotes extensive cytolipidaemia-induced osteopaenia in animal models of type 2 diabetes [33], although the relevance of this potential mechanism to humans without leptin or leptin receptor mutations has not been evaluated. Other metabolic changes that may reduce bone strength in T2DM include, disturbance of vitamin D, calcium and parathyroid hormone metabolism, diabetic nephropathy and changes associated with glucose-lowering therapy [19]. It is reassuring to note that, despite the renal tubular mechanism of action of dapagliflozin and its effect on weight, no influence on markers of bone turnover, BMD, serum calcium, 25-hydroxy vitamin D or parathyroid hormone levels or estimated glomerular filtration rate was evident.

This study has a number of methodological strengths. First, the study was prospectively designed, adequately powered and of sufficient duration to assess both clinically relevant long-term BMD changes and short-term and long-term changes in markers of bone turnover. Combined assessment of markers of bone turnover and BMD is recommended [14] because bone turnover markers are more sensitive to rapid changes, whereas changes in BMD are slow. Second, the inclusion criteria were designed to avoid possible confounding changes in BMD and to maximize the long-term retention of patients with minimal need for rescue therapy. Third, all three of the most important anatomical sites were evaluated for DXA BMD changes and rigorous quality control was undertaken at each DXA site, with scans sent for central reading and analysis at the end of the 50-week treatment period. Fourth, all blood samples for markers of bone turnover were sent to a single central laboratory for processing and analysis.

A number of potential limitations can be identified. First, the use of concomitant nutritional supplements at baseline was not

Table 3. Bone mineral density: (A) adjusted mean percent change from baseline to week 50 and (B) adjusted proportion of patients with at least a 3% decrease from baseline at week 50 (safety analysis set).

	Lumbar spine (L1–4)		Femoral neck		Total hip	
	PLA + MET (N = 91)	DAPA 10 mg + MET(N = 91)	PLA + MET (N = 91)	DAPA 10 mg + MET(N = 91)	PLA + MET (N = 91)	DAPA 10 mg + MET(N = 91)
(A) Adjusted mean percent change in BMD from baseline to week 50						
All patients, n	83	81	83	81	83	81
Baseline mean (s.d.), g/cm^2	1.19 (0.19)	1.18 (0.20)	0.94 (0.14)	0.97 (0.14)	1.06 (0.11)	1.10 (0.14)
Adjusted mean percent change from baseline*	0.15	0.25	0.15	−0.47	−0.23	−0.02
95% CI	−0.50 to 0.81	−0.41 to 0.92	−0.51 to 0.81	−1.13 to 0.19	−0.94 to 0.48	−0.73 to 0.70
Difference from PLA + MET		0.10		−0.62		0.22
95% two-sided CI of difference		−0.83 to 1.04		−1.54 to 0.32		−0.79 to 1.24
p value of difference		0.8318		0.1926		0.6713
Male patients, n	45	45	45	45	45	45
Baseline mean (s.d.), g/cm^2	1.22 (0.71)	1.21 (0.16)	0.97 (0.14)	1.02 (0.13)	1.08 (0.11)	1.15 (0.13)
Adjusted mean percent change from baseline*	0.55	0.63	−0.03	−0.20	−0.17	0.21
95% CI	−0.35 to 1.46	−0.26 to 1.54	−0.92 to 0.86	−1.11 to 0.71	−1.13 to 0.79	−0.78 to 1.21
Difference from PLA + MET		0.08		−0.17		0.38
95% two-sided CI of difference		−1.17 to 1.35		−1.42 to 1.10		−0.98 to 1.76
Female patients, n	38	36	38	36	38	36
Baseline mean (s.d.), g/cm^2	1.15 (0.21)	1.15 (0.24)	0.90 (0.13)	0.91 (0.13)	1.02 (0.11)	1.05 (0.13)
Adjusted mean percent change from baseline*	−0.24	−0.12	0.36	−0.80	−0.28	−0.26
95% CI	−1.21 to 0.73	−1.21 to 0.88	−0.62 to 1.35	−1.78 to 0.20	−1.34 to 0.78	−1.32 to 0.82
Difference from PLA + MET		0.12		−1.16		0.03
95% two-sided CI of difference		−1.26 to 1.52		−2.51 to 0.22		−1.45 to 1.53
(B) Adjusted proportion of patients with ≥3% decrease in BMD from baseline at week 50						
All patients, x/n	10/83	13/81	14/83	15/81	8/83	9/81
Adjusted proportion with ≥3% decrease†	12.1%	15.9%	16.4%	18.8%	9.0%	12.2%
95% CI	5.1 to 19.1%	7.8 to 23.9%	8.3 to 24.5%	10.4 to 27.3%	2.6 to 15.3%	5.5 to 18.9%
Difference from PLA + MET		3.8%		2.5%		3.3%
95% two-sided CI of difference		−7.0 to 14.6%		−9.3 to 14.3%		−6.1 to 12.6%
Male patients, x/n	4/45	8/45	8/45	6/45	5/45	3/45
Adjusted proportion with ≥3% decrease†	9.0%	17.6%	16.8%	13.5%	11.1%	6.7%
95% CI	0.7 to 17.3%	6.5 to 28.7%	5.7 to 27.9%	3.6 to 23.4%	NC	NC
Difference from PLA + MET		8.6%		−3.2%		−4.4%
95% two-sided CI of difference		−5.3 to 22.5%		−18.1 to 11.6%		−18.9 to 8.8%
Female patients, n	6/38	5/36	6/38	9/36	3/38	6/38
Adjusted proportion with ≥3% decrease†	15.8%	13.7%	15.8%	25.3%	7.9%	16.7%
95% CI	4.2 to 27.4%	2.5 to 24.9%	4.2 to 27.4%	11.3 to 39.4%	NC	NC
Difference from PLA + MET		−2.1%		9.6%		8.8%
95% two-sided CI of difference		−18.2 to 14.0%		−8.7 to 27.8%		−7.3 to 26.4%

BMD, bone mineral density; DAPA, dapagliflozin; MET, metformin; N, number of patients in the safety analysis set; n, number of patients in the safety analysis set with non-missing baseline and week 50 values; NC, not calculable; PLA, placebo.

