

Supplementary material

Type 2 diabetes and Change in Total Hip Bone Area and Bone Mineral Density in Elderly Swedish Men and Women

Adam Mitchell ¹, Tove Fall ², Håkan Melhus ³, Lars Lind ⁴, Karl Michaëlsson ¹, Liisa Byberg ¹

Institutions of origin:

1. Department of Surgical Sciences, Medical Epidemiology, Uppsala University, Sweden
2. Department of Medical Sciences, Molecular Epidemiology, Uppsala University, Sweden
3. Department of Medical Sciences, Clinical Pharmacogenomics and Osteoporosis, Uppsala University, Sweden
4. Department of Medical Sciences, Clinical Epidemiology, Uppsala University, Sweden

Corresponding author:

Adam Mitchell

Epihubben, MTC, Uppsala Science Park

751 85 Uppsala, Sweden

adam.mitchell@surgsci.uu.se

Phone: +46 762561548

ORCID: 0000-0003-3222-2970

Supplementary Methods

In SMCC, Fasting plasma glucose concentrations were analysed using three different methods depending on date of collection; glucose dehydrogenase reagent (Bergman & Beving, instrument Advia 1650, Stockholm, Sweden), glucose oxidase method (Bayer, instrument Advia 1650, Leverkusen, Germany), and hexokinase method (Abbott, Abbott Architect, Illinois, USA).

In PIVUS, fasting plasma glucose concentrations were analysed using the standard method at Uppsala University Hospital

In ULSAM, fasting plasma glucose concentrations (mmol/l) were measured by the glucose dehydrogenase method (Gluc-DH, Merck, Darmstadt, Germany).

Supplementary Table 1. Additional population characteristics at baseline and follow-up in the three cohorts.

	SMCC			PIVUS			ULSAM		
	No T2DM	Incident T2DM	Prevalent T2DM	No T2DM	Incident T2DM	Prevalent T2DM	No T2DM	Incident T2DM	Prevalent T2DM
<i>Anthropometric factors</i>									
Weight at follow-up, kg	67.7 (11.5)	74.6 (14.2)	74.7 (13.3)	74.0 (13.9)	81.4 (16.1)	80.5 (14.4)	75.4 (10.6)	77.9 (11.0)	81.7 (16.1)
Weight difference, kg	-0.1 (1.3)	0.1 (0.9)	-0.2 (1.1)	-1.1 (5.2)	0.2 (5.0)	-0.4 (7.0)	-2.8 (4.9)	-2.7 (5.0)	-3.3 (4.4)
Total body fat at baseline, g	25786 (8648)	30923 (10302)	31355 (9702)	24793 (8317)	26952 (10690)	26510 (6899)	21752 (7253)	23191 (7677)	26342 (9253)
Total body lean mass at baseline, g	38908 (4023)	40018 (4437)	41034 (5006)	47447 (10106)	51382 (10023)	50891 (9592)	51098 (5385)	52404 (3702)	51864 (6756)
Total body fat at follow-up, g	25710 (8794)	31024 (10507)	30622 (9245)	25110 (8751)	27559 (11301)	27622 (8326)	21605 (7640)	22581 (7782)	27005 (8621)
Total body lean mass at follow-up, g	39026 (3957)	40359 (4505)	41461 (5824)	46280 (9660)	50237 (9495)	49685 (9271)	51257 (5270)	51732 (4678)	52245 (6340)
Total body fat difference, g	-76 (4453)	101 (5428)	-733 (6162)	331 (3334)	606 (3051)	487 (4158)	-147 (3691)	-610 (3377)	663 (3782)
Total body lean mass difference, g	118 (2021)	341 (1980)	427 (3170)	-1176 (1883)	-1145 (1728)	-951 (1778)	159 (2666)	-672 (2718)	381 (2560)
<i>Diabetes treatment</i>									
Insulin at baseline, <i>n</i> (%)	0 (0)	0 (0)	6 (13.0)	0 (0)	0 (0)	2 (5.3)	0 (0)	0 (0)	1 (4.5)
Oral antidiabetics at baseline, <i>n</i> (%)	0 (0)	0 (0)	20 (43.5)	0 (0)	0 (0)	19 (50.0)	0 (0)	0 (0)	11 (50.0)
Insulin at follow-up, <i>n</i> (%)	0 (0)	2 (3.1)	11 (23.9)	0 (0)	3 (10.0)	12 (32.4)			
Oral antidiabetics at follow- up, <i>n</i> (%)	0 (0)	31 (48.4)	30 (65.2)	0 (0)	11 (36.7)	33 (86.8)			
Insulin or oral antidiabetics at follow-up, <i>n</i> (%)							0 (0)	8 (32.0)	9 (40.9)

Dietary factors at baseline

Subsample n	945	63	46	369	28	35	138	23	13
Adherence to the modified Mediterranean diet score, <i>n</i> (%)									
low (0-2 points)	75 (7.9)	7 (11.1)	3 (6.5)						
medium (3-5 points)	559 (59.2)	35 (55.6)	24 (52.2)						
high (6-8 points)	311 (32.9)	21 (33.3)	19 (41.3)						
Beta carotene, mg/day				1911 (1141)	1752 (1238)	1937 (1270)	2695 (1903)	2527 (1719)	2746 (2295)
Zinc, mg/day				10.3 (2.5)	10.5 (2.6)	11.2 (3.9)	9.4 (2.7)	9.6 (2.8)	10.3 (4.9)
Saturated fat, g/day				30.7 (12.3)	30.1 (9.8)	29.6 (11.4)	32.2 (11.1)	31.4 (11.2)	32.0 (13.9)
Monounsaturated fat, g/day				24.5 (8.7)	24.7 (7.3)	25.2 (9.6)	24.7 (7.9)	24.8 (8.1)	27.1 (10.9)
Polyunsaturated fat, g/day				9.0 (3.0)	8.8 (2.6)	10.0 (3.9)	9.7 (3.2)	9.7 (3.1)	11.6 (4.6)
Servings of fruit and vegetables/day							4.1 (2.2)	4.6 (2.5)	4.8 (2.0)

T2DM, type 2 diabetes mellitus; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men.