* Data are adjusted mean percent changes from baseline derived from an ANCOVA model with terms for treatment and gender as fixed effects and baseline value as fixed covariate.

† Data are adjusted proportions derived from logistic regression using the methodology of Zhang et al. [30] with adjustment for baseline BMD and gender.

controlled during this study. Although three patients in each treatment group were receiving vitamin D_3 supplementation at baseline, a further three patients in the dapagliflozin group were receiving multivitamins at baseline, which may have contained variable amounts of vitamin D. However, no changes in concomitant supplement use were allowed after study commencement, so their stable use is unlikely to have affected BMD changes from baseline upon which these analyses were based. Second, 96.7% of recruited patients had either normal or mild impairment of renal function. Given the mechanism of action of dapagliflozin, further data are required to assess potential effects of dapagliflozin on bone metabolism in patients with clinically significant renal impairment. Third, in order to achieve adequate power to assess dapagliflozin effects on bone metabolism it was necessary to recruit patients from multiple centres, which used 3 different types of DXA equipment. However, standardization procedures were rigorously followed and monitored, DXA phantom scans were performed to minimize inter-centre variability prior to study start, and individual patients were always assessed using the same DXA equipment. Given, that change from baseline scores were analysed, variation in DXA equipment across centres is highly unlikely to have affected the results. Finally, the study recruited patients exclusively from the European Union, which could potentially limit the generalizability of these findings. However, racial or ethnic origin is unlikely

Table 4. Selected laboratory values of interest: change from baseline at week 50 (safety analysis set). Data are mean (s.d.) or mean [s.e.].

	Placebo + metformin (N = 91)			Dapagliflozin 10 mg + metformin (N = 91)		
	Baseline mean	n at week 50	Mean change at week 50	Baseline mean	n at week 50	Mean change at week 50
Urine glucose, mmol/l	1.25 (3.56)	83	−0.04 [0.62]	1.72 (6.70)	81	120.67 [9.03]
Estimated GFR*, ml/min/1.73m^2	82.6 (16.1)	83	1.2 [1.2]	86.0 (14.2)	80	0.4 [1.0]
Calcium, mmol/l	2.38 (1.99)	83	0.01 [0.01]	2.38 (0.10)	80	0.04 [0.01]
Magnesium, mmol/l	0.80 (0.09)	83	−0.04 [0.01]	0.79 (0.08)	81	0.03 [0.01]
Inorganic phosphorus, mmol/l	1.12 (0.18)	83	0.01 [0.02]	1.11 (0.18)	80	0.06 [0.01]
Alkaline phosphatase, U/l	68.9 (18.8)	83	−4.4 [0.82]	67.6 (21.1)	80	−0.4 [1.20]
25-hydroxy vitamin D, nmol/l	56.4 (16.6)	81	−1.2 [1.6]	54.2 (14.1)	80	0.7 [1.6]
Parathyroid hormone, ng/l	30.3 (14.7)	81	0.7 [1.3]	36.1 (19.8)	78	0.9 [1.5]

N, number of patients in the safety analysis set and including data after rescue therapy; n, number of patients in the safety analysis set with non-missing baseline and week 50 values and including data after rescue therapy.

* Calculation of glomerular filtration rate (GFR) based upon the Modification of Diet in Renal Disease formula; eGFR (ml/min/1.73 m^2) = 186 × [serum creatinine (mg/dl)]$^{-1.154}$ × (Age)$^{-0.203}$ × (0.742 if female) × (1.21 if black). Normal values: calcium, 2.05–2.55 mmol/l; magnesium, 0.65–1.05 mmol/l; inorganic phosphorus, 0.74–1.52 mmol/l; 25-hydroxy vitamin D, 35–150 nmol/l; parathyroid hormone, 10–65 ng/l.

to have affected the mechanism of action of dapagliflozin as clinical studies of dapagliflozin have not shown differences in efficacy by geographical area [5].

In conclusion, compared with placebo, no changes in relevant markers of bone formation and resorption or BMD could be demonstrated during the course of 50 weeks of dapagliflozin treatment in patients whose T2DM was inadequately controlled on metformin.

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Conflict of Interest

J. B., J. L., J. W., A. M. L. and S. P. participated in the study concept and design. J. B. and Ö. L. performed data acquisition. A. M. L. and C. D. S. supervised the study. J. B., Ö. L., J. K., L. J., J. W., A. M. L., C. D. S., J. S. and S. P. analysed and interpreted the data. J. S. performed statistical verification of data. J. B., Ö. L., J. K., L. J., J. W., A. M. L., C. D. S., J. S. and S. P. contributed to writing and revising the report.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Exclusion criteria.

Appendix S2. Change in urinary glucose.

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