Supplementary Table 2. Femoral neck diameter (d3) and cross-sectional moment of inertia (CSMI) at baseline, follow-up and the change between examinations

	SMCC			PIVUS			ULSAM		
	No T2DM	Incident T2DM	Prevalent T2DM	No T2DM	Incident T2DM	Prevalent T2DM	No T2DM	Incident T2DM	Prevalent T2DM
d3 at baseline, mm	33.07 (2.35)	33.09 (2.13)	32.91 (2.16)	35.83 (3.46)	36.79 (3.98)	35.22 (3.51)	39.06 (2.88)	38.33 (2.34)	38.65 (3.21)
d3 at follow-up, mm	33.07 (2.35)	33.06 (2.21)	33.07 (2.06)	36.10 (3.53)	36.98 (4.42)	35.55 (3.77)	38.89 (2.28)	38.00 (1.87)	41.53 (2.74)
d3 difference, mm	-0.005 (1.257)	-0.037 (1.336)	0.163 (1.559)	0.270 (0.711)	0.192 (1.839)	0.332 (0.955)	-0.075 (1.413)	-0.040 (0.698)	0.180 (1.139)
CSMI at baseline, mm ⁴	9890 (2675)	10027 (1852)	10160 (2310)	13738 (5446)	15375 (5945)	13876 (5351)	17238 (4298)	17426 (3582)	18738 (4165)
CSMI at follow-up, mm ⁴	9867 (2606)	10104 (1944)	10502 (2253)	13747 (5521)	15326 (6307)	14033 (5682)	17360 (3765)	17186 (4333)	22848 (4233)
CSMI difference, mm ⁴	-22 (1525)	77 (1372)	342 (1592)	9 (1082)	-50 (1950)	157 (803)	-119 (1683)	-412 (1127)	-35 (1837)

T2DM, type 2 diabetes mellitus; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men.

Supplementary Table 3. Change in bone measurements in those with incident and prevalent T2DM relative to those without T2DM in each of the cohorts and in a pooled analysis, Model 1.

Cohort	Incident T2DM		Prevalent T2DM		No T2DM		<i>p</i>
	N	β (95% CI)	N	β (95% CI)	N	β (95% CI)	
Bone area of the total hip, cm ²							
SMCC	64	-0.17 (-0.33, -0.02)	46	0.06 (-0.12, 0.24)	950	(reference)	0.0769
PIVUS	30	-0.07 (-0.28, 0.14)	38	-0.03 (-0.22, 0.16)	415	(reference)	
ULSAM	25	-0.19 (-0.68, 0.30)	22	-0.04 (-0.56, 0.48)	156	(reference)	
pooled	119	-0.15 (-0.27, -0.02)	106	0.02 (-0.11, 0.16)	1521	(reference)	
BMD of the total hip, g/cm ²							
SMCC	64	0.013 (-0.008, 0.034)	46	0.026 (0.001, 0.050)	950	(reference)	0.0320
PIVUS	30	0.007 (-0.007, 0.020)	38	0.008 (-0.004, 0.020)	415	(reference)	
ULSAM	25	-0.017 (-0.042, 0.008)	22	0.008 (-0.020, 0.035)	156	(reference)	
pooled	119	0.007 (-0.007, 0.020)	106	0.018 (0.004, 0.032)	1521	(reference)	
Diameter of the femoral neck, mm							
SMCC	64	-0.037 (-0.348, 0.275)	46	0.148 (-0.216, 0.512)	950	(reference)	0.6583
PIVUS	30	-0.075 (-0.389, 0.239)	38	0.050 (-0.232, 0.333)	413	(reference)	
ULSAM	25	-0.048 (-0.574, 0.477)	22	0.176 (-0.381, 0.733)	156	(reference)	
pooled	119	-0.038 (-0.253, 0.176)	106	0.095 (-0.133, 0.322)	1519	(reference)	
Cross sectional moment of inertia (CSMI), mm ⁴							
SMCC	64	111 (-253, 474)	46	422 (-3, 846)	950	(reference)	0.1584
PIVUS	30	-54 (-477, 369)	38	168 (-211, 547)	413	(reference)	
ULSAM	25	-282 (-960, 397)	22	254 (-470, 977)	156	(reference)	
pooled	119	11 (-252, 274)	106	272 (-6, 550)	1519	(reference)	

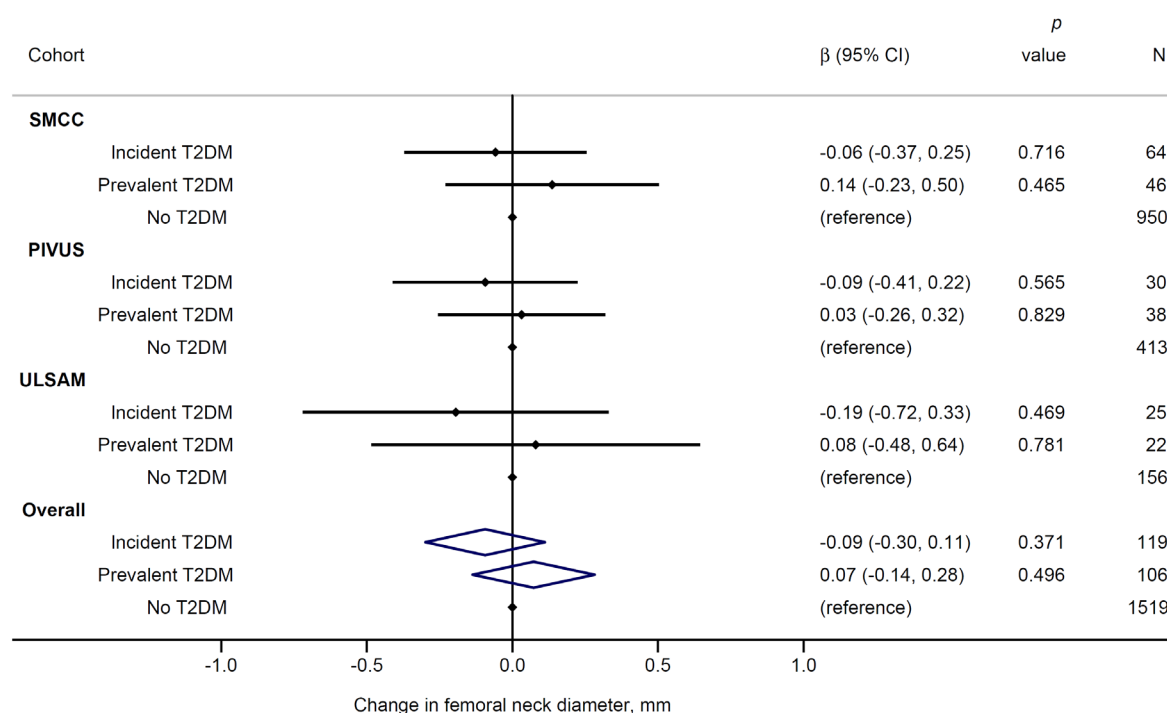
P values from the overall test. Model 1 includes as covariates: age, time between baseline and follow-up examination, baseline measurement of the outcome variable, and sex (for PIVUS and the pooled estimate). Cohort was additionally included in the model for the pooled estimate. T2DM, type 2 diabetes mellitus; BMD, Bone mineral density; CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men

Supplementary Table 4. Prescribed drugs before baseline and during follow-up and plasma concentrations at baseline in the Swedish Mammography Cohort Clinical (SMCC)

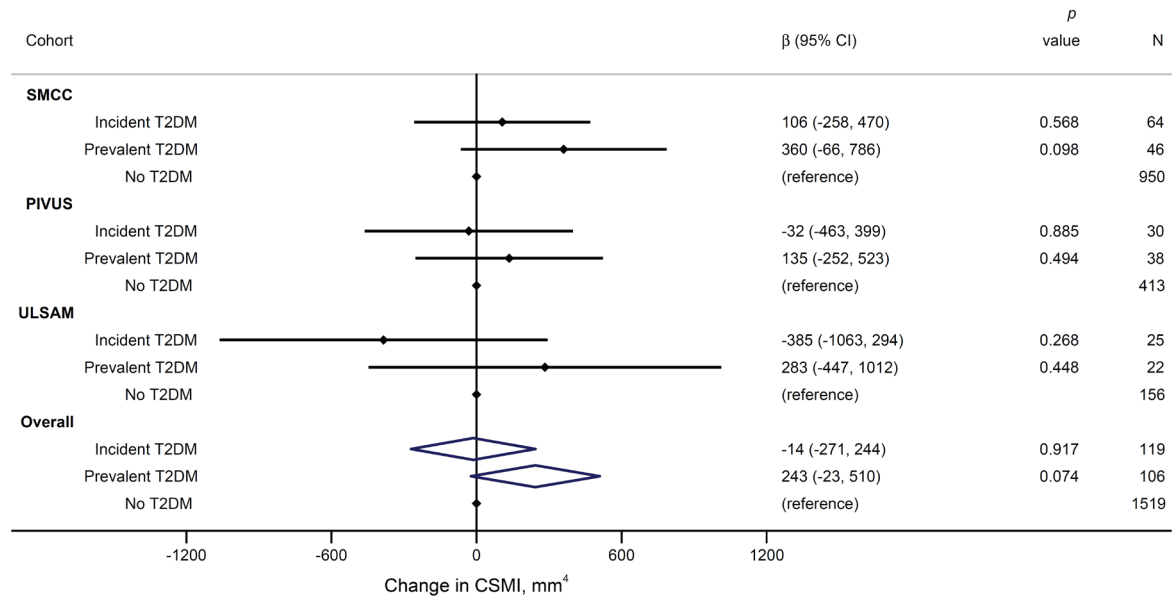
	SMCC		
	No T2DM	Incident T2DM	Prevalent T2DM
Prescribed drugs			
Subsample, n ^a (total n=777)	699	44	34
<i>Prescribed drugs at baseline, n(%)</i>			
Cardiovascular drugs (ATC C1-C9)	176 (25.2)	16 (36.4)	18 (52.9)
Lipid lowering drugs (ATC C10)	70 (10.0)	10 (22.7)	12 (35.3)
Drugs affecting thyroid function (ATC H03)	67 (9.6)	3 (6.8)	7 (20.6)
Drugs with effects on bone metabolism (ATC M05B)	22 (3.1)	0 (0.0)	2 (5.9)
Estrogen replacement therapy (ATC G03C G03F)	265 (37.9)	16 (36.4)	10 (29.4)
<i>Prescribed drugs at follow-up, n(%)</i>			
Cardiovascular drugs (ATC C1-C9)	336 (48.1)	30 (68.2)	22 (64.7)
Lipid lowering drugs (ATC C10)	127 (18.2)	16 (36.4)	12 (35.3)
Drugs affecting thyroid function (ATC H03)	54 (7.7)	8 (18.2)	3 (8.8)
Drugs with effects on bone metabolism (ATC M05B)	74 (10.6)	4 (9.1)	1 (2.9)
Estrogen replacement therapy (ATC G03C G03F)	171 (24.5)	10 (22.7)	14 (41.2)
Serum concentrations at baseline			
Subsample n (total n=1056)	946	64	46
Calcium, mmol/l (SD)	2.32 (0.10)	2.31 (0.09)	2.34 (0.10)
Vitamin D, nmol/l (SD)	60.1 (17.2)	55.6 (14.8)	57.6 (16.5)
Parathyroid hormone, pmol/l (SD)	4.83 (1.61)	5.34 (1.92)	5.16 (1.80)
Season of blood draw at baseline			
Winter, Dec-Feb	242 (25.5)	13 (20.3)	9 (19.6)
Spring, Mar-May	311 (32.7)	21 (32.8)	10 (21.7)
Summer, Jun-Aug	158 (16.6)	16 (25.0)	16 (34.8)
Autumn, Sep-Nov	239 (25.2)	14 (21.9)	11 (23.9)

^a Prescribed drugs from the Swedish Prescribed Drug Registry, including women attending their baseline examination after 1 July 2005

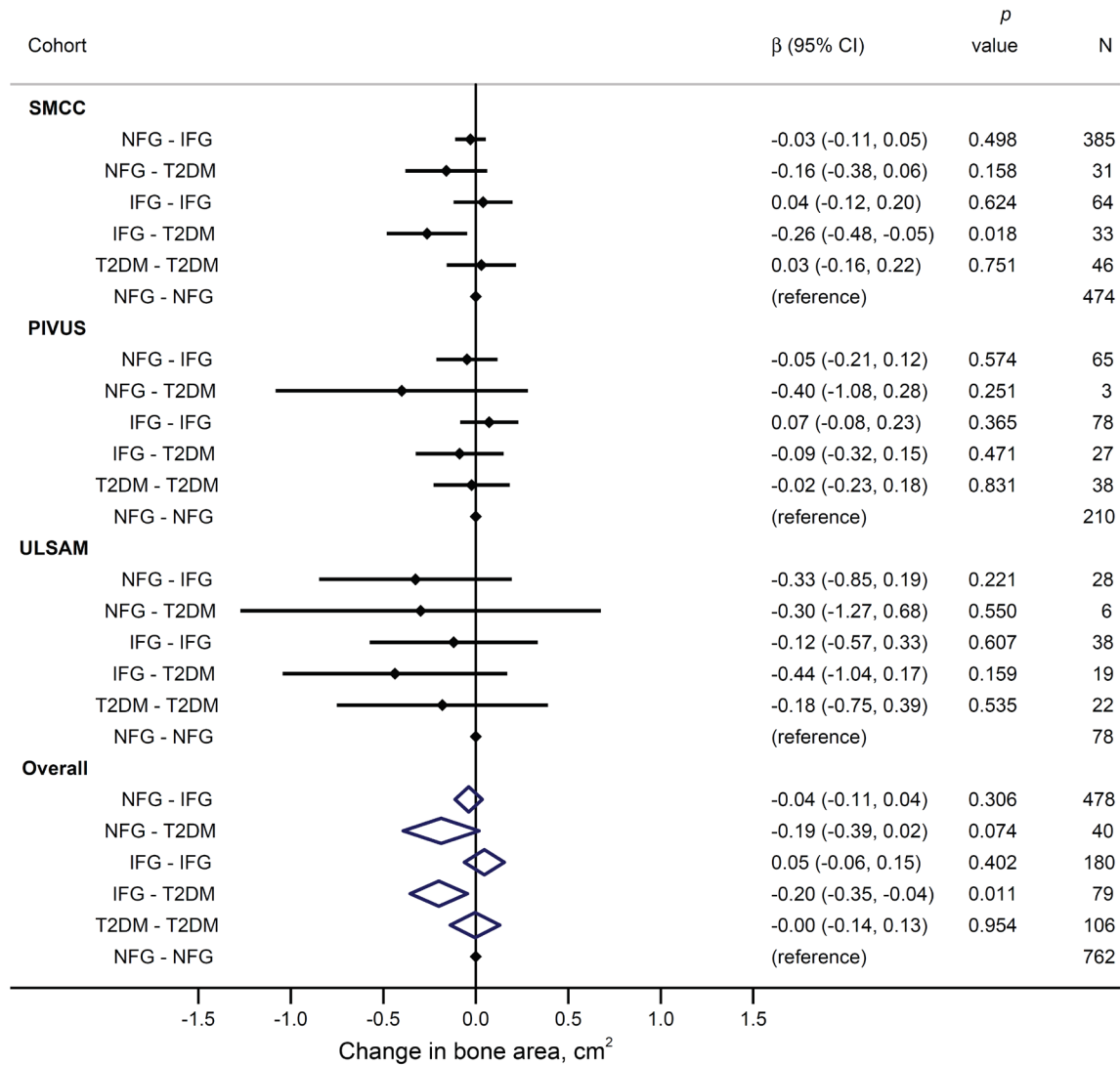
ATC, (Anatomical Therapeutic Chemical Classification) categories; T2DM, type 2 diabetes mellitus



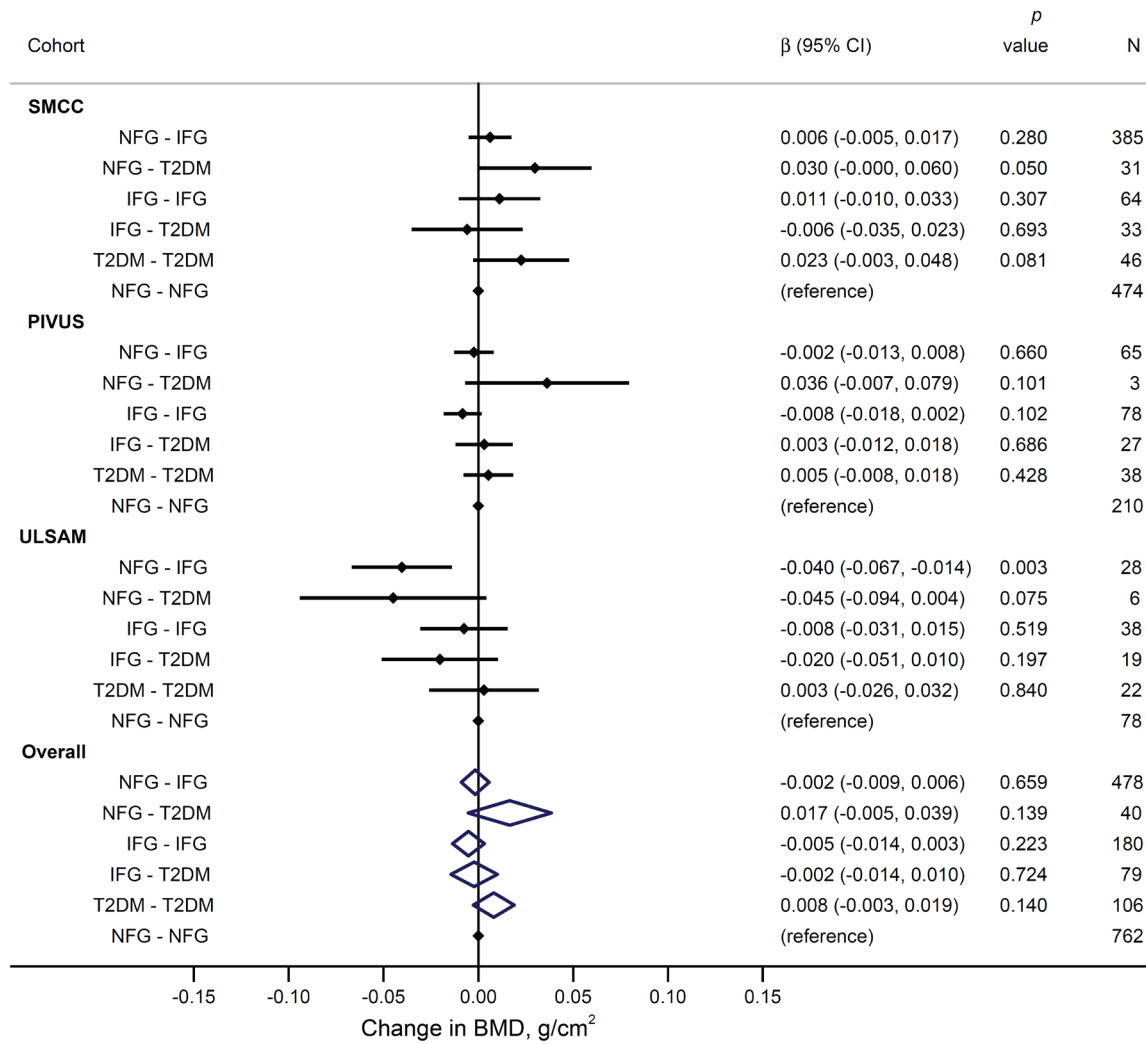
Supplementary Fig. 1. Change in femoral neck diameter (d3; mm) in those with incident and prevalent T2DM relative to those without T2DM in each of the cohorts and as meta-analysed overall estimates. Estimates were adjusted for age, sex, time between baseline and follow-up examinations, baseline d3, height, body mass index, education, smoking status, physical activity, and comorbidity (Charlson's comorbidity index in SMCC and ULSAM and cardiovascular disease diagnosis in PIVUS). T2DM, type 2 diabetes mellitus; CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men.



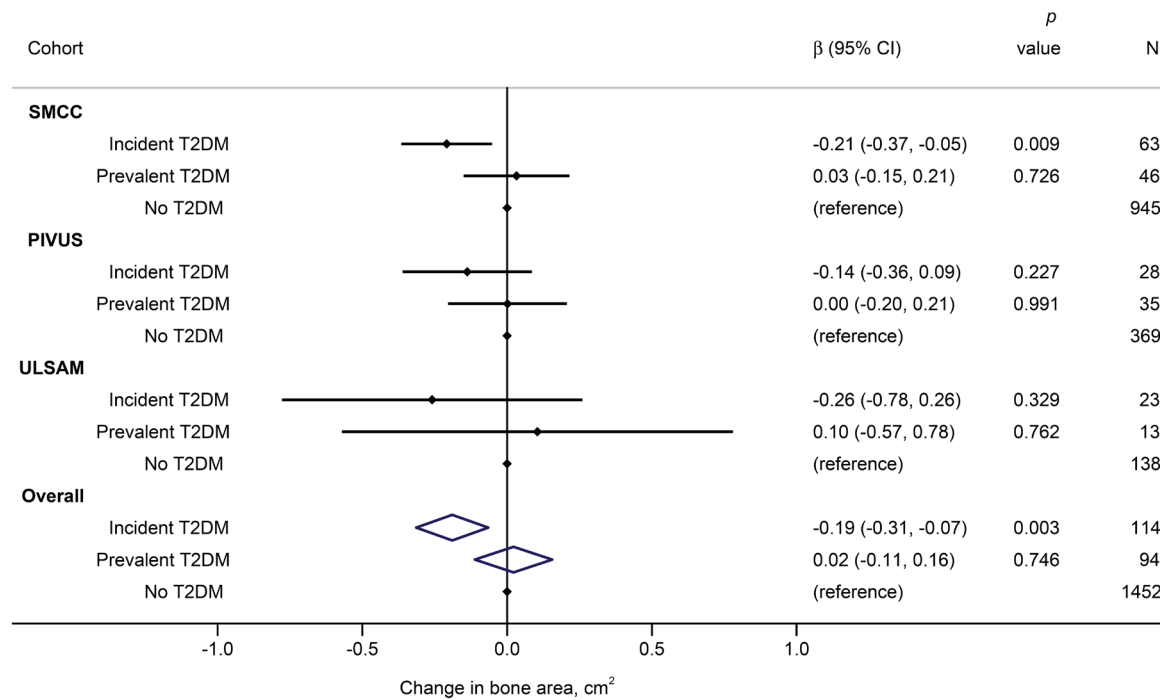
Supplementary Fig. 2. Change in cross-sectional moment of inertia (CSMI, mm⁴) in those with incident and prevalent T2DM relative to those without T2DM in each of the cohorts and as meta-analysed overall estimates. Estimates were adjusted for age, sex, time between baseline and follow-up examinations, baseline CSMI, height, body mass index, education, smoking status, physical activity, and comorbidity (Charlson's comorbidity index in SMCC and ULSAM and cardiovascular disease diagnosis in PIVUS). T2DM, type 2 diabetes mellitus; CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men.



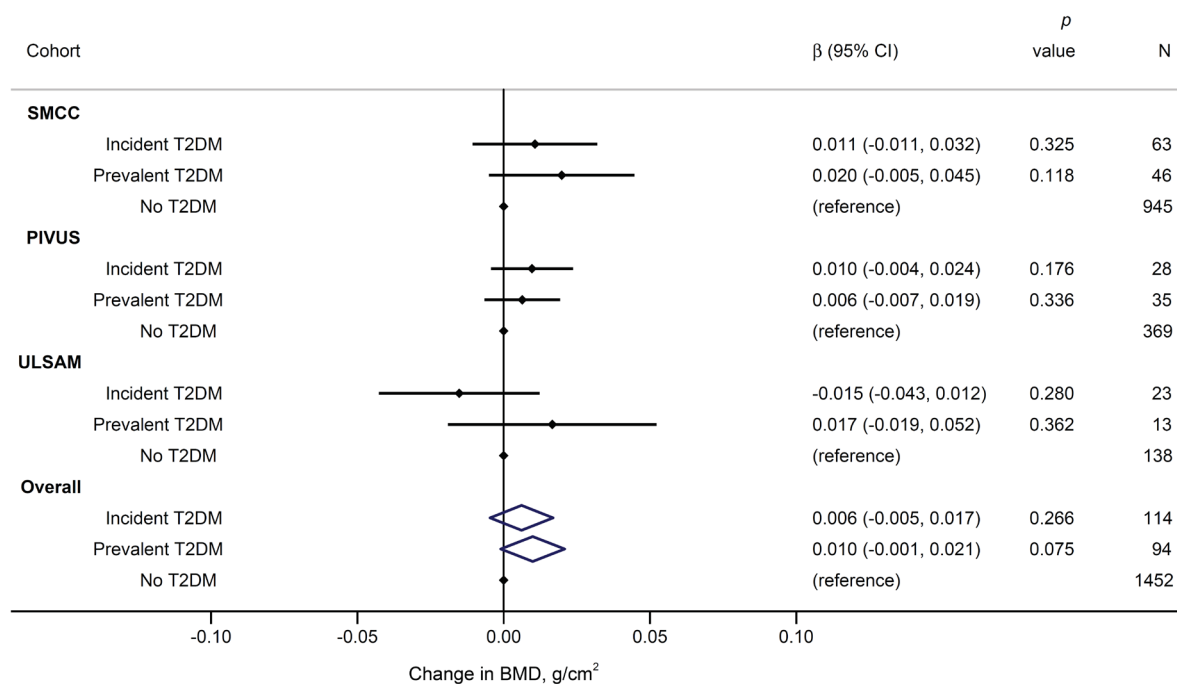
Supplementary Fig. 3. Change in bone area at the total hip (cm^2) by categories of normal fasting glucose (NFG), impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM) at baseline and follow up in each of the cohorts and as meta-analysed overall estimates. NFG at both baseline and follow-up (NFG-NFG) was used as reference category. Estimates were adjusted for age, sex, time between baseline and follow-up examinations, baseline bone area at the total hip, height, body mass index, education, smoking status, physical activity, and comorbidity (Charlson's comorbidity index in SMCC and ULSAM and cardiovascular disease diagnosis in PIVUS). CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men.



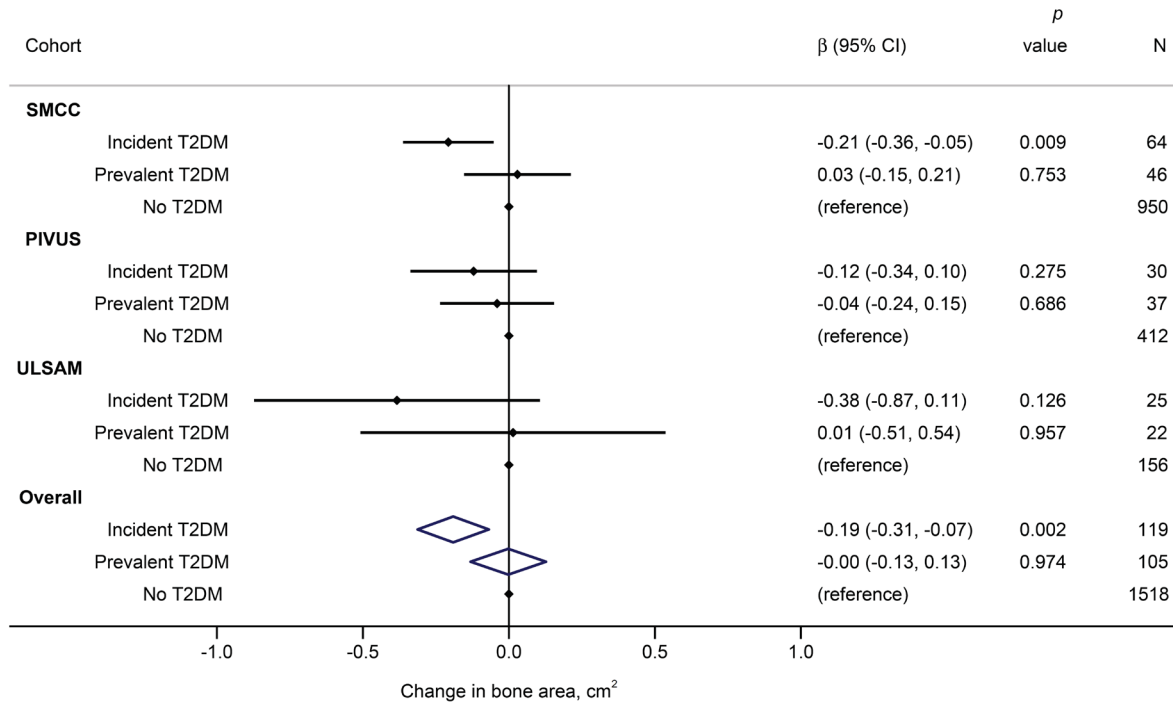
Supplementary Fig. 4. Change in bone mineral density (BMD) at the total hip (g/cm^2) by categories of normal fasting glucose (NFG), impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM) at baseline and follow up in each of the cohorts and as meta-analysed overall estimates. NFG at both baseline and follow-up (NFG-NFG) was used as reference category. Estimates were adjusted for age, sex, time between baseline and follow-up examinations, baseline BMD at the total hip, height, body mass index, education, smoking status, physical activity, and comorbidity (Charlson's comorbidity index in SMCC and ULSAM and cardiovascular disease diagnosis in PIVUS). CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men.



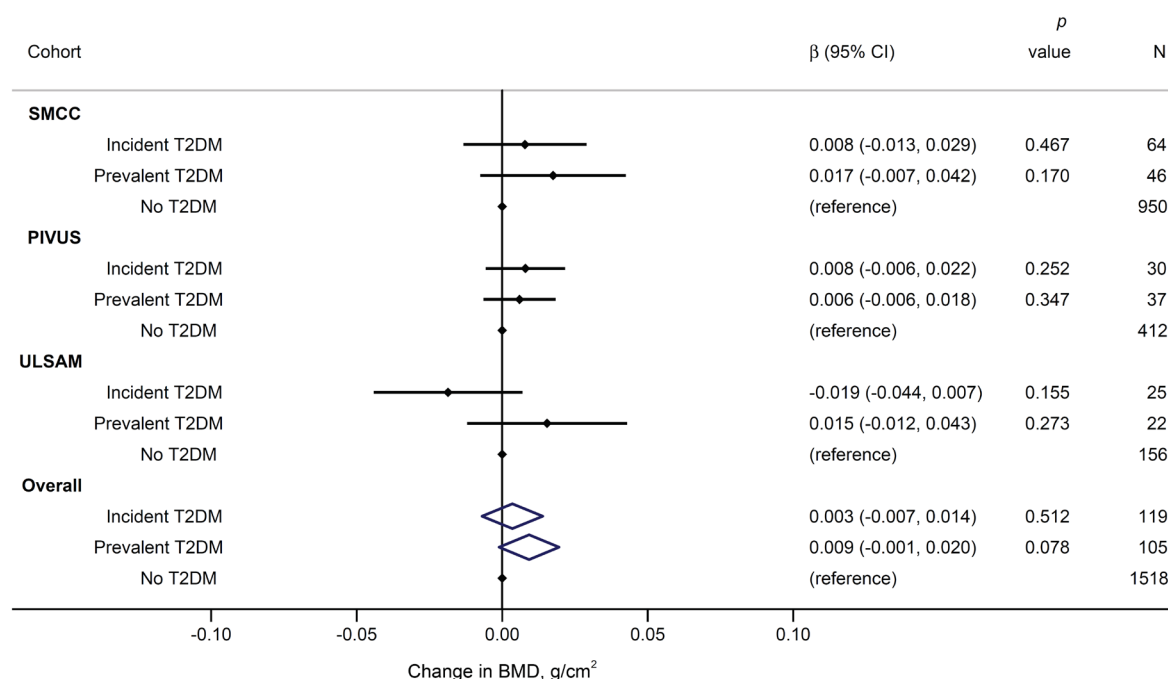
Supplementary Fig. 5. Change in bone area at the total hip (cm²) in those with incident and prevalent T2DM relative to those without T2DM in each of the cohorts and as meta-analysed overall estimates, with additional adjustment for dietary factors. Estimates were further adjusted for age, sex, time between baseline and follow-up examinations, baseline bone area at the total hip, height, body mass index, education, smoking status, physical activity, and comorbidity (Charlson's comorbidity index in SMCC and ULSAM and cardiovascular disease diagnosis in PIVUS). T2DM, type 2 diabetes mellitus; CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men.



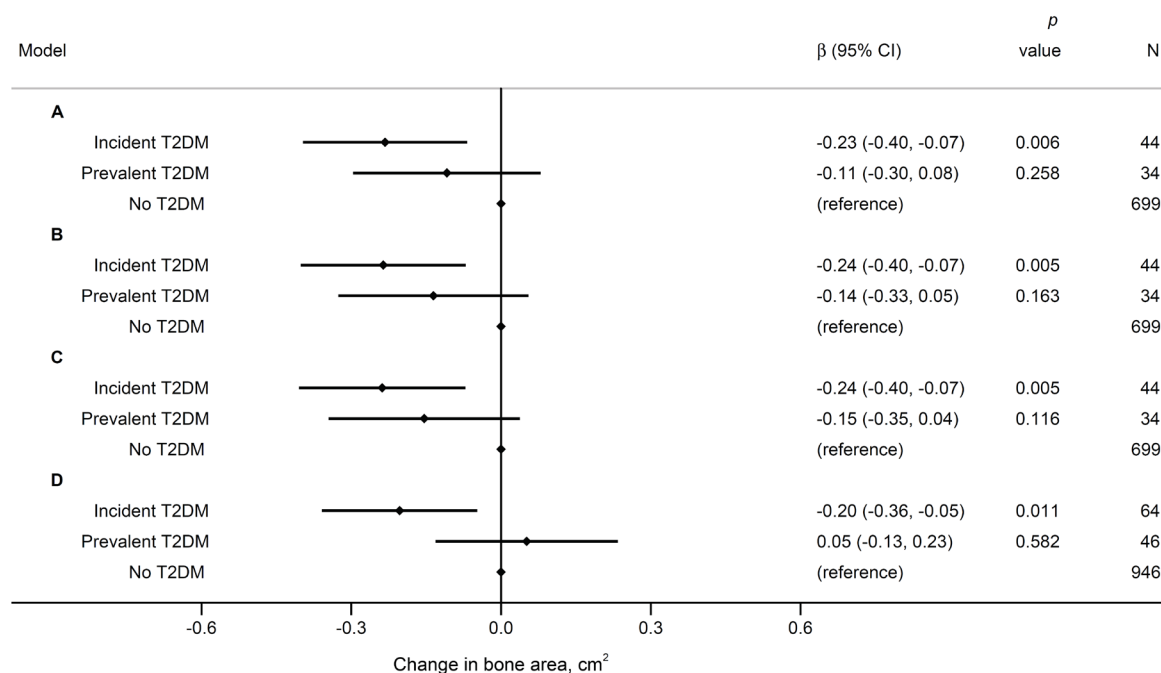
Supplementary Fig. 6. Change in bone mineral density (BMD) at the total hip (g/cm²) in those with incident and prevalent T2DM relative to those without T2DM in each of the cohorts and as meta-analysed overall estimates, with additional adjustment for dietary factors. Estimates were further adjusted for age, sex, time between baseline and follow-up examinations, baseline BMD at the total hip, height, body mass index, education, smoking status, physical activity, and comorbidity (Charlson's comorbidity index in SMCC and ULSAM and cardiovascular disease diagnosis in PIVUS). T2DM, type 2 diabetes mellitus; CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men.



Supplementary Fig. 7. Change in bone area at the total hip (cm²) in those with incident and prevalent T2DM relative to those without T2DM in each of the cohorts and as meta-analysed overall estimates, with additional adjustment for total fat mass, total lean mass, and the difference in total fat mass and lean mass between the first and second examination. Estimates were further adjusted for age, sex, time between baseline and follow-up examinations, baseline bone area at the total hip, height, body mass index, education, smoking status, physical activity, and comorbidity (Charlson's comorbidity index in SMCC and ULSAM and cardiovascular disease diagnosis in PIVUS). T2DM, type 2 diabetes mellitus; CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men.



Supplementary Fig. 8. Change in bone mineral density (BMD) at the total hip (g/cm²) in those with incident and prevalent T2DM relative to those without T2DM in each of the cohorts and as meta-analysed overall estimates, with additional adjustment for total fat mass, total lean mass, and the difference in total fat mass and lean mass between the first and second examination. Estimates were further adjusted for age, sex, time between baseline and follow-up examinations, baseline BMD at the total hip, height, body mass index, education, smoking status, physical activity, and comorbidity (Charlson's comorbidity index in SMCC and ULSAM and cardiovascular disease diagnosis in PIVUS). T2DM, type 2 diabetes mellitus; CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; ULSAM, Uppsala Longitudinal Study of Adult Men.



Supplementary Fig. 9. Change in bone area at the total hip (cm^2) in those with incident and prevalent T2DM relative to those without T2DM in the SMCC, with additional adjustments for drug use and serum concentrations of calcium, vitamin D, and parathyroid hormone.

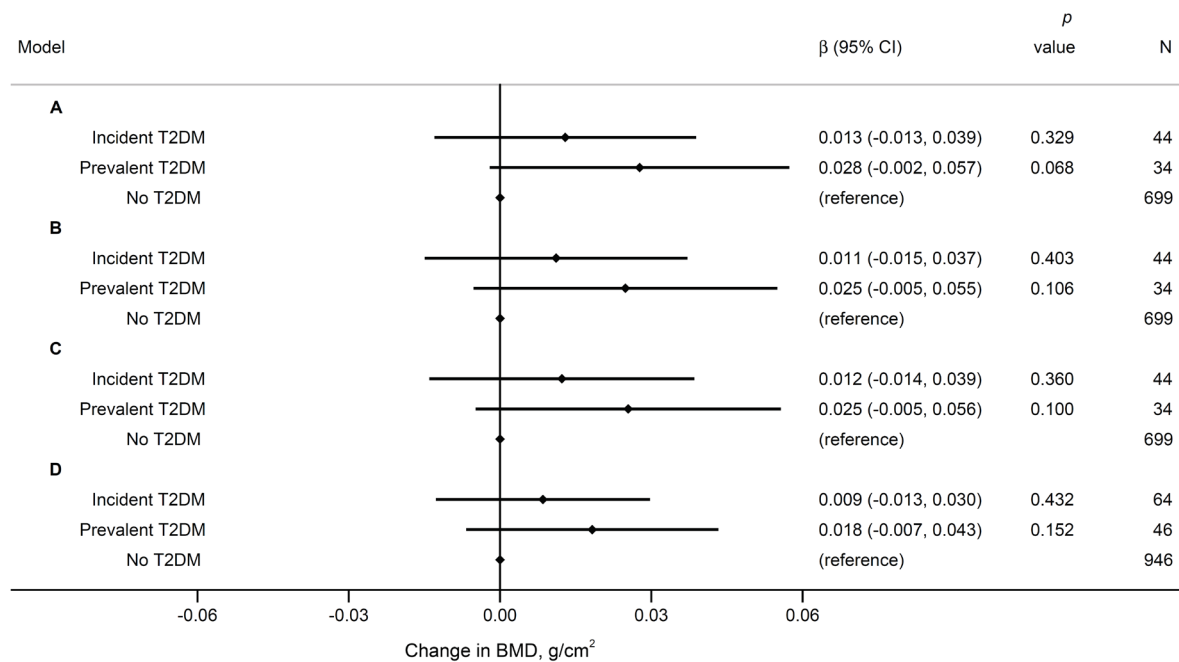
A. Estimates were adjusted for age, sex, time between baseline and follow-up examinations, baseline bone area at the total hip, height, body mass index, education, smoking status, physical activity, and Charlson's comorbidity in the subgroup with available information from the prescribed drug registry (including those examined after 1 July 2005).

B. Covariates as in A and baseline prescribed drugs: cardiovascular drugs (ATC C1-C9), lipid lowering drugs (ATC C10), drugs affecting thyroid function (ATC H03), drugs with effects on bone metabolism (ATC M05B), estrogen replacement therapy (ATC G03C G03F).

C. Covariates as in B and prescribed drugs during follow-up (same categories as in B).

D. Covariates as in A and baseline concentrations of calcium, vitamin D, and parathyroid hormone, and season of blood draw.

ATC, Anatomical Therapeutic Chemical Classification; T2DM, type 2 diabetes mellitus; CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical.



Supplementary Fig. 10. Change in bone mineral density (BMD) at the total hip (g/cm^2) in those with incident and prevalent T2DM relative to those without T2DM in the SMCC, with additional adjustments for drug use and serum concentrations of calcium, vitamin D, and parathyroid hormone.

A. Estimates were adjusted for age, sex, time between baseline and follow-up examinations, baseline bone area at the total hip, height, body mass index, education, smoking status, physical activity, and Charlson's comorbidity in the subgroup with available information from the prescribed drug registry (including those examined after 1 July 2005).

B. Covariates as in A and baseline prescribed drugs: cardiovascular drugs (ATC C1-C9), lipid lowering drugs (ATC C10), drugs affecting thyroid function (ATC H03), drugs with effects on bone metabolism (ATC M05B), estrogen replacement therapy (ATC G03C G03F).

C. Covariates as in B and prescribed drugs during follow-up (same categories as in B).

D. Covariates as in A and baseline concentrations of calcium, vitamin D, and parathyroid hormone, and season of blood draw.

ATC, Anatomical Therapeutic Chemical Classification; T2DM, type 2 diabetes mellitus; CI, confidence interval; SMCC, Swedish Mammography Cohort Clinical.